Enhancing quality in translational research

Learning from experiences in cardiac repair

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Learning from experiences in cardiac repair

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(met een samenvatting in het Nederlands)

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CHAPTER 1

Introduction and thesis outline

INTRODUCTION

Cardioreparative research studies the potential to use cell-based or cell-free therapeutics to heal the damaged heart, often targeting ischemic heart diseases. New therapeutic strategies are studied throughout the entire translational axis simultaneously: from basic research to clinical trials (figure 1). Promising results obtained in preclinical testing do not always translate to clinical successes. Several vulnerabilities in preclinical research may contribute to this limited translation.

In the first part of this thesis we discuss key issues in the design, conduct and reporting of translational research that hamper the progress of translation: 1) poor internal study validity, 2) incomplete reporting and 3) selective reporting of favourable results. These issues are observed in animal studies in the field of cardiac repair, but are not specific for this field of research. We then provide clear recommendation as a solution for these issues. In the second part of this thesis we focus on cardiac repair, more specifically on cell delivery techniques to optimize cardiac retention. We aimed to perform rigorous research and therefore optimized study designs based on the findings from part one of this thesis.

Translational failure

Biomedical research aims to improve quality of life and life expectancy of humans. New treatment strategies are developed through in vitro studies and laboratory animal science. However, translation of promising results to clinical successes is limited: only

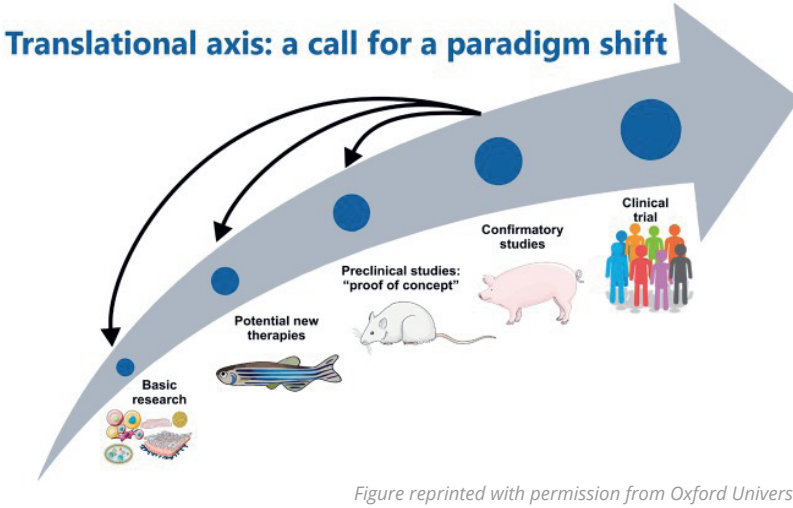

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Figure 1. Translational axis showing the process from in vitro research to clinical trials. Study results from each stage could help to advance to next stage, but could also require further research from an earlier stage.

11% of therapies tested in first-in-man studies becomes a registered therapy[2–5]. This might partly be explained by increased model complexity from mouse to man, but both impaired internal study validity and incomplete data reporting contribute also to this translational failure.

Risk of bias

Studies are vulnerable for systematic errors that cause results to deviate from the truth. For example, people have the tendency to search for, interpret, favour and recall data that support their hypothesis (confirmation bias). Specific subjects can be favoured to one specific study arm (selection bias) and data supporting one's hypothesis can be shared selectively and quicker (reporting bias). Optimizing study designs can limit the risk of bias and optimize the internal study validity. The most reliable study design is a randomized, blinded, controlled trial and systematic reviews on such studies (table 1)[6]. Randomization and blinding are often lacking in preclinical trials in the field of cardioreparative research, in accordance with a broad range of research fields[3,7–9]. Additionally, complete and transparent sharing of details of experiments is relevant to be able to fully interpret the results and to reproduce findings[10]. In other words, results of studies should be shared to properly inform colleagues and allow the study to contribute to the search for new treatments. This is true for studies in which the *a priori* hypothesis is confirmed, but also sharing of unexpected or unfavourable results is important to inform the research field and to prevent colleagues to repeat an experiment already performed. Currently, not all performed studies are published and not all findings are presented[11,12]. Instead, results with positive or more beneficial results have a higher probability of being published and are published quicker[13]. This effects systematic reviews, which use results of multiple individual studies to provide stronger evidence. Results of systematic reviews are influenced by missing studies and may lead to overestimation of effect sizes[14].

Table 1. Measurements to reduce the risk of bias in randomized, blinded, controlled trials.

Measurement	Effect
Controlled trials	Subjects undergoing the intervention are compared to a group treated identically except for the experimental intervention
Randomisation	Ensures subjects in the study arms are comparable
Blinding	Either the subjects or the researchers are unaware of the treatment allocation
Double blinding	Both the subjects and the researchers are unaware of the treatment allocation
Blinding of outcome assessment	Outcome assessors are unaware of the treatment allocation

Ethical aspect of animal studies

Specifically in animal testing, where animal lives are sacrificed for human benefits, ethical considerations play an important role. The estimated global use of animals for scientific procedures is 80 million animals on a yearly base (all species included)[15,16]. To put that in perspective, with a world population of circa 8 billion people, overall per year one laboratory animal is used for every 100 humans. In some cases it is mandatory to test in animals (e.g. safety testing), in addition animals are used for teaching purposes, to generate new pathophysiological theories of disease and to demonstrate treatment effects[17]. If we use animal lives to improve life expectancy or quality of life of human beings, experiments and production of data should be conducted as robust as possible.

Ischemic heart disease

Ischemic heart disease is the leading cause of death worldwide, responsible for 16% of global deaths. The incidence is increasing, with an estimated global mortality up to 8.9 million deaths in 2019[18]. Ischemic heart disease, also called coronary artery disease, involves all cardiac diseases induced by inadequate blood supply to the heart, and is often caused by atherosclerosis in the epicardial coronary arteries. Ischemic heart disease includes acute coronary syndromes (e.g. myocardial infarction), chronic coronary syndromes and ischemic cardiomyopathy. Myocardial infarction is defined as myocardial cell death due to prolonged ischaemia[19]. The most important treatment of an acute myocardial infarction is timely reperfusion therapy to reduce injury of the heart[19]. However, revascularization does not heal the already infarcted tissue and the restoration of blood flow induces additional damage called reperfusion injury[20]. This is caused by reactive oxygen and the inflammatory response, which leads to cellular dysfunction and cell death. Infarcted tissue will ultimately be replaced with scar tissue. The heart adapts to the new situation by both physiological (adaptive) and pathological remodelling, leading to changes in size, mass and function. This cardiac remodelling can cause left ventricular dysfunction and can consequently induce heart failure[19,21,22]. Ischemia is the most common aetiology of heart failure, as about 60% of all cardiomyopathies are induced by ischaemia[23,24]. Common non-ischemic aetiologies of heart failure include hypertension, toxic, infection, genetic or idiopathic cardiomyopathy[23]. Paradoxically, lifestyle adjustments and pharmacological treatment strategies have increased survival after myocardial infarction, which has led to an increased incidence of heart failure[25,26]. Nowadays, approximately 6-10% of patients who have suffered from a ST-segment elevation myocardial infarction develop heart failure over time[24,27]. Heart failure occurs in 1-2% of the adult population in developed countries, rising to over 10% among people >70 years of age and the prevalence of heart failure is expected to grow progressively due to ageing of the population and improved treatments[21,28].

Cardioreparative therapy

Rationale

The heart has long been assumed to be a post-mitotic organ, meaning that cardiomyocytes are not able to proliferate and thus damaged cardiac tissue cannot be replaced with new cardiomyocytes. However, this assumption has been questioned, for example after researchers showed that the zebrafish is capable of regenerating the heart[29]. Additionally, radiocarbon dating studies showed postnatal cardiomyocyte renewal in humans[30]. These findings opened doors for a new research field: regenerative cardiology. The initial approach of regenerative cardiology was to heal the heart by differentiation of transplanted stem cells to form new cardiomyocytes. This concept was supported by breakthrough research in mice showing newly formed myocardium after bone marrow transplantation[31,32]. The first clinical steps followed rapidly and the first clinical study was published only 1 year later. Although this treatment appeared to be safe, despite a lower infarct size in the cell therapy group there was no effect on left ventricular ejection fraction (LVEF)[33]. In the following years researchers failed in their attempts to replicate seminal studies, questioning the base for cardiac regeneration[34]. Eventually over 30 studies in the field of cardiac regeneration, including landmark studies, were called to be retracted as evidence showed that data was fabricated and images manipulated[35,36].

In the meanwhile, many researchers had already attempted to regenerate cardiac tissue, considering different cell types, dose, route of administration, timing of administration and patient selection. At least 50 clinical trials were published in the first decade of regenerative cardiology, showing only a modest beneficial effect. Most were randomised controlled trials, and also some cohort studies were performed. Protocols between studies differed widely: most studies included patients with acute myocardial infarction or chronic ischemic heart failure. Different cell types were tested, most studies used bone marrow mononuclear cells. Cell preparation was performed in different ways, as well as timing of transplantation after myocardial infarction, cell doses and administration method. Sample sizes were small (range 10-204 patients) and the total number included in these trials is approximately 2600 patients[37]. In the following years it seemed like more meta-analyses on regenerative therapy were performed than clinical trials[38]. These meta-analyses showed moderate beneficial effects at most, with an improvement in LVEF of 2-4%[39,40].

Although the field was damaged by fraudulent results and current clinical results are disappointing, the potential to cure ischemic heart failure remains an unmet clinical need and the field developed new strategies to move cardiac regeneration forward. The original mechanism of cell therapy, true regeneration by differentiation of transplanted cells, was questioned by preclinical research[34]. An alternative hypothesis was acknowledged to be more likely: stem cells produce cytokines and growth factors that stimulate neovascularisation, decrease apoptosis, improve metabolism, increase

contractility and reduce cardiac remodelling[41]. This hypothesis was called the paracrine hypothesis. The focus shifted from true regeneration to cardiac repair. Researchers are focusing on different strategies to trigger heart repair, for example by alternative cell-based and cell-free therapeutics and optimizing delivery strategies (table 2).

Table 2. Several strategies to optimize cardiac repair.

Strategies	Examples
Cell-based therapeutics	Allogeneic cells, cardiomyocytes derived from pluripotent stem cells
Cell-free therapeutics	Exosomes, mRNA
Mode of delivery	Administration methods, use of cell carriers, heart tissue patches

Cell retention

One factor potentially contributing to low efficacy of cardiac cell therapy is the high outwash of transplanted cells from the myocardium. Two routes of administration are commonly used to transplant cells to the myocardium, intracoronary infusions or intramyocardial injections.

Intracoronary infusion are performed via a percutaneous route. The coronary artery occluded during the myocardial infarction (the 'culprit') or supplying the damaged myocardium is often selected. This artery can be selected in advance based on the patients history. Blood flow in the selected artery is temporarily stopped by balloon occlusion, this allows diffusion of the therapeutic into the myocardium. The advantage of intracoronary infusions is its similarity to percutaneous coronary interventions used for revascularisation after acute myocardial infarction, and therefore interventional cardiologists are experienced with the technique and the procedure is quick and relatively safe.

Intramyocardial injections can be performed via two different approaches. The epicardial approach requires a thoracotomy. This approach is often chosen when patients are undergoing a planned thoracotomy, for example with coronary artery bypass graft surgery. The advantage is that surgeons have direct visualisation of the area of interest and the injection. The endocardial approach is performed percutaneously. Cells are preferable transplanted into the closest region of viable myocardium in the vicinity of the infarct area, also called the infarct border zone. An electromechanical map of the heart can be made prior to the injection to identify the infarct border zone[42]. This procedure is more time consuming and requires dedicated training of interventional cardiologists.

Previous studies showed that most transplanted cells, independent of route of administration, are immediately washed out of the heart via venous drainage and only 10-15% of transplanted cells remain in the heart[43,44]. Improving cardiac cell retention

could therefore be key in improving efficacy in reparative cardiology. In the second part of this thesis we focus on new transplantation methods to increase cardiac cell retention and enhance the beneficial effects of cell transplantation.

OUTLINE OF THIS THESIS

Part I - Enhancing quality in translational research

In **chapter 2** we discuss vulnerabilities that have entered biomedical research causing translational failure. We then discuss several solutions to optimize animal research, more specifically the value of registering of research protocols before the start of a study (preregistration). In **chapter 3** we show that preclinical meta-analyses need to be tailored to their specific purpose and statistics. In **chapter 4** we analyse the percentage of performed animal studies that are published. In addition we determined the number of animals used of which outcomes are not reported in publications. This study underlines the importance of sharing animal study protocols to provide an overview of all performed studies. In **chapter 5** we describe the development of the first registry dedicated to animal research (www.preclinicaltrials.eu) and evaluate it's 3-year existence. In **chapter 6** we describe a survey among animal researchers, aiming to better understand researchers' considerations on translational failure and their opinion towards preregistration as a solution for this problem.

Part II - Optimizing delivery techniques for cardiac repair

In part two we focus on optimizing delivery techniques for cardiac repair and aim to optimize our preclinical research based on the findings from part one of this thesis. In **chapter 7** we review literature on the efficacy of cardiovascular repair in the clinical arena and discuss shortcomings that need further research to contribute to improved efficacy of cardiovascular repair. In **chapter 8** we perform a Cochrane review and meta-analysis on bone marrow-derived cell therapy for acute myocardial infarction. In the following chapters we look at ways to improve cardiac retention after cell transplantation. In **chapter 9** we systematically review current literature on the effect of two different delivery methods on cardiac retention. In **chapter 10** we test the efficacy of both techniques in a head-to-head comparison in a confirmatory large animal study. In **chapter 11** we aim to show increased cardiac retention by using a cell barrier in a pig study.

In conclusion, this thesis evaluates strategies to improve translational success, focusing on cardiac repair. This is further explained in **chapter 12**, where we discuss the value of this thesis for current knowledge and how this might impact translational and human research.

REFERENCES

1. Fernández-Avilés F, Sanz-ruiz R, Climent AM, et al. Global position paper on cardiovascular regenerative medicine. *Eur Heart J*. 2017;38:1-20. doi:10.1093/eurheartj/ehx248
2. Zwetsloot PP, Végh AM, Jansen Of Lorkeers SJ, et al. Cardiac Stem Cell Treatment in Myocardial Infarction: A Systematic Review and Meta-Analysis of Preclinical Studies. *Circ Res*. 2016;118(8):1223-1232. doi:10.1161/CIRCRESAHA.115.307676
3. Jansen Of Lorkeers SJ, Eding JEC, Van Der Spoel TIG, et al. Similar effect of autologous and allogeneic cell therapy for ischemic heart disease: Results from a meta-analysis of large animal studies. *Eur Hear J*. 2013;34((Jansen Of Lorkeers S.J.; Eding J.E.C.; Van Der Spoel T.I.G.; Doevendans P.A.; Chamuleau S.A.J.) University Medical Center Utrecht, Department of Cardiology, Utrecht, Netherlands):280. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L71258557.
4. Fisher S a, Doree C, Mathur A, Martin-Rendon E. Meta-analysis of cell therapy trials for patients with heart failure. *Circ Res*. 2015;116(8):1361-1377. doi:10.1161/CIRCRESAHA.116.304386
5. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. 2004;3(8):711-715. doi:10.1038/nrd1470.
6. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Br Med J*. 2011;343(7829):d5928. doi:10.1136/bmj.d5928
7. Ramirez DD, Motazedian P, Jung RG, et al. Methodological Rigor in Preclinical Cardiovascular Studies: Targets to Enhance Reproducibility and Promote Research Translation. *Circ Res*. 2017;120(9):1916-1926. doi:doi: 10.1161/CIRCRESAHA.117.310628
8. Jonker SJ, Menting TP, Warlé MC, Ritskes-Hoitinga M, Wever KE. Preclinical evidence for the efficacy of ischemic postconditioning against renal ischemia-reperfusion injury, a systematic review and meta-analysis. *PLoS One*. 2016;11(3):e0150863. doi:10.1371/journal.pone.0150863
9. Hooijmans CR, Geessink FJ, Ritskes-Hoitinga M, Scheffer GJ. A systematic review of the modifying effect of anaesthetic drugs on metastasis in animal models for cancer. *PLoS One*. 2016;11(5):e0156152. doi:10.1371/journal.pone.0156152
10. Landis SC, Amara SG, Asadullah K, et al. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature*. 2012;490(7419):187-191. doi:10.1038/nature11556
11. Ross JS, Mulvey GK, Hines EM, Nissen SE, Krumholz HM. Trial publication after registration in ClinicalTrials.gov: A cross-sectional analysis. *PLoS Med*. 2009;6(9):e1000144. doi:10.1371/journal.pmed.1000144
12. Goldacre B, Drysdale H, Dale A, et al. COMPare : a prospective cohort study correcting and monitoring 58 misreported trials in real time. *Trials*. 2019;20(118). doi:10.1186/s13063-019-3173-2
13. Hopewell S, Clarke MJ, Stewart L, Tierney J. Time to publication for results of clinical trials. *Cochrane Database Syst Rev*. 2007;18(2):MR000011. doi:10.1002/14651858.MR000011.pub2
14. Sena ES, Bart van der Worp H, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*. 2010;8(3):e1000344. doi:10.1371/journal.pbio.1000344
15. Taylor K, Alvarez LR. An Estimate of the Number of Animals Used for Scientific Purposes Worldwide in 2015. *Altern to Lab Anim*. 2019;47(5-6):196-213. doi:10.1177/0261192919899853
16. Taylor K, Gordon N, Langley G, Higgins W. Estimates for Worldwide Laboratory Animal Use in 2005. *ATLA - Altern to Lab Anim*. 2008;36(3):327. doi:10.1177/026119290803600310
17. Kimmelman J, Mogil JS, Dirnagl U. Distinguishing between Exploratory and Confirmatory Preclinical Research Will Improve Translation. *PLoS Biol*. 2014;12(5):e1001863. doi:10.1371/journal.pbio.1001863
18. World Health Organization. The top 10 causes of death. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. Published 2020.
19. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth universal definition of myocardial infarction (2018). *Eur Heart J*. 2019;40(3):237-269. doi:10.1093/eurheartj/ehy462
20. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox Biol*. 2015;6:524-551. doi:10.1016/j.redox.2015.08.020
21. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2016;37(27):2129-2200. doi:10.1093/eurheartj/ehw128
22. Azevedo PS, Polegato BF, Minicucci MF, Paiva SAR, Zornoff LAM. Cardiac Remodeling: Concepts, Clinical Impact, Pathophysiological Mechanisms and Pharmacologic Treatment. *Arq Bras Cardiol*. 2016;106(1):62-69. doi:10.5935/abc.20160005
23. Shore S, Grau-Sepulveda M V., Bhatt DL, et al. Characteristics, Treatments, and Outcomes of Hospitalized Heart Failure Patients Stratified by Etiologies of Cardiomyopathy. *JACC Hear Fail*. 2015;3(11):906-916. doi:10.1016/j.jchf.2015.06.012
24. Gho JMIH, Postema PG, Conijn M, et al. Heart failure following STEMI: a contemporary cohort study of incidence and prognostic factors. *Open Hear*. 2017;4:e000551. doi:10.1136/openhrt-2016-000551

25. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J*. 2018;39(2):119-177. doi:10.1093/eurheartj/ehx393
26. Velagaleti RS, Pencina MJ, Murabito JM, et al. Long-term trends in the incidence of heart failure after myocardial infarction. *Circulation*. 2008;118:2057-2062. doi:10.1161/CIRCULATIONAHA.108.784215
27. Otero-García O, Cid-Álvarez AB, Juskova M, et al. Prognostic impact of left ventricular ejection fraction recovery in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: analysis of an 11-year all-comers registry. *Eur Hear Journal Acute Cardiovasc Care*. 2021;Jul 29:zuab058. doi:10.1093/ehjacc/zuab058
28. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. *Card Fail Rev*. 2017;3(1):7-11. doi:10.15420/cfr.2016:25:2
29. Poss KD, Wilson LG, Keating MT. Heart Regeneration in Zebrafish. *Science (80-)*. 2002;298(5601):2188-2190. doi:10.1126/science.1077857
30. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science (80-)*. 2009;324(5923):98-102. doi:10.1126/science.1164680
31. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature*. 2001;410(April):701-705.
32. Beltrami AP, Urbanek K, Kajstura J, et al. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med*. 2001;344(23):1750-1757. doi:10.1056/NEJM200106073442303
33. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation*. 2002;106(15):1913-1918. doi:10.1161/01.CIR.0000034046.87607.1C
34. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004;428(6983):664-668. doi:10.1038/nature02446
35. Drazen JM. Expression of Concern: Beltrami AP et al. Evidence That Human Cardiac Myocytes Divide after Myocardial Infarction. N Engl J Med 2001;344:17050-7 and Quaini F et al. Chimerism of the Transplanted Heart. N Engl J Med 2002;346:5-15. *N Engl J Med*. 2018;379(19):1870-1870. doi:10.1056/NEJMe1813801
36. Oransky I, Marcus A. Harvard and the Brigham call for more than 30 retractions of cardiac stem cell research. *Stat*. 2018;10(14):1-7. https://www.statnews.com/2018/10/14/harvard-brigham-retractions-stem-cell/.
37. Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. *Circulation*. 2012;126(5):551-568. doi:10.1161/CIRCULATIONAHA.111.086074
38. Martin-Rendon E. Meta-analyses of human cell-based cardiac regeneration therapies: What can systematic reviews tell us about cell therapies for ischemic heart disease? *Circ Res*. 2016;118(8):1264-1272. doi:10.1161/CIRCRESAHA.115.307540
39. Hirsch A, Nijveldt R, van der Vleuten P a, et al. Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE . *Eur Heart J*. 2011;32(14):1736-1747. doi:10.1093/eurheartj/ehq449
40. Gyöngyösi M, Wojakowski W, Navarese EP, Moye LA. Meta-analyses of human cell-based cardiac regeneration therapies: Controversies in meta-analyses results on cardiac cell-based regenerative studies. *Circ Res*. 2016;118(8):1254-1263. doi:10.1161/CIRCRESAHA.115.307347
41. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*. 2008;103(11):1204-1219. doi:10.1161/CIRCRESAHA.108.176826
42. van Slochteren FJ, van Es R, Gyongyosi M, et al. Three dimensional fusion of electromechanical mapping and magnetic resonance imaging for real-time navigation of intramyocardial cell injections in a porcine model of chronic myocardial infarction. *Int J Cardiovasc Imaging*. 2016. doi:10.1007/s10554-016-0852-x
43. van den Akker F, Feyen DAM, van den Hoogen P, et al. Intramyocardial stem cell injection: go(ne) with the flow. *Eur Hear*. 2017;38(3):184-186. doi:10.1093/eurheartj/ehw056
44. van der Spoel TI, Lee JC, Vrijsen K, et al. Non-surgical stem cell delivery strategies and in vivo cell tracking to injured myocardium. *Int J Cardiovasc Imaging*. 2011;27(3):367-383. doi:10.1007/s10554-010-9658-4

PART I

Enhancing quality in translational research

CHAPTER 2

Translational research in cardiovascular repair
A call for a paradigm shift

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ABSTRACT

The international consortium TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes) has recently addressed key priorities in the field of cell-based therapy for cardiac repair, identifying the efficacy of translational research as one of the main challenges to ultimately improve the quality of life of patients with ischemic disease. Much of the controversy and confusion surrounding cardiac regenerative therapy stems from insufficient rigor in the conduct of preclinical studies, and there is an increasing recognition of a number of problems that undermine its quality that may contribute to translational failure. Here, we introduce well defined stages for preclinical research, and put forth proposals that should promote more rigorous preclinical work, in an effort to improve its quality and translatability. To augment the utility of preclinical research and its translation, it is necessary to (1) improve the quality of preclinical research, (2) promote collaborative efforts, and (3) enhance the sharing of knowledge and protocols. In particular, confirmatory (stage III) preclinical studies should be considered as a preamble to clinical studies and therefore must adhere to their standards of quality (including internal validity, standardization of protocols, and multicenter design). To increase transparency and minimize bias, these studies should be prospectively registered in an independent, open database. Ultimately, these recommendations should be implemented in the daily routine of investigators and in the policies of institutions, journals, and funding agencies.

The international consortium TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes; https://www.tacticsalliance.org/) has recently addressed key priorities in the field of cell-based therapy for cardiac repair,[1] identifying the efficacy of translational research as one of the main challenges to ultimately improve the quality of life of patients with ischemic disease. With this article, written on behalf of the TACTICS consortium, we aim to increase awareness of several important issues in preclinical research and put forth proposals that should promote more rigorous preclinical work, in an effort to improve its quality and translatability. Ultimately, these recommendations should be implemented in the daily routine of investigators and in the policies of, institutions, journals, and funding agencies. Preclinical studies are necessary not only to understand basic mechanisms and thereby provide targets for new therapeutic strategies, but also to furnish information about the safety and efficacy of these new strategies. Furthermore, animal studies provide opportunities to test the feasibility of interventional or invasive approaches without the risk of harming humans. Obviously, these studies are performed in simplified models that do not completely reflect the clinical situation. Nevertheless, there is a correlation between effect size in preclinical studies and clinical trials for cardiac regeneration, although efficacy tends to drop when one moves from small animal models (Δ left ventricular ejection fraction, 12%)[2] to large animal models (Δ left ventricular ejection fraction, 8%)[3] and to humans (Δ left ventricular ejection fraction, 4%).[4] This is partly explained by increased model complexity.[5] However, it is also clear that research methodology in preclinical studies also needs to be optimized. There is an increasing recognition of several problems that undermine the quality of preclinical research.[3,6–8] Basic features of sound research such as randomization, blinding, and a priori sample size calculation, were found to be reported in less than one third of studies.[9,10] Qualitatively suboptimal research typically overestimates the true effect size, and hypotheses based on this research are not tested in rigorous models before entering the clinical arena. Consequently, these scientific flaws may well contribute to translational failure. To augment the utility of preclinical research and its translation, and to advance cardiac cell therapy, it is necessary to (1) improve the quality of preclinical research, (2) promote collaborative efforts, and (3) enhance the sharing of knowledge and protocols.

Translational Research: A Call for a Paradigm Shift

To date, the majority of preclinical work in the field of cell therapy has consisted of mechanistic studies in small animal models. Although this approach has yielded important insights into the effects of cells and cell products at a cellular and tissue level, the wide range of models and protocols used has inadvertently led to heterogeneous results of both preclinical and clinical studies that are difficult to compare, thereby limiting translatability.[4,11,12] Notwithstanding these limitations, cardiac

reparative efforts have advanced to the clinical arena, and clinical studies have shown that cell therapy is safe.[4] However, the efficacy of cell therapy in patients remains uncertain, principally because the results of clinical trials have been inconsistent or conflicting.[4,11,12]

We think that at least part of the uncertainty about cardiac regenerative therapy is caused by suboptimal preclinical research and that work at the preclinical level needs to be strengthened and standardized to increase reproducibility and enable data comparison. To advance the field, there is an increasing need for a novel preclinical framework with a stronger translational focus. Preclinical studies can be subdivided in 3 stages: (1) discovery and development of a lead cell product, (2) exploratory phase, and (3) confirmatory phase (Table 1). Each stage should focus on its specific strengths, and careful consideration should be given to choosing proper experimental models and protocols to address a particular research question.

Table 1. Classification of preclinical stages for cardiac regenerative/reparative research. *Typical examples of the different stages of preclinical research. These examples can be complemented by many other examples.

	Stage I	Stage II	Stage III
Aim	Fundamental information to understand biology	Exploratory studies	Confirmatory studies
Models (most frequently used)	In vitro studies Zebrafish Genome-wide assocation studies	Rodent models	Large animal models
Goals	• Understand the basic mechanisms involved in cardiac regeneration/repair • Discover new proteins and pathways	• Hypothesis- generating research • Dose-response relationships • Cell-tracking; cell state and phenotype • Improving retention rate	• Confirm or reject well-thought out hypotheses • Demonstration of safety and indication of efficacy
Examples*	In vitro study to investigate the role of a specific protein in stem cell function[24]	Mouse study to examine whether a specific cell type differentiates into cardiomyocytes[25]	Randomized, placebo-controlled, blinded pig study to investigate the safety and efficacy of a specific cell type[26]

Stage I: Discovery and Development of Lead Cell Products

The emphasis in stage I studies is to provide fundamental information for advancing our understanding of biology (Table 1). This stage is the cornerstone for the discovery and development of new therapeutic products. In vitro studies are of great value at this stage, because a reductionistic model gives the opportunity to focus on a specific element, and human cells can be studied (eg, for the ability to transdifferentiate and

migrate). Besides in vitro studies, the zebrafish is an interesting model because of the great regenerative capacity of its heart, which can help us gain insights into the mechanisms of endogenous regeneration.[13] Additionally, genome-wide association studies can be performed to discover single-nucleotide polymorphisms related to disease, and to investigate mechanisms of cardiac diseases including the role of genes and proteins involved in cardiac regeneration and repair. Last, rodent models may contribute to the development of new products, in particular because genetic modifications can be introduced to elucidate and confirm mechanistic insights.

Stage II: Exploratory Studies

The goal of stage II studies is to perform hypothesis-generating research, providing feedback that guides the creative efforts of the scientist (Table 1). Stage II studies should be based on a sound scientific rationale and should be relatively easy and affordable. This stage of preclinical work is predominantly performed in rodent models, which are well-suited to serve as screening tools. Systematic reviews of relevant data in the literature, including meta-analyses, are also considered exploratory studies. When a product is found promising, the presence of a dose–response relationship should be investigated to avoid under- or overdosing in further research, potentially leading to ineffectiveness or harmful side effects, respectively. Furthermore, different cells, cell products, or combinations of cell therapies can be properly compared only if optimal doses are used. At present, a significant dose–response relationship has been observed in several clinical trials, but the nature of this relationship is still unknown for most cell types.[14] Furthermore, body weight should be taken into account when a dosage is applied to another model or to humans.[2,15]

Another important aspect that can be addressed in exploratory studies is the ability to monitor cell state and phenotype. Tracking of long-term cell fate after delivery to the heart can be performed adequately in preclinical studies, thereby providing important insights, but most cell labeling techniques used in preclinical settings cannot be safely translated to human studies.[16] In the majority of clinical trials performed to date, cells have been delivered to the myocardium using percutaneous approaches by either intracoronary or transendocardial injection.[17] Each technique has its advantages and disadvantages and is associated with different myocardial cell retention rates.[18] Although improving the retention rate requires explorative research, this issue can only be investigated in a large animal model, because the similarity in heart size allows catheter handling in these animal models. This example nicely illustrates that not every exploratory study has to be conducted in a small animal model.

Stage III: Confirmatory Studies

Confirmatory studies are the final step in the translational axis, before entering the clinical arena. Once a product has successfully passed stage II studies, confirmation of

safety, feasibility, and efficacy is critical. These confirmatory studies should be considered as the preamble to clinical studies. Therefore, they should adhere to the general standards of clinical studies about quality, including registration (Table 2). Furthermore, to enhance transparency and reproducibility, these studies should be performed in a standardized manner, in accordance with scientific consensus in the field on best practices, and methods should be properly reported.[7] With this approach, sound hypotheses can be either confirmed or rejected. Such investigations help tremendously to define and underpin the next step in the translational axis. Only large animal models enable testing of intracoronary or transendocardial delivery. Although studies in large animal models are typically complex, time-consuming, expensive, and technically demanding, they offer the advantage of being conducted in settings as close as possible to the human situation.[5] Thus, they are preferred in stage III research on cardiac repair and are essential to justify the risks and costs of clinical trials.

Preclinical investigations should be conducted in a standardized and reproducible manner to yield robust data. However, reproducibility rates in biomedical research are very low.[7] For example, in hematology and oncology, only 11% of landmark studies can be reproduced.[19] When investigated with computer simulations, a research claim is more likely to be false than true.[20] Replicating genetic association studies by reperforming statistics led to a statistical reproducibility of only 44%, although the same data were being analyzed.[21] These findings are worrisome. Because the degree of reproducibility reflects both the quality of research and the quality of reporting, efforts need to be made in both of these areas. In this respect, standardized protocols (eg, induction of myocardial infarction, route of product delivery, etc) should be shared and rigorous statistical methods should be applied.

Following these recommendations opens the door to a challenging, but very important, aspect of confirmatory studies: multicenter preclinical studies. In the clinical arena, multicenter trials obviate the confounding influences of differences in work environment, technical details, and caregiving among centers. Moreover, clinical single-center randomized controlled trials are prone to overestimate the effect compared with multicenter randomized controlled trials.[22,23] Analogous considerations apply to the preclinical arena. It is particularly important that such joined studies be performed by specialized laboratories with relevant expertise to ensure data quality and reproducibility.[24–27] These collaborative networks should perform blinded, randomized, and adequately-powered studies in relevant disease models and species. Data analysis should be performed by blinded and independent core centers. With this approach, reproducibility of outcomes can be carefully verified among different laboratories. End points, sample size, exclusion criteria, and methods for handling missing data must be established a priori. Furthermore, these collaborations should be transparent in describing and justifying the animal models and protocols used and should put effort into optimizing them (eg, to increase translatability and decrease mortality). Obviously,

the centers participating in these consortia should have considerable experience and expertise in the complex models and methods involved in large animal research.

The CAESAR (Consortium for Preclinical Assessment of Cardioprotective Therapies) consortium,[24,26] which provided an infrastructure for multicenter studies of cardioprotective therapies, was a good example of a preclinical network in which experienced laboratories worked together to study potentially effective therapies with a level of rigor similar to that of clinical multicenter randomized controlled trials. CAESAR was a public resource, available to all National Institutes of Health funded investigators. Together with other multicentre preclinical consortia in other fields,[25] it embodied the current paradigm shift in preclinical research. The experience of this consortium has demonstrated that several drugs claimed to be effective in single-center studies were ineffective when subjected to the rigorous CAESAR test.[28,29] Identifying therapies that are truly effective and reproducible at a preclinical level is essential to avoid inappropriate, costly, and potentially harmful clinical trials that are unlikely to show any treatment effect. Future confirmatory preclinical studies of cardiac regeneration/repair should be performed using infrastructures similar to that of CAESAR. To ensure better translation of preclinical research, it is essential that the end points of preclinical studies be defined in a manner similar to those of phase II/III clinical trials.[30] For cardiac regeneration/repair, this implies that these end points are anticipated to be affected by the hypothesized mechanism of action of cell therapy. Furthermore, proper standardized outcome assessments should be performed for these carefully selected end points. The duration of follow-up after cell administration should be adequate to allow for similar event rates as expected in phase II clinical trials.

Table 2. Features of a rigorous confirmatory study performed in multicenter consortia

Solid null hypothesis, based on	- Stage II studies - Preclinical meta-analyses
Multicenter consortium	- Experience and expertise in the field - Establish and optimize animal models - Independent data analyses by core center - Validated reproducibility - Standardized protocols
Quality and rigor	- Multicenter studies - Blinded - Randomized - Adequately powered - *A priori* establishment of sample size, exclusion criteria and handling of missing data - Endpoints and follow-up time comparable to phase II clinical trials - Standardized protocols and outcome assessment
Reporting	- Prospective registration - Share protocols - Use ARRIVE guidelines

Strategies to Improve the Quality of Preclinical Research

The translation of preclinical work into clinical success is a great challenge in every research field, because only 11% of all first-in-man studies lead to the registration of a therapy.[33] Overall, an estimated 85% of all research investments is wasted.[34] Although this translational failure can be partially improved by setting the right research agenda and choosing the proper model and protocol, methodological flaws take part of the blame as well. Figure 1 shows strategies to improve the translational axis of preclinical research.

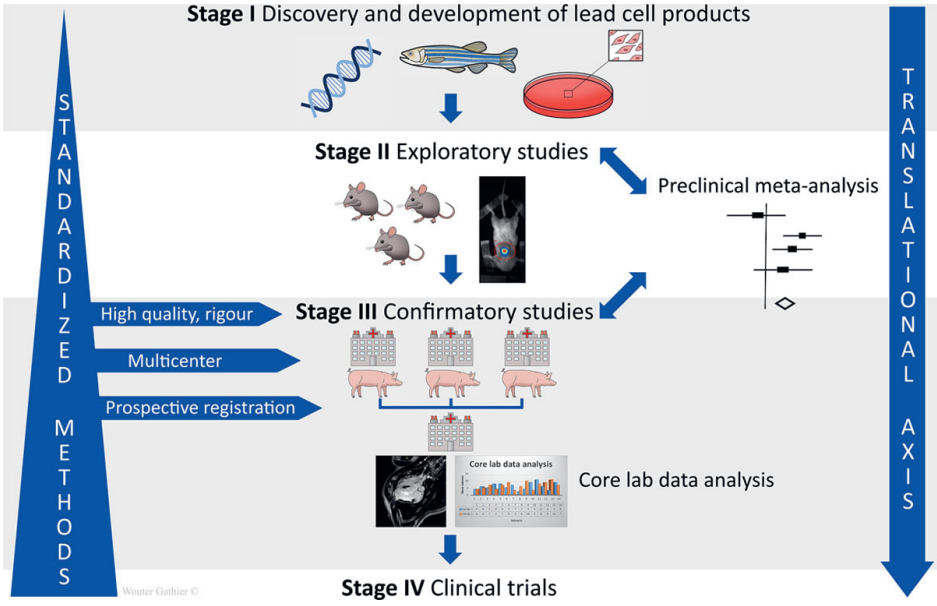

Figure 1. Translational axis, with **stage I studies** (including *in vitro* studies and zebrafish models) to investigate basic mechanisms and discover new proteins and pathways; **stage II studies** (exploratory studies), predominantly in mouse models, to conduct hypothesis-generating research and initial *in vivo* testing; and **stage III studies** (confirmatory studies), as the last step before entering the clinical arena, involving rigourous research in a multicentre setting, with prospective registration of the key features of the study. Pre-clinical meta-analyses are performed with data from both exploratory and confirmatory studies, with the goal of giving an overview of available data, pointing out the gaps, and providing a focus for future preclinical research. The level of standardization of methodology increases through the translational axis.

Publication Bias

Positive or significant results are more likely to be published[35,36] and are published faster.[37] This undesirable phenomenon, referred to as, publication bias, leads to an overestimation of the effects of therapies.[38,39] The causes of publication bias are found within the research team at the editorial board or funders/institute level (Figure 2). It is conceivable that there can be selective data presentation by the researchers (based on belief or external influences) and that, at times, publication may not be seen as

rewarding, because it can be more attractive to follow-up on positive results than to invest time, money, and energy in trying to publish negative results (which can be arduous). But biased reviewing and decision-making by reviewers and editors who prioritize positive studies may also play a role. Also, funders and institutes could mandate publication of results. In general, all of these reasons are not acceptable and the resulting publication bias should be avoided. To limit publication bias, it is important that the scientific community be open to a radical change in the way researchers are evaluated and how knowledge is shared. To promote sound preclinical research and limit unnecessary animal use, it is highly advisable that all results and data be available to the community. The classic publication strategy (ie, peer review) needs to be evaluated and optimized to achieve such an outcome. Although the proliferation of journals has made it easier to publish, it is hard to publish negative results in high-impact journals. The failure to publish negative data in journals not only distorts the perception of an issue, but also causes researchers worldwide to repeat studies unnecessarily, because they are unaware of the original study results. This can be prevented if protocols and results are shared. Novel publication strategies (open access journals; data sharing on dedicated websites), as well as focused issues for such studies in existing journals as proposed by Dirnagl et al,[40] can overcome this problem. It is essential that journals adhere to the National Institutes of Health recommendation about refutation, meaning that a paper that contradicts previous positive findings will not be penalized by the editors for not being the first if its quality is consistent with the journal's standards.[8]

The concept of Registered Reports is another novel publishing strategy that seems worthy of consideration by journals. In this scenario, an experimental design, instead of the final results, is peer-reviewed. Approved study proposals are offered in-principle acceptance and authors can then start collecting the data. The manuscript will be published under the condition that the authors follow through strictly with the registered methodology, independently of the direction or significance of the results.[41] This system would promote unbiased publication of high-quality research. Interestingly, studies funded by the Health Technology Assessment program of the National Institute for Health Research (United Kingdom) stand out in the field, with a publication rate of 98%. This high publication rate is stimulated by the fact that part of the grant is withheld until a report has been submitted. This financial reward serves as an incentive, and encourages investigators to write and publish their results. Naturally, caution must be taken to ensure that the financial stimulus is not abused, leading to fraudulent studies and poor quality papers. The Health Technology Assessment program provides a robust monitoring process of manuscripts submitted for publication; the existence of a dedicated journal (Health Technology Assessment; impact factor, 5.116) also contributes to the high publication rate.[42] If major journals are reluctant to publish negative studies, funding agencies (eg, the National Institutes of Health) may consider producing a journal in which these studies can be published.

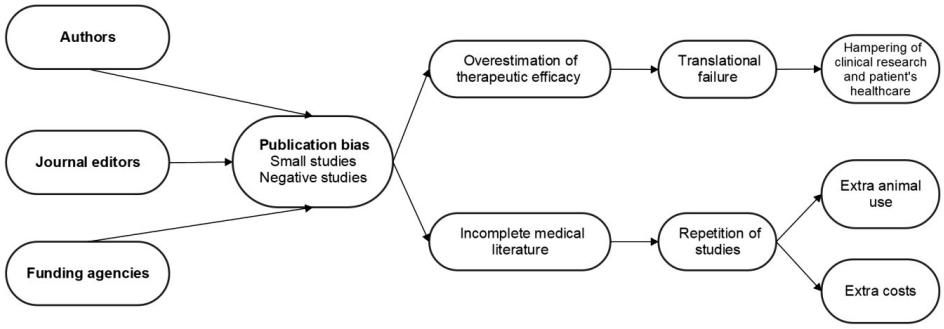

Figure 2. Causes and consequences of publication bias.

Table 3. Possible strategies that could contribute to a decrease in publication bias

Remedies suggested for publication bias	
Platforms for datasharing	Prospective registration Publication of study design Novel publication strategies (open access journals, negative results issues, registered reports) Journals provided by funders
Incentives to share data	Withhold part of the grant until after publication Re-evaluate ranking of researchers

Validity and Reproducibility

When testing a scientific hypothesis, it is of the utmost importance that the design, execution, analysis, and reporting be optimized.[43,44] A study should be conducted with minimized bias (internal validity) and should be generalizable to the target population (external validity).[43] Poorly-designed studies lead to an overestimation of the treatment effect.[9,43] Preclinical studies in the field of cardiac regenerative medicine need to improve with respect to internal validity and reporting.[3,7,9,10,45]

A detailed set of guidelines aimed at increasing rigor and reproducibility in preclinical research has recently been published.[8] As indicated therein, researchers should always report whether blinding and randomization were performed, and should justify when they choose not to do so. Moreover, preclinical studies should use sample size calculations to produce adequately-powered results; decisions about sample size should be made before the start of the study and exclusion criteria and outcomes should be predefined. Besides performing rigorous studies, it is also important to improve the quality of reporting. The *Circulation Research* guidelines or the ARRIVE guidelines for proper animal research publication should be incorporated in the study design.[8,46] These guidelines provide recommendations on several items (eg, title, abstract, objectives, study design, experimental animals, baseline data) to guide researchers in writing a manuscript that contains the information that is most essential

for reproducibility and transparency. Furthermore, the models and methods used in the study should always be published (eg, as an online supplement) with a level of detail that is sufficient for others to reproduce the published study. Minute details can have a major impact and should be included.

Preclinical Systematic Reviews and Meta-Analyses

Exploratory preclinical studies are often performed in theinitial phase of new ideas or strategies. Consequently, it is not uncommon that individual studies are performed without sample size calculation and are underpowered. In the clinical setting, systematic reviews and meta-analyses are used to provide an overview of the available evidence, increase power, and achieve a more robust end point estimate. Preclinical systematic reviews, often including meta-analysis, are becoming more common in general as well as within the field of cardioprotection.[47–49] Compared with the abundance of clinical meta-analyses in the field of cardiac regeneration/repair, there is a striking scarcity of preclinical meta-analyses. In the context of a systematic review, preclinical meta-analyses can be used to investigate the overall efficacy of a therapeutic agent in animal studies, and can therefore inform the researcher about the appropriateness of potential stage III preclinical studies or even clinical trials.[3,6] However, it should be noted that preclinical meta-analyses cannot guarantee that outcomes in human studies will show a comparable level of efficacy. When interpreting meta-analysis results, it is important to be aware of the possible biases in preclinical evidence (eg, publication bias).[50] Furthermore, heterogeneity is rather common. However, analysis of this heterogeneity can help identify factors that influence treatment efficacy—information that can then be used to improve the design of future preclinical and clinical studies. Systematic reviews can also point out areas in which there is a lack of solid evidence as well as promising areas on which further experiments should focus. In addition, systematic reviews can reveal flaws in study quality and therefore show the value of performed research.[51] At present, systematic reviews in the field of cardiac regeneration/repair are available for large animal studies, but not for research in small animal models.[3,52]

The CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data From Experimental Studies) framework is a powerful collaboration providing support for writing preclinical systematic reviews and meta-analysis. The collaboration has experience with performing preclinical systematic reviews and meta-analyses and offers assistance to researcher doing this type of research. This group hosts a database containing data from systematically performed meta-analyses. The CAMARADES group assists by providing quality control. Thanks to the large amount of data included, this database is an excellent platform to investigate study quality and bias.[9] Resources related to the conduct, training, and coaching of preclinical systematic reviews are available on the websites of CAMARADES (http://www.dcn.ed.ac.uk/camarades) and its national coordinating center SYRCLE (http://www.syrcle.nl) in The Netherlands.

Prospective Registration

Besides preclinical systematic reviews and meta-analyses, prospective registration of preclinical trials also increases transparency and public awareness of performed research. Moreover, a prospective registration will enable publishers, funders, and readers in general to assess whether the authors performed the study they set out to do. Sharing knowledge of research experience and available data is essential to the advancement of science. However, even in the realm of clinical trials, not even half of the studies are published.[53,54] Registration of planned preclinical studies should be encouraged to allow transparency and sharing of important information.[55,56] Such a registry provides an overview of ongoing/completed research and also offers a platform for subsequent study results. The registration could facilitate awareness of the parameters that are essential to increase study quality, by requiring investigators to address relevant issues about blinding, randomization, sample size calculation and power, and predetermined exclusion criteria. This will also give insight into reporting bias. Furthermore, the registry will lead to a reduction of unnecessarily repeated studies, animal use, and costs. A similar approach is currently considered the standard for clinical trials (http://www.clinicaltrials.gov), and now even serves as a prerequisite for publication. To increase participation, journals should implement the policy adopted by the International Committee of Medical Journal Editors for preclinical trials.[57] Possible disadvantages of patent sharing should be obviated by a fixed time lock until protocols are disclosed and by the assignment of an independent surveillant to control this collaborative web-based database. Pros and cons are summarized in Table 4. Although this registry should be implemented for research in general, it could very well start as a pilot program within the field of cardiac reparative therapy, where many stakeholders are supporting this initiative. This multidisciplinary, burgeoning, and rapidly evolving area involves the participation of basic scientists, translational scientists, and clinicians and is characterized by a rapid flow from bench to bedside. Because of these characteristics, in this field it is particularly important to have a clear overview of research that has already been performed and to use a prospectively described and shared protocol which, ideally, includes the features detailed above (blinding, randomization, prospective declaration of exclusion criteria, adequate sample size, etc). We have designed an example of such a register on https://preclinicaltrials.eu, a full checklist for registration included in https://preclinicaltrials.eu is provided as Supplementary Table 1. Therefore, we strongly recommend that all researchers involved in preclinical research register at least their stage III (confirmatory) animal studies on https://preclinicaltrials.eu or via the TACTICS website http://www.tacticsalliance.org/.

Table 4. Pros and cons of prospective registration of preclinical trials

PRO	CON
Datasharing	Sharing your (confidential) ideas/plans (with both scientists and public)
Increased transparency	Patents
Less animal use and therefore lower costs	Costs (management, website, hosting)
Increased methodological quality	Workload
Decreased publication bias	Limited flexibility for creativity of researchers
Improved translation into clinical research	
Better justification for the sacrifice of animals	
Advanced welfare of patients	

Research Integrity

Perhaps the most important factor in the conduct of proper science is the researcher's responsibility to follow the moral code of research integrity,[58] which is a sine qua non to move the field forward. In continuation of this, scientific decisions should not be conditioned by conflicts of interest. The National Institutes of Health defines research integrity as (1) the use of honest and verifiable methods in proposing, performing, and evaluating research; (2) reporting research results with particular attention to adherence to rules, regulations, and guidelines; and (3) following commonly accepted professional codes or norms. Unfortunately, there are numerous known cases in which an article was published that resulted from scientific misconduct or even falsification.[59] An even greater number of cases of scientific misconduct probably go undetected.[7] Not only should one have research integrity herself or himself, one should also teach young researchers this code. This requires an open ambiance in any research group, one that promotes a culture of research integrity.[41] In the Netherlands, all PhD students have to adhere to this policy through an official oath. It is also important that group leaders do not expect young investigators to generate data that necessarily validate the leaders' working hypothesis, and that data challenging the leaders' hypothesis be welcome. Furthermore, universities and scientific organizations should also contribute to promote integrity. Evaluation and ranking of researchers should for instance go beyond the count of their publications, and proper credit should be given for work that has been reproduced by others.[60]

Implementation in the Research Community

In this document, we have pointed out several important issues that may hamper successful translational preclinical research. We propose concrete recommendations to lower the risk of publication bias and increase the quality of experiments and study results, such as preregistration of trials, formation of multicentre consortia, and adherence to guidelines for scientific rigor. We realize that our proposed, transformative paradigm shift might be difficult to implement in a research culture in which publication

bias is deeply ingrained, and creativity and independence of investigators (rather than sharing, transparency, standardization, and confirmation) are incentivized.

Therefore, radical changes will be required at multiple levels (investigators, institutions, regulatory agencies, journals, and funding agencies). Obviously, actual implementation of our proposals in the research community is not within the power of this article, but improving the system starts with acknowledging the problems and promoting public awareness of their implications. We feel confident that leaders in this field of research will adopt our recommendations. Several recent developments support our optimism. Both the TACTICS consortium and the recently inaugurated European Society of Cardiology Working Group on Cardiovascular Regenerative and Reparative Medicine are very supportive of this endeavour, thereby establishing a best practice example from within. It is anticipated that in the near future these ideas will also positively affect funding agencies and other journal editors.

Conclusions

Researchers should carefully consider the proper stage, model, and protocol to address the research question at hand. Much of the controversy and confusion surrounding cell therapy stems from insufficient rigor in the conduct of preclinical studies. Confirmatory preclinical studies should be considered as a preamble to clinical studies and therefore should adhere to their standards of quality (including rigor, internal validity, standardization of protocols, and multicentre design). Rigorous and reproducible preclinical work gives a better estimate of the true effect size. Therefore, this is critical to reduce the number of negative clinical trials with their attendant cost, effort, risks to patients, and adverse impact on the public perception of the entire field of cell therapy. To increase transparency and minimize bias, all confirmatory preclinical studies should be prospectively registered in an independent, open database. Knowledge of performed research can be increased by preclinical systematic reviews and meta-analysis, which also give insight into promising areas that should be the focus of further research. We make a plea to establish multicentre networks to perform confirmatory preclinical studies with a level of rigor comparable to clinical trials, after the example of the CAESAR network in cardioprotection. If the field of cardiac regeneration/repair is to advance, it is essential that researchers follow rigorous research practices, collaborate, and share data and experiences.

REFERENCES

1. Fernández-Avilés F, Sanz-Ruiz R, Climent AM, et al; TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes) Writing Group; Authors/Task Force Members. Chairpersons; Basic Research Subcommittee; Translational Research Subcommittee; Challenges of Cardiovascular Regenerative Medicine Subcommittee; Tissue Engineering Subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee; Clinical Trials Subcommittee; Regulatory and funding strategies subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee. Global position paper on cardiovascular regenerative medicine. Eur Heart J. 2017;38:2532–2546. doi: 10.1093/eurheartj/ehx248.
2. Zwetsloot PP, Végh AM, Jansen Of Lorkeers SJ, van Hout GP, Currie GL, Sena ES, Gremmels H, Buikema JW, Goumans M-J, Macleod MR, Doevendans PA, Chamuleau SA, Sluijter JP. Cardiac stem cell treatment in myocardial infarction: a systematic review and meta-analysis of preclinical studies. Circ Res. 2016;118:1223–1232.
3. Jansen Of Lorkeers SJ, Eding JEC, Vesterinen HM, van der Spoel TIG, Sena ES, Duckers HJ, Doevendans PA, Macleod MR, Chamuleau SAJ. Similar effect of autologous and allogeneic cell therapy for ischemic heart disease: systematic review and meta-analysis of large animal studies. Circ Res. 2015;116:80–86.
4. Fisher SA, Doree C, Mathur A, Martin-Rendon E. Meta-analysis of cell therapy trials for patients with heart failure. Circ Res. 2015;116:1361–1377.
5. Bolli R, Ghafghazi S. Cell therapy needs rigorous translational studies in large animal models. J Am Coll Cardiol. 2015;66:2000–2004. doi:10.1016/j.jacc.2015.09.002.
6. Van Hout GPJ, Jansen Of Lorkeers SJ, Wever KE, Sena ES, Kouwenberg LHJ, van Solinge WW, Macleod MR, Doevendans PA, Pasterkamp G, Chamuleau SAJ, Hoefer IE. Translational failure of anti-inflammatory compounds for myocardial infarction: a meta-analysis of large animal models. Cardiovasc Res. 2016;109:240–248.
7. Bolli R. Reflections on the irreproducibility of scientific papers. Circ Res. 2015;117:665–666. doi: 10.1161/CIRCRESAHA.115.307496.
8. Bolli R. New initiatives to improve the rigor and reproducibility of articles published in circulation research. Circ Res. 2017;121:472–479.
9. Sena E, van der Worp HB, Howells D, Macleod M. How can we improve the pre-clinical development of drugs for stroke? Trends Neurosci.2007;30:433–439. doi:0.1016/j.tins.2007.06.009.
10. Ramirez FD, Motazedian P, Jung RG, Di Santo P, MacDonald ZD, Moreland R, Simard T, Clancy AA, Russo JJ, Welch VA, Wells GA, Hibbert B. Methodological rigor in preclinical cardiovascular studies: targets to enhance reproducibility and promote research translation. Circ Res.2017;120:1916–1926. doi: 10.1161/CIRCRESAHA.117.310628.
11. Gyöngyösi M, Wojakowski W, Lemarchand P, et al; ACCRUE Investigators. Meta-Analysis of Cell-based CaRdiac stUdiEs (ACCRUE) in patients with acute myocardial infarction based on individual patient data. Circ Res. 2015;116:1346–1360. doi: 10.1161/CIRCRESAHA.116.304346.
12. Bolli R, Ghafghazi S. Stem cells: cell therapy for cardiac repair: what is needed to move forward? Nat Rev Cardiol. 2017;14:257–258.
13. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. Science. 2002;298:2188–2190. doi: 10.1126/science.1077857.
14. Golpanian S, Schulman IH, Ebert RF, Heldman AW, DiFede DL, Yang PC, Wu JC, Bolli R, Perin EC, Moyé L, Simari RD, Wolf A, Hare JM; Cardiovascular Cell Therapy Research Network. Concise review: review and perspective of cell dosage and routes of administration from preclinical and clinical studies of stem cell therapy for heart disease. Stem Cells Transl Med. 2016;5:186–191. doi:10.5966/sctm.2015-0101.
15. Jansen of Lorkeers SJ, Gho JMIH, Koudstaal S, van Hout GPJ, Zwetsloot PPM, van Oorschot JWM, van Eeuwijk ECM, Leiner T, Hoefer IE, Goumans M-J, Doevendans PA, Sluijter JPG, Chamuleau SAJ. Xenotransplantation of human cardiomyocyte progenitor cells does not improve cardiac function in a porcine model of chronic ischemic heart failure. Results from a randomized, blinded, placebo controlled trial. PLoS One. 2015;10:e0143953.
16. Kupfer ME, Ogle BM. Advanced imaging approaches for regenerative medicine: emerging technologies for monitoring stem cell fate in vitro and in vivo. Biotechnol J. 2015;10:1515–1528. doi: 10.1002/biot.201400760.
17. Dib N, Menasche P, Bartunek JJ, et al; International Society for Cardiovascular Translational Research. Recommendations for successful training on methods of delivery of biologics for cardiac regeneration: a report of the International Society for Cardiovascular Translational Research. JACC Cardiovasc Interv. 2010;3:265–275. doi: 10.1016/j.jcin.2009.12.013.
18. van der Spoel TIG, Lee JC-T, Vrijsen K, Sluijter JPG, Cramer MJM, Doevendans PA, van Belle E, Chamuleau SAJ. Non-surgical stem cell delivery strategies and in vivo cell tracking to injured myocardium. Int J Cardiovasc Imaging. 2011;27:367–383.

19. Begley CG, Ellis LM. Drug development: raise standards for preclinical cancer research. Nature. 2012;483:531–533. doi: 10.1038/483531a.
20. Ioannidis JPA. Why most published research findings are false. PLoS Med. 2005;2:e124.
21. Patsopoulos NA, Tatsioni A, Ioannidis JP. Claims of sex differences: an empirical assessment in genetic associations. JAMA. 2007;298:880–893. doi: 10.1001/jama.298.8.880.
22. Friedman LM, Furberg CD, Demets DL. Fundamentals of Clinical Trials. New York: Springer-Verlag; 2010:1–445.
23. Dechartres A, Boutron I, Trinquart L, Charles P, Ravaud P. Single-center trials show larger treatment effects than multicenter trials: evidence from a meta-epidemiologic study. Ann Intern Med. 2011;155:39–51. doi:10.7326/0003-4819-155-1-201107050-00006.
24. Lefer DJ, Bolli R. Development of an NIH consortium for preclinical AssESsment of CARdioprotective therapies (CAESAR): a paradigm shift in studies of infarct size limitation. J Cardiovasc Pharmacol Ther. 2011;16:332–339.
25. Dirnagl U, Fisher M. International, multicenter randomized preclinical trials in translational stroke research: it's time to act. J Cereb Blood Flow Metab. 2012;32:933–935.
26. Jones SP, Tang XL, Guo Y, et al. The NHLBI-sponsored Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR): a new paradigm for rigorous, accurate, and reproducible evaluation of putative infarct-sparing interventions in mice, rabbits, and pigs. Circ Res.2015;116:572–586. doi: 10.1161/CIRCRESAHA.116.305462.
27. Howells DW, Sena ES, Macleod MR. Bringing rigour to translational medicine. Nat Rev Neurol. 2014;10:37–43.doi: 10.1038/nrneurol.2013.232.
28. Kukreja R, Tang X-L, Lefer D, et al. Administration of sildenafil at reperfusion fails to reduce infarct size: results from the CAESAR Cardioprotection Consortium (LB650). FASEB J. 2014;28:LB650.
29. Lefer D, Jones S, Steenbergen C, et al. Sodium nitrite fails to limit myocardial infarct size: results from the CAESAR Cardioprotection Consortium (LB645). FASEB J. 2014;28:LB645.
30. Hare JM, Bolli R, Cooke JP, et al; Cardiovascular Cell Therapy Research Network. Phase II clinical research design in cardiology: learning the right lessons too well: observations and recommendations from the Cardiovascular Cell Therapy Research Network (CCTRN). Circulation. 2013;127:1630–1635. doi: 10.1161/CIRCULATIONAHA.112.000779.
31. Hariharan N, Quijada P, Mohsin S, Joyo A, Samse K, Monsanto M, De La Torre A, Avitabile D, Ormachea L, McGregor MJ, Tsai EJ, Sussman MA. Nucleostemin rejuvenates cardiac progenitor cells and antagonizes myocardial aging. J Am Coll Cardiol. 2015;65:133–147.
32. van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SJ, Middleton RC, Marba E, Molkentin JD. c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature. 2014;509:337–341.
33. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov. 2004;3:711–715. doi: 10.1038/nrd1470. 34. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. Lancet. 2009;374:86–89.
35. Dwan K, Gamble C, Williamson PR, Kirkham JJ; Reporting Bias Group. Systematic review of the empirical evidence of study publication bias and outcome reporting bias - an updated review. PLoS One. 2013;8:e66844. doi: 10.1371/journal.pone.0066844.
36. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke. 2004;35:1203–1208.
37. Hopewell S, Clarke MJ, Stewart L, Tierney J. Time to publication for results of clinical trials. Cochrane Database Syst Rev. 2007;MR000011.
38. Sena ES, van der Worp HB, Bath PM, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. PLoS Biol. 2010;8:e1000344. doi: 10.1371/journal.pbio.1000344.
39. Cuijpers P, van Straten A, Bohlmeijer E, Hollon SD, Andersson G. The effects of psychotherapy for adult depression are overestimated: a metaanalysis of study quality and effect size. Psychol Med. 2010;40:211–223. doi: 10.1017/S0033291709006114.
40. Dirnagl U, Lauritzen M. Fighting publication bias: introducing the negative results section. J Cereb Blood Flow Metab. 2010;30:1263–1264. doi:10.1038/jcbfm.2010.51.
41. Chambers CD, Dienes Z, McIntosh RD, Rotshtein P, Willmes K. Registered reports: realigning incentives in scientific publishing. Cortex. 2015;66:A1–A2. doi: 10.1016/j.cortex.2015.03.022.
42. Turner S, Wright D, Maeso R, Cook A, Milne R. Publication rate for funded studies from a major UK health research funder: a cohort study. BMJ Open. 2013;3:e002521.
43. Jüni P, Altman DG, Egger M. Assessing the quality of controlled clinical trials. BMJ. 2001;323:42–46.
44. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c332.
45. van Hout GP, Jansen of Lorkeers SJ, Wever KE, Sena ES, Kouwenberg LH, van Solinge WW, Macleod MR, Doevendans PA, Pasterkamp G, Chamuleau SA, Hoefer IE. Translational failure of anti-inflammatory compounds for myocardial infarction: a meta-analysis of large animal models. Cardiovasc Res.

2016;109:240–248. doi: 10.1093/cvr/cvv239.
46. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Animals. 2013;4:35–44.
47. Korevaar DA, Hooft L, ter Riet G. Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments. Lab Anim. 2011;45:225–230.
48. Wever KE, Hooijmans CR, Riksen NP, Sterenborg TB, Sena ES, Ritskes-Hoitinga M, Warlé MC. Determinants of the efficacy of cardiac ischemic preconditioning: a systematic review and meta-analysis of animal studies. PLoS One. 2015;10:e0142021. doi: 10.1371/journal.pone.0142021.
49. Bromage DI, Pickard JMJ, Rossello X, Ziff OJ, Burke N, Yellon DM, Davidson SM. Remote ischaemic conditioning reduces infarct size in animal in vivo models of ischaemia-reperfusion injury: a systematic review and meta-analysis. Cardiovasc Res. 2016;113:288–297.
50. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:43. doi: 10.1186/1471-2288-14-43.
51. Macleod MR, Ebrahim S, Roberts I. Surveying the literature from animal experiments: systematic review and meta-analysis are important contributions. BMJ. 2005;331:110. doi:10.1136/bmj.331.7508.110-b.
52. van der Spoel TI, Jansen of Lorkeers SJ, Agostoni P, van Belle E, Gyöngyösi M, Sluijter JP, Cramer MJ, Doevendans PA, Chamuleau SA. Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. Cardiovasc Res. 2011;91:649–658. doi: 10.1093/cvr/cvr113.
53. Ross JS, Mulvey GK, Hines EM, Nissen SE, Krumholz HM. Trial publication after registration in clinicaltrials.gov: a cross-sectional analysis. PLoS Med. 2009;6:e1000144. doi: 10.1371/journal.pmed.1000144.
54. Chalmers I, Glasziou P, Godlee F. All trials must be registered and the results published. BMJ. 2013;346:f105.
55. Jansen of Lorkeers SJ, Doevendans PA, Chamuleau SA. All preclinical trials should be registered in advance in an online registry. Eur J Clin Invest. 2014;44:891–892.
56. Kimmelman J, Anderson JA. Should preclinical studies be registered? Nat Biotechnol. 2012;30:488–489. doi: 10.1038/nbt.2261.
57. Brown T. It's time for alltrials registered and reported. Cochrane Database Syst Rev. 2013;4. doi: 10.1002/14651858.ED000057.
58. Hale C, Arrowsmith J, Bayes S, Bodmer H, Garman A, Hubbard P, Perrin N, Sooben P. The Concordat to Support Research Integrity. http://www.universitiesuk.ac.uk/policy-and-analysis/reports/Documents/2012/theconcordat-to-support-research-integrity.pdf. Accessed February 24, 2017.
59. Moylan EC, Kowalczuk MK. Why articles are retracted: a retrospective cross-sectional study of retraction notices at BioMed Central. BMJ Open. 2016;6:e012047.
60. Benedictus R, Miedema F, Ferguson MWJ. Fewer numbers, better science. Nature. 2016;538:453–455.

SUPPLEMENTARY TABLE

Supplementary table 1. Full checklist requsupplred for registration on preclinicaltrials.eu

Nr	Item	Field options	Information field	Mandatory [y/n]
General information				
1	Unique ID	Allocated by preclinicaltrials.eu	Each record is allocated a unique ID once it is registered	Allocated by site
2	Title of the study	Open field	Enter the full title of the study	Yes
3	Acronym/short title	Open field	Enter optional acronym/short title for the	No
4a	Contact details - Name	Open field	Give the name of the main administrative contact for the study	Yes
4b	Contact details - Role	Open field	What is the role of the main contact in the study (e.g. executive researcher, research group supervisor)	Yes
4c	Contact details - Email address	Open field	Provide the email address of the main contact	Yes
5a	Study center details - Name	Open field	Give the details of the institutions where the experiments will be undertaken. Add additional lines if there is more than one.	Yes
5b	Study center details - City	Open field	Indicate the city in which the study will be undertaken.	Yes
5c	Study center details - Country	Dropdown menu	Indicate the country in which the study will be undertaken.	Yes
6	Source of support	drop down menu; industry/investigator driven/grants/other [open field]	Give the sources of financial support for the study	Yes
7	Start date	dd/mm/yyyy	The date the study started or is expected to start	Yes
8	Expected end date	dd/mm/yyyy	The date the study ended or is expected to end	Yes
9	Study status	Not started/ active/ completed published, completed and published	Please indicate what the current status of the study is	Yes
Study design				
10	Field of medicine	Open field	To what field of medicine does this study relate?	Yes
11	Health condition/ problem studied	Open field	Give the health condition or problem the study investigates	Yes
12	Intervention type	compound/application method/retention/model optimalisation/surgery/ other [open field	What type of intervention is being tested in the study?	Yes

Supplementary table 1. Continued

Nr	Item	Field options	Information field	Mandatory [y/n]
13	Study stage	Drop down menu: Stage 1 – fundamental information to understand biology Stage 2 – exploratory study Stage 3 – confirmatory study	Please indicate the stage of the study Ad 1 Investigate the understanding of biology to discover and develop new therapeutic products (e.g. in vitro studies, genetic studies) Ad 2 Hypothesis-generating research Ad 3 Final study confirming (or rejecting) a single hypothesis, these are normally blinded, randomized, controlled trials	Yes
14	Hypotheses	Open field	Formulate the hypotheses for this study.	If stage 3, yes. Otherwise not.
15	Primary endpoint(s)	Safety/feasibility/efficacy + open field	What is the primary endpoint of the study? For example, efficacy based on LVEF after 4 weeks.	Yes
16	Secondary endpoint(s)	Open field	What is the secondary endpoint of the study?	No
17	Species	Cats/dogs/ferrit/goats/ guinea pig/hampsters/ horses/mice/monkeys/ oxen/pigs/rabbit/rats/ sheep/other [open field]	Select the species category then the appropriate species for the study	Yes
18	Strain	Open field	Provide the strain or other specifications on the species	No
19	Sex	Male/female/both	Indicate the sex of the animals in the study	Yes
20	Animal model used	Open field	What animal model was used for the study	Yes
21	Sum of animals in study arms	Number input	Indicate the total number of animals which are expected to be analysed in total (exclude expected procedural drop-out)	Yes
22	Expected drop-out due to mortality or other causes	Number input + unknown	Indicate the number of animals which are expected to drop-out due to procedural complications (e.g. mortality) or other animals not expected to participate in analysis (e.g. not fulfilling in- and/or exclusion criteria	Yes
23a	Randomisation - Randomly allocated	Randomly allocated/ method used/further details on randomisation	Are the animals randomly allocated to the experimental groups	Yes

Supplementary table 1. Continued

Nr	Item	Field options	Information field	Mandatory [y/n]
23b	Randomisation - Method used	Computer-generated random number sequence/random number table/shuffled blinded envelopes/ coin-toss/other [open field]	If randomisation is applied, please indicate the method used	If 23a is answered with yes
23c	Randomisation - Further details on randomisation	Simple randomisation/ block randomisation/ stratified randomisation	Provide further details on randomisation	No
24a	Blinding of the investigators	Yes Partially, because [open field] No	Are the investigators involved in the experiment blinded to the allocation of the animals to the experimental groups	Yes
24b	Blinded assessment of outcome	Yes Partially, because [open field] No	Are the outcome assessors (e.g. performing echocardiography or MRI) blinded to the allocation of the (samples from) animals to the experimental groups?	Yes
25	Placebo-controlled	Yes/no	Was one of the arms of the study a placebo arm?	Yes
26a	Sample size calculation Was a sample size calculation performed	Yes/no	Please indicate if a sample size calculation was performed in advance.	Yes
26b	Sample size calculation Specifications - Alpha - Beta - Mean effect group 1/proportion of success - Estimated number of drop-outs	 - Number - Number - Number - Number	Give details of the sample size calculation and the results	No
27	Follow-up duration	open field + drop down menu min/h/d/w/m/y	How long will the follow-up be?	Yes
28	Groups - Group 1 - Possibility to add additional groups	Field 1: sham/control/ intervention/other (open field) Field 2: Number input Field 3: Intervention	Please indicate all of the study groups/arms and their purpose	Yes
29	Original animal ethics committee application or number of application	Open field	Please upload the original animal ethics committee application for this study, provide a link to an online copy or provide the number of the application	No
30	Additional information	Open field	Please give any other information about the study that is not covered elsewhere in the form	No
31	Link to publication	Open field	Please provide links to any published articles relating to the study	No

CHAPTER 3

Standardized mean differences cause funnel plot distortion in publication bias assessments

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ABSTRACT

Meta-analyses are increasingly used for synthesis of evidence from biomedical research, and often include an assessment of publication bias based on visual or analytical detection of asymmetry in funnel plots. We studied the influence of different normalisation approaches, sample size and intervention effects on funnel plot asymmetry, using empirical datasets and illustrative simulations. We found that funnel plots of the Standardized Mean Difference (SMD) plotted against the standard error (SE) are susceptible to distortion, leading to overestimation of the existence and extent of publication bias. Distortion was more severe when the primary studies had a small sample size and when an intervention effect was present. We show that using the Normalised Mean Difference measure as effect size (when possible), or plotting the SMD against a sample size-based precision estimate, are more reliable alternatives. We conclude that funnel plots using the SMD in combination with the SE are unsuitable for publication bias assessments and can lead to false-positive results.

INTRODUCTION

Systematic reviews are literature reviews intended to answer a particular research question by identifying, appraising and synthesizing all research evidence relevant to that question. They may include a meta-analysis, a statistical approach in which outcome data from individual studies are combined, which can be used to estimate the direction and magnitude of any underlying intervention effect, and to explore sources of between-study heterogeneity. Simultaneously, meta-analysis can be used to assess the risk of publication bias: the phenomenon that published research is more likely to have positive or statistically significant results than unpublished experiments[1]. Meta-analyses are routinely used in clinical research to guide clinical practice and healthcare policy, reduce research waste and increase patient safety[2]. The use of meta-analysis continues to increase[3] and it has become more common to apply these approaches to the synthesis of preclinical evidence[4]. Importantly, preclinical studies are, generally, individually small, with large numbers of studies included in meta-analysis, and large observed effects of interventions. This contrasts with clinical research, where meta-analyses usually involve a smaller number of individually larger experiments with smaller intervention effects. This calls for methodological research to ascertain whether approaches to data analysis routinely used in the clinical domain are appropriate in the pre-clinical domain and for resources that guide and inform researchers, reviewers and readers on best practice. In this light, we present findings which show that the use of the standardized mean difference (SMD) measure of effect size in funnel plots can introduce a risk of incorrect assessment of publication bias, particularly in meta-analyses of preclinical data characterised by a large number of individually small studies with large observed effects.

Formulation of raw mean difference, standardized mean difference and normalized mean difference

To combine data statistically on *e.g.* the effects of an intervention which has been tested in several studies, outcome measures first need to be expressed on a common scale. Such scales include (for binary outcomes) the risk or odds ratios; and for continuous data a raw mean difference (RMD), SMD or normalized mean difference (NMD). The RMD can be used when all outcome data are in the same measurement unit, and the interpretation of the outcome is the same in all settings (*i.e.* the reported measurement unit of the change in outcome has the same meaning in all studies). The RMD is calculated by subtracting the mean outcome value in the control group (M_{ctrl}) from the mean in the intervention group (M_{int}):

$$RMD = M_{int} - M_{ctrl}. \quad (1)$$

The observed standard deviation (SD) is likely to differ between experimental groups, and therefore the standard error (SE) of the RMD is calculated as:

$$SE_{RMD} = \sqrt{\frac{SD_{int}^2}{n_{int}} + \frac{SD_{ctrl}^2}{n_{ctrl}}}, \quad (2)$$

where n is the sample size per group.

In cases where the measurement unit, or the interpretation of the outcome, or both differ between studies (*e.g.* a given change in infarct size measured in mm^3 has a different consequence in the mouse brain than in the rat brain), the intervention effect may be expressed as an SMD. For each study the SMD is obtained by dividing the RMD by that study's pooled standard deviation (SD_{pooled}) to create an effect estimate that is comparable across studies:

$$SMD = d = \frac{M_{int} - M_{ctrl}}{SD_{pooled}} \quad (3)$$

, where SD_{pooled} is:

$$SD_{pooled} = \sqrt{\frac{(n_{ctrl}-1)\,SD_{ctrl}^2 + (n_{int}-1)\,SD_{int}^2}{n_{ctrl} + n_{int} - 2}} \quad (4)$$

Thus, the SMD expresses the intervention effect in all studies in the same new unit: the SD.

For each study, the standard error (SE) of the SMD can be approximated using the sample sizes (n) and the effect estimate (SMD):

$$SE_{SMD} = \sqrt{\frac{(n_{ctrl}+n_{int})}{n_{ctrl} * n_{int}} + \frac{SMD^2}{2*(n_{ctrl}+n_{int})}} \quad (5)$$

Of note, equations 3 and 5 estimate the SMD using the approach of Cohen[5]; this estimate is therefore termed Cohen's *d*. However, Cohen's *d* tends to overestimate the 'true' SMD and its variance when the sample sizes in the primary studies are small (e.g. <10). This bias can be corrected using the approach of Hedges[6], which adjusts both the SMD estimate and its variance by a correction factor based on the total sample size. The resulting estimate is the unbiased SMD known as Hedges' *g* (see Supplementary file 2 for full equations). In many clinical meta-analyses, Hedges' *g* will be almost identical to Cohen's *d*, but the difference between the estimates can be larger in preclinical meta-analyses, where small sample sizes are more common.

A third effect measure commonly used for continuous data in preclinical meta-analyses is the normalised mean difference (NMD), which relates the magnitude of effect in the intervention group to that seen in untreated animals, with reference to the outcome in a normal, healthy animal[7]. A condition for using the NMD is that the

baseline measurement in an untreated, unlesioned 'sham' animal is known, or can be inferred. For each study, the NMD is calculated as:

$$NMD = 100\% \times \frac{(M_{int} - M_{sham}) - (M_{ctrl} - M_{sham})}{(M_{ctrl} - M_{sham})} \quad (6)$$

where M_{sham} is the mean score for normal, unlesioned and untreated subjects. The corresponding SE is calculated as:

$$SE_{NMD} = \sqrt{\frac{(100 * \frac{SD_{ctrl}}{M_{ctrl} - M_{sham}})^2}{n_{ctrl}} + \frac{(100 * \frac{SD_{int}}{M_{int} - M_{sham}})^2}{n_{int}}} \quad (7)$$

(see Supplementary file 2 for additional equations and[7] for a comprehensive overview of (preclinical) meta-analysis methodology).
Note that Equation 5 dictates that the SE_{SMD} is correlated to the SMD effect size, whereas the SEs of the RMD (Equation 2) and NMD (Equation 7) are independent of the corresponding effect sizes.

Funnel plots and publication bias

Funnel plots are scatter plots of the effect sizes of the included studies versus a measure of their precision, usually the SE or 1/SE. In the absence of bias and heterogeneity, funnel plots should be funnel-shaped and symmetrically centred around the summary effect estimate of the analysis, since 1) imprecise (smaller) studies will deviate further from the summary effect compared to precise (larger) studies and 2) studies are equally likely to overestimate or underestimate the true effect (Figure 1A). Assessment of the possible presence of publication bias frequently relies on a visual or analytical evaluation of funnel plot asymmetry. If studies showing small, neutral or controversial effects are more likely to remain unpublished, publication bias may occur. As a result, the funnel plot will become asymmetrical, and the summary effect estimate will shift accordingly (Figure 1B). Importantly, there are other causes of asymmetry in funnel plots. For instance, the true effect size in smaller (and therefore less precise) studies may be genuinely different from that in large studies (for instance because the intensity of the intervention was higher in small studies). For this reason, funnel plot asymmetry is often referred to as a method to detect small study effects, rather than being a definitive test for publication bias[8]. In addition, artefacts and chance may cause asymmetry (as shown e.g. in this study).

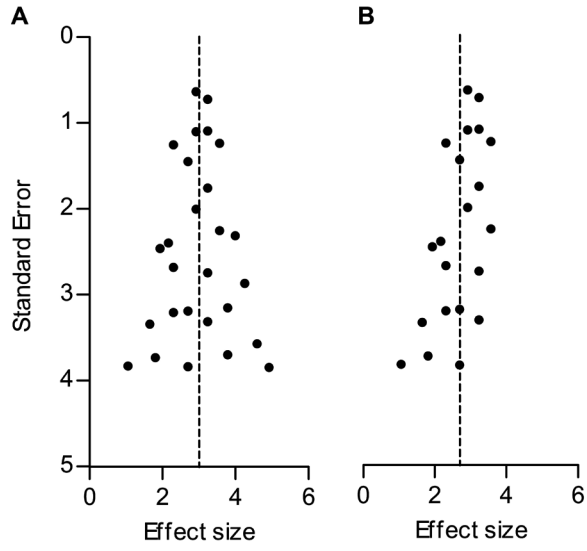


Figure 1. Hypothetical funnel plots in the absence (A) and presence (B) of bias. The precision estimate used is the standard error (SE). Dashed lines indicate the summary effect estimate.

Theoretical explanation of SMD funnel plot distortion

In a meta-analysis using the SMD as effect measure, in the absence of publication bias, observed SMDs in a funnel plot will be scattered around the true underlying SMD. However, the dependency of the SE_{SMD} on the observed SMD will impact the appearance of the funnel plot. When we review the equation for the SESMD, (Equation 5) the first component on the right of the '=' sign reflects the variance of the difference between the two group means, rescaled into pooled standard deviation units. Consequently, in this first part only n_{ctrl} and n_{int} play a role. The second component includes the squared SMD, and reflects the variation in the within-groups standard deviation as measured by SD_{pooled} (Equation 4).

If there is no intervention effect, the SMD (and the second component) will be zero, and the SE will therefore depend solely on the sample size (Equation 5 and Figure 2A). If an intervention effect is present, the SE will increase, as the size of SMD^2 in the equation will increase. This is no problem if the observed SMD is similar to the true SMD. However, a study with an observed SMD larger than the true SMD will have a larger SE. On the other hand, a study with an observed SMD smaller than the true SMD (but >0) will have a relatively small SE (Figure 2B). This will cause funnel plot distortion: studies with a relatively small effect size (and associated SE) will skew towards the upper left region of the plot, while studies with a relatively large effect size and SE will skew towards the bottom right region of the plot, as the associated SE of these studies will be relatively large. Because the SMD is squared in the equation for the SE, this

holds true for both positive and negative SMDs (Figure 2C). The smaller the first component of Equation 5, the larger the influence of the SMD on the size of the SE, worsening the distortion when sample sizes are small. Of note, this component is smallest when group sizes are unequal. The effect of the second component on the SE, and the resulting distortion, is largest if the sample size is small and the SMD is large (Figure 2D).

In summary, a funnel plot using both the SMD and its SE may become asymmetrical in the absence of publication bias. When funnel plot distortion is assessed by visual inspection, this skewing might cause the plot to be interpreted as being asymmetrical and lead the observer to erroneously conclude that publication bias is present. Furthermore, funnel plot asymmetry is often tested statistically using Egger's regression[9] or Duval and Tweedie's trim and fill analysis[10], but neither of these analyses take the phenomenon described above into account, and their use may lead to erroneous conclusions that publication bias is present.

AIM OF THIS STUDY

We investigated the reliability of RMD, SMD and NMD-based funnel plots for the assessment of publication bias in meta-analyses, using both empirical datasets and data simulations. We investigate the effect on the severity of funnel plot distortion of the study sample size, the number of studies in the meta-analysis and the magnitude of the intervention effect. We assess whether distortion can be avoided by using a precision estimate based on the sample size of the primary studies, as previously suggested for mean difference outcome measurements[11].

Our findings have important implications for the meta-research field, since authors may have reached incorrect conclusions regarding the existence of publication bias based on funnel plots using the SMD measure of effect size.

MATERIALS AND METHODS

We performed data simulations and re-analyses of empirical data using R statistical software (version 3.1.2; RRID:SCR_001905) and the most recent MBESS, xlsx, meta and metafor packages[8,12–15] (See Supplementary file 3 for all R scripts). For all analyses involving RMD and SMD the primary outcome of interest was the number of asymmetrical funnel plots as detected by Egger's regression[9]. As a secondary outcome, we assessed the number of missing studies as imputed by Duval and Tweedie's trim and fill analysis[10]. This method provides an estimate of the number of missing studies in a meta-analysis, and the effect that these missing studies may have had on its

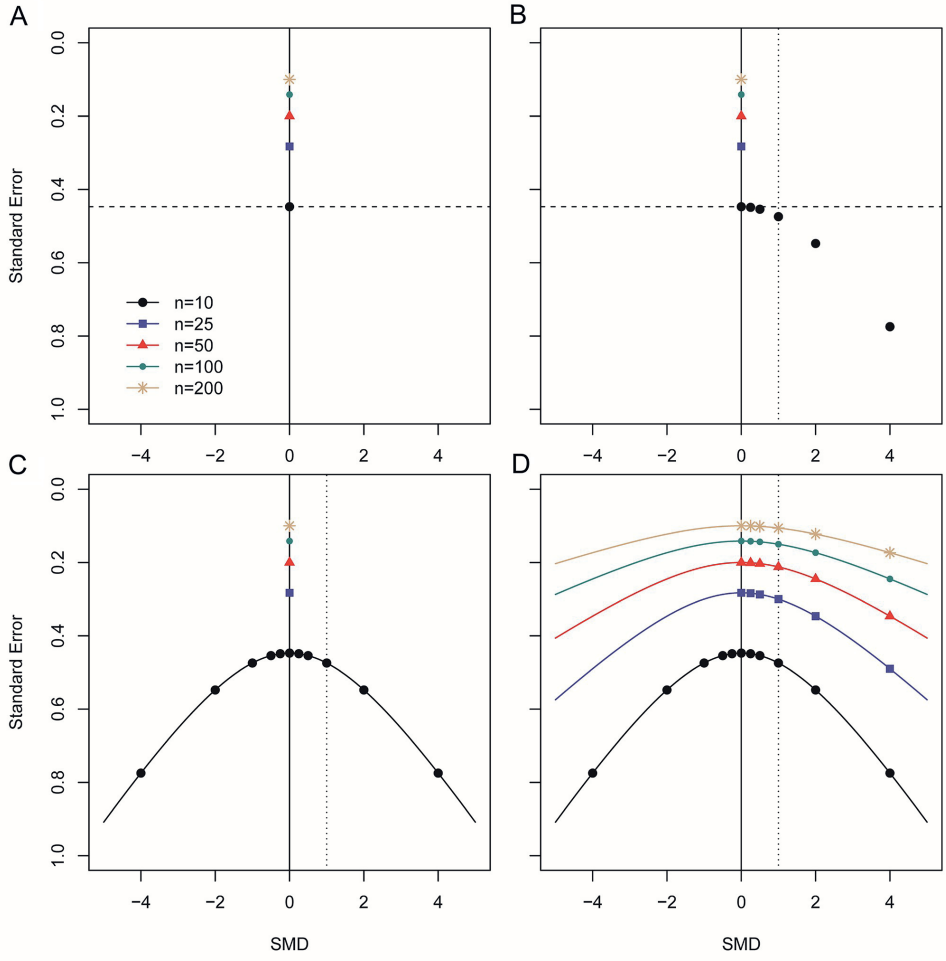


Figure 2. Step-wise illustration of distortion in SMD versus SE funnel plots. (A) Depicted are simulated studies with a sample size of respectively 10 (large black circles), 25 (blue squares), 50 (red triangles), 100 (small green circles) and 200 (gold asterisks) subjects per group, and an SMD of zero. The SE of these studies (indicated by the dashed line for studies with n = 10) solely depends on their sample size, as SMD2 = 0 and therefore does not contribute to the equation for the SE. As expected, the SE decreases as the sample size increases. (B) Five data points from simulated studies with n = 10 and a stepwise increasing SMD are added to the plot. For these studies, the SMD2 contributes to the equation for the SE, and the SE will decrease even though the sample size is constant. The dotted line represents a hypothetical summary effect of SMD = 1 in a meta-analysis. Note that when assessing a funnel plot for asymmetry around this axis, the data points with an SMD < 1 have skewed to the upper left-hand region, whereas studies with an SMD > 1 are in the lower right region of the plot. This distortion worsens as the SMD increases. (C) Because the SMD is squared in the equation for the SE, the same distortion pattern is observed for negative SMDs. Thus, funnel plots will be distorted most when the study samples sizes are small and SMDs are either very positive or very negative. (D) The same deviation is observed for simulated studies with larger sample sizes, however, the deviation decreases as the sample size increases, because the sample size will outweigh the effect of SMD2 in the equation for the SE.

outcome. In brief, the funnel plot is mirrored around the axis represented by the overall effect estimate. Excess studies (often small, imprecise studies with a neutral or negative effect size) which have no counterpart on the opposite side of the plot are temporarily removed (trimmed). The trimmed plot is then used to re-estimate the overall effect estimate. The trimmed data points are placed back into the plot, and then a paired study is imputed with the same precision but reflected to have an effect size reflected around the adjusted overall estimate, and plotted in a different color or symbol from the observed data points. The analysis is re-run and repeated until no further asymmetry is observed. We used trim and fill analysis and a random effects model in R to seek evidence for publication bias overstating the effectiveness of the interventions, based on the proposed direction of the intervention effect. Because of its superior performance in studies with small sample sizes, Hedges' *g* was used in the main analyses throughout this manuscript. We considered a p-value of <0.05 to be significant for Egger's regression in individual simulations.

Empirical data published as RMD re-analyzed as SMD

In our first re-analysis of empirical data from published preclinical meta-analyses[16,17], we constructed funnel plots using the unbiased SMD (Hedges' g[6]) vs. SE, and compared these to funnel plots using the RMD vs. SE (as in the original publication).

Data simulation methods

In our first simulation, we tested the estimation of publication bias using the unbiased SMD (Hedges' *g*) in simulated data where there was no publication bias. As a sensitivity analysis, all scenarios of simulation 1 were also performed using Cohen's *d*. We generated simulated meta-analyses by simulating the desired number of individual studies, each with a control group and an intervention group. The control groups were simulated by randomly sampling individual subject data from a normal distribution with a mean (M_{ctrl}) of 30 and an SD of 10 (Table 1); these values were based on outcome data for functional imaging in myocardial infarction studies[17]. Individual subject data for the intervention group was sampled from a normal distribution with mean M_{ctr} +ES (effect size). To assess the effect of differences in overall intervention effects on funnel plot distortion, we simulated meta-analyses for an ES of respectively 0, 5, or 10 (Table 1). To assess the effect of study sample size on funnel plot distortion, we simulated two types of study sizes: small (12–30 subjects per study), as is more common in animal studies, and large (60–320 subjects per study), as is more common in human studies. For each simulated study, we determined the number of subjects by sampling the group sizes from the uniform distribution within the ranges of study sizes given (Table 1). Of note, an intervention effect of SMD = 1 may appear large to those experienced in meta-analyses of clinical data, but is typical of those observed in animal studies, as are the group sizes reported (see e.g. Figure 2 and Table 4).

Table 1. Simulation characteristics.

	Small studies			Large studies			RMD	SMD	NMD
Experimental groups	***N***	***Mean***	***SD***	***N***	***Mean***	***SD***			
Intervention 1 (no effect)	7–14	30	10	40–150	30	10	0	0	0
Intervention 2 (RMD = 5)	7–14	35	10	40–150	35	10	5	0.5	0.125
Intervention 3 (RMD = 10)	7–14	40	10	40–150	40	10	10	1	0.25
Control	5–16*	30	10	20–170*	30	10			
Sham	4–6	70	4						

n = sample size; ND = normal distribution; SD = standard deviation; *control group sample size = intervention group sample size ±≤2 (small studies) or ±≤20 (large studies).

Simulation and aggregation of individual subject data into study-level data was repeated until the desired number of studies to be included in the meta-analysis was obtained. We assessed the influence of the number of included studies on funnel plot distortion by simulating meta-analyses containing either 30, 300, or 3000 studies. Although there is no consensus on the minimal number of studies required for publication bias analysis, 30 has been previously proposed as the minimal number to obtain sufficient power for asymmetry testing[18]. We chose 3000 studies for the largest meta-analysis as this is substantially larger than any meta-analysis of which we know, and any effects of study number are likely to be saturated at that number of studies. Importantly, we did not introduce publication bias to any of these datasets and the funnel plots should therefore be symmetrical. We repeated each simulation 1000 times, and we compared the effects of expressing the meta-analysis results as RMD or SMD, and used funnel plots with the effects size plotted on the x-axis and the SE as precision estimate plotted on the y-axis (RMD vs. SE and SMD vs. SE plots). As a second sensitivity analysis, we assessed the robustness of our findings using Egger's test by re-testing all scenario's of simulation 1 using Begg and Mazumdar's test[19].

Informed by the outcomes of simulation 1, in our second simulation we selected the conditions introducing the most prominent distortion in SMD vs. SE funnel plots to investigate the performance of alternatives including SMD vs. 1/√n funnel plots and NMD funnel plots. Thus, all simulations were performed with a small study sample size, in the presence of an intervention effect (see Table 1) and with 3000 studies per meta-analysis. Under these conditions, we constructed RMD vs. SE and SMD vs. SE funnel plots as described above, as well as funnel plots of the SMD against the inversed square root of the total sample size (1/√n) in each study, and of the NMD against the SE. For the NMD, sham group data were simulated to have a mean of 70 and an SD of 4 (Table 1). Group size was selected to be 4–6 subjects, which is a typical sample size for sham groups in preclinical experiments. We performed the simulations once and compared outcomes across all four funnel plots.

In our final simulation we investigated the effects of a modelled publication bias on the performance of the SMD vs. SE and alternative approaches. We simulated meta-

analyses containing 300 and 3000 studies with a small individual sample size and an intervention effect present (Δμ = difference in means between control and intervention group = 10; see Table 1). RMD vs. SE, RMD vs. 1/√n, SMD vs. SE, SMD vs. 1/√n and NMD vs. SE funnel plots were constructed and tested for asymmetry using Egger's regression. We then introduced publication bias in these meta-analyses using a stepwise method, Publication bias was introduced stepwise, by removing 10% of primary studies in which the difference between the intervention and control group means was significant at $p<0.05$ (Student-t test), 50% of studies where the significance level was $p\geq0.05$ to $p<0.10$, and 90% of studies where the significance level was $p\geq0.10$. Funnel plot asymmetry testing was performed as above, and the results were compared to the unbiased simulations and between different funnel plot types. All simulations were repeated 1000 times. Of note, this simulation was not performed for meta-analyses of studies with a large sample size, since pilot data showed that the large sample size will cause only very few studies to be removed from the 'biased' meta-analysis.

Re-analysis of empirical data using an n-based precision estimate

Finally, to assess the usefulness and impact of using a sample size-based precision estimate in SMD funnel plots of empirical data, we re-analysed data from five published preclinical meta-analyses that used SMD vs. SE funnel plots to assess publication bias. The selected datasets were from our own groups, or from recent collaborations, which allowed for easy identification of meta-analyses using SMD vs. SE funnel plots, and easy access to the data. There were no selection criteria in terms of *e.g.* the number of studies in the analysis, or the outcome of the publication bias assessment. The distribution of the total number of subjects per data point in the selected studies is (in median (min-max): 11.7 (6–38) for Wever et al[20], 20(12-46) for Groenink et al[21], 11(4-24) for Yan et al[22], 14.5 (6–35) for Kleikers et al[23] and 12(4-66) for Egan et al[24]. For these data sets, we compared the outcome of Egger's regression and trim and fill analysis when using SMD vs. SE funnel plots to that of SMD vs. 1/√n funnel plots. We obtained the corresponding author's consent for re-analysis.

RESULTS

Publication bias assessment using RMD versus SMD funnel plots of two preclinical RMD datasets

Dataset 1 (ischaemic preconditioning) contains 785 individual effect sizes[16]. In the original analysis using the RMD as effect measure, funnel plot asymmetry was detected by Egger's regression ($p=1.7\times10-5$), but no additional studies were imputed in trim and fill analysis (Figure 3A). When expressing the same data as SMD, funnel plot asymmetry increased substantially (Figure 3B; $p<1.0\times10-15$, Egger regression) and 196 missing

studies were imputed by trim and fill analysis, leading to adjustment of the estimated SMD effect size from 2.8 to 1.9.

Dataset 2 (stem cell treatments) contained 95 individual effect sizes[17]. Funnel plot asymmetry was detected in the original analysis using RMD (p=0.02) and trim and fill analysis suggested a reduction in effect estimate of 0.1% after filling two additional studies (Figure 3C). In contrast, a funnel plot of the same data expressed as SMD showed asymmetry at a higher level of statistical significance (p=3.4×10−10, Egger regression), but no missing studies were imputed (Figure 3D).

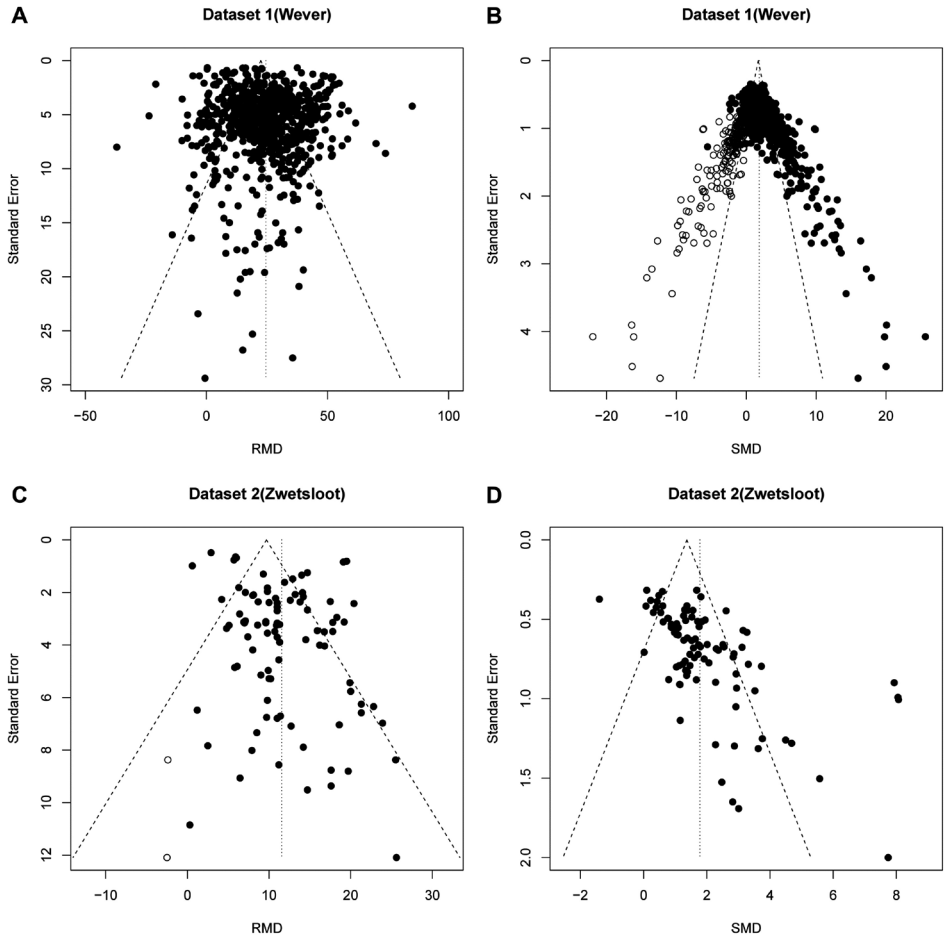


Figure 3. Reanalysis of data from Wever et al. (A,B) and Zwetsloot et al. (C,D), with funnel plots based on raw mean difference (RMD; A,D) or standardized mean difference (SMD; B,D). Filled circles = observed data points; open circles = missing data points as suggested by trim and fill analysis.

Data simulation results

Results of our first simulation (in the absence of publication bias) are shown in Table 2, and representative funnel plots of these simulations in Figure 4 (small study sample size) and Figure 4—figure supplement 1 (large study sample size). When we simulated no intervention effect, neither Egger's regression nor trim and fill analysis gave different results for the RMD vs. SE and SMD vs. SE analyses (Table 2, Figure 4A,B,E and F and Figure 4—figure supplement 1, panel A, B, E and F) and in ~95% of cases there was no evidence of asymmetry. Most simulated funnel plots were assessed as symmetrical, however, as expected, around 5% of the cases were considered asymmetrical by chance.

When we simulated the presence of an intervention effect ($\Delta\mu = 10$; RMD = 10 and SMD = 1 or $\Delta\mu = 5$; RMD = 5 and SMD = 0.5), again around 5% of the RMD funnel plot analyses were judged asymmetrical (Table 2, Figure 4C and G, and Figure 4—figure supplement 1, panel C and G). In contrast, when using the SMD, funnel plot asymmetry was detected in over 60% of the simulated funnel plots with $\Delta\mu = 10$, where the size of contributing studies was small (Figure 4D and H and Figure 4—figure supplement 1, panel D and H), increasing as the number of individual studies contributing to the meta-analysis increased. When we modelled larger individual contributing studies (n = 60–320 subjects), respectively 9%, 34% and 100% of the SMD funnel plots with 30, 300 or 3000 studies were assessed as asymmetrical (Table 2, Figure 4—figure supplement 1). Trim and fill analysis resulted in on average 7% extra studies filled in preclinical simulation scenarios using the RMD. Adjusting the overall effect estimate based on these filled data points improved the estimation of the simulated RMD in all scenarios. However, when using the SMD, the number of filled studies was much higher in many scenarios (up to 21% extra studies filled). As a result, the adjusted overall effect estimate after trim and fill in SMD funnel plots tended to be an underestimation of the true effect size. Finally, through visual inspection, distortion could be seen in all SMD funnel plots that incorporated a true effect, most prominent in the preclinical (small study) scenarios (Figure 4 and Figure 4—figure supplement 1).

When repeating the simulations using Cohen's *d* SMD instead of Hedges' *g*, or using Begg and Mazumdar's test, we found highly similar results in all scenarios simulated (see Supplementary file 1 and exemplary funnel plots in Figure 4—figure supplement 2).

Next, we assessed the impact of censoring non-significant simulated experiments (to simulate publication bias) and the performance of SMD vs. $1/\sqrt{n}$ funnel plots and NMD funnel plots in the presence of an intervention effect as alternatives to the SMD vs. SE funnel plot. As in simulation 1, SMD vs. SE funnel plots of unbiased simulations were identified as asymmetrical by Egger's test (Table 3). However, when the precision estimate was changed from SE to $1/\sqrt{n}$, the prevalence of false positive results fell to the expected 5% (Table 3). For the NMD, Egger's test performed correctly when using either the SE or $1/\sqrt{n}$ as precision estimate. In all scenario's, approximately 50 out of 1000 simulated funnel plots appeared to be asymmetrical by chance (Table 3). The

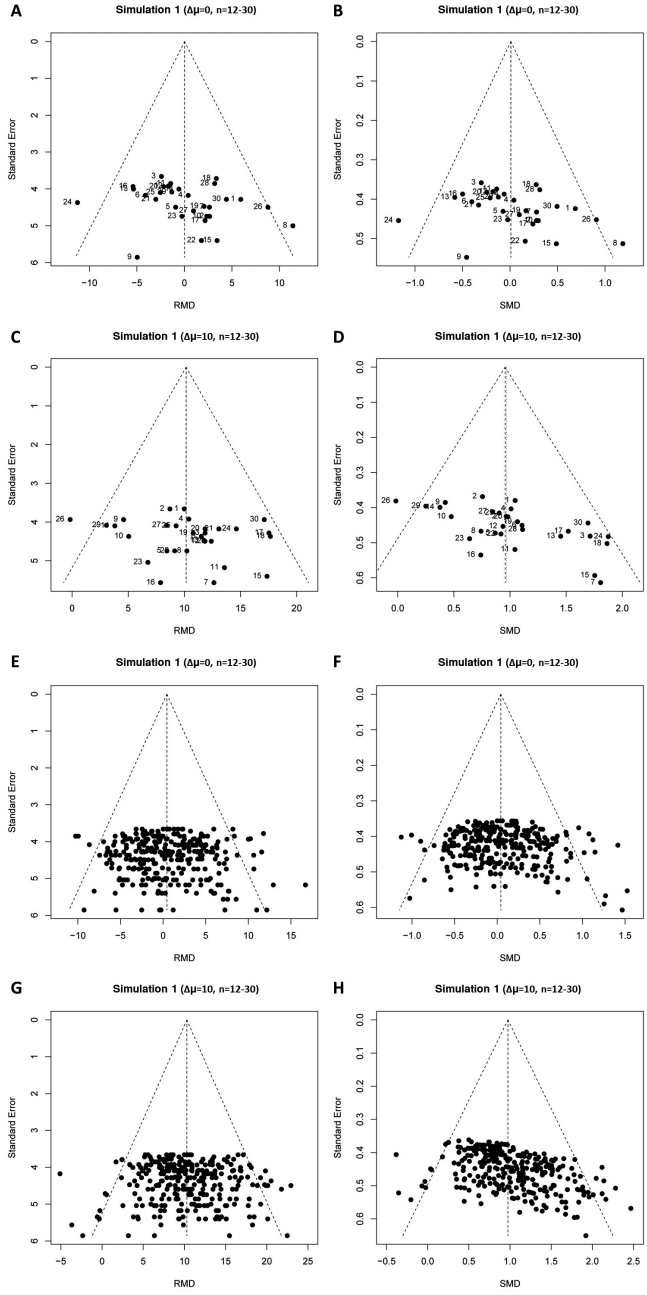


Figure 4 . Representative raw mean difference (RMD; A, C, E, G) and standardized mean difference (Hedges' g SMD; B, D, F, H) funnel plots for simulated unbiased meta-analyses containing thirty (A–D) or 300 (E–H) studies with a small sample size (total study n = 12–30). Simulations were performed without an intervention effect (Δμ = 0; A–B and E–F), or with an intervention effect (Δμ = 10; C–D and G–H). Δμ = difference in normally distributed means between control and intervention group. Representative funnel plots for studies with a large sample size (total study n = 60–320) are shown in Figure 4—figure supplement 1. Representative funnel plots for the comparison between Hedges' *g* and Cohen's *d* are shown in Figure 4—figure supplement 2.

results of Egger's test are supported by visual inspection of funnel plots of these unbiased scenario's (Figure 5). The typical left-upward shift of the small SMD datapoints and right-downward shift of the large SMD data points is clearly visible in the SMD vs. SE plot (Figure 5B), but not in the RMD, SMD vs. 1/√n or NMD plots.

Table 2. Study characteristics in relation to publication bias assessment in simulation of unbiased meta-analyses (simulation 1).

Total study N	Δμ	No. of studies in MA	Effect measure	% of simulations with Egger's p<0.05	No. of studies filled by T&F (mean(min - max))	Overall effect size (mean(min - max))	Overall effect size after T&F (mean(min - max))
12–30	0	30	RMD	6.2%	2.1 (0–11)	0.74(−12.2–11.3)	0.0(−3.8–3.6)
			SMD(*g*)	9.3%	1.6 (0–10)	0.1(−1.1–1.4)	0.0(−0.36–0.33)
12–30	5	30	RMD	4.9%	2.1 (0–10)	5.3(−3.4–19.1)	5.0 (1.2–9.6)
			SMD(*g*)	19.5%	2.4 (0–10)	0.55(−0.4–2.2)	0.43 (0.11–0.74)
12–30	10	30	RMD	4.6%	2.0 (0–10)	11.2 (1.2–20.4)	10.0 (5.4–13.5)
			SMD(*g*)	67.2%	4.4 (0–10)	1.16 (0.2–2.4)	0.85 (0.5–1.2)
12–30	0	300	RMD	4.8%	25.4 (0–62)	0.0(−15.2–12.3)	0.0(−2.1–2.3)
			SMD(*g*)	9.8%	18.8 (0–57)	0.0(−1.9–1.6)	0.0(−0.2–0.2)
12–30	5	300	RMD	5.5%	25.1 (0–65)	5.5(−10.2–23.7)	5.0 (3.0–6.8)
			SMD(*g*)	96.0%	47.3 (0–70)	0.55(−1.1–2.3)	0.37 (0.28–0.50)
12–30	10	300	RMD	5.9%	25.8 (0–61)	10.3(−11.1–29.0)	10.0 (7.9–12.3)
			SMD(*g*)	100%	61.5 (40–76)	1.0(−1.4–3.1)	0.80 (0.70–0.89)
12–30	0	3000	RMD	5.4%	249 (0–453)	0.0(−18.6–17.9)	0.0(−1.4–1.3)
			SMD(*g*)	8.7%	175.1 (0–386)	0.0(−2.1–2.6)	0.0(−0.1–0.1)
12–30	5	3000	RMD	4.4%	252 (0–475)	4.9(−13.0–21.1)	5.0 (3.7–6.4)
			SMD(*g*)	100%	492(417 - 565)	0.49(−1.7–2.9)	0.36 (0.33–0.39)
12–30	10	3000	RMD	5.0%	250 (0–456)	10.0(−7–27)	10.0 (8.6–11.3)
			SMD(*g*)	100%	620(568 - 669)	1.0(−0.7–4.5)	0.79 (0.8–0.8)
60–320	0	30	RMD	4.7%	2.4 (0–10)	−0.2(−3.8–3.3)	0.0(−1.3–1.3)
			SMD(*g*)	5.0%	2.4 (0–10)	0.0(−0.4–0.4)	0.0(−0.1–0.1)
60–320	5	30	RMD	3.8%	2.2 (0–10)	4.8 (1.9–7.6)	5.0 (3.8–6.1)
			SMD(*g*)	5.2%	2.4 (0–13)	0.48 (0.2–0.8)	0.5 (0.4–0.6)
60–320	10	30	RMD	5.9%	2.4 (0–10)	10.0 (6.7–14.0)	10.0 (8.7–11.2)
			SMD(*g*)	7.9%	2.6 (0–10)	1.0 (0.6–1.3)	1.0 (0.8–1.1)
60–320	0	300	RMD	4.4%	18.9 (0–58)	0.1(−3.7–5.5)	0.0(−0.5–0.6)
			SMD(*g*)	4.6%	17.3 (0–58)	0.0(−0.4–0.5)	0.0(−0.1–0.1)
60–320	5	300	RMD	4.7%	17.8 (0–63)	4.9 (0.0–9.7)	5.0 (4.4–5.6)
			SMD(*g*)	11.8%	20.7 (0–60)	0.49 (0.0–0.9)	0.49 (0.4–0.5)
60–320	10	300	RMD	6.2%	18.4 (0–63)	10.1 (4.8–16.5)	10.0 (9.4–10.6)
			SMD(*g*)	33.9%	29.5 (0–71)	1.0 (0.5–1.7)	0.97 (0.9–1.0)
60–320	0	3000	RMD	5.3%	140.0 (0–367)	0.0(−6.5–5.6)	0.0(−0.3–0.3)
			SMD(*g*)	5.4%	136.6 (0–348)	0.0(−0.7–0.6)	0.0 (0.0–0.0)
60–320	5	3000	RMD	4.7%	143 (0–331)	5.0(−1.4–11.3)	5.0 (4.7–5.3)
			SMD(*g*)	69.0%	243 (0–391)	0.5(−0.1–1.2)	0.48 (0.46–0.51)
60–320	10	3000	RMD	5.0%	135.8 (0–340)	10.0 (4.6–16.2)	10.0 (9.7–10.3)
			SMD(*g*)	99.7%	334.5(168–464)	1.0 (0.47–1.61)	0.97 (0.95–0.98)

n = sample size; Δμ = difference in normally distributed means between intervention and control group; no. = number; MA = meta analysis; T and F = trim and fill analysis; RMD = raw mean difference; SMD(*g*)=Hedges' *g* standardized mean difference; SD = standard deviation

Table 3. Publication bias assessments in unbiased and biased simulations using the RMD, SMD or NMD in combination with an SE or sample size-based precision estimate (simulation 3).

	Precision estimate SE		Precision estimate 1/√n		
Effect measure	**Bias?**	**% of sims with Egger's p<0.05**	**Median p-value (range)**	**% of sims with Egger's p<0.05**	**Median p-value (range)**
RMD	No	5.1	0.51 (0.001–1.0)	5.1%	0.50 (0.001–1.0)
RMD	Yes	69.1%	0.01 ($2.7*10^{-8}$ - 0.99)	69.6%	0.01 ($1.6*10^{-8}$ - 0.97)
SMD	No	100%	$2.9*10^{-13}$(0–$8.1*10^{-6}$)	4.3%	0.51 (0.001–1.0)
SMD	Yes	100%	$4.4*10^{-16}$(0–$1.8*10^{-6}$)	72.4%	0.01 ($5.4*10^{-10}$ - 0.99)
NMD	No	6.4%	0.51 (0.001–1.0)	6.4%	0.50 (0.001–1.0)
NMD	Yes	60.5%	0.02 ($7.1*10^{-8}$ - 0.99)	60.4%	0.02 ($8.0*10^{-8}$ - 0.98)

Simulated meta-analyses contained 300 studies (total study n = 12–30 subjects) and the difference in normally distributed means between control and intervention group was 10. Publication bias was introduced stepwise, by removing 10% of primary studies in which the difference between the intervention and control group means was significant at p<0.05, 50% of studies where the significance level was p≥0.05 to p<0.10, and 90% of studies where the significance level was p≥0.10. SE = standard error; RMD = raw mean difference; SMD = standardized mean difference (Hedges' g); NMD = normalized mean difference; sims = simulations.

In our final simulation we tested the performance of these different approaches in the presence of simulated publication bias. In the majority of these simulations of meta-analyses of individually small studies, asymmetry was detected both visually (Figure 6), and using Egger's regression (Supplementary file 1). When the size of individual studies was small, SMD vs.1/√n funnel plots performed as well as the RMD vs. SE funnel plots, in both biased and unbiased simulations (Table 3). The NMD also behaved similar to the RMD with either an SE or 1/√n precision estimate.

Re-analyses of SMD funnel plots from published meta-analyses

Since a sample size-based precision estimate might be more suitable for asymmetry analysis, we used data from five previously published meta-analyses which used an SMD vs. SE funnel plot and claimed funnel plot asymmetry as a result of publication bias. In the original publications, all five of these funnel plots were asymmetrical according to Egger's regression test. In three out of five cases, this asymmetry was not present in funnel plots using 1/√n as a precision estimate (Table 4 and Figure 7). Furthermore, three out of five papers reported several missing data points, as detected by trim and fill analysis. Missing data points were not detected when using SMD vs. 1/√n funnel plots for trim and fill analysis (Table 4 and Figure 7).

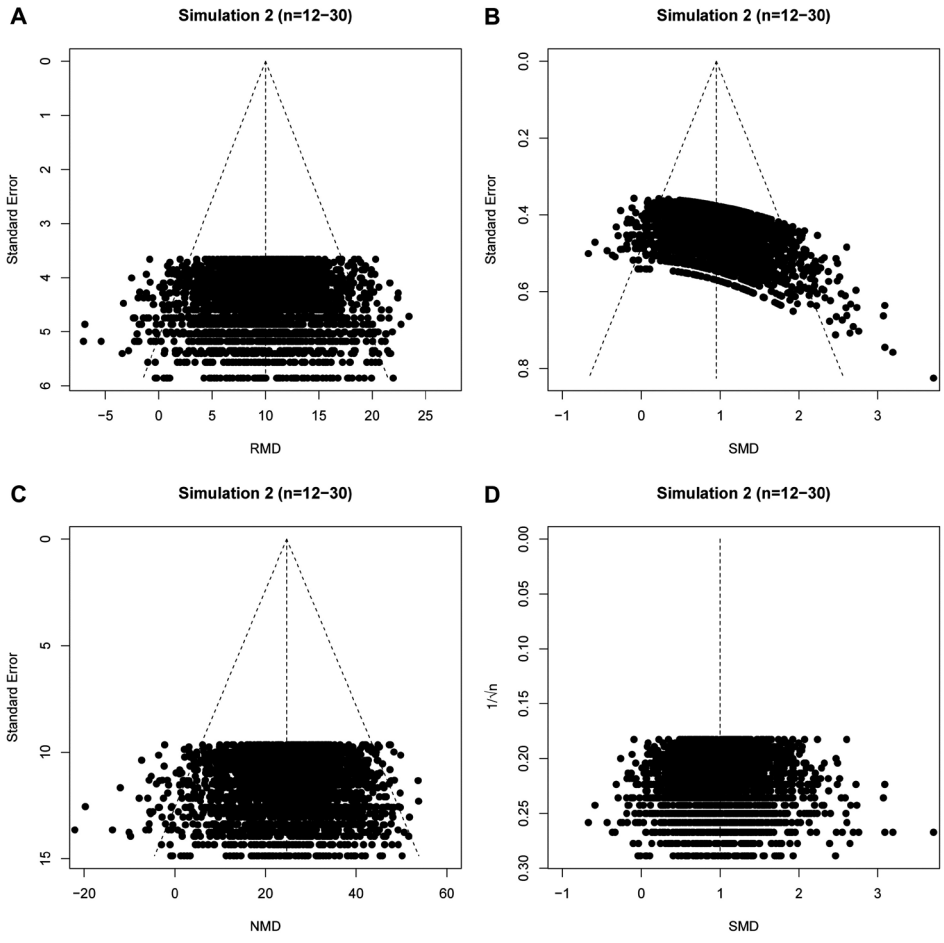
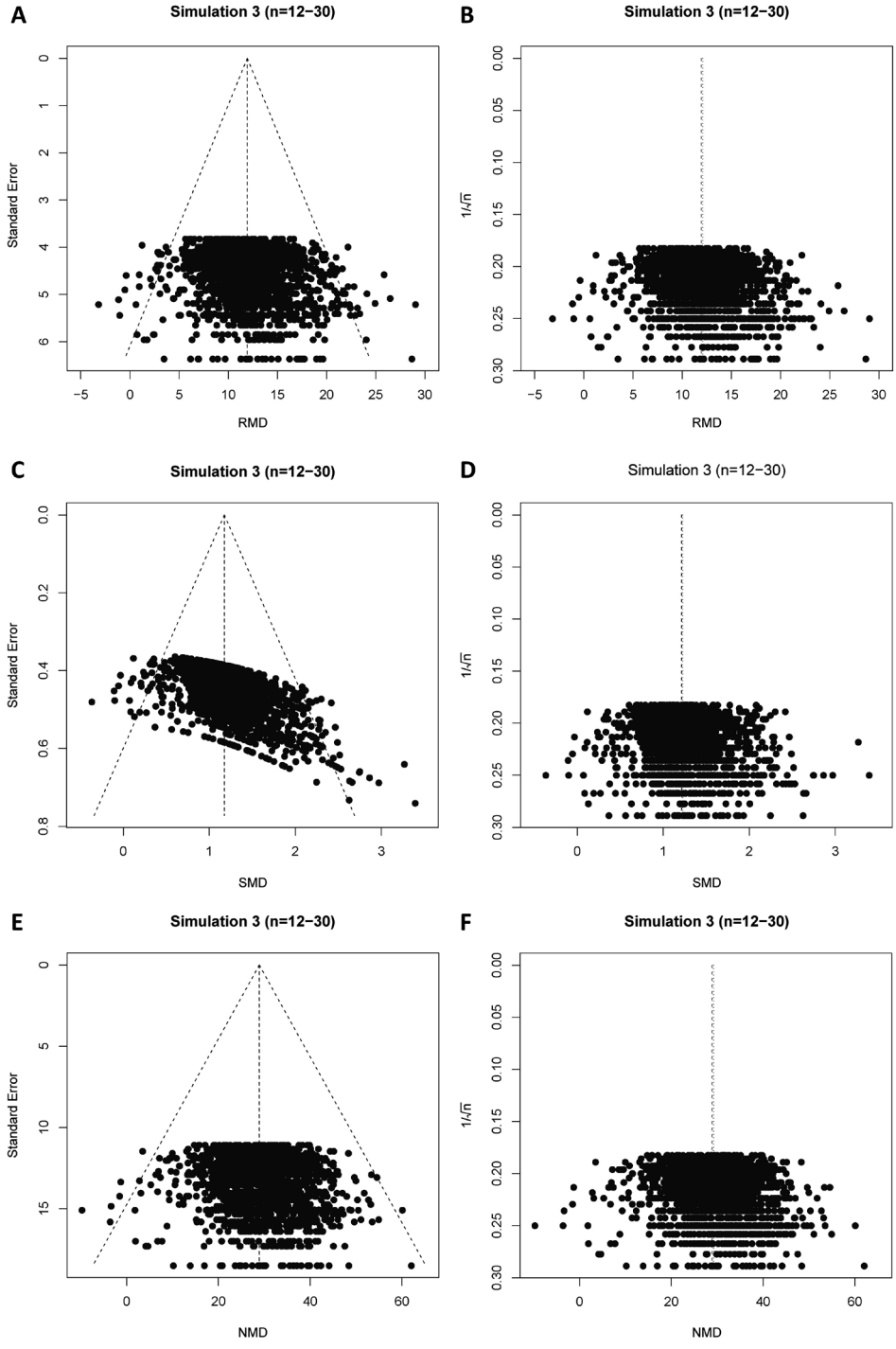


Figure 5 Raw mean difference (RMD; A), standardized mean difference (SMD; B), normalized mean difference (NMD; C) with SE as precision estimate, and SMD funnel plots using 1/√n as precision estimate (D). All plots show the same simulated meta-analysis containing 3000 studies with small sample sizes (n = 12–30) and an overall intervention effect of Δμ = 10. Δμ = difference in normally distributed means between control and intervention group.

DISCUSSION

Using data from both simulated and empirical meta-analyses, we have shown that the use of Egger's regression test for funnel plot asymmetry based on plotting SMD against SE is associated with such a substantial over-estimation of asymmetry as to render this approach of little value, particularly when the size of contributing studies is small. This distortion occurs whenever an intervention effect is present, in meta-analyses both with and without publication bias. The severity of distortion and the risk of misinterpretation are influenced by the sample size of the individual studies, the


A
Simulation 3 (n=12−30)
Standard Error
RMD
B
Simulation 3 (n=12−30)
1/√n
RMD
C
Simulation 3 (n=12−30)
Standard Error
SMD
D
Simulation 3 (n=12−30)
1/√n
SMD
E
Simulation 3 (n=12−30)
Standard Error
NMD
F
Simulation 3 (n=12−30)
1/√n
NMD

number of studies in the meta-analysis, and the presence or absence of an intervention effect. Thus, the use of SMD vs. SE funnel plots may lead to invalid conclusions about the presence or absence of publication bias and should not be used. Since it is the association between the SMD and its SE that leads to funnel plot distortion, it almost inevitable that the issues described will occur with any test for publication bias that relies on an assessment of funnel plot asymmetry (*e.g.* Begg and Mazumdar's test[19]). When using trim and fill analysis, funnel plot distortion introduces the risk of incorrectly adjusting the summary effect estimate. Previous reports of the presence of publication bias based on this approach should be re-evaluated, both for pre-clinical and clinical meta-analyses. Importantly, distortion does not occur in NMD vs. SE funnel plots, which formed the basis of a recent analysis showing evidence for substantial publication bias in the animal stroke literature[25].

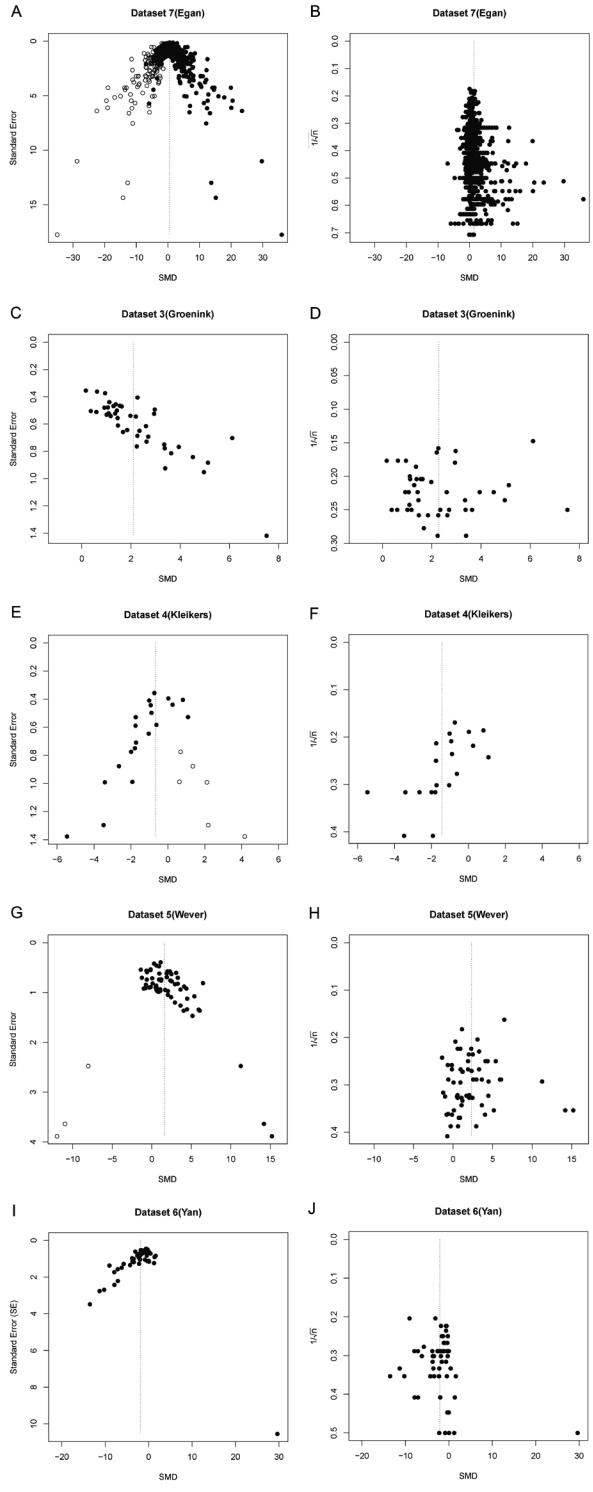
As the use of meta-analysis to summarize clinical and preclinical data continues to increase, continuous evaluation and development of research methods is crucial to promote high-quality meta-research[26]. To our knowledge (see also Sterne et al[11]), potential problems in tests for funnel plot asymmetry have not been extensively studied for SMDs, and guidance is limited. For instance, the Cochrane Handbook for Systematic

Table 4. Re-analysis of published preclinical meta-analyses using SMD.

		Precision estimate						
			Standard Error			1/√n		
Study	**n**	**Observed SMD [95% CI]**	**Egger's p**	**filled**	**Adjusted SMD**	**Egger's p**	**filled**	**Adjusted SMD**
Egan et al[24]	1392	0.75 [0.70, 0.80]	$<2.2\times10^{-16}$	252	0.42 [0.37,0.47]	2.2×10^{-11}	0	N/A
Groenink et al[21]	43	−1.99[−2.33,−1.64]	8.5×10^{-10}	0	N/A	0.68	0	N/A
Kleikers et al[23]	20	−1.15[−1.67; −0.63]	3.5×10^{-4}	6	?	2.9×10^{-3}	0	N/A
Wever et al[20]	62	1.54 [1.16, 1.93]	7.8×10^{-6}	3	?	0.62	0	N/A
Yan et al[22]	60	1.58 [1.19, 1.97]	6.5×10^{-6}	0	N/A	0.19	0	N/A

n = number of studies; SMD = standardized mean difference; CI = confidence interval; Egger's p=p value for Egger's regression; adjusted SMD = SMD after trim and fill analysis; N/A = not applicable.

◂ **Figure 6.** Simulation 3 Funnel plots of biased meta-analyses**.** Representative funnel plots of simulated biased meta-analyses using a raw mean difference (RMD; **A–B**), a standardized mean difference (SMD; **C–D**), or a normalised mean difference (NMD; **E–F**) effect measure. The present example contains 3000 studies with a small study sample size (n = 12–30) and an intervention effect present (difference in normal distribution means between control and intervention group = 10). Publication bias was introduced stepwise, by removing 10% of primary studies in which the difference between the intervention and control group means was significant at $p<0.05$, 50% of studies where the significance level was $p\geq0.05$ to $p<0.10$, and 90% of studies where the significance level was $p\geq0.10$. Precision estimates are standard error (**A, C, E**) or sample size-based (**B, D, F**), where n = total primary study sample size.


A
Dataset 7(Egan)
Standard Error
SMD
B
Dataset 7(Egan)
1/√n
SMD
C
Dataset 3(Groenink)
Standard Error
SMD
D
Dataset 3(Groenink)
1/√n
SMD
E
Dataset 4(Kleikers)
Standard Error
SMD
F
Dataset 4(Kleikers)
1/√n
SMD
G
Dataset 5(Wever)
Standard Error
SMD
H
Dataset 5(Wever)
1/√n
SMD
I
Dataset 6(Yan)
Standard Error (SE)
SMD
J
Dataset 6(Yan)
1/√n
SMD

◂ **Figure 7** Funnel plots of re-analysis of empirical meta-analyses. Funnel plots of empirical meta-analyses plotted as standardized mean difference (SMD) *versus* standard error, as in the original publications (left hand panels), and as SMD *versus* 1/√n after re-analysis. n = total primary study sample size; filled circles = observed data points; open circles = missing data points as suggested by trim and fill analysis.

Reviews of Interventions states that artefacts may occur and that firm guidance on this matter is not yet available[22]. It is disquieting that publication bias analyses using SMD funnel plots have been published in clinical and preclinical research areas, presumably because both the authors and the peer reviewers were unaware of the risk of spurious publication bias introduced by this methodology. Accepted papers from our group and others using SMDs for publication bias assessments have passed the peer review system, with no additional questions and or comments on this potential problem.

A similar phenomenon has been reported for the use of odds ratios in funnel plots, which also induces artificial significant results in Egger's regression (Peters et al., 2006). Here, too, an alternative test based on sample size has been proposed to circumvent this problem[27], and we suggest to extend this recommendation to SMDs.

However, given the relative performance of the RMD, NMD and SMD approaches, it is reasonable to consider whether SMD should ever be used. The RMD approach is limited because there are many instances (for example across species) where, although the same units of measurement are used, a given change may have very different biological importance. The NMD approach is preferred, but – because it expresses the effects of an intervention as a proportion of lesion size – there may be circumstances where outcome in a non-lesioned animal is not reported or cannot be inferred, and here the NMD approach is not possible. Further, the relative performance of RMD, NMD and SMD approaches in identifying heterogeneity between groups of animal studies (partitioning of heterogeneity) or in meta-regression is not known.

Taken with the increased distortion seen when contributing studies are individually small, this means our findings may be especially relevant for preclinical meta-analyses. The SMD is frequently used in preclinical meta-analyses to overcome expected heterogeneity between data obtained from different animal species. Nevertheless, the SMD is also used in clinical meta-analyses and the degree of distortion cannot be readily predicted. In any case, distortion causes the threshold for determining publication bias to be artificially lowered when using SMDs and their SE, increasing the chance of false-positive results.

Of note, trim and fill analysis may not always be reliable when the number of studies in a meta-analysis is large; in half of the cases of our unbiased simulations with 300 and 3000 studies, many studies were deemed missing, even if no intervention effect was introduced. Still, the SMD simulations were always more susceptible to the addition of imputed studies if a true effect was introduced, and the effect size reduction was larger compared to RMD measurements.

Limitations of this study

We designed our data simulations to closely resemble empirical data in terms of the range of sample sizes, effect sizes and numbers of studies in a meta-analyses. We acknowledge that our current range of simulation scenarios does not enable us to predict the impact of funnel plot distortion in every possible scenario, but we present those scenarios which most clearly illustrate the causes and consequences of funnel plot distortion. Furthermore, our simulations may still be improved by *e.g.* studying the effects of unequal variances between treatment groups, sampling data from a non-normal distribution, or introducing various degrees of heterogeneity into the simulation. However, research on how to optimally simulate these parameters is first needed, and was beyond the scope of this study. instead, we used re-analyses of empirical data to test our proposed solutions on a number of real-life meta-analyses which include all of the aforementioned aspects.

Recommendations

We recommend that, where possible, investigators use RMD or NMD instead of SMD when seeking evidence of publication bias in meta-analyses. Where it is necessary to use SMD, assessment for publication bias should use a sample size-based precision estimate such as 1/√n. In a given analysis it may be possible to calculate an NMD effect size for some but not all studies. In these circumstances there is a trade-off between the reduced number of included studies and an improved estimation of publication bias, and sensitivity analysis may be used to compare the meta-analysis outcome using the NMD versus the SMD. Of note, other methods to investigate publication bias in a dataset may be used in addition to funnel plots (*e.g.* fail-safe N, Excess Significance Test[28], or selection method / weight funcion model approaches[27], but the performance of these approaches in the context of SMD, RMD and NMD estimates of effect size is not known.

In conclusion, funnel plots based on SMDs and their SE should be interpreted with caution, as the chosen precision estimate is crucial for detection of real funnel plot asymmetry.

REFERENCES

1. Dwan K, Gamble C, Williamson PR, Kirkham JJ. Systematic Review of the Empirical Evidence of Study Publication Bias and Outcome Reporting Bias - An Updated Review. *PLoS One*. 2013;8(7):e66844. doi:10.1371/journal.pone.0066844
2. Chalmers I, Bracken MB, Djulbegovic B, et al. How to increase value and reduce waste when research priorities are set. *Lancet (London, England)*. 2014;383(9912):156-165. doi:10.1016/S0140-6736(13)62229-1
3. Bastian H, Glasziou P, Chalmers I. Seventy-five trials and eleven systematic reviews a day: How will we ever keep up? *PLoS Med*. 2010;7(9). doi:10.1371/journal.pmed.1000326
4. Korevaar D a, Hooft L, ter Riet G. Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments. *Lab Anim*. 2011;45(4):225-230. doi:10.1258/la.2011.010121
5. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd editio. LAWRENCE ERLBAUM; 1988.
6. Hedges L V. Distribution Theory for Glass's Estimator of Effect size and Related Estimators. *J Educ Stat*. 1981;6(2):107-128. doi:10.3102/10769986006002107
7. Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: A practical guide. *J Neurosci Methods*. 2014;221:92-102. doi:10.1016/j.jneumeth.2013.09.010
8. Rothstein HR, Sutton AJ, Borenstein M. *Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments*. (Edition 1st, ed.). John Wiley and sons Ltd.; 2005.
9. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315(7109):629 LP - 634. doi:10.1136/bmj.315.7109.629
10. Duval S, Tweedie R. Trim and Fill: A Simple Funnel-Plot–Based Method of Testing and Adjusting for Publication Bias in Meta-Analysis. *Biometrics*. 2000;56(2):455-463. doi:https://doi.org/10.1111/j.0006-341X.2000.00455.x
11. Sterne JAC, Sutton AJ, Ioannidis JPA, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*. 2011;343:d4002. doi:10.1136/bmj.d4002
12. Kelley K. MBESS: The MBESS R Package. https://cran.r-project.org/package=MBESS.
13. Schwarzer G. Meta: General Package for Meta-analysis. 2016. https://cran.r-project.org/web/packages/meta/index.html.
14. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. *J Stat Softw*. 2010;36(3 SE-Articles):1-48. doi:10.18637/jss.v036.i03
15. Dragulescu A. Read, write, format Excel 2007 and Excel 97/2000/XP/2003 files. 2014. https://cran.r-project.org/package=xlsx.
16. Wever KE, Hooijmans CR, Riksen NP, et al. Determinants of the efficacy of cardiac ischemic preconditioning: A systematic review and meta-analysis of animal studies. *PLoS One*. 2015;10(11):1-17. doi:10.1371/journal.pone.0142021
17. Zwetsloot PP, Vegh AM, Jansen Of Lorkeers SJ, et al. Cardiac Stem Cell Treatment in Myocardial Infarction: A Systematic Review and Meta-Analysis of Preclinical Studies. *Circ Res*. 2016;118(8):1223-1232. doi:10.1161/circresaha.115.307676
18. Lau J, Ioannidis JP, Terrin N, Schmid CH, Olkin I. The case of the misleading funnel plot. *BMJ*. 2006;333:597-600. doi:10.1136/bmj.38946.501215.68
19. Begg CB, Mazumdar M. Operating Characteristics of a Rank Correlation Test for Publication Bias. *Biometrics*. 1994;50(4):1088-1101. doi:10.2307/2533446
20. Wever KE, Menting TP, Rovers M, et al. Ischemic Preconditioning in the Animal Kidney, a Systematic Review and Meta-Analysis. *PLoS One*. 2012;7(2):e32296. https://doi.org/10.1371/journal.pone.0032296.
21. Groenink L, Verdouw PM, Bakker B, Wever KE. Pharmacological and methodological aspects of the separation-induced vocalization test in guinea pig pups; A systematic review and meta-analysis. *Eur J Pharmacol*. 2015;753:191-208. doi:10.1016/j.ejphar.2014.10.062
22. Yan XZ, Yang F, Jansen JA, de Vries RB, van den Beucken JJ. Cell-Based Approaches in Periodontal Regeneration: A Systematic Review and Meta-Analysis of Periodontal Defect Models in Animal Experimental Work. *Tissue Eng Part B Rev*. 2015;21(5):411-426. doi:10.1089/ten.TEB.2015.0049
23. Kleikers PWM, Hooijmans C, Göb E, et al. A combined pre-clinical meta-analysis and randomized confirmatory trial approach to improve data validity for therapeutic target validation. *Sci Rep*. 2015;5:13428. doi:10.1038/srep13428
24. Egan KJ, Vesterinen HM, Beglopoulos V, Sena ES, Macleod MR. From a mouse: systematic analysis reveals limitations of experiments testing interventions in Alzheimer's disease mouse models. *Evidence-based Preclin Med*. 2016;3(1):e00015. doi:https://doi.org/10.1002/ebm2.15
25. Sena ES, Bart van der Worp H, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*. 2010;8(3):e1000344. doi:10.1371/journal.pbio.1000344

26. Ioannidis JPA, Fanelli D, Dunne DD, Goodman SN. Meta-research: Evaluation and Improvement of Research Methods and Practices. *PLOS Biol*. 2015;13(10):e1002264. https://doi.org/10.1371/journal.pbio.1002264.
27. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of Two Methods to Detect Publication Bias in Meta-analysis. *JAMA*. 2006;295(6):676-680. doi:10.1001/jama.295.6.676
28. Ioannidis JPA, Trikalinos TA. An exploratory test for an excess of significant findings. *Clin Trials*. 2007;4:245-253. doi:10.1177/1740774507079441

CHAPTER 4

Publication rate in preclinical research: a plea for preregistration

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ABSTRACT

Objectives The ultimate goal of biomedical research is the development of new treatment options for patients. Animal models are used if questions cannot be addressed otherwise. Currently, it is widely believed that a large fraction of performed studies are never published, but there are no data that directly address this question.
Methods We have tracked a selection of animal study protocols approved in the University Medical Center Utrecht in the Netherlands, to assess whether these have led to a publication with a follow-up period of 7 years.
Results We found that 60% of all animal study protocols led to at least one publication (full text or abstract). A total of 5590 animals were used in these studies, of which 26% was reported in the resulting publications.
Conclusions The data presented here underline the need for preclinical preregistration, in view of the risk of reporting and publication bias in preclinical research. We plea that all animal study protocols should be prospectively registered on an online, accessible platform to increase transparency and data sharing. To facilitate this, we have developed a platform dedicated to animal study protocol registration: www.preclinicaltrials.eu.

INTRODUCTION

Biomedical research is performed to gain an understanding of (patho)physiological mechanisms and ultimately to use this knowledge to develop new therapies for patients. However, limitations in the design and reporting of experiments are known to cause avoidable research waste[1,2] and it has been estimated that 85% of all research costs and efforts is wasted[3–5]. One important factor leading to avoidable waste is publication bias, in which the outcome of a study influences the chance of publishing. This has been recognised as an important problem in the biomedical sciences for several decades[6,7]. The systematic over-representation of statistically significant study results leads to an overestimation of effect sizes, threatens the validity of systematic reviews and meta-analyses and can influence the development of guidelines and recommendations, or the decision to proceed to a clinical trial[6,8].

The publication rate (ie, the percentage of conducted studies that is eventually published) is an important indicator of publication bias, and has been extensively studied for clinical trials, for example, by tracking studies from their initiation to publication or non-publication. Such studies report a wide variation in publication rates, ranging from 12.5%[8] to 93%[9], depending on for example the source of identification of the trials (eg, institutional ethics committee approvals vs entry in a clinical trial protocol registry), the trial phase and the source of funding[8–11]. The statistical significance of the trial outcomes is associated with both the publication rate and the time to publication[10,12].

Although not as extensively evaluated, there is also reason for concern regarding the selective publication of preclinical animal studies[13–15]. As in clinical research, systematic review and meta-analysis has been instrumental in making publication bias in animal research transparent. Between 46% and 62% of preclinical systematic reviews find evidence of publication bias[15]. In preclinical neurology research, the number of animal studies reporting statistically significant beneficial treatment effects far exceeds the expected number of animals studies with such positive results[16]. Furthermore, an estimated 14% of animal studies in stroke is performed but not published, possibly causing a relative overestimation of the overall effect of treatment of 31%[17]. In a survey among Dutch animal researchers, respondents estimated the publication rate of animal studies to be on average 50% in non-for-profit organisations, and 10% in for-profit organisations[1]. Important reasons of non-publication indicated by the respondents were lack of statistical significance, the opinions of supervisors and peer reviewers, and technical problems during the experiment.

However, compared with clinical studies, measuring publication and reporting bias (selective reporting of results) in animal studies directly by assessing their publication rate from initiation is more difficult, because few accessible registries of animal study protocols exist and publications of animal studies rarely refer to a study protocol. Here,

we present the first study investigating the publication rate in animal research, by tracking a set of research protocols from their approval by an animal ethics committee, to publication or non-publication.

METHODS

Study protocol selection

We tracked animal studies performed at three research departments at the University Medical Center Utrecht, for which study applications were approved by the animal ethics committee in 2008 or 2009. Applications from commercial parties were not included. At that time all applications for animal studies in the Netherlands were approved by local institutional animal experiment committees. Applications are confidential, and mainly consist of the study protocol, which includes background information, hypotheses, a sample size calculation and a detailed description of the experimental procedures. We were granted access to applications only after consent from at least one of the researchers listed on the application.

Searching and selecting publications

We performed systematic searches using the names of all investigators listed on the 67 applications, in PubMed and EMBASE on 14 March 2016. The search string therefore included all researchers' names. For example, for PubMed the search was as follows: "last name researcher #1 initials"[author] OR "last name researcher #2 initials"[author] OR "last name researcher #3 initials"[author] OR" etc. Search results were limited to articles published after 01/01/2007. The search results were screened for eligibility based on their title and abstract by either MvdN or SW. Based on a title/abstract screening, publications on animal studies related to any of the three involved research departments were included. Publications were included if they were (1) primary reports on animal study data and (2) related to any of the three involved research departments. Full-text screening was performed in duplicate by two independent reviewers (MvdN and SW). Exclusion criteria were as follows: (1) not a primary report of an animal study, (2) not related to (experimental) cardiology or medical physiology, (3) use of slaughterhouse material only, (4) the animal experiment committee reported in the article was not from the institute of interest, (5) study was performed in accordance to non-Dutch legislation, (6) the animal ethics committee application number format did not match that of the institute of interest and (7) the author's full name did not match the name of the researcher on the application (eg, same initials, but different full first name). Included articles were sorted per animal species (figure 1).

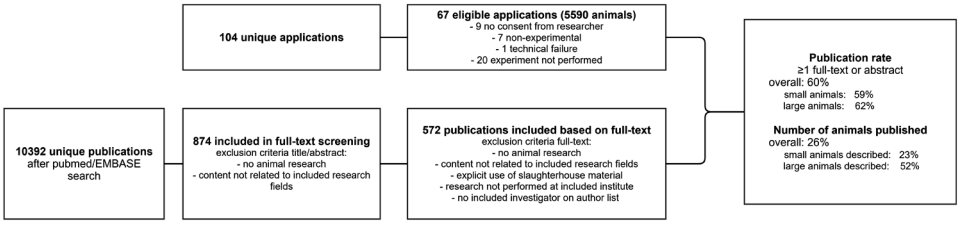

Figure 1. Flow chart showing the total number of applications, the number of included applications, the number of publications in the PubMed/EMBASE search, in the full-text screening and those included in the linking to the applications.

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Matching of study protocol to publications

Two reviewers (MvdN and SW) independently identified whether the application has led to a publication. For each application, possible publications were identified in the publication database by matching animal species, involved researchers with the author list and performing a detailed comparison of the research question, animal model, intervention(s) and experimental procedures described in the application versus the publication. Discrepancies were solved by a third reviewer (KW).

Endpoints

The aim of this study was to determine the publication rate of animal studies within the three participating departments. Publication rate is assessed using the following definitions: (1) the number of applications which led to ≥1 publications (full-text only), (2) the number of applications which led to ≥1 publications (abstracts included) and (3) the number of animals published as an percentage of the total number of animals sacrificed for the performed studies.

Count of animals

Data from the local animal welfare bodies were used to identify the number of animals sacrificed per application. The total of number of animals (including those reported as 'excluded' or 'deceased') reported in each matched publication was extracted by two independent researchers (MvdN and SW). When publications lacked details on the exact number of animals used for a specific experiment, we used the biggest number mentioned (eg, when a group size was mentioned as a range from 3 to 5, we noted 5 animals).

Limited updated search

On 7 June 2019, the search was repeated in PubMed and Embase. For PubMed, results were filtered by publication date after 1 March 2016 and species 'Other Animals'. For Embase, the following filters were applied: publication year 2016–2019 and study types:

'animal experiment', 'animal model', 'animal tissue', 'disease model', 'feasibility study', 'in vivo study', 'model', 'mouse model', 'nonhuman' and 'preclinical study'. Title and abstract screening and matching to research applications were performed as described above by one researcher (SW). This resulted in 1286 unique publications. After title and abstract screening 133 publications remained. The publications were only compared with research applications for which no publications had been identified yet. Ultimately, no new matches were made.

Post hoc survey

A post hoc survey was conducted in March 2018 among the involved researchers. Goal of this survey was to verify if the tracing was done correctly, if any publications were missing and to assess why data were not published. Researchers were sent an overview of the applications, including the number of animals used according to the institutional data as well as the publications identified by our search. They were asked (1) if the found publications were correct, (2) if all data were published, (3) if not, why data were not published, (4) if the study was explorative or confirmative and (5) if the study result was significant or not-significant.

RESULTS

A total of 104 unique applications were approved by the three selected research departments in 2008 and 2009 at our institution. Part of the protocols that were approved in 2008 or 2009 were continuations of research that was originally started in 2007. These applications were included. We obtained consent to access the study protocols from at least one of the researchers listed on the applications for 95 (91%) of these applications. Seven applications were excluded based on their non-experimental character (ie, applications for training or educational purposes), and one application was not accessible due to a technical failure. Local animal welfare bodies documented the number of animals sacrificed per application. According to this data, 20 of the 87 (23%) remaining applications were never carried out. Thus, study protocols from 67 applications were included in our analysis (figure 1). There were four applications for which assessment by a third reviewer (KW) was needed to determine whether the publication matched the application. Assessment by a third reviewer (KW) was also needed three times to decide on the number of animals mentioned in a publication.

A total of 30 full-text papers and 41 conference abstracts were found to be produced from these 67 applications. Our search identified at least one full-text publicationresulting from the research application for 46% (31/67) of the applications. Sixty per cent (40/67) was published when conference abstracts were also taken into

account. After stratifying for species, the publication rate (full text or abstract) for small animal models (mice, rats and rabbits) was 59% (24/41), compared with 62% (16/26) for large animal models (pigs, dogs and sheep; figure 1).

According to institutional administration, a total of 5590 animals were used in the 67 applications. In total, 26% (1471/5590) of the animals were described in the publications resulting from these applications. This percentage was considerably lower for small animals (23% (1190/5014) of animals published), than for large animals (52% (299/576) of animals published; figure 1).

The 40 applications that were published accounted for 79% of the total animals used (4402/5590). Out of these published applications, reports on small animals described on average 30% of the animals used in the applications (1190/3979, range 6%–100%). For studies involving large animals, this was on average 71% (299/423 range 8%–100%).

The average time between approval of a project by the animal ethics committee and the first resulting publication was 30.7 months (median 27.5). In this sample, the longest time between approval of a project and the first publication (either full text of abstract) was 65 months. In one case, the first full-text manuscript was published after 90 months, but an abstract had already been published after 35 months.

A post hoc survey conducted in March 2018 among the involved researchers. The survey was sent out to all researchers that gave permission for their 67 included applications. We received a response for 53 (79%) of the applications. We discovered one publication that was not identified by our search; this publication is included in our analysis. One survey participant informed us that a manuscript was in preparation, but had not yet been published; this manuscript is not included in our analysis. The most frequently reported reasons for non-publication were a lack of statistical significance, the study being a pilot study and technical problems with the animal model.

CONCLUSION AND DISCUSSION

With this study we attempt to determine the publication rate of animal research. To the best of our knowledge, this is the first report tracking the number of animals used, providing a percentage of animal published. The results show that 60% of the animal studies were ultimately published, but a considerable number of the animals used is not reported in these publications, as only 26% of the used animals were reported.

Sample size

Although our sample size was relatively small, we believe that these data are likely to be representative of the field of preclinical research (and not specific to our institution).

These findings are consistent with a previously published survey among 454 laboratory animal researchers in the Netherlands, that estimated that approximately 50% of animal experiments is published.1 A recent study reporting the publication rate of animal studies in Germany showed a publication rate of 67%, which is in line with our findings[18]. The most frequently reasons not to publish mentioned in our post hoc survey are similar to the reasons reported in a previous survey (lack of statistical significance, technical problems and objections from supervisors and peer reviewers)[1]. Although we expected the publication rate of animal studies to be lower the numbers, we found are comparable to publication rates reported in the clinical domain.

Animal tracking

Our post hoc survey identified only a single publication not identified by our search, indicating that our search and matching approach was able to correctly identify and match the vast majority of publications to the corresponding application. We noticed that publications often included experimental groups that could not have originated from the same application. These animals were not included in the number of published animals. Because results from separate research applications (with similar animal experiments) are frequently combined in a single report we cannot exclude that some of the animals described in the identified publications originated from other applications (which, eg, could have been performed before our study period). In that case the publication rate may be lower than reported here.

Follow-up period

The research applications analysed here were performed in 2007, 2008 and 2009, which allowed researchers up to 7 years to publish their findings. It is therefore unlikely that results from these experiments will be reported on in future publications if they have not been shared with the research community thus far. Concordantly, the survival publication curve (figure 2) reaches a plateau after 60 months. Due to the ethically and thus politically sensitive nature of our findings, over 3 years have passed since the systematic search and submission of the manuscript. However, as argued above, it is unlikely that the publication rate has significantly increased in the intervening period. This is also supported by the fact that our post hoc survey (performed in 2018) did not identify any papers published after our study period and the fact that our additional search did not identify any new matches.

Over the past decade, many studies have highlighted the importance of good research practices, among which is the sharing of data and publishing negative results[19–25]. Although there is growing awareness of the importance of good research practices, we are not aware of any data indicating that publication rates have significantly increased between 2008 and present day. Especially in the preclinical domain, there is a paucity of data available on this subject which this report in part is meant to address.

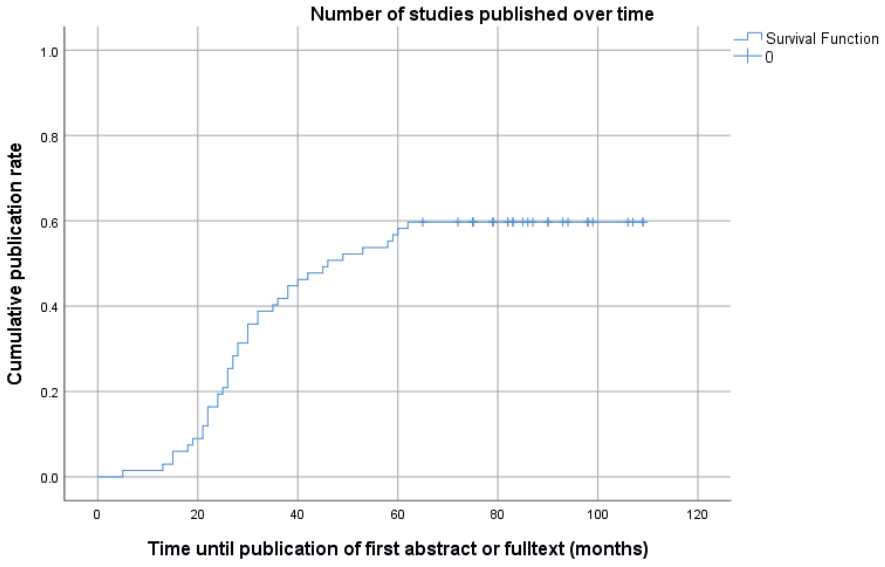

Figure 2. Kaplan-Meier curve with time between approval by the animal ethics committee until first publication (abstract or full-text).

Sharing of data

We believe that results of virtually all animal experiments should be shared with the research community. Animal studies are performed for the benefit of human health, and the ethical justification for the use of animals rests on those benefits. Increasing transparency and data sharing in animal research are essential to ensure a valuable contribution of animal experiments to advancing human health(care). The sharing of non-significant results or technical failures is important for scientific progress, for example, by improving methodology of animal models, as well as to prevent research waste in the form of unnecessary replications by others who are unaware of your results. There may also be a (perceived) lack of interest from scientific journals to publish non-significant data (lack of statistical significance was named as an important reason not to publish).

Preregistration

Prospective registration of animal study protocols—as is already common practice in the clinical arena—may increase sharing of data. If all animal studies are preregistered, researchers can use the animal study protocol database for a comprehensive overview of all experiments that have been performed to aid in answering research questions and designing new studies. It may allow researchers to identify colleagues who are working on the same topic or with experience with similar animal models and it can provide a platform where researchers can share unpublished data. Furthermore,

prospective registration can improve study design by emphasising the importance of rigour. Finally, it creates transparency around key elements of the experiment that was originally planned (eg, sample size calculations primary outcome) and enables comparison of the original protocol with the study as it was ultimately reported[26–28].

Implementation of preclinicaltrials.eu

To facilitate preregistration, we developed www. preclinicaltrials.eu: the first online accessible, international register dedicated to the (pre)registration of animal studies (launched 11 April 2018)[27,29,30]. The register aims to provide a comprehensive listing of animal studies to help avoid unplanned duplication, minimise publication bias and increase transparency. The platform allows registrants to link their protocols to published or unpublished data, thus enabling others to identify unpublished studies and data, for example, for the purpose of a systematic review or meta-analysis.

All stakeholders involved in animal studies and translational research (ie, researchers, institutions, funders and journals) should underscore the importance of preregistration of animal studies in order to incorporate this in routine practice. In this respect, it is very promising that the Dutch parliament recently unanimously accepted a motion declaring that all animal studies should be (prospectively) registered, and that all their results should be made publicly available. In addition, multiple policy makers, Dutch institutes (including the Netherlands Heart Institute) and funding agencies are taking steps towards implementation of preregistration. Utrecht University and University Medical Centre Utrecht have decided to make such preregistration mandatory. Various international scientific communities, such as the Transnational AllianCe for regenerative Therapies In Cardiovascular Syndromes (TACTICS) consortium and several working groups of the European Society for Cardiology, are committed to implement preregistration within their research fields, journal editors are discussing the possibilities to implement preregistration within their author guidelines and other countries and researchers are discussing and working on animal study registration[27,30,31]. In the meantime, we encourage individual researchers to take responsibility and actively contribute to prospective registration of preclinical trials.

Strengths and limitations of this study

- This study directly traces animal study protocols to potential publications and is the first study to assess the number of animals used and the number of animals published.
- We had full access to all documents submitted to the animal experiment committee of the University Medical Center Utrecht from the selected protocols.
- There is a sufficient follow-up period for researchers to publish their animal study.
- Due to privacy reasons, we are not able to publish the exact search terms used.
- A delay has occurred between the start of this project and time of publishing, this is related to the political sensitivity of this subject.

REFERENCES

1. ter Riet G, Korevaar DA, Leenaars M, et al. Publication Bias in Laboratory Animal Research: A Survey on Magnitude, Drivers, Consequences and Potential Solutions. *PLoS One*. 2012;7(9):e43404. doi:10.1371/journal.pone.0043404
2. Ioannidis JPA, Greenland S, Hlatky MA, et al. Increasing value and reducing waste in research design, conduct, and analysis. *Lancet*. 2014;383(9912):166-175. doi:10.1016/S0140-6736(13)62227-8
3. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. *Lancet*. 2009;374(9683):86-89. doi:10.1016/S0140-6736(09)60329-9
4. Chalmers I, Glasziou P, Chalmers I, Glasziou P, Chalmers I. Research waste is still a scandal — an essay by Paul. 2018;4645(November):10-12. doi:10.1136/bmj.k4645
5. Macleod MR, Michie S, Roberts I, et al. Biomedical research: increasing value, reducing waste. *Lancet*. 2014;383(9912):101-104. doi:10.1016/S0140-6736(13)62329-6
6. Chan AW, Song F, Vickers A, et al. Increasing value and reducing waste: Addressing inaccessible research. *Lancet*. 2014;383(9913):257-266. doi:10.1016/S0140-6736(13)62296-5
7. Dickersin K. Publication Bias: Recognizing the Problem, Understanding Its Origins and Scope, and Preventing Harm. In: *Publication Bias in Meta-Analysis: Prevention, Assessment, and Adjustments*. ; 2006:9-33. doi:10.1002/0470870168.ch2
8. Song SY, Koo DH, Jung SY, Kang W, Kim EY. The significance of the trial outcome was associated with publication rate and time to publication. *J Clin Epidemiol*. 2017;84:78-84. doi:10.1016/j.jclinepi.2017.02.009
9. Dickersin K, Min YI. NIH clinical trials and publication bias. *Online J Curr Clin Trials*. 1993;Doc No 50.
10. Suñé P, Suñé JM, Montoro JB. Positive Outcomes Influence the Rate and Time to Publication, but Not the Impact Factor of Publications of Clinical Trial Results. *PLoS One*. 2013;8(1):e54583. doi:10.1371/journal.pone.0054583
11. Pich J, Carne X, Arnaiz J-A, Gomez B, Trilla A, Rodes J. Role of a research ethics committee in follow-up and publication of results. *Lancet (London, England)*. 2003;361(9362):1015-1016. doi:10.1016/S0140-6736(03)12799-7
12. Ioannidis JPA. Effect of the Statistical Significance of Results on the Time to Completion and Publication of Randomized Efficacy Trials Characteristics of Registered Trials. 1998;279(4).
13. Jonker SJ, Menting TP, Warlé MC, Ritskes-Hoitinga M, Wever KE. Preclinical evidence for the efficacy of ischemic postconditioning against renal ischemia-reperfusion injury, a systematic review and meta-analysis. *PLoS One*. 2016;11(3):e0150863. doi:10.1371/journal.pone.0150863
14. Wever KE, Bruintjes MHD, Warlé MC, Hooijmans CR. Renal perfusion and function during pneumoperitoneum: A systematic review and meta-analysis of animal studies. *PLoS One*. 2016;11(9):e0163419. doi:10.1371/journal.pone.0163419
15. Korevaar D a, Hooft L, ter Riet G. Systematic reviews and meta-analyses of preclinical studies: publication bias in laboratory animal experiments. *Lab Anim*. 2011;45(4):225-230. doi:10.1258/la.2011.010121
16. Tsilidis KK, Panagiotou OA, Sena ES, et al. Evaluation of Excess Significance Bias in Animal Studies of Neurological Diseases. *PLoS Biol*. 2013;11(7):1-10. doi:10.1371/journal.pbio.1001609
17. Sena ES, Bart van der Worp H, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*. 2010;8(3):e1000344. doi:10.1371/journal.pbio.1000344
18. Wieschowski S, Biernot S, Deutsch S, Glage S, Strech D. Publication rates in animal research. Extent and characteristics of published and non- published animal studies followed up at two German university medical centres. *PLoS One*. 2019;14(11):e022375. doi:10.1371/journal.pone.0223758
19. Ramirez DD, Motazedian P, Jung RG, et al. Methodological Rigor in Preclinical Cardiovascular Studies: Targets to Enhance Reproducibility and Promote Research Translation. *Circ Res*. April 2017. http://circres.ahajournals.org/content/early/2017/03/30/CIRCRESAHA.117.310628.abstract.
20. Glenn Begley C, Ellis LM. Drug development: Raise standards for preclinical cancer research. *Nature*. 2012;483(7391):531-533. doi:10.1038/483531a
21. Ioannidis JP a. Why most published research findings are false. *PLoS Med*. 2005;2(8):e124. doi:10.1371/journal.pmed.0020124
22. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *Animals*. 2013;4(1):35-44. doi:10.3390/ani4010035
23. Ross JS, Mulvey GK, Hines EM, Nissen SE, Krumholz HM. Trial publication after registration in ClinicalTrials.gov: A cross-sectional analysis. *PLoS Med*. 2009;6(9):e1000144. doi:10.1371/journal.pmed.1000144
24. Chalmers I, Glasziou P, Godlee F. All trials must be registered and the results published. *Br Med J*. 2013;346(7890):f105. doi:10.1136/bmj.f105
25. Sena E, van der Worp HB, Howells D, Macleod M. How can we improve the pre-clinical development of drugs for stroke? *Trends Neurosci*. 2007;30(9):433-439. doi:10.1016/j.tins.2007.06.009

26. Goldacre B, Drysdale H, Powell-Smith A, et al. *The COMPare Trials Project*.; 2016. www.COMPare-trials.org.
27. Chamuleau SAJ, van der Naald M, Climent AM, et al. Translational Research in Cardiovascular Repair A call for a paradigm shift. *Circ Res*. 2018;122(2):310-318. doi:10.1161/CIRCRESAHA.117.311565
28. Kimmelman J, Anderson JA. Should preclinical studies be registered? *Nat Biotechnol*. 2012;30(6):488-489. doi:10.1038/nbt.2261
29. Jansen of Lorkeers SJ, Doevendans PA, Chamuleau SA. All preclinical trials should be registered in advance in an online registry. *Eur J Clin Invest*. 2014;44(9):891-892. doi:10.1111/eci.12299
30. Baker M. Animal registries aim to reduce bias. *Nature*. 2019;Sep;573(7773):297-298. doi:10.1038/d41586-019-02676-4
31. Bert B, Heinl C, Chmielewska J, et al. Refining animal research: The Animal Study Registry. *PLoS Biol*. 2019;17(10):e3000463. doi:10.1371/journal.pbio.3000463

CHAPTER 5

Preregistration of animal research protocols: development and 3-year overview of preclinicaltrials.eu

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ABSTRACT

Open, prospective registration of a study protocol can improve research rigour in a number of ways. Through preregistration, key features of the study's methodology are recorded and maintained as a permanent record, enabling comparison of the completed study with what was planned. By recording the study hypothesis and planned outcomes a priori, preregistration creates transparency and can reduce the risk of several common biases, such as hypothesising after results are known and outcome switching or selective outcome reporting. Second, preregistration raises awareness of measures to reduce bias, such as randomisation and blinding. Third, preregistration provides a comprehensive listing of planned studies, which can prevent unnecessary duplication and reduce publication bias. Although commonly acknowledged and applied in clinical research since 2000, preregistration of animal studies is not yet the norm. In 2018 we launched the first dedicated, open, online register for animal study protocols: wwwpreclinicaltrialseu. Here, we provide insight in the development of preclinicaltrials. eu (PCT) and evaluate its use during the first 3 years after its launch. Furthermore, we elaborate on ongoing developments such as the rise of comparable registries, increasing support for preregistration in the Netherlands—which led to the funding of PCT by the Dutch government—and pilots of mandatory preregistration by several funding bodies. We show the international coverage of currently registered protocols but with the overall low number of (pre) registered protocols.

INTRODUCTION

Although controversial, animal experiments are still considered essential in many fields of biomedical and toxicological research. Unfortunately, concerns are raised about their validity and robustness, especially when new therapies based on promising animal studies fail to show clinical efficacy, safety and return on investment.[1,2] A thorough investigation of the causes of translational failure is currently hampered by the lack of rigorous science. Key requirements for highly robust experimental data are adequate statistical power, a study design which maximises external validity and high internal study validity. Furthermore, reporting on all performed experiments should be complete and transparent, regardless of their outcome. In addition, studies should be optimised by previous findings, and thus new experiments should be preceded by an evaluation of relevant literature. Unfortunately, preclinical animal studies currently show major deficits in all of these areas, causing key findings to be difficult to reproduce and translate.[3,4] Perhaps the most important (and highly common) problem in individual animal studies is poor reporting of study methodology.[5–7] This includes incomplete reporting of details of the study design and animal characteristics relevant to external validity, as well as measures to reduce bias and details of the statistical analysis.[8–10] As such, poor reporting affects all key requirements and obscures the true state of affairs in animal studies, rendering external validity, internal validity and statistical robustness and power largely unclear. Meta-research shows that studies failing to report measures to reduce bias tend to report larger effect sizes, suggesting an overestimation of the true effect size due to low internal validity.[11,12] The limitations found within studies are further exacerbated by reporting biases such as publication bias and selective outcome reporting. The publication rate of animal studies has been shown to be limited to 60%–67%,[13,14] and especially studies yielding neutral results or results contradicting existing evidence remain unpublished.[15–18] Simultaneously, the under-reporting of animals in publications suggests that data are reported selectively, which can cause outcome reporting bias.[13] Finally, outcome switching and hypothesising after results are known (HARKing) are additional forms of bias that affect research. These arise when researchers deviate from their research questions and/or plans as originally set up or when no a priori plan is in place at all. Research into these forms of bias has been dependent on open access registration of clinical trial protocols and comparing them with their subsequent publications.[19] Animal study protocols are not registered or inaccessible, and therefore hardly any evidence on outcome switching or HARKing in preclinical research exists. However, there is no reason to assume that animal research would be immune to these biases.

Preclinicaltrials.eu (PCT): an online international register of preclinical trial protocols

Our vision is to optimise the efficacy of preclinical research for improving human health. We propose that registration of a protocol before starting an experiment (preregistration) can play an essential role in improving the robustness and transparency of animal studies and lead to more reliable research. Such preregistration of preclinical studies has four main benefits[20,21]:

1. Disclosing the a priori study intention, that is, hypothesis, exploratory or confirmatory character and key elements of its design, including primary and secondary outcomes and sample size calculations.
2. Promoting the use of methods to reduce risks of bias (i.e., blinding and randomisation) and creating transparency about their use.
3. Providing a complete overview of all performed studies (including those that remain unpublished) and the possibility to share or link to related data.
4. Creating transparency and accountability within the research community and towards society.

Several other initiatives have been developed to improve animal study robustness, for example, guidelines for planning (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines) and reporting (Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines). Compared with guidelines for reporting, the added value of preregistration is its timing. Requesting the ARRIVE-checklist (or any other reporting guideline) at the submission stage may improve reporting, but for that particular research project it is too late to optimise the study design.[6] Akin to the PREPARE guidelines[22], preregistration supports scientists much earlier in the research process, that is, during planning and execution of the study, thereby improving research rigour and robustness. For instance, researchers who are unfamiliar with measures to reduce bias can be made aware of implementing these measures within their study protocol. Importantly, preregistration requires sharing of key elements of the proposed outcome measures and a prespecified statistical analysis plan, enabling insight in a priori versus post-hoc analyses. Compliance with preregistration can be monitored by multiple stakeholders (i.e., funders, institutes, journal editors, reviewers), whereas reporting guidelines are mostly checked by reviewers only. Importantly, preregistration can reduce unnecessary repetition of animal studies, since new animal studies should be preceded by a (systematic) search to prevent repetition, help formulate relevant research questions and optimise the animal model. Similarly, consulting an animal study registry can be useful when searching for potential collaborators. Of note, study protocols are already widely used in the approval process of animal studies, although the protocol format and the level of detail required may differ per country or even per institute. However, in general, we expect most information required for registration in an animal study registry to also be included in the study's application for local approval.

The development of PCT

In 2014 we first published a review suggesting an online registry for preclinical trial protocols.[23] In the following years, we developed the first registry dedicated to animal studies to facilitate preregistration: PCT (figure 1). This initiative was developed with the help of several stakeholders to create a solid, robust base. We assembled a steering committee and attracted the Netherlands Heart Institute as an independent party responsible for hosting and reviewing submitted protocols. Subsequently, the University Medical Center Utrecht formed the legal entity. The PCT advisory board was established in 2018 to provide solicited and unsolicited advice to the steering committee regarding, for example, the future direction of the registry and the implementation of preregistration. Board members are based in various countries, various research fields and multiple disciplines, in particular (but not limited to) animal research and meta-research. Current members are Professor John Ioannidis (Stanford University, USA), Professor Jonathan Kimmelman (McGill University, Canada), Professor Paul Glasziou (Bond University, Australia), Professor Lina Badimon (IR- Hospital de la Santa Creu i Sant Pau, Autonomous University Barcelona, Spain) and Professor Thomas Eschenhagen (University Medical Center Hamburg Eppendorf, Germany). We have organised yearly meetings of the steering committee with the advisory board.

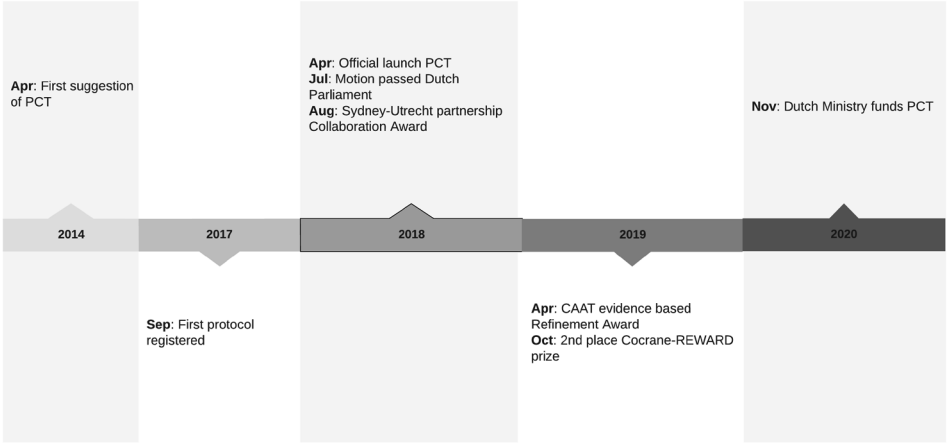

Figure 1. Timeline of the development of preclinicaltrials.eu (PCT).

The format of the protocol registration form was discussed with fellow researchers from the Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes (TACTICS) group, the Radboud University Medical Center, University Medical Center Utrecht, the University of Sydney and several animal welfare bodies within the Netherlands. Based on this, we optimised the level of detail of the information required

for registration (including which information should be mandatory vs optional) and aimed to determine the minimal amount of detail required to have an impact on research rigour, thereby minimising the additional administrative burden for researchers (a common concern regarding preregistration among researchers, see table 1). Most information required for registration would likely already be documented in a study's experimental protocol, which is often required for approval by a local committee, as per our experience with such applications in the Netherlands. We simultaneously set out to further reduce the administrative burden for researchers by enabling an automatic transfer of the required information from local digital systems to the PCT format. After reaching out to developers of such software, this function is now in place for PRIS, a system used in several institutes in the Netherlands for animal study protocols submission to local animal welfare bodies. This allows researchers to copy most of the required information from their local application form to PCT with the click of a button. Discussions with other software developers are ongoing.

Table 1. Concerns often mentioned in discussions with colleagues during development of preclinicaltrials.eu and our solutions.

Concerns	Solutions
Cost	• Free submission of protocol • Free use of database
Administrative Burden	• Export data from existing study protocols
Limited flexibility of creativity	• Tracked-changed adjustments are allowed
Misuse by animal activists	• Login required • Personal details anonymized
Data theft	• Embargo
Threat to intellectual property	• Embargo • Time-stamped protocols

After optimising the registration form, we added functional options to the registry to overcome two other well-known concerns among researchers, namely (1) the privacy of researchers submitting protocols and (2) the risk of intellectual theft of research ideas or loss of intellectual property. Regarding privacy, personal details of the researcher submitting the protocol are anonymised, except for the institution where the experiments are performed. It is possible to contact the submitting researcher through an encrypted email message to facilitate contact and collaboration. To prevent abuse, detailed information of study protocols can only be accessed after creating an account and logging in. Without an account only limited data (titles, study centre details)

of studies are visible. Regarding the fear of sharing preliminary ideas, PCT provides the option to register a protocol under embargo. The full details of the protocol remain hidden until revealed by the investigator or after a release date which is automatically set at 1 year after registration. We feel that even though an embargo delays our aim to create full transparency, the other benefits of preregistration outweigh this downside. Also, we propose that the option to register under embargo is necessary at this stage, until preregistration becomes the gold standard and the research community comes to view preregistration as a safeguard against intellectual theft of scientific ideas and intellectual property (since preregistration in fact 'claims' an idea), rather than a risk.

Results after 3 years of PCT

The first protocol on PCT was published in September 2017 (PCTE0000098). A position paper from TACTICS supporting PCT and discussing the importance of preregistration was published in January 2018 (figure 1).[20] Subsequently, PCT was officially launched in April 2018, at the scientific session 'Promoting Transparency in Preclinical Research' held at the Netherlands Heart Institute.[24] In November 2019, the Netherlands Heart Institute organised a round table discussion to explore possibilities to implement preregistration within the Netherlands. Over 20 participants from different universities, funders and the government were present.

The Royal Netherlands Academy of Arts and Sciences stated in 2018 that funders and journals should make preregistration mandatory for hypothesis-testing research.[25] After the launch of PCT, the discussion on preregistration in the Netherlands intensified substantially. On 28 June 2018, members of the Dutch parliament unanimously accepted a motion stimulating preregistration for all animal research in the Netherlands.[26] In response, the Dutch government supported the PCT initiative and in November 2020 the Dutch Ministry of Agriculture, Nature and Food quality provided funding for its maintenance and further development.[27,28] The board of directors of the University Medical Center Utrecht agreed to stimulate preregistration of animal studies within their facilities, focusing principally on preregistration of confirmatory studies as defined by Kimmelman et al.[29] Several funding agencies (including the Collaborating Health Foundations) within the Netherlands support preregistration, and the Netherlands Organisation for Health Research and Development (ZonMw) made preregistration a requirement for funding of animal studies in several pilot programmes.[30]

Since its launch, PCT has been internationally recognised for its importance in promoting rigour in animal studies. In 2018, we received the University of Sydney–Utrecht Partnership Collaboration Award, together with Dr Kieron Rooney, to empower collaboration on preregistration. In April 2019, PCT received the Science-based Refinement Award from Johns Hopkins University Center for Alternatives to Animal Testing. In August 2019, we were awarded second place in the Cochrane- REWARD

prize. Three years after the official launch of PCT, there are over 1563 active accounts. Users originate from institutions in industry and academia in 30+ countries all over the world. Despite international recognition and encouraging engagement of stakeholders in, for example, the Netherlands, the number of registered protocols is still low.[31] As of 20 January 2022, 107 protocols have been submitted, all of which have eventually been approved. The 87 non-embargoed protocols originate from 23 countries. They consist of both small animal (n=48, 55%) and large animal (n=39, 45%) studies and 54 studies (62%) are confirmatory studies (figure 2). Only a limited number of the overall protocols were registered before the start of the study (n=36, 33.5%). Of note, in January 2019, the German Centre for the Protection of Laboratory Animals (Bf3R) launched a comparable platform for registration of animal studies (www.animalstudyregistry.org).[32] After 3 years, 102 studies from 14 different countries have been registered on this platform. Similarly, a low percentage of these studies was preregistered (n=21, 20.5%). Most studies are under embargo (n=81, 79.5%). Of the available non-embargoed protocols, 4 studies (19%) have a confirmatory character and 3 (14%) involve large animal models. Other platforms for preregistration exist, but they are not free of charge or do not focus primarily on animal research. In total, only 209 protocols have been registered on the dedicated animal study platforms over the last 3 years. Taking into account that over 58 million animals are used for scientific purposes globally, the amount of registered studies is still extremely low.[33]

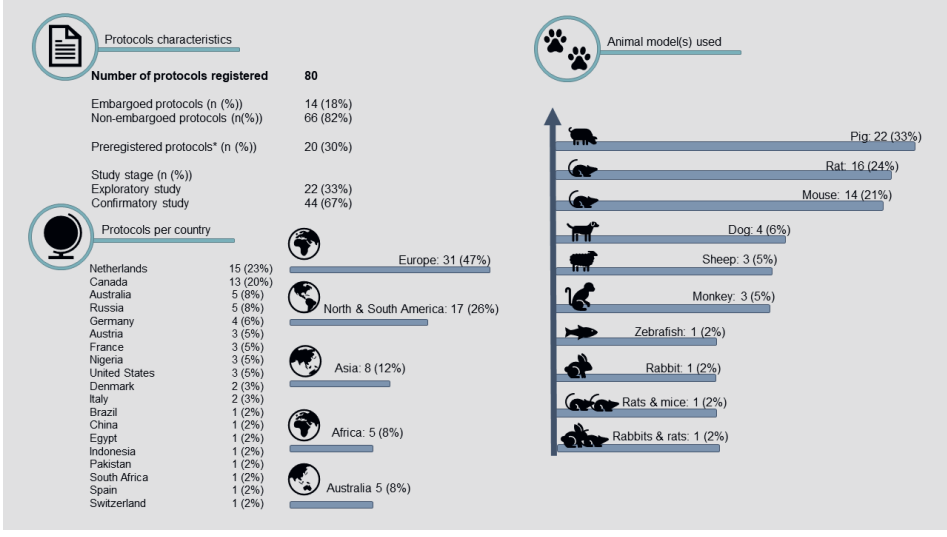

Figure 2. Protocols published on preclinicaltrials.eu on 20 January 2022. Note that only details of non-embargoed protocols are shown. *Preregistration is based on the reported study status at the first version of the submitted.

Preregistration of clinical trials

In comparison to preclinical registration, clinical trials registration is widely accepted and embraced by journals.[34] The first clinical trial registries were established in the 1980s, mostly in the field of HIV-AIDS research.[35] In 1989, the US government required the dissemination of information on HIV research, treatment and prevention, leading to the development of the AIDS Clinical Trials Information Service in 1989.[36] In 1997, the US government required the National Institute of Health to provide a database of information on clinical trials for drugs for serious or life-threatening diseases and conditions, which resulted in the launch of clinicaltrials.gov in 2000.[37,38] In 2005, the International Committee of Medical Journal Editors required all clinical trials to be registered in a public trial registry as a requirement for publication,[34] resulting in an increasing number of trial registration.[39] Over time, more than 15 clinical trial registries have arisen, which prompted the WHO to establish the International Clinical Trials Registry Platform (WHO ICTRP), a meta-search engine that allows searching through individual clinical trials registries. Over the years, clinical trial registries have provided us with opportunities for meta-research, for example, by providing insight into the frequency of reporting bias.[19,40] These initiatives have been instrumental to improve the quality of biomedical research. Also, clinical trial registries are regularly searched for systematic reviews and provide additional data for meta-analysis.[41]

Following the example set by clinical trials registries, we may speculate that preclinical registration would need incentives from journal editors or governmental agencies to encourage researchers to preregister their studies.

The future of preclinical preregistration

Preregistration might not be the only approach for improving translational research, but it is generally an easily implemented solution that will contribute to addressing various problems that currently reduce the impact of translational research. Ideally, preregistration would not be limited at all (e.g., by an embargo), but the provided solutions act to lower the threshold for stakeholders to embrace preregistration and are therefore necessary at this phase of preregistration. We have learnt from our experiences so far and are continuously working on improving the platform. At this point, two free and public databases dedicated to preclinical registration exist, but this could increase, as has been the case for clinical trial registration platforms. A meta-search engine, like the WHO ICTRP, could be an added value for researchers. To carry our ambitions and increase the number of registered protocols, we designed a strategy for focusing on three main action points: promote, facilitate and understand. To promote preregistration among researchers, we will provide webinars and aim to develop e-learning tools. We also create promotional material and publish relevant information online, in collaborators' newsletters or via short communications.[42] Institutions, animal ethics committees and animal welfare bodies will be approached

to aid in promoting preregistration and reward good behaviour. They can educate and encourage researchers to preregister their protocols. For this purpose, we recently developed a short video explaining preregistration (PCT).[43]
Moreover, an international ambassador network was started to further promote preregistration worldwide. Ambassadors commit to showing the example by preregistering themselves, promoting preregistration in their teams and institute and helping us reach out to important stakeholders in their countries.

To facilitate and ease preregistration, we are currently focusing on minimising the administrative burden for researchers.[44,45] The obvious step to link data from locally required protocols to PCT should be further developed. Moreover, we will provide personal guidance with protocol registration when requested. To better understand stakeholders, we aim to gain knowledge about current practice and evaluate experiences with PCT. In collaboration with the University of Sydney, we are currently working on a survey among researchers on the believed benefits and concerns of preregistration. The results will provide us with additional information on how to improve the motivation of researchers to preregister. We will continue to discuss issues on preregistration and PCT with relevant stakeholders and evaluate the platform if necessary. In addition to this bottom-up approach, several stakeholders play a pivotal role in a top-down approach for the implementation of preregistration. Funders can guard quality in research by making preregistration mandatory for provided funding and journals can stimulate preregistration by setting it as a requirement for publication, just like they did for clinical preregistration.[34] Committees and institutions involved in animal research can require accountability of previously provided animals as part of a new application. Journals can reward researchers who preregister, for example, with preregistration badges that are currently implemented by BMJ Open Science and the Journal of Neuroscience Research among others.[46,47] In addition, journals play an important role in monitoring compliance. Institutes and funders can stimulate preregistration by incorporating preregistration in their reward system and monitor compliance by reviewing preregistration in applications.

Concluding remarks

Preregistration increases transparency and contributes to more effective preclinical research. Multiple platforms to facilitate preregistration have been developed, but the number of registered protocols is still low. We show in this paper the development of and considerations behind PCT and highlight the growing interest for preregistration of animal studies and the role of multiple stakeholders in this endeavour. Several Dutch stakeholders have taken the lead in implementing preregistration. We are encouraging other stakeholders to follow these examples and thereby increase the number of registered protocols. At the same time, we keep putting preregistration on the agenda in all our discussions with relevant stakeholders. We believe it is time for the scientific community to take responsibility and move towards more effective animal research.

REFERENCES

1. Steedman M, Taylor K, Stockbridge M, Korba C, Shah S, Thaxter M. *Unlocking Productivity R&D Productivity. Measuring the Return of from Pharmaceutical Innovation 2018*.; 2018.
2. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. 2004;3(8):711-715. doi:10.1038/nrd1470.
3. Freedman LP, Cockburn IM, Simcoe TS. The economics of reproducibility in preclinical research. *PLoS Biol*. 2015;13(6):e1002165. doi:10.1371/journal.pbio.1002165
4. Leenaars C, Stafleu F, de Jong D, et al. A Systematic Review Comparing Experimental Design of Animal and Human Methotrexate Efficacy Studies for Rheumatoid Arthritis: Lessons for the Translational Value of Animal Studies. *Animals*. 2020;10:1047. doi:10.3390/ani10061047
5. Hooijmans CR, Leenaars M, Ritskes-Hoitinga M. A Gold Standard Publication Checklist to Improve the Quality of Animal Studies, to Fully Integrate the Three Rs, and to Make Systematic Reviews More Feasible. *Altern to Lab Anim*. 2010;38(2):167-182. doi:10.1177/026119291003800208
6. Percie Du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *BMJ Open Sci*. 2020;4(1):e100115. doi:10.1136/bmjos-2020-100115
7. Ramirez DD, Motazedian P, Jung RG, et al. Methodological Rigor in Preclinical Cardiovascular Studies: Targets to Enhance Reproducibility and Promote Research Translation. *Circ Res*. April 2017. http://circres.ahajournals.org/content/early/2017/03/30/CIRCRESAHA.117.310628.abstract.
8. Wever KE, Hooijmans CR, Riksen NP, et al. Determinants of the efficacy of cardiac ischemic preconditioning: A systematic review and meta-analysis of animal studies. *PLoS One*. 2015;10(11):1-17. doi:10.1371/journal.pone.0142021
9. Jonker SJ, Menting TP, Warlé MC, Ritskes-Hoitinga M, Wever KE. Preclinical evidence for the efficacy of ischemic postconditioning against renal ischemia-reperfusion injury, a systematic review and meta-analysis. *PLoS One*. 2016;11(3):e0150863. doi:10.1371/journal.pone.0150863
10. Hesen NA, Riksen NP, Aalders B, Ritskes-Hoitinga M, Messaoudi S El, Wever KE. A systematic review and meta-analysis of the protective effects of metformin in experimental myocardial infarction. *PLoS One*. 2017;12(8):e0183664. doi:10.1371/journal.pone.0183664
11. Crossley NA, Sena E, Goehler J, et al. Empirical evidence of bias in the design of experimental stroke studies: a metaepidemiologic approach. *Stroke*. 2008;39(3):929-934. doi:10.1161/STROKEAHA.107.498725
12. Hirst TC, Vesterinen HM, Conlin S, et al. A systematic review and meta-analysis of gene therapy in animal models of cerebral glioma: why did promise not translate to human therapy? *Evidence-based Preclin Med*. 2014;1(1):e00006. doi:10.1002/ebm2.6
13. van der Naald M, Wenker S, Doevendans PA, Wever KE, Chamuleau SAJ. Publication rate in preclinical research: a plea for preregistration. *Br Med J Open Sci*. 2020;4:e100051. doi:10.1136/bmjos-2019-100051
14. Wieschowski S, Biernot S, Deutsch S, Glage S, Strech D. Publication rates in animal research. Extent and characteristics of published and non- published animal studies followed up at two German university medical centres. *PLoS One*. 2019;14(11):e022375. doi:10.1371/journal.pone.0223758
15. ter Riet G, Korevaar DA, Leenaars M, et al. Publication Bias in Laboratory Animal Research: A Survey on Magnitude, Drivers, Consequences and Potential Solutions. *PLoS One*. 2012;7(9):e43404. doi:10.1371/journal.pone.0043404
16. Macleod MR, O'Collins T, Howells DW, Donnan G a. Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke*. 2004;35(5):1203-1208. doi:10.1161/01.STR.0000125719.25853.20
17. Sena ES, Bart van der Worp H, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*. 2010;8(3):e1000344. doi:10.1371/journal.pbio.1000344
18. Perrin S. Make mouse studies work. *Nature*. 2014;507:423-425.
19. Goldacre B, Drysdale H, Dale A, et al. COMPare : a prospective cohort study correcting and monitoring 58 misreported trials in real time. *Trials*. 2019;20(118). doi:10.1186/s13063-019-3173-2
20. Chamuleau SAJ, van der Naald M, Climent AM, et al. Translational Research in Cardiovascular Repair A call for a paradigm shift. *Circ Res*. 2018;122(2):310-318. doi:10.1161/CIRCRESAHA.117.311565
21. Kimmelman J, Anderson JA. Should preclinical studies be registered? *Nat Biotechnol*. 2012;30(6):488-489. doi:10.1038/nbt.2261
22. Smith AJ. Guidelines for planning and conducting high-quality research and testing on animals. *Lab Anim Res*. 2020;36:21. doi:10.1186/s42826-020-00054-0
23. Jansen of Lorkeers SJ, Doevendans PA, Chamuleau SA. All preclinical trials should be registered in advance in an online registry. *Eur J Clin Invest*. 2014;44(9):891-892. doi:10.1111/eci.12299
24. Netherlands Heart Institute Homepage. www.heart-institute.nl/index.php?pagina=News%25id=45. Accessed April 4, 2020.

25. Royal Netherlands Academy of Arts and Sciences. *Replication Studies - Improving Reproducibility in the Emperical Sciences*.; 2018. https://www.knaw.nl/shared/resources/actueel/publicaties/pdf/20180115-replication-studies-web.
26. De Groot, Dik-Faber, Von Martels. *Motie van Het Lid De Groot c.s. over Het Registreren van Alle Individuele Dierproeven*.; 2018:ISSN 0921-7371. https://www.tweedekamer.nl/kamerstukken/.
27. Schouten C. *BRIEF VAN DE MINISTER VAN LANDBOUW, NATUUR EN VOEDSELKWALITEIT*.; 2019:ISSN 0921-7371.
28. van Engelshoven I. *Pilot Stimulering Transparant Proefdieronderzoek En Voortgang van Transitie Naar Minder Proeven Met Apen En Meer Proefdiervrije Innovaties in Het BRPC*.; 2020. https://www.tweedekamer.nl/kamerstukken/detail?id=2020Z20890&did=2020D44732.
29. Kimmelman J, Mogil JS, Dirnagl U. Distinguishing between Exploratory and Confirmatory Preclinical Research Will Improve Translation. *PLoS Biol*. 2014;12(5):e1001863. doi:10.1371/journal.pbio.1001863
30. Zonmw. Transparency on research projects, findings and data. https://www.zonmw.nl/en/research-and-results/fair-data-and-data-management/2-transparency-on-research-projects-findings-and-data/.
31. Baker M. Animal registries aim to reduce bias. *Nature*. 2019;Sep;573(7773):297-298. doi:10.1038/d41586-019-02676-4
32. Bert B, Heinl C, Chmielewska J, et al. Refining animal research: The Animal Study Registry. *PLoS Biol*. 2019;17(10):e3000463. doi:10.1371/journal.pbio.3000463
33. Taylor K, Gordon N, Langley G, Higgins W. Estimates for Worldwide Laboratory Animal Use in 2005. *ATLA - Altern to Lab Anim*. 2008;36(3):327. doi:10.1177/026119290803600310
34. De Angelis C, Drazen JM, Frizelle FA, et al. Clinical Trial Registration: A Statement from the International Committee of Medical Journal Editors. *N Engl J Med*. 2004;351(12):1250-1251.
35. Easterbrook PJ. Directory of registries of clinical trials. *Stat Med*. 1992;11(3):345-359.
36. Katz DG, Dutcher GA, Toigo TA, Bates R, Temple F, Cadden CG. The AIDS Clinical Trials Information Service (ACTIS): A Decade of Providing Clinical Trials Information. *Publich Heal Reports*. 2002;117(March-April):123-130.
37. US Government. *Food and Drug Administration Modernization Act (FDAMA) of 1997*.; 1997.
38. clinicaltrials.gov homepage. https://www.clinicaltrials.gov/. Accessed April 5, 2020.
39. Zarin DA, Tse T, Ide NC. Trial Registration at ClinicalTrials.gov between May and October 2005. *N Engl J Med*. 2005;353:2779-2787.
40. Devito NJ, Bacon S, Goldacre B. Compliance with legal requirement to report clinical trial results on ClinicalTrials.gov: a cohort study. *Lancet*. 2020;395:361-369. doi:10.1016/S0140-6736(19)33220-9
41. Baudard M, Yavchitz A, Ravaud P, Perrodeau E, Boutron I. Impact of searching clinical trial registries in systematic reviews of pharmaceutical treatments: methodological systematic review and reanalysis of meta-analyses. *BMJ*. 2017;(356):j448. doi:10.1136/bmj.j448
42. van der Naald M, Chamuleau SAJ, Menon JML, et al. A 3-year evaluation of preclinicaltrials.eu reveals room for improvement in preregistration of animal studies. *PLOS Biol*. 2021;19(9):e3001397. doi:10.1371/journal.pbio.3001397
43. preclinicatrials.eu. https://preclinicaltrials.eu/.
44. Wieschowski S, Silva DS, Strech D. Animal Study Registries: Results from a Stakeholder Analysis on Potential Strengths, Weaknesses, Facilitators, and Barriers. *PLoS Biol*. 2016;14(11):e2000391. doi:10.1371/journal.pbio.2000391
45. Wieschowski S, Laser H, Sena ES, Bleich A, Tolba R, Strech D. Attitudes towards animal study registries and their characteristics: An online survey of three cohorts of animal researchers. *PLoS One*. 2020;15(1):e0226443. doi:10.1371/journal.pone.0226443
46. Journal of Neuroscience Research. Preregistered badge Journal of Neuroscience Research. https://onlinelibrary.wiley.com/page/journal/10974547/homepage/open_research_badges.html.
47. BMJ Open Science. Preregistered badge BMJ Open Science. https://openscience.bmj.com/pages/policies/#Ethics.

CHAPTER 6

Considerations regarding translational failure and preregistration: a survey among animal researchers

Submitted

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ABSTRACT

Background Translational research suffers from multiple issues hampering its efficiency. Preregistration of animal studies is proposed to limit translational failure by increasing internal study validity, decreasing reporting bias and increasing transparency. Although the scientific community is slowly endorsing preregistration, the number of registered animal study protocols is limited. We aimed to investigate why animal researchers do not preregister their study protocols.

Methods We distributed a survey among animal researchers in Australia and the Netherlands to identify proposed challenges hindering translational research. We additionally identified benefits and challenges of preregistration to investigate whether animal researchers believe preregistration can limit translational failure.

Results Sixty-two respondents participated in this survey. There were 6 key issues identified to hamper translational research: 1) the use of animal models that are not translatable to human patients 2) flawed study designs 3) issues related to publication (incomplete reporting, publication bias) 4) irreproducibility 5) conditions under which experiments take place (e.g. insufficient training of staff) 6) pressure to succeed. Preregistration is claimed to improve study design, reduce the number of animals used and increase reproducibility, collaboration and transparency. The fear of sharing preliminary ideas, threat to intellectual property, the lack of flexibility and administrative burden are concerns expressed by respondents.

Conclusion We confirmed several issues hampering translational research. Preregistration can contribute to limit translational failure and increase collaboration. Support among animal researchers can be increased by protecting researchers' ideas and by limiting the administrative burden. Although awareness can help to increase the number of registered protocols, researchers require a top-down strategy to implement preregistration.

INTRODUCTION

Translational failure of animal studies is been discussed over the past decades[1]. Alarming reports regarding the minimal success rate of translating promising results from animal studies into clinical benefit include Lancet's 2014 *Increasing value, reducing waste* series[2], the 2016 Nature survey on the reproducibility crisis[3] and more recently a scoping review summarizing the extent of the problem[4]. Time and again, the absence or incompleteness of a study protocol, avoidable weaknesses in study design, and selective outcome reporting have been identified as important contributors to translational failure and lack of reproducibility[5–8].

Open access registration of a date-stamped study protocol preceding data collection and analysis (preregistration) has been proposed as an important safeguard against "sloppy animal science" by both meta-research experts[2,9,10] and researchers themselves[11,12] for nearly a decade. Preregistration is believed to limit part of the problems causing translational failure, by reducing publication bias and selective outcome reporting and contributing to improved internal study validity. Additionally, preregistration should increase transparency and help to avoid unnecessary duplication[13,14].

Several developments were made in the research community that support preregistration. In 2017, the Australian National Health and Medical Research Council (NHMRC) published a guideline on best practice methodology in the use of animals for scientific purposes. This guideline articulated actions for institutions and researchers, including the prospective registration of study protocols[15]. In 2018, the Royal Dutch Academy of Arts and Sciences (KNAW) proposed mandatory preregistration as part of a strategy to improve research reproducibility[16]. In the same year, Dutch researchers developed the first registry dedicated to preregistration of animal studies: www.preclinicaltrials.eu[13]. Dutch politicians pleaded for mandatory preregistration and the Dutch government expressed its support and dedicated funding to the registry[17]. In addition, several Dutch funders have endorsed preregistration and are piloting mandatory preregistration[18,19]. Dutch research institutes are slowly following this development, encouraging researchers to preregister. Simultaneously, the German Centre for the Protection of Laboratory Animals launched another register (www.animalstudyregistry.org). The United States National Institute of Health recently recommended to raise awareness and evaluate effects of preregistration in their statement on enhancing rigor, transparency and translatability in animal research[20].

Despite this extending line of support for preregistration of animal studies, the number of registered protocols remains limited[12]. Three years after launch, only 167 study protocols have been registered on preclinicaltrials.eu and animalstudyregistry.org combined[21]. In relation to the estimated tens of millions of animals used for scientific purposes yearly, this number is extremely low[22,23]. Explanations for these low

numbers are presently unclear. A stakeholder analysis identified several strengths and weaknesses of preregistration, which were confirmed in a survey among animal researchers[24,25]. However, these studies did not broadly identify hurdles in translational research. Therefore, it is unclear to what extent preregistration addresses translational failure.

The aim of this study was to investigate the attitude of Australian and Dutch animal researchers towards the problem of translational failure and prospective registration of animal study protocols as a strategy for improving practice. We developed a survey which first explores animal researchers' opinions on translational failure and identifies factors hampering translational research. Secondly, we investigate whether preregistration is believed to be a solution for the hurdles identified. For this purpose we identify possible benefits of preregistration and possible risks. We prospectively recruited participants from two populations (Australia and The Netherlands), because the Dutch seem more progressive in promoting preregistration practices as compared to Australia.

MATERIALS AND METHODS

Survey

The survey (see appendix 1) was designed to collect data on three major themes; 1) perceived challenges in translational research, 2) perceived benefits of preregistration and 3) perceived challenges of preregistration. Additional questions were based on respondents characteristics and on optimal characteristics of platforms for preregistration. The survey contained both closed and open-ended questions. The survey was designed by three researchers (KN, KR, MN), reviewed by three authors (KW, SC, VW) and piloted within our research groups.

Surveys in Australia were preceded by a mandatory informed consent question. Although informed consent was not mandatory in The Netherlands, we did provide the same information in Dutch surveys. We used an identical survey in English for both countries. Due to local institutional guidelines, we used Explora Zorg (Newcom, https://exploratio.nl/) in The Netherlands and REDCap (Vanderbilt University, https://projectredcap.org/) in Australia.

Ethical approval

In Australia, approval from the ethics committee was obtained (University of Sydney, project number 2019/163). In The Netherlands, ethical approval and informed consent was waived as the study did not subject to the Medical Research Involving Human Subjects Act (WMO). This study was not preregistered.

Recruitment

Only researchers actively performing or supervising the conduct of animal studies were included. In Australia, advertisements / flyers were posted on community noticeboards at departments of researchers involved in preclinical research around the University of Sydney. A link to the online survey, including information sheet and consent form was further circulated via email to all chief investigators of active animal studies of the University of Sydney in this period. In The Netherlands, all animal welfare bodies throughout the country were asked to forward an email to researchers known to be involved in animal research. This email contained a request to join the survey and provided background information on the survey and a link to the survey. The survey was open from March 2019 until June 2019.

Statistical analysis

All statistical analyses are descriptive. For questions based on a 5-point Likert scale, answers were recorded on a scale of 1 (strongly disagree) to 5 (strongly agree) points. Median and interquartile ranges are presented in text, individual responses in figures. Percentages were rounded to even.

Thematic analysis

Two authors (KR, MN) performed thematic analysis of the answers provided to the survey's open questions. First, both authors independently read the answers given per open question and separated individual items (quotes) per answer where applicable (table 1). Both authors independently formulated themes based on these quotes and assigned all items to one or more themes. Then, the authors reviewed and reconciled a final set of themes and reassigned quotes if necessary. Next, subthemes were discussed within the identified themes by both authors independently and differences were solved. If a single quote was assigned to two or more themes, the quote was counted for both topics. Themes and subthemes were not pre-specified. A table containing all identified quotes and allocated themes and subthemes is provided in the appendix 2-4.

Table 1. Example of thematic analyses. Column 1 shows the original answer. In the second column the separate quotes that were identified were shown. Afterwards de themes and subthemes that were agreed on were shown.

Original answer	Individual quotes	Agreed themes	Agreed subthemes
The insane pressure to publish more than 5 papers a year, resulting in poor quality studies. The culture of journals favouring studies with positive results. The lack of accountability.	1. The insane pressure to publish more than 5 papers a year, resulting in poor quality studies. 2. The culture of journals favouring studies with positive results. 3. The lack of accountability.	1. "Publication" and "methodology" 2. "Publication" 3. "Other"	1. "Publication pressure" and "poor study quality" 2. "Publication bias" 3. "Other"

Subgroup analysis

We performed subgroup analyses for Australian *versus* Dutch respondents and less experienced *versus* more experienced researchers. For the latter, the cut-off point for "more experienced" was set at ≥5 years of experience. For these analyses we used the percentage of quotes per subgroup in relation to the total of quotes within that (sub) theme. We did not perform the planned subgroup analyses for researchers supervising animal studies *versus* researchers who perform hands-on animal research, because a high percentage of respondents was involved in both. Subgroup analyses for medical doctors *versus* non-medical doctors, and for various fields of research were not performed due to the limited number of responses per category.

RESULTS

Respondent characteristics

Sixty-two respondents completed the survey (table 2; AUS n=28, NL n=34). Respondents had been involved in animal research for a median period of 10 years (range 54 years) and were predominantly active in the fields of neurosciences (26%), behavioural science (21%) and immunology (19%). Eighty-seven percent of the respondents were active in performing animal handling/data collection at the time of the survey, whilst 73% were involved in supervising animal studies. The most frequently used species were mice (n=37, 60%), rats (n=21, 34%) and pigs (n=15, 24%). Five respondents (8%) had preregistered a study in the past.

Table 2. Respondent characteristics.

	Australia	Netherlands	Total
Completed surveys	28 (45%)	34 (55%)	62 (100%)
Age in years (mean±SD)	39 ± 14	40 ± 12	39 ± 13
Academic position			
- BSc/MSc student (no MD or veterinarian)	5 (18%)	1 (3%)	6 (10%)
- PhD (no MD or veterinarian)	4 (14%)	5 (15%)	9 (15%)
- PhD (MD or veterinarian)	0 (0%)	3 (9%)	3 (5%)
- Post-doctoral researcher	6 (21%)	10 (29%)	16 (26%)
- Research assistant/technical officer/technician	4 (14%)	5 (15%)	9 (15%)
- Principle Investigator / group leader	11 (39%)	9 (26%)	20 (32%)
- Member of a body involved in animal research approval or animal research policy	0 (0%)	3 (9%)	3 (5%)
- Veterinarian	0 (0%)	3 (9%)	3 (5%)
- Other*	0 (0%)	2 (6%)	2 (3%)
Years involved in animal studies (median [min-max])	9.5 [0.8-55]	11 [2-41]	10 [0.8-55]
- Little experience (<5 years)	9 (32%)	7 (21%)	16 (26%)
- More experience (≥5 years)	19 (68%)	27 (79%)	46 (74%)

Table 2. Continued

	Australia	Netherlands	Total
Main field of research			
- Behavioural science	10 (36%)	3 (9%)	13 (21%)
- Cardiovascular medicine and haematology	1 (4%)	6 (18%)	7 (11%)
- Immunology	5 (18%)	7 (21%)	12 (19%)
- Medical biochemistry and metabolomics	6 (21%)	2 (6%)	8 (13%)
- Medical microbiology	1 (4%)	1 (3%)	2 (3%)
- Medical physiology	2 (7%)	2 (6%)	4 (6%)
- Neurosciences	9 (32%)	7 (21%)	16 (26%)
- Nutrition and dietetics	3 (11%)	2 (6%)	5 (8%)
- Oncology and carcinogenesis	1(4%)	7 (21%)	8 (13%)
- Ophthalmology and optometry	0 (0%)	1 (3%)	1 (2%)
- Pharmacology and pharmaceutical sciences	2 (7%)	2 (6%)	4 (6%)
- Toxicology	1 (4%)	1 (3%)	2 (3%)
- Other**	3 (11%)	9 (26%)	12 (19%)
Currently directly involved in handling of animals and collection of data from animal studies	25 (89%)	29 (85%)	54 (87%)
Supervision of animal studies	20 (71%)	25 (74%)	45 (73%)
Involved with both hands-on work as well as supervision of animal studies	18 (64%)	20 (59%)	38 (61%)
Using animal models for understanding human disease and/or the development of new therapeutic and diagnostic approaches	25 (89%)	27 (79%)	52 (84%)
Typically used animal model***			
- Mouse	17 (61%)	20 (59%)	37 (60%)
- Rat	12 (43%)	9 (26%)	21 (34%)
- Rabbit	0 (0%)	3 (9%)	3 (5%)
- Pig	2 (7%)	13 (38%)	15 (24%)
- Dog	0 (0%)	4 (12%)	4 (6%)
- Chicken	0 (0%)	6 (18%)	6 (10%)
- Sheep	3 (11%)	1 (3%)	4 (6%)
- Cattle	1 (4%)	2 (6%)	3 (5%)
Other****	3 (11%)	3 (9%)	6 (10%)
Preregistered an animal study in the past			
- No	26 (93%)	31 (91%)	57 (92%)
- Yes; preclinicatrials.eu	1 (4%)	0 (0%)	1 (2%)
- Yes; Open Science Framework	0 (0%)	0 (0%)	0 (0%)
- Yes; different platform*****	1 (4%)	3 (9%)	4 (6%)

All data are in n (%) unless indicated otherwise. SD = standard deviation; BSc = bachelor of science; MSc = master of science; PhD = Doctor of Philosophy; MD = medical doctor.

*Researcher at research institute (n=1), director animal facility (n=1)

** Ecology (n=1), microbiological (n=1), applied reproduction (n=1), diabetes and metabolic complications (n=1), transfer studies (n=1), animal nutrition and welfare (n=1), animal sciences (n=1), cardiology (n=1), regenerative medicine (n=2), physiology (n=1), tissue engineering and reconstructive medicine (n=1)

***Note that some respondents use multiple animal models.

****Wild animals (n=2), horse (n=1), cat (n=1), ferret (n=1), primate (n=1), goat (n=1), guinea pig (n=1), hamster (n=1)

***** As predicted (n=1), Layman's summary on website university (n=1), JOVE (n=1), CCD application (n=1).

Challenges in translational research

Animal researchers were asked what the biggest challenging in translational research are in both open responses and closed questions. Based on their responses we formulated six key issues that are challenging translational research: 1) the use of animal models that are not translatable to human patients 2) flawed study designs 3) issues related to publication (incomplete reporting, publication bias) 4) irreproducibility 5) conditions under which experiments take place (e.g. insufficient training of staff) 6) pressure to succeed (figure 1).

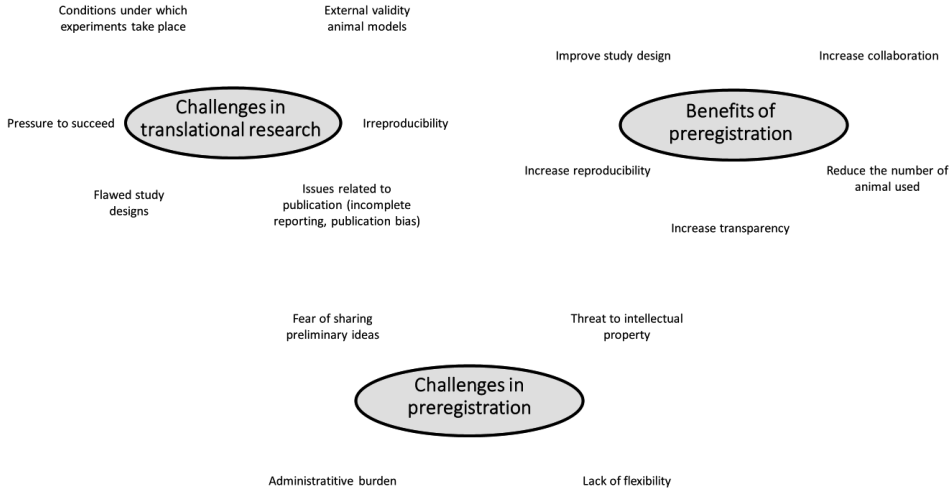


Figure 1. Key issues challenging translational research and benefits and challenges of preregistration.

Open responses

Respondents provided a total of 176 quotes related to challenges in translational research (appendix 2). We identified 10 themes and 20 additional subthemes (table 3).

Animal models

Half of the respondents (n=31) stated that animal models are challenging translational research. Almost one quarter (n=43, 24%) of all quotes was related to this theme. Respondents indicated that they perceive the external validity of animal models as uncertain: some respondents doubt if data from an animal experiment can be translated to the human patients at all. Others suggested that models with higher external validity might be available, but are not always used. Respondents stated there is a "*widespread lack of acceptance that mice are not best model for humans*" (AUS45) and animal models "*lack co-morbidities*" (NL19549932). More appropriate models are not

Table 3. Themes and subthemes identified in the open question about the biggest challenges in translational research.

Theme	Subthemes	Respondents	Quotes
Animal models		31	43
	External validity of models	20	22
	Heterogeneity	3	4
	Non-animal alternatives	4	5
	Other	11	12
Bureaucracy		21	25
	Approval committees	7	7
	Administrative burden	3	3
	Legislation	5	5
	Time to approval	4	4
	Lack of freedom	2	2
	Other	4	4
Conditions		15	20
	Training	8	8
	Standardization	5	5
	Animal distress	3	3
	Other	4	4
Publication		15	17
	Transparency	6	6
	Publication bias	6	6
	Publication pressure	3	3
	Influence of journals	2	2
Methodology		13	14
	Biostatistics	6	6
	Poor quality studies	2	2
	Data interpretation	2	2
	Other	4	4
Reproducibility		12	13
Funding		10	11
	Funding structure	4	5
	Costs	3	3
	Other	3	3
Perception		4	5
Researchers' attitudes		3	3
Incentives		2	2
Other		17	21
No comment		2	2
TOTAL NUMBER		62	176

Respondents = number of respondents reporting on this (sub)theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes.

always preferred, are too expensive and "*it is hard to apply a better model, since the new model is unknown and you cannot publish about it* (NL19547003)". Large animal models are suggested to have higher translational value. In addition, alternatives to animal

testing should be further developed. Respondents stated that experiments are influenced by the lack of heterogeneity in standardized lab animals, but also by the differences between animal species and heterogeneity in behaviour and environment.

Bureaucracy

Over one-third of the respondents (n=21, 34%) provided at least one quote related to the theme bureaucracy and 14% (n=25) of the quotes were related to this theme. Respondents reported that approval committees influence the study design and choice of animal model and can "*limit the effectiveness of an experiment*" (AUS57). *"Unreasonable amounts of paperwork"*, the time to approval and strict legislation are perceived as a burden in animal research. In addition, the flexibility within granted applications is too limited.

Conditions

Fifteen respondents (24%) provided 25 quotes (14% of total quotes) on circumstances under which experiments are performed. Training of staff involved in animal research is insufficient, "*as a result the skill levels vary widely and often students with little practical skill or knowledge will attempt to complete a research project with animals. This results in excessive waste of animals and essentially unusable data*" (AUS59). Respondents report that experiments are not performed in a standardized matter. The infrastructure of animal facilities need to improve (e.g. soft close doors and better ventilation), and experimental procedures should be optimized to minimize suffering (e.g. more stimulation and fewer instances of single-housing).

Publication

Fifteen respondents (24%) provided 17 quotes (10% of total quotes) on publication, stating that research is not transparent, reporting of studies is incomplete and translation is hampered by publication bias. Respondents claim the pressure to publish is "*insane"* (AUS48), resulting in poor quality studies and it "*perpetuates research for the sake of publication rather than to contribute to our understanding*" (AUS77). "*Grants are generally short-term and heavily influenced by a researcher's publication record, which results in scientists cutting corners to publish faster and more frequently in order to simply keep their jobs*" (AUS59). Journals influence the choice of animal models and favour studies with positive results.

Methodology

Thirteen respondents (21%) provided 14 quotes (8% of total quotes) about flaws in research methodology. Researchers' skills in biostatistics are insufficient and samples sizes are not always adequate. Study quality is poor and data is not interpreted properly.

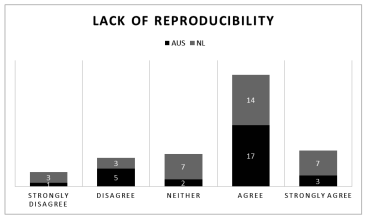
Reproducibility
Twelve respondents (19%) provided 13 quotes (7% of total quotes) on reproducibility. Even though research cannot be replicated "*it remains on a pedestal*" (AUS35), and irreproducibility makes it hard to rely on previously published work.

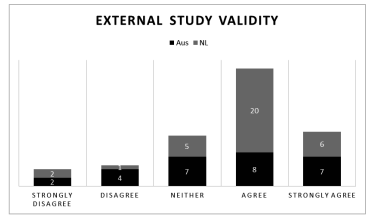
Funding
Ten respondents (16%) provided 11 quotes (6% of total quotes) on funding. Animal studies are believed to be too expensive and there is insufficient funding, especially funding for improvement of animal models or alternatives is insufficient. The current funding structure "*results in scientists cutting corners to publish faster and more frequently in order to simply keep their jobs*" (AUS59).

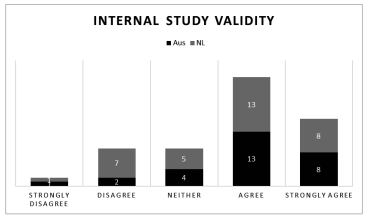
Perception, researchers' attitudes and incentives
The public perception is believed to challenge translational research (4 respondents (6%) provided 5 quotes (3%)). There is a need to better explain the goal and purpose of animal research to the public and politics. The poor history of translation perpetuates the idea that animal work is not highly relevant. Three respondents (5%) provided 3 quotes (2%) stating that the researchers' attitude should change. Two respondents (3%) provided 2 quotes (1%) reported the perverse incentives for research and *"desire to 'own' intellectual property shapes research direction too strongly"*.

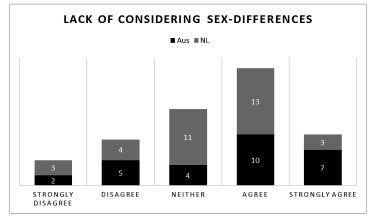
Other quotes
A total of 21 quotes (12% of total quotes) provided by 17 respondents could not be identified with the above-mentioned themes. Two respondents did not provide any comments on this question.

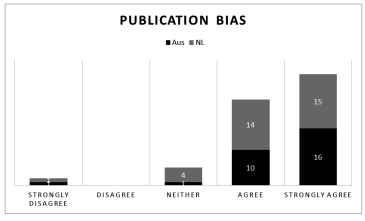
Closed response questions
When addressed in a closed question format, respondents agreed on multiple issues hampering the translation of findings from animal studies into clinical practice (figure 2). There is an overall acceptance that translation is hampered by publication bias (median 4.5, IQR 1), a lack of reproducibility (median 4, IQR 1), use of animal models that are not valid for understanding human diseases (median 4, IQR 1), lack of considering sex differences (median 4, IQR 1), selective publication of animals studies (median 4, IQR 1), selective reporting of outcomes (median 4, IQR 1), publication of underpowered studies (median 4, IQR 1), lack of published attempts to replicate previously published results (median 4, IQR 1) and poor internal study validity (median 4, IQR 1.75). Researchers are less convinced that lack of systematic reviews prior to study conduct (median 3, IQR 2) and a lack of predefined statistical plan (median 3, IQR 1.75) contribute to translational failure.

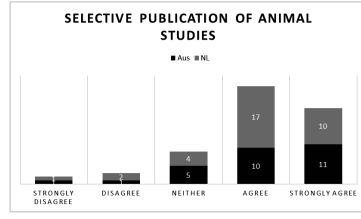


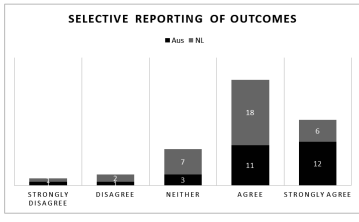


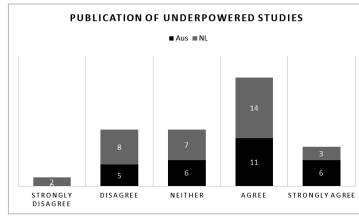


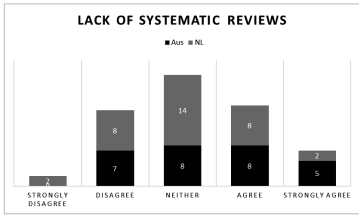


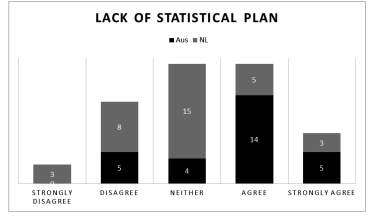












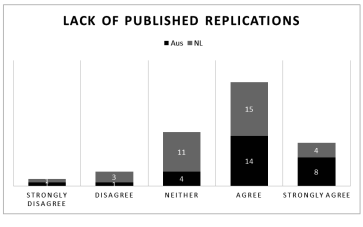
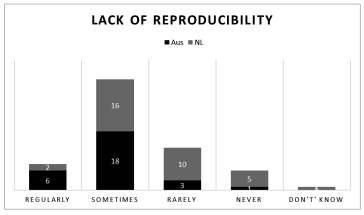
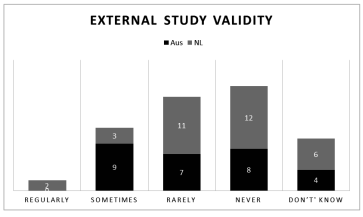
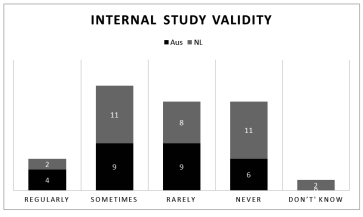
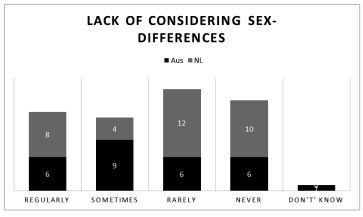


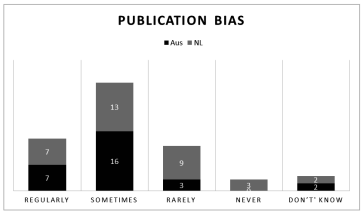
Figure 2. Responses to survey question 11. "To what extent do you agree these issues hamper translation of findings from animal studies into clinical practice?", on a 5-point Likert scale. Numbers indicate the number of responses.

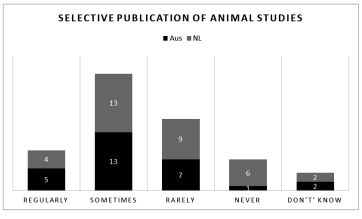


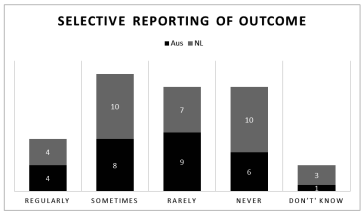


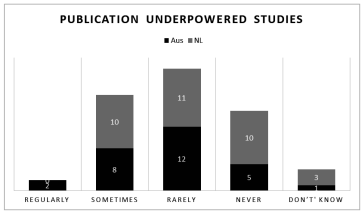


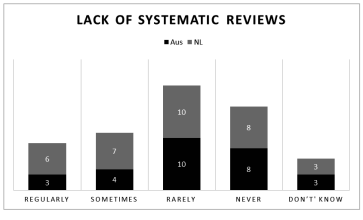


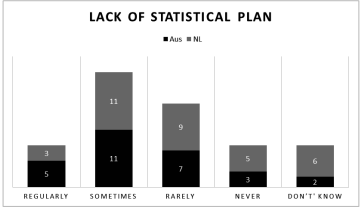












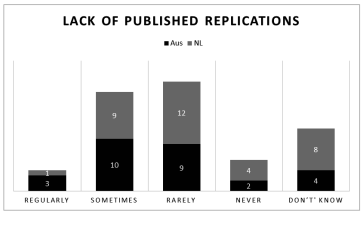


Figure 3. Responses to survey question 13 "How often have the following issues occurred for you or your group that relate to the conduct, reporting or translational capacity of your work involving animal studies?". Numbers indicate the number of responses.

Prevalence of issues hampering translational research

Almost all respondents (97%) admit at least one form of suboptimal research is performed in their research group (figure 3). Research is performed without a predefined statistical analysis plan, animal studies are published selectively, and lack attempts to replicate previously published work. On a smaller level, researchers also admit to the use of animal models which are not valid for the understanding of human disease, perform studies with a risk of bias, selectively report outcomes, publish underpowered studies, lack to perform a systematic review prior to study conduct and have problems with reproducibility either between or within research groups.

Benefits of preregistration

Based on the open responses and the responses on the closed questions we formulated five major benefits of preregistration. Preregistration is claimed to improve study design, reduce the number of animals used and increase reproducibility, transparency and collaboration (figure 1).

Open responses

Respondents were asked what the benefits of mandatory preregistration would be for their work. The researchers provided a total of 99 quotes, for which we identified 7 major themes and 7 additional subthemes (table 3, appendix 3).

Study design

Over one third of the respondents (n=23, 37%) provided 25 quotes (25% of total quotes) stating that preregistration improves study design. Several arguments on how preregistration contributes to more robust studies are reported. First, researchers see preregistration as a mechanism that will force them to think through their work better and they will be better prepared. Preregistration leads to "*a better chance to accurately addressing the hypotheses of the study*" (AUS83). Second, preregistration provides an overview of protocols, allowing researchers to search for examples to improve their study designs. Third, preregistered protocols are open for peers, which allows feedback to improve study design.

Reduce number of animals and wasted time

Almost one in five respondents (n=12, 19%) provided 14 quotes (14%) on the reduction in number of animals used and the time that can be saved. Preregistration could "*prevent wasted time trying to repeat experiments that have been performed elsewhere*" (AUS35). Better planning and more robust studies reduce the number of animals used. In addition, respondents state that preregistration can speed up research as it gives better insight in performed studies and their methods.

Collaboration
Almost one fifth of the respondents (n=11, 18%) provided 13 quotes (13% of all quotes) stating that preregistration improves collaboration. Registration of protocols allows feedback on study design, but it can also help researchers to identify collaborators.

Reproducibility, accountability, transparency and data interpretation
Researchers state that preregistration contributes to transparency (11 respondents, 11 quotes), accountability (7 respondents, 7 quotes), reproducibility (4 respondents, 4 quotes) and better interpretation of data (5 respondents, 5 quotes).

Others
Thirteen researchers (21%) do not see advantages of mandatory preregistration for their work.

Closed Responses

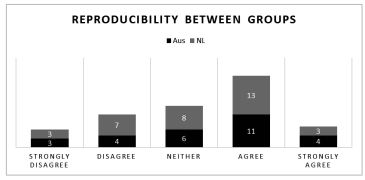
In response to closed questions, respondents overall agree that preregistration reduces unnecessary repetition of animal studies (median 4, IQR 1), the lack of engagement with a priori sample size calculations (median 4, IQR 1) and the lack of reproducibility between research groups (median 3.5, IQR 2) (figure 4). Researchers do not believe preregistration affects bias in study design (median 3, IQR 1), the use of non-valid models (median 3, IQR 2), the lack of reproducibility within research groups (median 3, IQR 2), the under representation of female animals (median 3, IQR 2), underpowered studies (median 3, IQR 1.75), publication bias (median 3, IQR 2), underreporting of data from animal studies (median 3, IQR 2), the rate of conducting systematic reviews (median 3, IQR 2), the practice of p-hacking, data dredging or hypothesizing after results are known (median 3, IQR 2) and poor translational capacity of animal studies to human clinical trials (median 3, IQR 2).

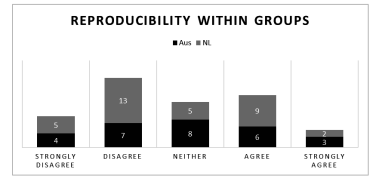
Challenges in preregistration

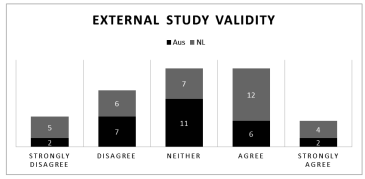
Four major concerns in preregistration were formulated based on the open and closed responses (figure 1). Researchers fear sharing preliminary ideas, believe preregistration can threaten intellectual property and worry that it will be an additional administrative burden and limit the scientific freedom.

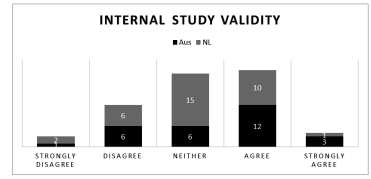
Open responses

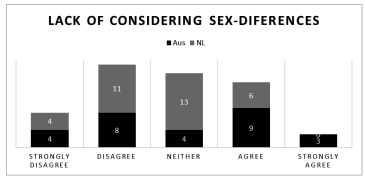
Seven themes and 6 subthemes were identified in the 105 open responses to the potential risks of preregistration (table 4, appendix 5).

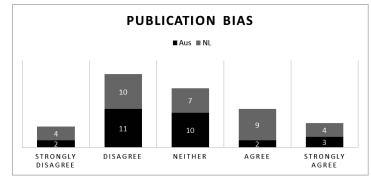


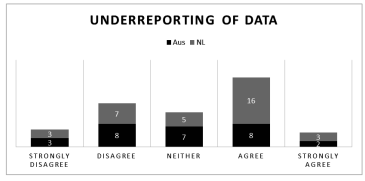


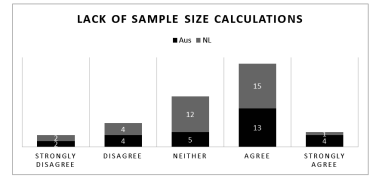


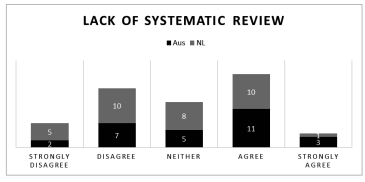


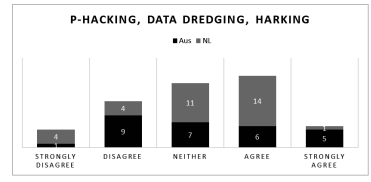


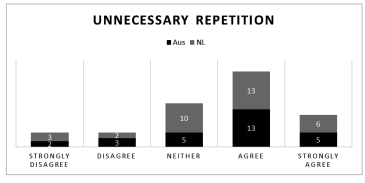


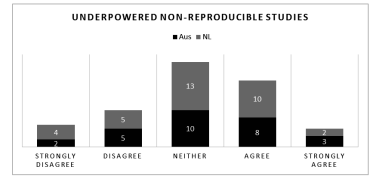












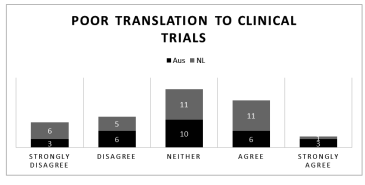


Figure 4. Responses to question 17 "To what extent do you agree preregistration of animal studies will have an effect on reducing the following issues believed to occur in the conduct of animal studies?" on a 5-point Likert scale. Numbers indicate the number of responses.

Table 4. Themes and subthemes identified in the open question about the benefits of mandatory preregistration.

Theme	Subthemes	Resp	Quotes
Study design		23	25
	More thought on design	9	9
	Access to examples	5	5
	Better review of design	3	3
	Other	8	8
Reduce waste of time/animals		12	14
	Reduce number of animals	11	11
	Reduce time	3	3
Collaboration		11	13
	Optimize design	5	5
	Identify collaborators	4	4
	Other	4	4
Reproducibility		4	4
Accountability		7	7
Transparency		11	11
Data interpretation		5	5
Other		6	7
Minimal or none		10	10
Negative		3	3
TOTAL		62	99

Resp = number of respondents reporting on this (sub)theme. Quote = number of quotes identified on this (sub) theme.

Fear of sharing ideas and risk to intellectual property

Almost one third of the respondents (n=20, 32%) provided 21 quotes (23% of total quotes) stating the fear for theft of ideas and the risk for intellectual property. Respondents state that competitors can "*take your idea and publish before you"* (AUS74). It could therefore "*foster competition, rather than collaboration" (AUS57)*, and is especially beneficial for research groups that work faster. It can also "*create problems with projects in collaboration with companies*" (NL19548961)

Time

Over one quarter of the respondents (n=17, 27%) provided 17 quotes (17%) on the time it requires to preregister a study and the delay it will cause. Respondents state that preregistration their research "*would be delayed incredibly because it would impose more 'hoops to jump through'*" (AUS 77). Stating it is "*another layer of administration, which will take a lot of work*" (NL19509558) leaving "less time/money for actual research" (NL19509558). Some respondents worry about the rift it can cause between smaller and larger research groups and others think they would be "*forced to complete a study that we know will fail, wasting time and precious resources*" (AUS59).

Lack of flexibility

Almost one fourth of the respondents (n=15, 24%) provided 17 quotes (17% of all quotes) on the lack of flexibility due to preregistration. Respondents state that is often necessary to adapt the protocol as "*many issues are impossible to anticipate prior to getting into the lab and often it is not until we are in the middle of a research project that we identify flaws in the original study design or hypothesis"* (AUS59). Preregistration could limit post-hoc analysis as it may "*prevent thinking outside the box*" *(AUS74)* and will limit the possibility to follow-up on unexpected results. It might limit the possibility to establish new models and, if everybody is working with similar protocols, it provides "*less opportunity for coincidental positive findings"* (NL19548388).

Administrative burden and bureaucracy

One fifth of the researchers (n=13, 21%) provided 13 quotes (13% of all quotes) on the administrative burden and bureaucracy around preregistration. The bureaucracy is already "*immense*" (NL19631709) and preregistration will create another barrier with additional paper work and longer processes, allowing researchers less time to perform research. One respondent states that bureaucracy has already led colleagues to perform animal studies abroad because "*it is way easier*" (NL1963709).

Table 5. Themes and subthemes identified in the open question about the risks of mandatory preregistration.

Theme	Subthemes	Resp	Quotes
Theft of ideas/IP		20	21
	Stealing of ideas	9	9
	Risk to IP	9	9
	Other	3	3
Time		17	17
Lack of flexibility		15	17
	Protocol adjustment	5	6
	Post-hoc analysis	4	5
	Other	6	6
Administrative burden / bureaucracy		13	13
	Bureaucracy	6	6
	Administrative burden	7	7
Resources		10	10
Pilot studies		4	4
Animal activists		2	2
Other		12	14
No risk		7	7
TOTAL		62	105

Resp = number of respondents reporting on this (sub)theme. Quote = number of quotes identified on this (sub) theme.

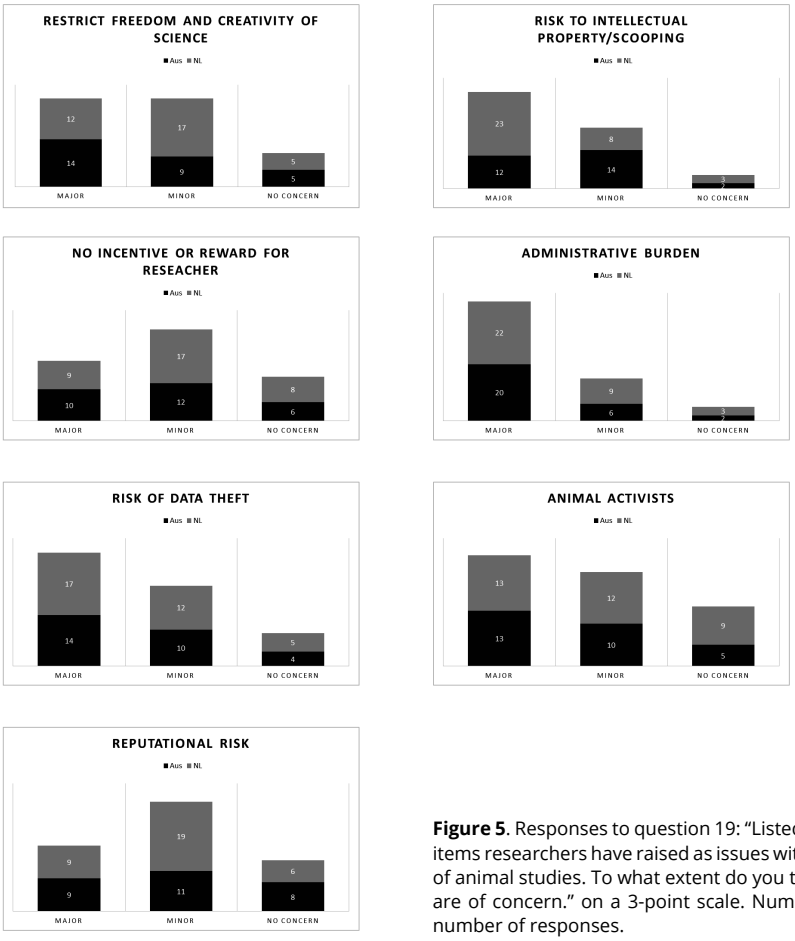


Figure 5. Responses to question 19: "Listed below are some items researchers have raised as issues with preregistration of animal studies. To what extent do you think these issues are of concern." on a 3-point scale. Numbers indicate the number of responses.

Resources, pilot studies and animal activists

Ten respondents (16%) provided 10 quotes (10% of all quotes) state that preregistration requires extra resources. It will take extra funding, resources to support preregistration and departments to monitor preregistration. Four respondents (6%) provided 4 quotes (4% of all quotes) reporting preregistration would limit the possibility to perform pilot studies. These studies are relevant to establish novel protocols and sample sizes are believed to be less important in these types of studies.

Other

Seven respondents (11%) see no risk of preregistration.

Closed responses

In response to closed questions respondents agree that the risk to intellectual property/ sharing of ideas, administrative burden, the restriction in freedom and the lack of incentives to preregister are challenging preregistration (Figure 5). The risk to reputation is marked as a minor concern.

Characteristics of registries and implementation of preregistration

If preregistration was to be mandatory, respondents feel that compliance to preregistration should be monitored by researchers, animal welfare bodies and research institutions (table 6). About half of the respondents (n=30) believe preregistration is relevant for all animal studies. Respondents have a broad view on the desired length of the embargo period (table 7). About one fifth (19%) of the respondents believe the embargo period should be between 0 to 6 months, 16% propose an embargo period of 1 year and 15% think the embargo period should be 2-5 years. Other respondents think the embargo should be related to time of publication or securing of the intellectual property. After completing the survey 29 respondents (47%) consider to preregister their studies, 19 respondents (31%) will only preregister if were mandatory, and 7 respondents (11%) do not consider preregistration at all (table 8).

Table 6. Party that should be responsible for monitoring compliance of preregistration.

	Resp (%)
Senior researchers	32 (52%)
Animal Welfare Body	32 (52%)
Research institutions	27 (44%)
Editors	15 (24%)
Reviewers	9 (15%)
Junior reseachers	8 (13%)
Funding agencies	6 (10%)
Others	6 (10%)
National politics	1 (2%)
Parliament	1 (2%)

Resp = number of respondents providing this answer, % = percentage of total respondents providing this answer.

Table 7. Ideal embargo time for embargoed protocols.

	Resp (%)
0-6 months	12 (19%)
1 year	10 (16%)
2-5 years	9 (15%)
> 5 years	3 (5%)
Optional 1-5-10 years	1 (2%)
Until after experiment is executed	3 (5%)
Until after publication of article	10 (16%)
Until after IP	4 (6%)
None	1 (2%)
Forever	1 (2%)
Don't know	3 (5%)
Other	6 (10%)

Resp = number of respondents providing this answer, % = percentage of total respondents providing this answer.

Table 8. Responses to the closed question whether respondents would consider preregistration of their studies.

		Resp (%)
Yes	I will retrospectively register all my studies and from now on preregister all my animal studies	1 (2%)
	I will register my next animal study	5 (8%)
	Only part of my animal studies	2 (3%)*
Possibly	Maybe, I will have to discuss with my colleagues	21 (34%)
	Only if journals, funders or institutes make it mandatory	19 (31%)
No	I don't trust the website	2 (3%)
	I will not register my animal study protocol	7 (11%)
Other		5 (8%)**

*translational studies (n=1) or late phase pre-clinical outcome studies (n=1). **It would compromise the nondisclosure agreement I have signed (n=1), we already do this on our own website (n=1), No, as I am not in the position to do so (n=1), i do not perform preclinical trials (n=1), for my field that website is not a good fit. But I would publish it on a website for my field (n=1).

Dutch versus Australian researchers

Challenges in translational research

Overall, both Australian and Dutch respondents experience comparable challenges in translational research, although small differences were observed. Australian respondents report more frequently on issues related to methodology (10 quotes by Australian vs 4 quotes by Dutch respondents), especially poor quality studies (2 quotes) and improper interpretation of data (2 quotes) which are only reported by Australian respondents. The pressure to publish (3 quotes), the attitudes of researchers and science (3 quotes) and perverse incentives for research (2 quotes) are only reported by Australian respondents. Furthermore, funding issues are reported more frequently by Australian respondents (7 quotes) compared to Dutch respondents (4 quotes). In response to the closed questions, Australian researchers believe more strongly that publication bias affects translational failure (median 5, IQR 1) and do feel that the lack of a predefined statistical analysis plan hampers translational research (median 4, IQR 1).

Dutch respondents report more frequently on issues related to bureaucracy (6 quotes by Australian respondents vs 19 by Dutch respondents), especially time to approval (4 quotes) and lack of freedom (2 quotes) which are only reported by Dutch respondents. The lack of alternatives to animal models (5 quotes) and the influence of journals (2 quotes) are only reported by Dutch respondents. In response to the closed questions, Dutch respondents doubt whether the lack of considering sex differences hampers translational research (median 3, IQR 1).

Benefits of preregistration

Australian respondents report more frequently that preregistration would improve preparation of the study design (7 quotes by Australian vs 2 by Dutch respondents)

and about the effect on reproducibility (3 quotes by Australian vs 1 by a Dutch respondent). They are more convinced preregistration reduces bias in studies (median 4, IQR 1.25) and the lack of performing systematic reviews before the conduct of a study (median 3.5, IQR 2).

Dutch respondents report more on the reduction of animals and wasted time (10 quotes by Dutch vs 4 quotes by Australian respondents) and on the effect on collaboration (10 quotes by Dutch and 3 quotes by Australian respondents). Identification of potential collaboration is only reported by Dutch respondents (4 quotes). Dutch researchers tend to disagree more that preregistration affects reproducibility between research groups (median 3, IQR 2) and within their own research group (median 2, IQR 2).

Challenges in preregistration

Australian respondents report more frequently on the limited flexibility (12 quotes by Australian vs 5 by Dutch respondents), and only Australian respondents report on the limited possibility for post-hoc analysis (5 quotes). Australians are also more worried about resources (7 quotes by Australian vs 3 by Dutch respondents) and pilot studies (3 quotes by Australian vs 1 by Dutch respondents), while only Dutch respondents worry about animal activists (2 quotes). In response to closed questions Australian respondents are more concerned about lack of freedom and less about the risk to intellectual property.

More experienced vs less experienced researchers

Challenges in translational research

There are no major differences in the reporting of challenges in translation research between the researchers with less compared to more experienced researchers (appendix 9, 10, 11), but small differences were observed. Less experienced researchers report relatively more often on animal models (14 quotes by less experienced vs 29 by more experienced respondents), especially on the subthemes heterogeneity (2 quotes by less experienced vs 2 quotes by more experienced respondents) and non-animal alternatives (3 quotes by less experienced vs 2 quotes by more experienced respondents). Less experienced researchers also worry more on animal distress (2 quotes by less experienced vs 1 quote by more experienced researchers) and the influence of journals (1 quote by a less experienced vs 1 quote by a more experienced respondent). In response to closed questions, respondents with <5 years of experience have a stronger opinion that internal study validity, publication bias, selective publication and lack of a statistical plan hamper translational research (appendix 5). The less experienced researchers report a higher prevalence of publication bias, selective publication of animal studies and a lack of considering sex-differences as compared to more experienced researchers (appendix 6).

Respondents with ≥5 years of experience reported more frequently on bureaucracy (22 quotes by more experienced vs 3 by less experienced respondents), within this theme the administrative burden (3 quotes), legislation issues (5 quotes) and lack of freedom (2 quotes) were only reported by respondents with ≥5 years of experience.

Benefits of preregistration

Less experienced researchers report relatively more on collaboration (6 quotes by less experienced vs 7 quotes by more experienced researchers) and only the less experienced researchers report on identifying collaborators (4 quotes). They also report more on transparency (10 quotes by less experienced vs 1 quote by more a more experienced researcher) and are more convinced preregistration affects reproducibility (median 4, IQR 2), reduces bias in study design (median 4, IQR 1) and reduced underreporting of data from animal studies (median 4, 1.25) (appendix 7).

Challenges in preregistration

Researchers with less experiment are more concerned about incentives for preregistration and reputational risk, but less about the lack of freedom (appendix 9, 10, 11). Only more experienced researchers worry about the administrative burden of preregistration (13 quotes), the effect on pilot studies (4 quotes) and about animal activists (2 quotes). They report more on the required resources (9 quotes by more experienced vs 1 quote by a researcher with <5 years of experience).

DISCUSSION

In this survey among animal researchers, we identified several issues that hamper translational research. In both open and closed questions, respondents report that current practice involves using animal models of which the translational value is unclear, using flawed study designs, selective and incomplete publication of animal studies, and struggling with irreproducibility. In addition, in open questions, one in three researchers reported suboptimal conditions under which experiments take place, including insufficient training of staff. One in three respondents experiences a high administrative burden and feels pressured by animal experiment approval committees, research institutes and journals to make decisions that threaten the validity of their experiments. The self-reported prevalence of questionable research practice is high, with 97% of the respondents admitting that at least one form of science misconduct occurred in their research group.

Animal researchers acknowledge several benefits of preregistration. In both open and closed responses, they state that preregistration will lead to more thorough and better reviewed study designs, a reduction in the number of animals used and an

increase in reproducibility. In addition, in open responses, one-fifth of respondents state preregistration will improve collaboration and another fifth appreciates the increased transparency. Identified risks of preregistration are the fear of sharing ideas and threat to intellectual property, the additional administrative burden and the restriction of freedom.

Preregistration addresses many of the identified hurdles and can therefore be a potential solution to improve translational research. However, two important issues are not directly affected by preregistration; the use of animal models with unclear translational value and insufficient training of staff. Measures to address these issues have not been investigated in this survey. In addition, the experienced work load and pressure to succeed are already believed to hamper research and preregistration is believed to increase the work load even more.

Our study shows that only a limited proportion of respondents (8%) has preregistered an animal study in the past. Almost half of the respondents (47%) would consider preregistration in the future, but a significant proportion (31%) would only do so if it were mandatory. This indicates that researchers awareness and intrinsic motivation for preregistration is still low. Although steps are taken to encourage preregistration through a top-down approach, engagement with registries currently largely depends on the awareness and motivation of the individual researcher. Simultaneously, the success of registries to positively enhance conduct of animal research is dependent on researcher engagement.

Australian versus Dutch researchers

The overall differences between Australian and Dutch researchers in their perception on the research crisis and risks and benefits of preregistration seem small. Overall, Australian researchers are more concerned about preregistration. That could possibly be explained by the higher political and institutional support for preregistration in the Netherlands.

Dutch researchers report that the time to ethical approval of animal studies by local regulators is hampering translational research, which is not mentioned by Australian researchers at all. This might be explained by the differences in regulations between both countries. Animal studies performed in Australia need to be approved by the local Animal Ethics Committees (AECs). The median time from submission to approval for animal ethics applications in 2018 for the University of Sydney was 34 calendar days. The approval rate of animal protocols for the University of Sydney in 2018 was 78% within 45 calendar days and 98% within 90 calendar days. In the Netherlands, as of 2014, all research projects involving laboratory animals need to be approved by the Central Authority for Scientific Procedures on Animals (CCD). After the project license is approved, a researcher has to submit a work protocol at the local Animal Welfare Body (AWB). The CCD has 40 business days to evaluate a project

proposal. The AWB has an additional 10 business days to respond to the work protocol. In 2018, 75% of the proposals was evaluated by the CCD within these 40 business days and 98% (404/414) of evaluated proposals were approved[26–29]. The approval rates between countries are comparable, but time to approval is longer in the Netherlands (75% in 50 business days) compared to Australia (78% in 32 business days). If compared with the average time between approval of a protocol and timing of publication, which was 31 months in a Dutch sample size, this delay seems relatively short[19].

Level of experience

In our sample, researchers with more than 5 years of experience (74%) were overrepresented compared to researchers with less experience. This was also seen in a previous study among animal researchers[24]. In Australia chief investigators were addressed per email, which might have contributed to this overrepresentation. More experienced researchers might also have been more willing to participate, as they might have a developed a stronger opinion about translational research and preregistration during their career.

Researchers with at least 5 years of experience are more worried about the extra time and administration it will take to preregister a study and claim more resources are needed to adhere to these standards in the future. The fact that more experienced researchers are often responsible for securing funding and employment of the research group, whereas less experienced researchers might be more focused on their own experiments, might contribute to this difference. This would also explain why less experienced researchers herald the positive effects of preregistration on internal study validity, publication bias, selective publication, reduction of unnecessary repetition of animal studies and predefined statistical analysis more, compared to more experienced researchers.

Researchers with more experience report more concerns about incentives in research compared to researchers with less than 5 years of experience. This could be explained by the differences in reward systems and the pressure individuals experience.

Questionable research practice in perspective

Suboptimal research practices are not specific for animal research. A recent survey among all academic researchers in the Netherlands showed that this is common among all fields of science[30]. In our study, 43 respondents (69%) report "positive" findings are more likely to be published in their research groups to a considerable degree. Previous surveys among respectively Dutch and international animal researchers estimated a lower prevalence of publication bias (50% and 34%)[9,24]. Our study findings are more in line with two studies tracing animal study protocols to investigate the publication rate. In these two studies the publication rate was 60 and 70%[19,31]. Twenty-six respondents (42%) report flaws in study designs occurs sometimes or even regularly in their

research. Poor reporting of animal obscures the true state of internal study validity in animal research[6,32]. Reporting of blinding, randomisation and sample size calculations occurs in less than half of the publications[6]. Our respondents question the translational value of animal models. Only 11% of therapies tested in first-in-man studies lead to a registered therapy and the translational success rate ranges from 0 to 100%[1,4]. Optimizing study designs, conduct and reporting might increase the translational success rate, but does not influence the translational capacity of an animal model.
In line with previous surveys, our study shows that preregistration of animal studies improves study designs, reduces the number of animals used, increases collaboration and increases transparency[24,25].

A stakeholder analysis, including a systematic review of literature and interviews with 21 key informants from four stakeholder groups, additionally showed the benefit of preregistration for meta-research[25]. A survey specifically among animal researchers confirmed these strengths[24]. Identified weaknesses of preregistration in both studies are the administrative burden, potential theft of ideas and reduced creativity and serendipity in animal studies.

Limitations

The response rate is unknown, as we do not know exactly how many people were addressed to participate in this study. The number of respondents is low and we did not have enough data to address our planned subgroup-analyses of medical doctors vs non-medical doctors. Most respondents are involved with both hands-on work, as well as supervising tasks, limiting the possibility to perform analyses between these groups. Nevertheless this study is the first to identify perceived hurdles of animal researchers in their work and their attitudes' towards preregistration as a solution for this problem. Although multiple open responses were included in this survey, further exploration of underlying arguments was not possible in this non-interactive set-up. In-depth interviews are required for further analyses. Interpretation of the provided quotes might be subjected to personal perception of the researchers. To minimize these effects all thematic analyses were performed in duplicate.

Characteristics of existing registries

There are currently, to the best of our knowledge, two free, open access platforms dedicated to the registration of protocols of animal studies: preclinicaltrials.eu and animalstudyregistry.org. Both platforms have addressed the concerns voiced by animal researchers. They allow researchers to register their protocol under embargo to protect their intellectual property. Preclinicaltrials.eu offers version control, allowing researchers to submit time-stamped amendments to their protocol after initial registration. There is no data available on time needed to complete registration for either of these registries. Harmonization of requirements between institutional review

and preregistration registries can ease preregistration. Registration forms from local approval committees are not publicly available, therefore it is unclear to what extent they differ from the registries' forms.

Future perspectives

With this study we showed several issues hampering translational research and how preregistration can be part of the solution to tackle these problems. One major concern is the expected additional administrative burden and time consumption of preregistration, although no details of experienced burden are currently available. Future research should study the additional burden of preregistration. To ease preregistration, institutions should share and harmonize their registration forms. Automatic transfer of data from these institutional protocols to registries should be considered. The scientific community should put efforts in increasing awareness of preregistration. In addition, institutions should require higher standards of training and further research should be performed to investigate the translational value of animal studies.

REFERENCES

1. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. 2004;3(8):711-715. doi:10.1038/nrd1470.
2. Ioannidis JPA, Greenland S, Hlatky MA, et al. Increasing value and reducing waste in research design, conduct, and analysis. *Lancet*. 2014;383(9912):166-175. doi:10.1016/S0140-6736(13)62227-8
3. Baker M. 1,500 scientists lift the lid on reproducibility. *Nature*. 2016;533:452-454. doi:10.1038/533452a
4. Leenaars CHC, Kouwenaar C, Stafleu FR, et al. Animal to human translation: A systematic scoping review of reported concordance rates. *J Transl Med*. 2019;17(1):223. doi:10.1186/s12967-019-1976-2
5. Jansen Of Lorkeers SJ, Eding JEC, Vesterinen HM, et al. Similar effect of autologous and allogeneic cell therapy for ischemic heart disease: systematic review and meta-analysis of large animal studies. *Circ Res*. 2015;116(1):80-86. doi:10.1161/CIRCRESAHA.116.304872
6. Ramirez DD, Motazedian P, Jung RG, et al. Methodological Rigor in Preclinical Cardiovascular Studies: Targets to Enhance Reproducibility and Promote Research Translation. *Circ Res*. 2017;120(9):1916-1926. doi:doi: 10.1161/CIRCRESAHA.117.310628
7. Villamor-Martinez E, Hundscheid T, Kramer BW, Hooijmans CR, Villamor E. Stem Cells as Therapy for Necrotizing Enterocolitis: A Systematic Review and Meta-Analysis of Preclinical Studies. *Front Pediatr*. 2020;8(December):578984. doi:10.3389/fped.2020.578984
8. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. *Lancet*. 2009;374(9683):86-89. doi:10.1016/S0140-6736(09)60329-9
9. ter Riet G, Korevaar DA, Leenaars M, et al. Publication Bias in Laboratory Animal Research: A Survey on Magnitude, Drivers, Consequences and Potential Solutions. *PLoS One*. 2012;7(9):e43404. doi:10.1371/journal.pone.0043404
10. Kimmelman J, Anderson JA. Should preclinical studies be registered? *Nat Biotechnol*. 2012;30(6):488-489. doi:10.1038/nbt.2261
11. Jansen of Lorkeers SJ, Doevendans PA, Chamuleau SA. All preclinical trials should be registered in advance in an online registry. *Eur J Clin Invest*. 2014;44(9):891-892. doi:10.1111/eci.12299
12. Baker M. Animal registries aim to reduce bias. *Nature*. 2019;Sep;573(7773):297-298. doi:10.1038/d41586-019-02676-4
13. Chamuleau SAJ, van der Naald M, Climent AM, et al. Translational Research in Cardiovascular Repair A call for a paradigm shift. *Circ Res*. 2018;122(2):310-318. doi:10.1161/CIRCRESAHA.117.311565
14. Bert B, Heinl C, Chmielewska J, et al. Refining animal research: The Animal Study Registry. *PLoS Biol*. 2019;17(10):e3000463. doi:10.1371/journal.pbio.3000463
15. National Health and Medical Research Council (NHMRC). *Best Practice Methodology in the Use of Animals for Scientific Purposes*. www.nhmrc.gov.au/guidelines-publications/ea20.
16. Royal Netherlands Academy of Arts and Sciences. *Replication Studies - Improving Reproducibility in the Emperical Sciences*.; 2018. https://www.knaw.nl/shared/resources/actueel/publicaties/pdf/20180115-replication-studies-web.
17. De Groot, Dik-Faber, Von Martels. *Motie van Het Lid De Groot c.s. over Het Registreren van Alle Individuele Dierproeven*.; 2018:ISSN 0921-7371. https://www.tweedekamer.nl/kamerstukken/.
18. van Engelshoven I. *Pilot Stimulering Transparant Proefdieronderzoek En Voortgang van Transitie Naar Minder Proeven Met Apen En Meer Proefdiervrije Innovaties in Het BRPC*.; 2020. https://www.tweedekamer.nl/kamerstukken/detail?id=2020Z20890&did=2020D44732.
19. van der Naald M, Wenker S, Doevendans PA, Wever KE, Chamuleau SAJ. Publication rate in preclinical research: a plea for preregistration. *Br Med J Open Sci*. 2020;4:e100051. doi:10.1136/bmjos-2019-100051
20. Advisory committee to the Director (National Institute of Health). ACD working group on enhancing rigor, transparency, and translatability in animal research - final report. *acd.od.nih.gov*. 2021. https://acd.od.nih.gov/documents/presentations/06112021_ACD_WorkingGroup_FinalReport.pdf.
21. van der Naald M, Chamuleau SAJ, Menon JML, et al. A 3-year evaluation of preclinicaltrials.eu reveals room for improvement in preregistration of animal studies. *PLOS Biol*. 2021;19(9):e3001397. doi:10.1371/journal.pbio.3001397
22. Taylor K, Gordon N, Langley G, Higgins W. Estimates for Worldwide Laboratory Animal Use in 2005. *ATLA - Altern to Lab Anim*. 2008;36(3):327. doi:10.1177/026119290803600310
23. Taylor K, Alvarez LR. An Estimate of the Number of Animals Used for Scientific Purposes Worldwide in 2015. *Altern to Lab Anim*. 2019;47(5-6):196-213. doi:10.1177/0261192919899853
24. Wieschowski S, Laser H, Sena ES, Bleich A, Tolba R, Strech D. Attitudes towards animal study registries and their characteristics: An online survey of three cohorts of animal researchers. *PLoS One*. 2020;15(1):e0226443. doi:10.1371/journal.pone.0226443
25. Wieschowski S, Silva DS, Strech D. Animal Study Registries: Results from a Stakeholder Analysis on Potential Strengths, Weaknesses, Facilitators, and Barriers. *PLoS Biol*. 2016;14(11):e2000391. doi:10.1371/

journal.pbio.2000391
26. Website Animal Welfare Body Utrecht. https://ivd-utrecht.nl/nl.
27. Website Centrale Commissie Dierproeven. https://www.centralecommissiedierproeven.nl/.
28. Centrale Commissie Dierproeven. *Jaarverslag Centrale Commissie Dierproeven 2018*.; 2018. www.centrale-commissiedierproeven.nl.
29. Centrale Commissie Dierproeven. *Jaarverslag Centrale Commissie Dierproeven 2014/2015*.; 2015. www.centralecommissiedierproeven.nl.
30. Gopalakrishna G, ter Riet G, Cruyff M, et al. Prevalence of questionable research practices, research misconduct and their potential explanatory factors: a survey among academic researchers in The Netherlands. *MetaArXiv*. doi:10.31222/osf.io/vk9yt
31. Wieschowski S, Biernot S, Deutsch S, Glage S, Strech D. Publication rates in animal research. Extent and characteristics of published and non- published animal studies followed up at two German university medical centres. *PLoS One*. 2019;14(11):e022375. doi:10.1371/journal.pone.0223758
32. Chan A-W, Song F, Vickers A, et al. Increasing value and reducing waste: addressing inaccessible research. *Lancet (London, England)*. 2014;383(9913):257-266. doi:10.1016/S0140-6736(13)62296-5

APPENDIX 1 QUESTIONNAIRE

An investigation into the perception of researchers on preregistration of animal studies

1) Are you actively performing animal studies or supervising the conduct of animal studies? [yes/no]
2) What is your age in years? [open question]
3) What is/are your current role(s)? [multiple choice]
   - Bachelor or Master student - no background as MD or veterinarian
   - Bachelor or Master student - with a background as MD or veterinarian
   - PhD student - no background as MD or veterinarian
   - PhD student - with a background as MD or veterinarian
   - Post - doctoral researcher
   - Research assistant / technical officer / technician
   - Principal investigator / group leader
   - Administrative worker
   - Member of a body involved with animal research approvals (Animal experiment/ethical committee) or animal research policy
   - Clinician / MD
   - Veterinarian
   - Other: please specify [open]
4) How many years in total have you been active in research involving animal studies? [open question]
5) What is your main field of research? [multiple choice]
   - Behavioral science
   - Cardiovascular medicine and haematology
   - Dentistry
   - Human movement and sports science
   - Immunology
   - Medical biochemistry and metabolomics
   - Medical Microbiology
   - Medical physiology
   - Neurosciences
   - Nursing
   - Nutrition and dietetics
   - Oncology and carcinogenesis
   - Ophthalmology and optometry
   - Paediatrics and reproductive medicine
   - Pharmacology and pharmaceutical sciences
   - Toxicology
   - Other: please specify [open]
6) Are you currently directly involved with the handling and collection of data from animal studies? [yes/no]
7) Do you supervise other researchers directly involved with the handling and collection of data from animal studies? [yes/no]
8) Are you using animals as potential models for understanding human disease and/or the development of new therapeutic and diagnostic approaches? [yes/no]
9) Which species do you typically use for animal studies?
   - Mouse
   - Rat
   - Guinea pig
   - Hamster
   - Rabbit
   - Pig
   - Dog
   - Chicken

- Zebrafish
- Other: please specify [open]

10) In your opinion what are the 3 biggest challenges to improving the conduct of animal studies for translational research? [open question]
11) The table below lists potential problems limiting the quality and translational capacity of animal studies. To what extent do you agree these issues hamper translation of findings from animal studies into clinical practice?

	Strongly disagree	Disagree	Neither disagree nor agree	Agree	Strongly agree
A lack of reproducibility either between or within research groups working in similar fields					
The use of animal models that are not valid for an understanding of human disease					
Bias in study design (such as a lack of randomization or blinding of investigators)					
The lack of consideration towards potential sex-differences and underrepresentation of female animals in experimental groups					
Publication bias (positive, significant results are more likely to be published than negative, non-significant results)					
Selective publication of animal studies (not all studies completed are submitted for publication)					
Selective reporting of outcomes in animal studies that are submitted for publication (not all data collected is reported)					
Publication of underpowered studies (i.e. sample size too small)					
Lack of systematic review prior to experiments to inform study design					
Lack of predefined statistical analysis plan (p-hacking, HARKing, etc)					
A lack of published attempts to replicate previously published results (successful or not)					

12) If you believe there are other issues not identified above please describe them here [open]
13) How often have the following issues occurred for you or your group that relate to the conduct, reporting or translational capacity of your work involving animal studies?

	Regularly	Sometimes	Rarely	Never	Don't know
A lack of reproducibility either between or within research groups working in similar fields					
The use of animal models that are not valid for an understanding of human disease					
Bias in study design (such as a lack of randomization or blinding of investigators)					
The lack of consideration towards potential sex-differences and underrepresentation of female animals in experimental groups					

Publication bias (positive, significant results are more likely to be published than negative, non-significant results)
Selective publication of animal studies (not all studies completed are submitted for publication)
Selective reporting of outcomes in animal studies that are submitted for publication (not all data collected is reported)
Publication of underpowered studies (i.e. sample size too small)
Lack of systematic review prior to experiments to inform study design
Lack of predefined statistical analysis plan (p-hacking, HARKing, etc)
A lack of published attempts to replicate previously published results (successful or not)

14) If there are any other issues relating to animal studies you or your group have experienced that relate to the conduct, reporting or translational capacity of your work not identified above, please describe them here: [open]

Preregistration of animal studies means that, before starting a study, the study protocol (i.e. hypothesis, experimental groups, sample sizes, species, strains, primary outcome, etc) is stored on a register (e.g., online database), which is publicly accessible from a verified personal log-in. Example in the case of human research are ANZCTR.org and clinicaltrials.gov and an example in the case of animal studies is preclinicaltrials.eu. These protocols can be anonymized with an optional embargo (i.e., protocol details are not published until a specific date).

15) In your opinion, if preregistration of animal studies was to be mandated at either the institutional, ethical, funding or publication approval level, what may be benefits involved for your work? [open question]
16) In your opinion, if preregistration of animal studies was to be mandated at either the institutional, ethical, funding or publication approval level, what may be risks involved for your work? [open question]
17) To what extent do you agree preregistration of animal studies will have an effect on reducing the following issues believed to occur in the conduct of animal studies?

	Strongly disagree	**Disagree**	**Neither disagree nor agree**	**Agree**	**Strongly agree**
The lack of reproducibility between groups in a common field of research					
The lack of reproducibility within research groups working together					
The use of non-valid animal (disease) models for understanding human disease					
Bias in study design resulting from the lack of randomized allocation of animals to intervention groups and/or absence of blinding of investigators or outcome assessors to interventions and groups					
The under representation of female animals in studies					

Publication bias towards positive, significant results being more likely to be published than negative, or non-significant results
Underreporting of data from animal studies
The lack of engagement with a priori sample size calculations
The rate of conducting systematic reviews to justify use of animal model or study design
The practice of p-hacking, data dredging, or Hypothesizing after results are known (HARKing)
Over use of animals in the unnecessary repetition of animal studies
Under use of animals through the publication of underpowered but non-reproducible data
Poor translational capacity of animal studies to human clinical trials

18) If there are any issues not identified above that you think preregistration of animal studies will impact, please describe them here: [open]
19) Listed below are some items researchers have raised as issues with preregistration of animal studies. To what extent do you think these issues are of concern.

	Major concern	**Minor concern**	**No concern**
Preregistration will restrict freedom and creativity of science			
Preregistration will increase the risk of losing intellectual property and being scooped by competitors			
There is no incentive or reward for individual researchers to preregister their studies			
Preregistration will increase the administrative burden of researchers			
Preregistration will increase the risk of data theft			
Preregistration will raise the awareness of animal activists and put researchers and institutions at increased risk of personal harm			
Preregistration will increase reputational risk of individuals and institutions if their data is seen to be negative, inconclusive or non-reproducible			

20) Other concerns:
21) If preregistration of animal studies was mandated, who should be responsible for monitoring compliance?
    - Senior researchers (PIs / group leaders / supervisors)
    - Junior researchers (research fellows / PhD students)
    - Scientific journal editors
    - Scientific journal reviewers
    - Research institutions / Academic hospitals
    - Animal welfare bodies / animal experimental committees / CCD
    - Funding agencies
    - National politics / parliament
    - European politics / parliament
    - Others: please specify
22) Do you think preregistration is relevant for all animal studies?
    - Yes, please explain [open]
    - No, please explain [open]
23) Have you ever preregistered or published methodologies or protocols prior to conducting an animal study?
    - No

- Yes, I used preclinicaltrials.eu
- Yes, I used Open science Framework
- Yes, I used a different platform

24) Would you preregister your animal study protocol on preclinicaltrials.eu?
    - Yes, I will retrospectively register all my studies and from now on preregister all my animal studies
    - Yes, I will register my next animal study
    - Yes, but only part of my animal studies, please specify [open[
    - Maybe, I will have to discuss this with my colleagues
    - Only if journals, funding agencies or institutes make it mandatory
    - No, I don't trust this website
    - No, I will not register my animal study protocol
25) If you were to choose an embargo that prevents your protocol to be publicly available immediately, how long should this embargo period be? [open question]

APPENDIX 2

Quotes biggest challenges to improving the conduct of animal studies for translational research

			ANIMAL MODEL **31 respondents, 43 quotes**
ID	**Nat**	**Exp**	**Quote**
			Validity of models **20 respondents, 22 quotes**
50	AUS	1	Establishing that the behaviours we study in animals can indeed be generalised to humans.
73	AUS	1	Having accurate models of human disease in animal
74	AUS	2	different genomes
19509517	NL	2	to build a modelsystem you need validation in an actual animal
19547003[p]	NL	3	Translational value of a model (sometimes a model is used in literature and it is hard to apply a better model since the new model is unknown and you cannot publish about it).
57	AUS	3	effectiveness of disease models; there are many variations of models for a number of diseases, the variety can make it hard to compare research of the same topic when different models are being used
35	AUS	9	Animal models often do not completely recapitulate the human disease
35	AUS	9	humans are diverse and animal models are usually very controlled so transitioning will always be hard.
19479561	NL	10	lack of good translational models
71	AUS	10	differences to human conditions
19564087	NL	10	mouse-to-human comprison not optimal -
19564193	NL	18	the use of relevant, often larger, animal models-
45	AUS	20	widespread lack of acceptance that mice are not best model for humans.
19611694	NL	20	Improved disease models (closer to human disease) and knowledge of the behaviour of drugs/molecules in animals
54	AUS	22	Models that recapitulate human immunopathology
54	AUS	22	The reliance on one or two strains of mice
19548961	NL	41	Recognize that large animals are more useful because of their similarity to man
19551752	NL	30	developing translational animal models and tests (mainly the latter)
19611581	NL	30	translatibility: animal model not representative for human disease-
19488046	NL	30	differences in physiology between humans and animals
19488062	NL	35	an animal experiment is just a model: how representative is it for certain diseases-
19631725	NL	35	Translation of toxicity pharmacokinetic behavior and efficacy of drugs.
			Heterogeneity **3 respondents, 4 quotes**
50	AUS	1	Finding ways to better understand the heterogeneity in animal's behaviours.
19425716	NL	4	Differences between animal species used in research.
19488046	NL	30	Variation in humans as compared to standardized lab animal breeds
19488046[c]	NL	30	Influence of environment en mental state affecting humans and this is difficult to mimic in lab circumstances
			Alternatives to animals **4 respondents, 5 quotes**
19462856	NL	3	Replacing (one of the 3Rs)-

19462856	NL	3	Collaborations to work towards the transition from animal-models to computer- or organ-on-a-chip-models.
19553604	NL	3	Replacement of animals with other (ex vivo of in vitro) experiments -
19432454	NL	10	Development of alternatives
19479561	NL	10	lack of good translational alternatives (in vitro/ in silico models)
			Other **11 respondents, 12 quotes**
72[m]	AUS	1	lack of information for designing robust study and which animal model for translation
74[f]	AUS	2	practicality of using animals with closer genomes (cost, availability)
19547003	NL	3	Using the right animal (some animals are not prefered wheras the are more suitable to serve as test-animals).
33	AUS	8	Lack of appropriate control models in experimental models (eg. Gender / age / littermate) matching
19535396[p]	NL	9	3. What are the most relevant models to use for all you can have positive and negative arguments to use them. And what do reviewers want? (PDX or not? humanized mice?)
19549932	NL	12	implementation of animal models with co-morbidities-
45[b]	AUS	20	access to (physcially and with ethics approval) animals that would be better models than mice
76	AUS	25	production and choice of valid animal models
44	AUS	28	finding the right model
44	AUS	28	knowledge and acceptance of the model
19488062	NL	35	defining the most valuable biomarkers - readout parameters to come to a valuable translation
19575974[f]	NL	36	Generate novel and improved animal models of cardiovascular disease that incorporate the various co-morbidities (ageing metabolic dysregulation hypertension dyslipidemia etc.) typically found in humans (this requires additional funding as these models are often more expensive).

Nat=nationality, Exp = experience of researchers in years, NL = Dutch, AUS = Australian Quotes are also classified as [a]animal model [b]bureaucracy, [c]conditions, [f]funding, [m]methodology, [i]incentives, [o]others, [pe]perception, [p]publication, [rep]replication, [ra]researcher's attitude

			BUREAUCRACY **21 respondents, 25 quotes**
ID	**Nat**	**Exp**	**Quote**
			Approval committees **7 respondents, 7 quotes**
19547003[m]	NL	3	sample size (If I conduct a in-man study I have 300-600 patients/volunteers whereas I do not get approval from the ethics board when I need this number of animals)
57	AUS	3	experimental design; what is allowed and not allowed by ethics committees can sometimes limit the effectiveness of an experiment
19548388	NL	5	Working with the IvD is very difficult at least in Maastricht. They should improve their knowledge about certain research topics or invite experts to avoid miscommunication delayed handling times irrelevant questions and increases in the amount of animals used for experiments.
35[rep,p]	AUS	9	reproducibility of experiments as animal experiments are not conducted the same way due to differing ethical views/committees and lack of detail in publications
19612957[b]	NL	9	No good reviewers for CCD application, decline applications because of having not enough scientific background

61	AUS	10	Having the right balance of researchers, vets and members of the public on AECs.
45[a]	AUS	20	access to (physcially and with ethics approval) animals that would be better models than mice
			Administrative burden **3 respondents, 3 quotes**
75	AUS	10	Unreasonable amounts of paperwork and bureaucracy in getting protocols approved.
19611694	NL	20	Decrease administrative burden
19586787	NL	25	Facilitating application for animal ethics approval
			Legislation **5 respondents, 5 quotes**
19447245	NL	8	Foreign authorities that demands specifix needs for animal study (more animal more injections different volumes different humane endpoints etc)
19549932	NL	12	strict animal regulations
19576659	NL	15	Dutch regulations
19613285	NL	17	Diminishing the regulatory hurdles related to (ethical) approval for experiments with laboratory animals.
53[o]	AUS	35	Most researchers in my area (developmental toxicology) are in drug companies. Their work is guided by legislation so fundamental research is often lacking. This gap is filled by underfunded academics such as myself.
			Time to approval **4 respondents, 4 quotes**
19588197[f]	NL	2	The process of getting approval for performing animal experiments is 1) expensive and 2) time consuming therefore it is challenging to perform numerous experiments within a project which is often necessary to come to explicit conclusions.
19611542	NL	9	long waiting times to get projects approved
19564087	NL	10	Long proces to get CCD licence approved
19621148	NL	21	the long process of application for animal studies
			Lack of freedom **2 respondents, 2 quotes**
19611542	NL	9	No flexibility
19586787	NL	25	improve flexibility within granted application
			Other **4 respondents, 4 quotes**
19631709	NL	5	Bureaucracy
19488230	NL	7	By giving very narrow possibilities in discomfort results can be skewed. In example when a scale is used for feather scores in laying hens: from 5 (naked area) to 1 (completely covered) the CCD never allows a score of 4 or 5. Meaning your results are already cat down in the possibilities and distribution. This could result in the end in more research on the same topic because big differences cannot be observed as a rule.
19447245	NL	8	European monograph where models are specified so in vitro is difficult
19612957[b]	NL	9	No good reviewers for CCD application, decline applications because of having not enough scientific background

			CONDITIONS **15 respondents, 20 quotes**
ID	**Nat**	**Exp**	**Quote**
			Training **8 respondents, 8 quotes**
62	AUS	2	Training
63	AUS	4	lack of proper hands on training
19631709	NL	5	Skills/professionality of the responsible researcher
59	AUS	9	Better practical training with animals used for research purposes. As it stands, our ethics seminars are virtually 100% theory, and each group determines the level of practical training that it imparts on new members who participate in animal research. As a result, the skill levels vary wildly and often students with little practical skill or knowledge will attempt to complete a research project with animals. This results in excessive waste of animals and essentially unusable data. Suggestion: the CPC has a team of experts for our microscope facility that go through extensive training exercises with new researchers before they embark on their projects. A similar approach could be taken to animal research.
75	AUS	10	Lack of formalised practical training in animal handling.
19547467	NL	15	technically experienced researchers performing experiments to avoid unclear results repetition of experiments and need of big experimental groups
19564193	NL	18	Continuity of experienced staf and transfer of knowledge of specific models
67	AUS	55	Need for better training in behavioural methods for many researchers who use such methods
			Standardization **5 respondents, 5 quotes**
50	AUS	1	Ensuring consistency of circumstances across testing procedures.
19509266	NL	2	Standardization of research conduct (in different groups cities countries)
63	AUS	4	non-standardised equipment across labs
75	AUS	10	Lack of controlled environments (prior to any experimenter intervention)
19488062[rep]	NL	35	reproducibility (results vary due to variations in experimental conditions between animal facilities)
			Animal distress **3 respondents, 3 quotes**
19553604	NL	3	Finding a minimal invasive surgical therapy to make suffering minimal
63	AUS	4	facilities with appropriate building features for animal research (e.g. soft close doors, soundproofing, ventilation etc)
59	AUS	9	The conditions required for animal housing could yet be improved (i.e. more stimulation, fewer instances of single-housing etc.) in order to minimize distress which is a major confounding factor in research studies.
			Other **4 respondents, 4 quotes**
73	AUS	1	Objective monitoring and care for animals, which may impact results or continued health of animals
19631709	NL	5	Communication between researcher and animal caretaker
19488230	NL	7	-housing regulations of animals is fare from practice in animal husbandry making it hard to relate the results found in research to practice.
19488046[a]	NL	30	Influence of environment en mental state affecting humans and this is difficult to mimic in lab circumstances

			PUBLICATION **15 respondents, 17 quotes**
ID	**Nat**	**Exp**	**Quote**
			Transparency **6 respondents, 6 quotes**
62[r]	AUS	2	Reproducibility (method reporting)
33	AUS	8	Lack of regulated publishing requirements regarding specifics in animal research and experimental models.
35[b,r]	AUS	9	reproducibility of experiments as animal experiments are not conducted the same way due to differing ethical views/committees and lack of detail in publications
34	AUS	20	Competitive science environment - people don't share resources or study design of fear of losing recognition.
19621148	NL	21	The transparancy of research
19548961[pe]	NL	41	More open communication to the public; there is nothing to be ashame of.
			Publication bias **6 respondents, 6 quotes**
19588197	NL	2	there is still a bias against publishing studies which find no significant results which can lead to unsuccesful "treatments" being studied multiple times. This can have a negative effect on the speed by which sucessful treatments are implemented."
48	AUS	8	The culture of journals favouring studies with positive results.
83	AUS	10	Publication biases
19576659	NL	15	publication bias
19621148	NL	21	the possibility to publish negative data
19611581	NL	30	selective publication
			Publication pressure **3 respondents, 3 quotes**
77	AUS	1	The 'publish or perish' attitude in science that perpetuates research for the sake of publication rather than to contribute to our understanding. Developing a 'news-worthy' story is more important than its content. The rest of my listed problems can be traced back to this point.
48[m]	AUS	8	The insane pressure to publish more than 5 papers a year, resulting in poor quality studies.
59[f]	AUS	9	Our current funding structure undermines the integrity of good research practice in general. Grants are generally short-term and heavily influenced by a researcher's publication record, which results in scientists cutting corners to publish faster and more frequently in order to simply keep their jobs. Validation experiments for new protocols or to confirm previous findings are often abandoned or cut short as they use up precious time that is necessary to generate publishable data.
			Influence of journal **2 respondents, 2 quotes**
19547003[a]	NL	3	Translational value of a model (sometimes a model is used in literature and it is hard to apply a better model since the new model is unknown and you cannot publish about it).
19535396[a]	NL	9	What are the most relevant models to use for all you can have positive and negative arguments to use them. And what do reviewers want? (PDX or not? humanized mice?)

			METHODOLOGY **13 respondents, 14 quotes**
ID	**Nat**	**Exp**	**Quote**
			Biostatistics **6 respondents, 6 quotes**
19553604	NL	3	Reducing the number of animals (number to treat) -
19547003[b]	NL	3	sample size (If I conduct a in-man study I have 300-600 patients/volunteers whereas I do not get approval from the ethics board when I need this number of animals)
19447245	NL	8	European monograph where animal numbers are specified so less animal is difficult-
76	AUS	25	improving expertise in data analysis of researchers
44	AUS	28	working with right number of animals
81	AUS	42	Deficits in training in biostatistics. Few researchers can design a study that has all controls and strong statistical power .
			Poor quality studies **2 respondents, 2 quotes**
48[p]	AUS	8	The insane pressure to publish more than 5 papers a year, resulting in poor quality studies.
67	AUS	55	Need to improve design of many studies
			Data interpretation **2 respondents, 2 quotes**
73	AUS	1	Consistent interpretation of results due to bias and a desire for good results
69	AUS	11	the biggest challenge in my opinion is that animal studies are too reliant on user interpretation, especially in pain studies. It needs more automation for unbiased profiling.
			Other **4 respondents, 4 quotes**
72[a]	AUS	1	lack of information for designing robust study and which animal model for translation
52	AUS	3	Methodological constraints
76	AUS	25	improving tools for in vivo longitudinal data collection in animals
19575974[f]	NL	36	Force investigators to work in a double blinded manner and multicenter setting in larger preclinical outcome trials (this requires additional personnel for coordinating such studies and hence more funding).

			FUNDING **10 respondents, 11 quotes**
ID	**Nat**	**Exp**	**Quote**
			Funding culture **4 respondents, 5 quotes**
77	AUS	1	A lack of interest in funding projects that explore alternative ways of testing e.g. refine medications in vitro and directly apply to human trials - we still don't know how Paracetamol works exactly, but we administer that readily. When we find out, will that change our attitude towards how we use it? Not the best example, but you get the idea.

59[p]	AUS	9	Our current funding structure undermines the integrity of good research practice in general. Grants are generally short-term and heavily influenced by a researcher's publication record, which results in scientists cutting corners to publish faster and more frequently in order to simply keep their jobs. Validation experiments for new protocols or to confirm previous findings are often abandoned or cut short as they use up precious time that is necessary to generate publishable data.
19498144	NL	12	funding to dedicate research to improvement of research methods (very hard to obtain from industry)
19498144[a]	NL	12	Generate novel and improved animal models of cardiovascular disease that incorporate the various co-morbidities (ageing metabolic dysregulation hypertension dyslipidemia etc.) typically found in humans (this requires additional funding as these models are often more expensive).
19575974[m]	NL	36	Force investigators to work in a double blinded manner and multicenter setting in larger preclinical outcome trials (this requires additional personnel for coordinating such studies and hence more funding).
			Costs **3 respondents, 3 quotes**
74[a]	AUS	2	practicality of using animals with closer genomes (cost, availability)
19588197[b]	NL	2	The process of getting approval for performing animal experiments is 1) expensive and 2) time consuming therefore it is challenging to perform numerous experiments within a project which is often necessary to come to explicit conclusions.
71	AUS	10	cost
			Other **3 respondents, 3 quotes**
45	AUS	20	cost of using models that are more appropriate.
81	AUS	42	The low and decreasing funding for scientists and universities. Researchers should be public servants, not beggars whose future salary requires attractive data.
67	AUS	55	Insufficient financial support

			REPRODUCIBILITY **12 respondents, 13 quotes**
ID	**Nat**	**Exp**	**Quote**
77[ra]	AUS	1	Even though the research cannot be replicated, it remains on a pedestal as some kind of gold standard for research. It is ironic that a community priding itself on rigorous testing and 'evidence' fails to change its ways in response to evidence, and that speaks volumes in saying that the scientific community is not as above-reproach as they want you to think.
62[p]	AUS	2	Reproducibility (method reporting)
19425716	NL	4	Reproducibility
33	AUS	8	Hard to replicate or rely on previously published research.
35[b,p]	AUS	9	reproducibility of experiments as animal experiments are not conducted the same way due to differing ethical views/committees and lack of detail in publications
19611542	NL	9	reproduction
61	AUS	10	Reproducibility
61	AUS	10	Replicability
19564087	NL	10	reproducibility
19611694	NL	20	Reproducibility
49	AUS	29	Reproducibility of results
19611581	AUS	30	Reproducibility
19488062[c]	NL	35	reproducibility (results vary due to variations in experimental conditions between animal facilities)-

INCENTIVES
2 respondents, 2 quotes

ID	Nat	Exp	Quote
34	AUS	20	Intellectual property in medicine - the desire to 'own' IP shapes research direction too strongly.
49	AUS	29	Perverse incentives for research

PERCEPTION
4 respondents, 5 quotes

ID	Nat	Exp	Quote
72	AUS	1	poor history of translation perpetuates idea that animal work not highly relevant
74	AUS	2	public perception
19586787	NL	25	increasing awareness of the usefulness/value of animal studies in public and politics
19548961	NL	41	Better explain the goal and purpose of the study to the public
19548961[p]	NL	41	More open communication to the public; there is nothing to be ashame of.

RESEARCHER'S ATTITUDE
3 respondents, 3 quotes

ID	Nat	Exp	Quote
72	AUS	1	changing attitudes of researchers
77[rep]	AUS	1	Even though the research cannot be replicated, it remains on a pedestal as some kind of gold standard for research. It is ironic that a community priding itself on rigorous testing and 'evidence' fails to change its ways in response to evidence, and that speaks volumes in saying that the scientific community is not as above-reproach as they want you to think.
81	AUS	42	The overall decline in average levels of ethics awareness and standards in the community.

OTHERS
17 respondents, 21 quotes

ID	Nat	Exp	Quote
52	AUS	3	lack of well-defined operationalisation of human behaviours being modelled
57	AUS	3	lack of collaboration; more collaboration by researchers in the same field or even in different fields could reduce the number of animals used
19425716	NL	4	Variability in results.
19488230	NL	7	Seperation of research that use animals as a model and research that has animals itself as the aim.
48	AUS	8	The lack of accountability
19535396	NL	9	protein half life
19535396	NL	9	Good translation of in vitro work to in vivo work
83	AUS	10	pressure to find differences
83	AUS	10	use of sub-optimal experimental approaches

19432454	NL	10	Development of alternatives check on previous studies benefit of results have to be significant and also benefit the used animal so no effect "It is already mandatory species"
19547467	NL	15	strong scientific background to make sure experiments are needed to confirm a good hypothesis already pro
19547467	NL	15	a good evaluation of the project by an independent committee composed of experienced scientists that can give advice and suggestions on how to reduce and refine the use of animals
19547467	NL	15	need of big experimental groups.
19564193	NL	18	the ability to adapt a model based on the observations
54	AUS	22	recognition of the value of animal models to interrogate mechanisms
49	AUS	29	Validity of theoretical constructs
19551752	NL	30	translational of animal findings to humans
19631725	NL	35	translation of newly developed tissue engineering approaches
53[b]	AUS	35	Most researchers in my area (developmental toxicology) are in drug companies. Their work is guided by legislation so fundamental research is often lacking. This gap is filled by underfunded academics such as myself.
19575974	NL	36	Improved registration of experiments (this requires additional funding to meet the increased administrative burden).
34	AUS	20	Big data techniques being thoughtlessly applied - much 'omics work is not founded in solid understanding of reality of biological variation.

NO COMMENT
2 respondents, 2 quotes

ID	Nat	Exp	Quote
40	AUS	6	?
19509558	NL	25	dont know

APPENDIX 3

Quotes benefits of preregistration

			Study design **23 respondents, 25 quotes**
ID	**Nat**	**Exp**	**Quote**
			More thought on design **9 respondents, 9 quotes**
50[a]	AUS	1	More accountability, which should allow for more rigorous research. Also would force researchers to have clear aims for what they want to achieve.
74	AUS	2	a solid plan know exactly what experiments to do what data to collect how to analysis the data
62	AUS	2	I would be forced to think through my work a little better, and better research how work has been done before (ystematic review or meta analysis eg)
33	AUS	8	Experimental planning reaching tool, forces researchers to consider parameters in experimental design prior to action.
83	AUS	10	Researchers would be better prepared for each experiment. Experiments would have fewer unforeseen variables and therefore a better chance of accurately addressing the hypotheses.
19576659	NL	15	This will get you to conduct the study in more detail in advance before performing the study
54	AUS	22	more thought on experimental design and statistical analysis.
76	AUS	25	It would definitely make us consider study design more carefully
19631725	NL	35	Possibly more thorough study design
			Access to examples 5 respondents, 5 quotes
19462856	NL	3	Our group and organisation will have more possibilities to review and compare study-designs in advance. There might be a more realistic and up-to-date knowledge of current comparable workingfield.
33	AUS	8	Source/database for accessing appropriately designed experimental protocols
35	AUS	9	It could help plan your experiment better as you may notice an error with the study design of previously performed experiments.
19564087	NL	10	Ideas about properly setting up your study
19547467[red,t]	NL	15	compare experimental protocols and study designs that have already been tested and proven to work would avoid experimenting" on conditions and speed up technical issues and thus refine and improve experimental outcomes"
			Better review of study design 3 respondents, 3 quotes
19588197[c]	NL	2	Other researchers/statisticians could comment on the planned experiments which will make them more robust.
63[c]	AUS	4	allow for standardisation across research groups and can receive feedback about quality of protocol from others
44	AUS	28	Better critical review and scrutiny of the study so long as it is done in ethical and professional way without vested interest.
			Other 8 respondents, 8 quotes
77	AUS	1	In the long term, my lab's published research would perhaps be more robust.

19553604	NL	3	the choosing the right animals for the experimenst
19631709	NL	5	The studies that are performed will be of higher quality
59[t]	AUS	9	It's not clear to me how openly accessible this database would be, but I'd hope that as a result, researchers would be facilitated to share more of their outcomes from failed experiments or experiments without a clear outcome. This would lead to better study design.
19564087	NL	10	Improved experiments
69	AUS	11	It may help with identifying how the flaws of the design
19564193	NL	18	The text of the protocol will be written with more care
19575974[a]	NL	36	Increased methodological rigor and reliability of study results.

[s]study design, [c]collaboration, [rep]reproducibility, [red]reduce waste of time/animals, [a]accountability, [t]transparency, [d]data interpretation, [min]minimal or none

			Collaboration **11 respondents, 13 quotes**
ID	**Nat**	**Exp**	**Quote**
			Optimize design 5 respondents, 5 quotes
19588197[s]	NL	2	Other researchers/statisticians could comment on the planned experiments which will make them more robust.
57	AUS	3	If we decided to change our animal model used we could see if any groups are using this model and ask how effective it is rather than also using animals to test its effectiveness.
63[s]	AUS	4	allow for standardisation across research groups and can receive feedback about quality of protocol from others
19425716	NL	4	Get to know the relevance of my study and probably get some feedback.
19611581[t]	NL	30	transparancy: inform other groups on our research activities insight in activities of other groups- stimulate discussion among researchers on design of studies
			Identify collaborators 4 respondents, 4 quotes
19488230[red]	NL	7	Making the design of an animal experiment easier could be easier. It would be easier to see if somebody already did the same work but maybe without results since it is not (yet) published. Either way you could contact the reseachers involved and be sure you are doing something useful with the animals.
19447245	NL	8	To enhance collaboration / exchange information. But than you need to now who is also working on this topic so It should be able to contact the person. Not completely anonymos
19611542	NL	9	could get some ideas from other projects or see with whom you can collaborate
19535396[t]	NL	9	It would be more clear what other experiments are performed more oppertunities for collaborations
			Other 4 respondents, 4 quotes
57[red]	AUS	3	It would significantly minimise pilot or trail studies for new techniques and could also encourage collaborations between groups.
19425716	NL	4	Sharing of expertise
19612957	NL	9	comments on these studies
19586787[rep]	NL	25	It is less likely to replicate studies that are already being performed people may want to join in art an earlier level

			Reproducibility **4 respondents, 4 quotes**
ID	**Nat**	**Exp**	**Quote**
73	AUS	1	More reliable results, more sound impact within the research field and easier to translate results to other fields/replicate the experiments.
19588197	NL	2	Also duplication of studies with insigifnicant p-values could be better avoided.
61[d]	AUS	10	Easier to identify variables responsible for poor experimental reproducibility
54[t]	AUS	22	Better reproducibility and transparency.

			Reduce Waste of Time / Animals **12 respondents, 14 quotes**
ID	**Nat**	**Exp**	**Quote**
			Reduce number of animals 11 respondents, 11 quotes
57[c]	AUS	3	It would significantly minimise pilot or trail studies for new techniques and could also encourage collaborations between groups.
19547003	NL	3	The odds that a study will be repeated without notice will be smaller.
19548388	NL	5	Less repetition of experiments that will lead to negative results and thus less waist of animals.
19488230[c]	NL	7	Making the design of an animal experiment easier could be easier. It would be easier to see if somebody already did the same work but maybe without results since it is not (yet) published. Either way you could contact the reseachers involved and be sure you are doing something useful with the animals.
35	AUS	9	It could prevent wasted time trying to repeat experiments that have been performed elsewhere.
19612957	NL	9	No duplicate studies
59	AUS	9	At a minimum, I think that those who lead the decisions to approve funding for various projects would be cross-referencing these databases to ensure that researchers do not attempt to repeat failed studies.
19564087	NL	10	Reduced number of animals by carefully thinking about setup of study
19611694	NL	20	Better insight into behaviour of molecules possibly less animal experiment necessary or early go/no go moment to decide to proceed with the experiment
19586787[c]	NL	25	It is less likely to replicate studies that are already being performed people may want to join in art an earlier level
19488046	NL	30	prevention of duplication of studies
			Safe time 3 respondents, 3 quotes
19548388	NL	5	Less time needed for finding the best protocols.
35	AUS	9	It could prevent wasted time trying to repeat experiments that have been performed elsewhere.

19547467[t,s]	NL	15	compare experimental protocols and study designs that have already been tested and proven to work would avoid experimenting" on conditions and speed up technical issues and thus refine and improve experimental outcomes"

Accountability
7 respondents, 7 quotes

ID	Nat	Exp	Quote
50[s]	AUS	1	More accountability, which should allow for more rigorous research. Also would force researchers to have clear aims for what they want to achieve.
77[min]	AUS	1	Immediately, there would be few. In the long term, my lab's published research would perhaps be more robust.
19509266[d]	NL	2	I would be nice to be able to see in published articles with which methods they set out to do the study (with pre-registration) and then to see how and why they deviated from their original protocol (if this was the case). Researchers would be forced to explain why they deviated from the original plan which is very useful information as a researcher from another group trying to perform the same research. Often I find I run into problems when trying to set up a study which I feel would have been problems other researchers in the field should have run into as well but often nothing is reported about such problems in the literature. So we are all trying to invent the wheel all over again in different groups. Maybe this could be prevented with pre-registration and the consequence of that in reporting methods in articles.
33	AUS	8	General accountability per individual experiments
48	AUS	8	Makes researchers more accountable i suppose, but won't stop someone who is really determined to lie or cheat or fabricate data.
19576659	NL	15	It will also get you to stay with the original plan.
19575974[s]	NL	36	Increased methodological rigor and reliability of study results.

Transparency
11 respondents, 11 quotes

ID	Nat	Exp	Quote
72	AUS	1	-greater transparency of other work in my field
40	AUS	6	The benefits would be more transparency between research groups working on or previously working on the same area of research.
59[s]	AUS	9	It's not clear to me how openly accessible this database would be, but I'd hope that as a result, researchers would be facilitated to share more of their outcomes from failed experiments or experiments without a clear outcome. This would lead to better study design.
19535396[c]	NL	9	It would be more clear what other experiments are performed more oppertunities for collaborations
19479561	NL	10	transparancy about protocols and samples size used by other groups
19547467[s,red]	NL	15	compare experimental protocols and study designs that have already been tested and proven to work would avoid experimenting" on conditions and speed up technical issues and thus refine and improve experimental outcomes"

54[r]	AUS	22	Better reproducibility and transparency.
19621148	NL	21	More transparancy in research.
19611581[c]	NL	30	transparancy: inform other groups on our research activities insight in activities of other groups- stimulate discussion among researchers on design of studies
19488046	NL	30	more insight in what is been done in the field
19488062	NL	35	More information on right dosage timing of read-out efficacy of certain interventions will be available reducing the number of 'mistakes due to not-knowing'. Especially technical information on experimentation is useful.

			Data interpretation **5 respondents, 5 quotes**
ID	**Nat**	**Exp**	**Quote**
19509266[a]	NL	2	I would be nice to be able to see in published articles with which methods they set out to do the study (with pre-registration) and then to see how and why they deviated from their original protocol (if this was the case). Researchers would be forced to explain why they deviated from the original plan which is very useful information as a researcher from another group trying to perform the same research. Often I find I run into problems when trying to set up a study which I feel would have been problems other researchers in the field should have run into as well but often nothing is reported about such problems in the literature. So we are all trying to invent the wheel all over again in different groups. Maybe this could be prevented with pre-registration and the consequence of that in reporting methods in articles.
19553604	NL	3	Beter understanding the work we perform
40	AUS	6	An ability to understand if results are not in agreement, that it may be due to trial design differences
61[rep]	AUS	10	Easier to identify variables responsible for poor experimental reproducibility
81	AUS	42	Understanding the detail of each experimental design would aid in discussions designed to understand differences in outputs of overlapping sets of data from different or the same labs.

			Minimal or none **10 respondents, 10 quotes**
ID	**Nat**	**Exp**	**Quote**
77[a]	AUS	1	Immediately, there would be few. In the long term, my lab's published research would perhaps be more robust.
52	AUS	3	Not many as my work is exploratory
19432454	NL	10	It is already mandatory, so no effect
19498144	NL	12	I don't see nay advantages
19549932	NL	12	none
45	AUS	20	Minimal.
19509558	NL	25	Hopefully shorter application procedure for licence for animal experiments but I doubt it...

49	AUS	29	I don't see any benefits to my own research programme. I routinely replicate findings of interest from my lab before attempting to publish.
53	AUS	35	nil
19548961	NL	41	none

Negative
3 respondents, 3 quotes

ID	Nat	Exp	Quote
19613285	NL	17	We do not need such laborious systems; if people do not trust what other people are doing they should come and take a look rather than overload the ones performing animal studies with tons of paperwork. Personally I think that institutional ethical committees are very suitable and reliable for deciding whether or not a proposed animal study is justified related to the importance of the expected outcome. There is no need for an additional review by CCD and the way the latter works in NL is completely different (and more cumbersome/ frustrating) compared to the regulatory procedures in the rest of Europe (same laws!!!) or outside Europe.
34	AUS	20	I cannot really conceive of much benefit from this at all. If anything I suspect it would retard progress and increase research costs. It might serve as a resource for full protocol details to be available, but journals could mandate for that w/o needing preregistration.
76	AUS	25	would make it difficult to complete key pilot experiments to source funding for animal studies

Other
6 respondents, 7 quotes

ID	Nat	Exp	Quote
72	AUS	1	improved standards for animal research across the board,
72	AUS	1	high rate of publication
19509517	NL	2	?
71	AUS	10	to be aware of planned similar studies to my own
75	AUS	10	Easier to find protocols
19551752	NL	30	When testing a manipulation (lesion drug etc.) in a validated model and/or test the results will be more acceptable.
67	AUS	55	I am involved in two kinds of animal research: 1. translational experiments on the effects of diet on metabolism and behaviour; such studies WOULD benefit from pre-registration; 2. 'pure science' studies concerned with .e.g. learning/ performance processes involved in changes in flavour preferences. These WOULD NOT benefit from pre-registration.

APPENDIX 4

Risk of preregistration

			Lack of flexibility **15 respondents, 17 quotes**
ID	**Nat**	**Exp**	**Quote**
			Protocol adjustment **5 respondents, 6 quotes**
50	AUS	1	Perhaps a lack of flexibility. If you notice results which might warrant a different form of testing to what you originally thought, then you'd be less able to change your methodology if your study was pre-registered.
33	AUS	8	Does not allow/account for often necessary changes or adaptation to protocols in experiments that might be ongoing.
59	AUS	9	The risks may be a loss of flexibility and creative license afforded to researchers. Many issues are impossible to anticipate prior to getting into the lab and often it is not until we are in the middle of a research project that we identify flaws in the original study design or hypothesis. We often need to be able to re-adjust our approach occasionally to reach our study goals.
59[t, r]	AUS	9	If there are too many hurdles to cross before we are allowed to change course, we may be forced to complete a study that we know will fail, wasting time and precious resources.
19564193	NL	18	Certain steps will probably require more specific descriptions This may lead to fewer opportunities to adapt the protocol to your observed data.
19631725	NL	35	Deviation from the preregistration study is quite likely and it is unclear how this will be evaluated.
			Post-hoc analysis **4 respondents, 5 quotes**
74	AUS	2	it may prevent thinking outside the box you may only look at the result that are in your plan and not consider others, and miss something that you may not have considered
52	AUS	3	Risk of not being approved to run experiments and follow-up on unexpected results that may be important
83	AUS	10	Difficulty in disseminating previously captured datasets or novel analyses of such datasets.
83	AUS	10	Restriction of insights gained from unexpected findings within the data that necessitate unplanned analyses.
67[t, r]	AUS	55	It could impose delays, extra expense and general lack of flexibility in studies of Type 2 above (i.e. non-translational experiments). the latter are normally quite short and the outcome of one experiment can be unexpected and require a follow up that was not planned ahead.
			Other **6 respondents, 6 quotes**
19548388	NL	5	less oppurtinity for coincidental positive findings because everybody is using similar protocols
69[t]	AUS	11	We can't always predict the outcomes of these studies. Will this move end up restricting the creativity or speed at which we can execute groundbreaking studies?
54[t]	AUS	22	lack of flexibility to change approach and time delays.
76[r]	AUS	25	I believe it would increase animal-based research costs and thus disadvantage high risk-high reward research

19551752	NL	30	The risk is that no new models or tests can be established anymore with room for errors ie learning. Thus a clinicaltrial.gov for animals should only apply to validated models and tests.
19488062	NL	35	It might be that certain experiments will no longer be allowed by the ethical committe as they seem performed before or elsewhere. However some experiments need optimazation within a specific animal facility or a deviation from a preregistered protocol might be seen as 'wrong' and not allowed. As a source of information it would be very suitable but it should not hamper the possibilities to perform certain experiments.

[l]lack of flexibility, [t]time, [a]administration/bureaucracy, [r]resources, [p]pilot studies, [o]other

Time
17 respondents, 17 quotes

ID	Nat	Exp	Quote
73	AUS	1	Time constraint
77	AUS	1	Our research would be delayed incredibly because it would impose more 'hoops to jump through,' as it were.
19588197	NL	2	Performing animal experiments would become even more time consuming which will lead to fewer experiments being performed.
48[r]	AUS	8	It would cause an even greater rift between labs that already have alot of funding and those that struggle to receive funding because it would slow down the research process, and as a result only large labs with lots of money and personnel will be able to regularly churn out publications. The rich get richer and the poor get poorer...
59[l,r]	AUS	9	If there are too many hurdles to cross before we are allowed to change course, we may be forced to complete a study that we know will fail, wasting time and precious resources.
75	AUS	10	Further delays than are already in place to get research done.
19564087	NL	10	Delay in start of research
69[l]	AUS	11	We can't always predict the outcomes of these studies. Will this move end up restricting the creativity or speed at which we can execute groundbreaking studies?
19549932	NL	12	waste of time
19613285[a]	NL	17	The entire regulatory path to obtain approval for performing animal studies takes too much time for any temporarily employed researcher (PhD-student/ postdoc).
45[a,r]	AUS	20	Another round of requirements and paper trails that consumes time and resources.
19621148[a]	NL	21	More paperwork, longer process.
54[l]	AUS	22	lack of flexibility to change approach and time delays.
19509558[r,a]	NL	25	another layer of administration, which will take a lot of work! Less time/ money for actual research
49	AUS	29	If i had to pre-register every experiment i ran, this would add considerably to the time and effort involved in my research. It would slow down my research considerably for very little gain.
19575974[r]	NL	36	If this were only to be mandated at our institution the risk would be that we would seem to be less 'productive' than other research groups that do not spend resources (time and personnel) on preregistration. Nevertheless this is a minor concern given the longterm benefits of preregistration.
67[r,l]	AUS	55	It could impose delays, extra expense and general lack of flexibility in studies of Type 2 above (i.e. non-translational experiments). the latter are normally quite short and the outcome of one experiment can be unexpected and require a follow up that was not planned ahead.

			Theft of ideas/IP **20 respondents, 21 quotes**
ID	**Nat**	**Exp**	**Quote**
			Stealing of ideas **9 respondents, 9 quotes**
74	AUS	2	if it is visible by other they may take your idea and publish before you
19509266	NL	2	Other researchers might steal your ideas.
19588197	NL	2	Stealing" of ideas could occur.
57	AUS	3	Like all good things there is potential for people to use this in a bad way such as to steal others experimental ideas which could foster competition rather than collaboration.
35	AUS	9	Reproducibility is sometimes poor and therefore repeating something yourself with a small group is sometimes beneficial. It could result in another person taking your idea and publishing something you are working on.
19611542	NL	9	Other people perform our experiments/ideas faster and we are scooped
19564087	NL	10	Scoop
19586787	NL	25	people may try to scope/ publish before
44	AUS	28	Loss of originality of your idea and concept in the public domain. it could be made a political, unethical football with vested interest eg. social media fights..
			Risk to IP **9 respondents, 9 quotes**
19553604	NL	3	risk on loosing intelectual property
57	AUS	3	I am not sure how sensitive/non patented information would be displayed in this data base but that could also raise concerns.
63	AUS	4	any IP sensitive work would be risky
19425716	NL	4	Steal intelectual property
40	AUS	6	potential risk to IP for projects, especially if external funding bodies are involved and may not want to disclose the intricacies of study designs
19535396	NL	9	ip problems
19479561	NL	10	loss of unicity of propriatory protocols and models
19547467	NL	15	the risks are related to intellectual property issues and using ideas by competitors working in the same field.
19548961	NL	41	jeopardizing the confidentiality of the study both allowing others to do the study and scooping the findings and create problems with projects in collaboration with companies
			Other **3 respondents, 3 quotes**
19548388	NL	5	Data theft
19488230	NL	7	Some specifics in the treatments (i.e. certain products etc) in animal nutrition field of work should be made unrecognizable. Concurrence can be nasty especially on the level of (inter)national funding outside of your research group.
19611581	NL	30	copy cat behaviour

			Administrative burden / bureaucracy **13 respondents, 13 quotes**
ID	**Nat**	**Exp**	**Quote**
			Bureaucracy **6 respondents, 6 quotes**
19631709[o]	NL	5	The immense bureaucracy that researchers have to deal with will be even greater. People are already stopping with animal studies in the NL because its way easier abroad.
83	AUS	10	Concerning policies are already being implemented at this University with regards to animal work (e.g. Faculty's prioritisation of some projects over others due to a dearth of space in animal facilities). Having an extra barrier to doing research without adequate and ongoing support from the University would be detrimental, especially to early-career researchers and students.
19498144	NL	12	Lot of bureaucracy I don't see the advantage to do this. Researchers should be assessed for the quality of their research in other ways.
19613285[t]	NL	17	The entire regulatory path to obtain approval for performing animal studies takes too much time for any temporarily employed researcher (PhD-student/ postdoc).
34	AUS	20	It would lead to more red tape and that almost inevitably leads to less thought, or thought that is designed to get around red tape rather than improve the experiment.
45[t,r]	AUS	20	Another round of requirements and paper trails that consumes time and resources.
			Administrative burden **7 respondents, 7 quotes**
19535396	NL	9	extra work does not outweigh the benifits
75	AUS	10	More paperwork than already is required.
19611694	NL	20	Administrative burden too high
19621148[t]	NL	21	More paperwork longer process.
54	AUS	22	More paper work.
19509558[t,r]	NL	25	another layer of administration, which will take a lot of work! Less time/ money for actual research
71	AUS	71	extra level of administrative work

			Resources **10 respondents, 10 quotes**
ID	**Nat**	**Exp**	**Quote**
73	AUS	1	funding constraints
48[t]	AUS	8	It would cause an even greater rift between labs that already have alot of funding and those that struggle to receive funding because it would slow down the research process, and as a result only large labs with lots of money and personnel will be able to regularly churn out publications. The rich get richer and the poor get poorer...
59[l,t]	AUS	9	If there are too many hurdles to cross before we are allowed to change course, we may be forced to complete a study that we know will fail, wasting time and precious resources.
83	AUS	10	A lot of support would be required to get all researchers up to speed with this approach.
45[a,r]	AUS	20	Another round of requirements and paper trails that consumes time and resources.
19621148	NL	21	There will be a need for yet another software program and a department for monitoring.

76	AUS	25	I believe it would increase animal-based research costs and thus disadvantage high risk-high reward research
19509558[t,b]	NL	25	another layer of administration, which will take a lot of work! Less time/ money for actual research
19575974[t]	NL	36	If this were only to be mandated at our institution the risk would be that we would seem to be less 'productive' than other research groups that do not spend resources (time and personnel) on preregistration. Nevertheless this is a minor concern given the longterm benefits of preregistration.
67[t,l]	AUS	55	It could impose delays, extra expense and general lack of flexibility in studies of Type 2 above (i.e. non-translational experiments). the latter are normally quite short and the outcome of one experiment can be unexpected and require a follow up that was not planned ahead.

Pilot studies
4 respondents, 4 quotes

ID	Nat	Exp	Quote
ID	Nat	Exp	Quote
75	AUS	10	Pilot/exploratory studies would be more difficult to run. Potential animal wastage as large, 'complete' experiments would be conducted start-to-finish before un-blinding to determine whether the hypothesis is supported/ disproved.
83	AUS	10	Stifling of exploratory/preliminary/pilot studies, especially non-invasive behavioural/observational experiments, that can be useful for establishing novel protocols
19509558	NL	25	How would pilot experiments be incorporated?
53[o]	AUS	35	Who gets to decide if a project is allowed to begin ? What about preliminary work where sample size is less important ?

Animal activists
2 respondents, 2 quotes

ID	Nat	Exp	Quote
ID	Nat	Exp	Quote
19548388	NL	5	problems with animal activists
19612957	NL	9	Animal activists

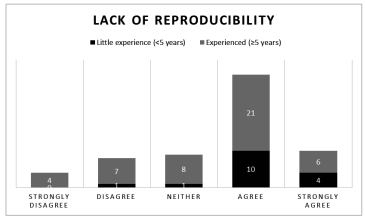
No risks
7 respondents, 7 quotes

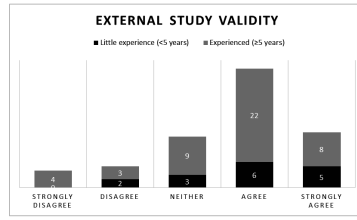
ID	Nat	Exp	Quote
ID	Nat	Exp	Quote
72	AUS	1	None that I can avail
62	AUS	2	None
19462856	NL	3	In my work not so much as I am in a controlling and supervising position (not researcher's position)
19547003	NL	3	I do not think there is a risk. My clinical studies are preregistrated and they all work
61	AUS	10	I see no risks. This is transparency at its best!
19432454	NL	10	It is already mandatory so no effect
19488046	NL	30	cannot think of one

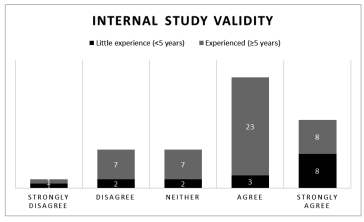
			Other **12 respondents, 14 quotes**
ID	**Nat**	**Exp**	**Quote**
ID	Nat	Exp	Quote
73	AUS	1	emotional attachment rather than viewing it as scientific requirement.
19509517	NL	2	?
19548388	NL	5	increased competition
19447245	NL	8	researchers do not use it as much as it should
33	AUS	8	May not be stringently adhered to post-planning by all researchers.
83	AUS	10	Prevention of use of animals that are in excess of requirements to validate non-invasive obervational/behavioural protocols.
19576659	NL	15	-
19613285	NL	17	Additionally the numbers on animal use in these overarching protocols do not correspond to the numbers of animals that are actually used.
34	AUS	20	It would increase risk of public availability of research before it has gone through institutional ethics procedures - this would likely result in misrepresentation of actual research practice.
19611694	NL	20	quality control data
19611694	NL	20	clarity of rules
19611694	NL	20	competition
53[P]	AUS	35	Who gets to decide if a project is allowed to begin ? What about preliminary work where sample size is less important ?
81	AUS	42	The 'specified date' for publication needs to be after public release of results. Publication often takes longer than originally envisaged.

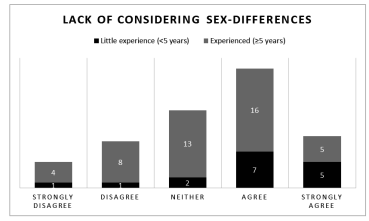
APPENDIX 5

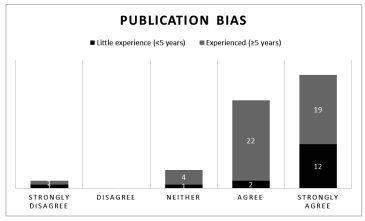
Issues hampering translation (less versus more experienced researchers)

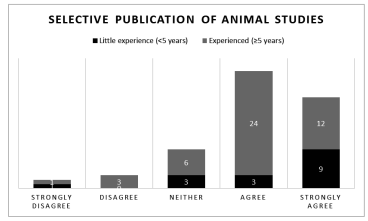


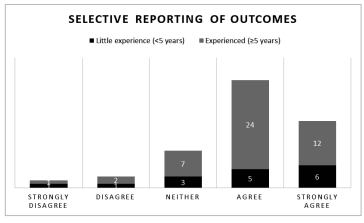


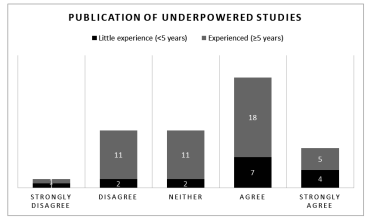


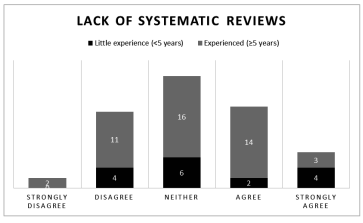


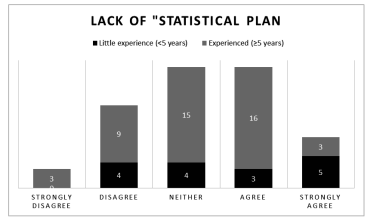


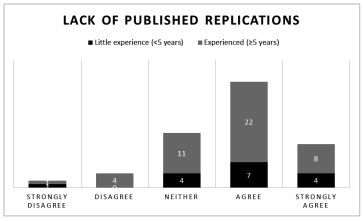








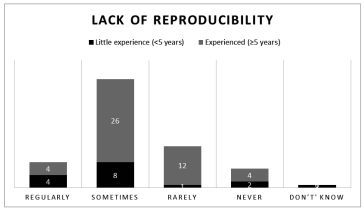


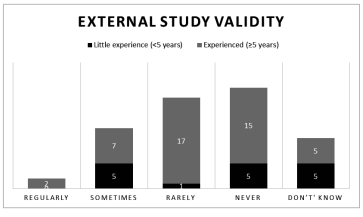


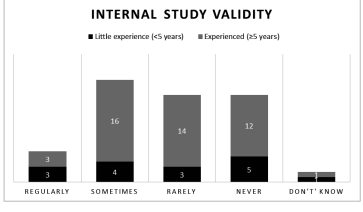
Responses to survey question 11: "To what extent do you agree these issues hamper translation of findings from animal studies into clinical practice?", on a 5-point Likert scale. Numbers indicate the number of responses. Subgroup less vs more experienced researchers.

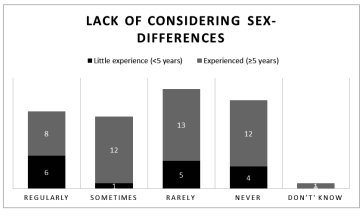
APPENDIX 6

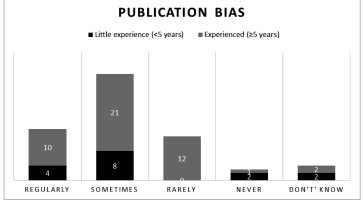
Prevalence of suboptimal research (less versus more experienced researchers)

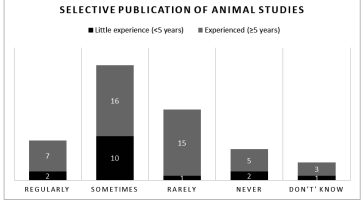


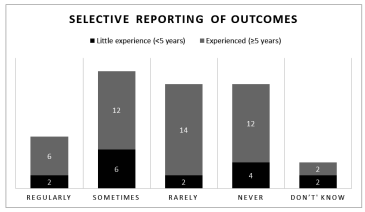


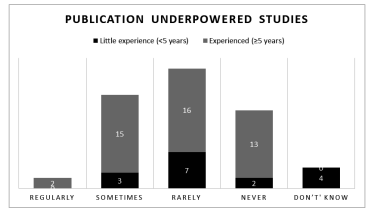


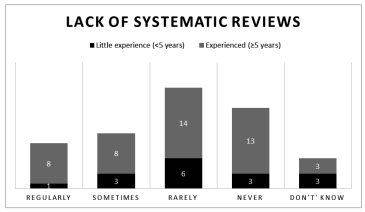


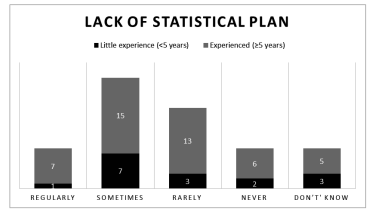


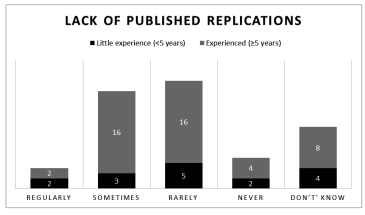








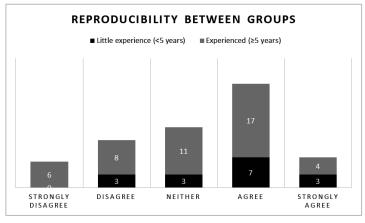


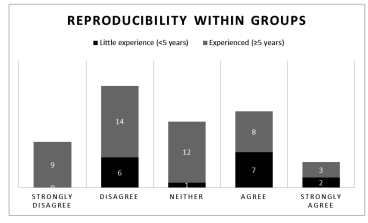


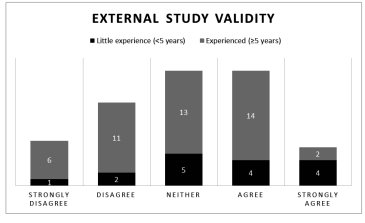
Responses to survey question 13"How often have the following issues occurred for you or your group that relate to the conduct, reporting or translational capacity of your work involving animal studies?". Numbers indicate the number of responses. Subgroup less versus more experienced researchers.

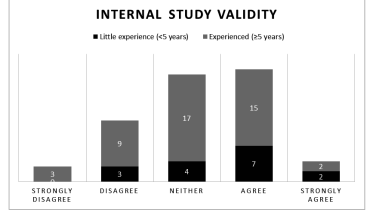
APPENDIX 7

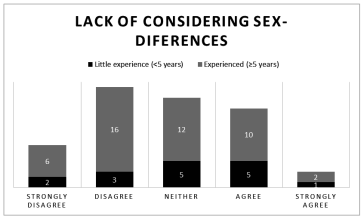
Effect of preregistration on issues (less vs more experienced researchers)

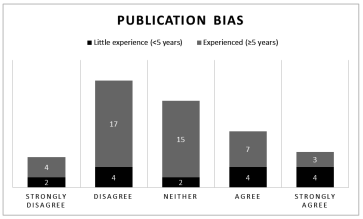


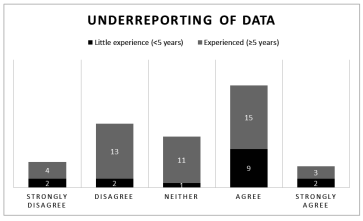


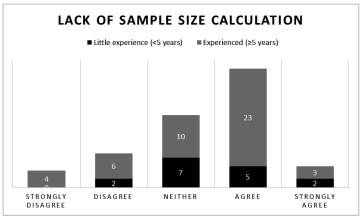


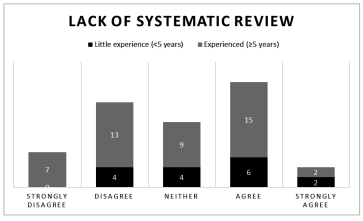


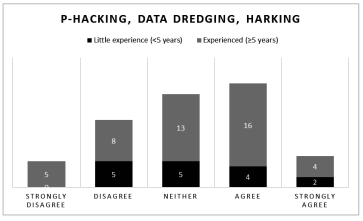


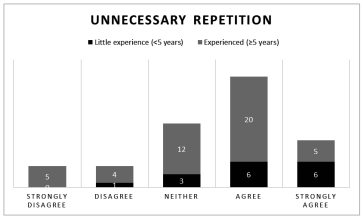


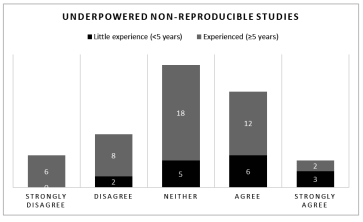


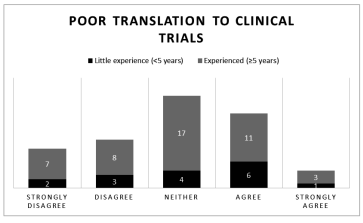








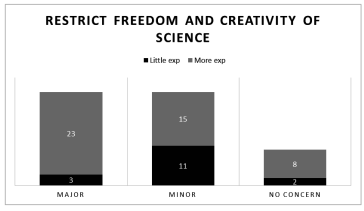


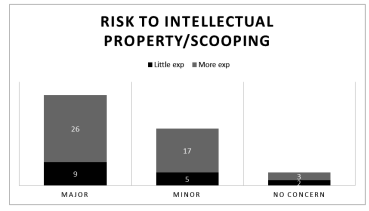


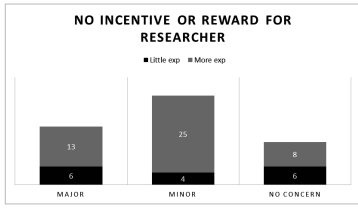
Responses to question 17: "To what extent do you agree preregistration of animal studies will have an effect on reducing the following issues believed to occur in the conduct of animal studies?" on a 5-point Likert scale. Numbers indicate the number of responses. Subgroup less versus more experienced researchers.

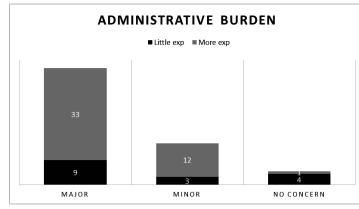
APPENDIX 8

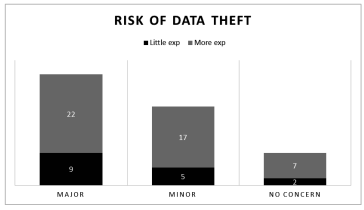
Challenges in preregistration (less vs more experienced researchers)

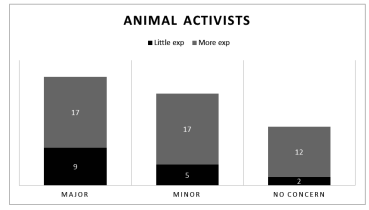


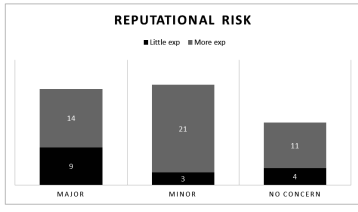












Responses to question 19: "Listed below are some items researchers have raised as issues with preregistration of animal studies. To what extent do you think these issues are of concern." on a 3-point scale. Numbers indicate the number of responses. Less versus more experienced researchers.

APPENDIX 9

Subgroup analysis - Biggest challenges in translation research

Table 1. Subgroup Australian versus Dutch respondents.

Theme	Subthemes	Resp AUS	Resp NL	Quotes AUS	Quotes NL
Animal models		12	19	18	25
	External validity of models	8	12	10	12
	Heterogeneity	1	2	1	3
	Alternatives to animals	0	4	0	5
	Other	6	5	7	5
Bureaucracy		6	15	6	19
	Approval committees	4	3	4	3
	Administrative burden	1	2	1	2
	Legislation	1	4	1	4
	Time to approval	0	4	0	4
	Lack of freedom	0	2	0	2
	Other	0	4	0	4
Conditions		7	8	11	9
	Training	5	3	5	3
	Standardization	3	2	3	2
	Animal distress	2	1	2	1
	Other	1	3	1	3
Publication		8	7	9	8
	Transparency	4	2	4	2
	Publication bias	2	4	2	4
	Publication pressure	3	0	3	0
	Influence of journals	0	2	0	2
Methodology		9	4	10	4
	Biostatistics	3	3	3	3
	Poor quality studies	2	0	2	0
	Data interpretation	2	0	2	0
	Other	3	1	3	1
Reproducibility		7	5	8	5
Funding		7	3	7	4
	Costs	2	1	2	1
	Funding structure	2	2	2	3
	Other	3	0	3	0
Perception		2	2	2	3
Incentives		2	0	2	0
Researchers' attitudes		3	0	3	0
Other		8	9	9	12
No comment		1	1	1	1
TOTAL NUMBER		28	34	86	90

Themes and subthemes identified in the open question about the biggest challenges to improve the conduct of animal studies for translational research. Respondents = number of respondents reporting on this (sub) theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes.

Table 2. Subgroup researchers with < 5 years of experience versus ≥ 5 years of experience.

Theme	Subthemes	Resp <5	Resp ≥5	Quotes <5	Quotes ≥5
Animal models		10	21	14	29
	External validity of models	6	14	6	16
	Heterogeneity	2	1	2	2
	Alternatives to animals	2	2	3	2
	Other	3	8	3	9
Bureaucracy		3	18	3	22
	Approval committees	2	5	2	5
	Administrative burden	0	3	0	3
	Legislation	0	5	0	5
	Time to approval	1	3	1	3
	Lack of freedom	0	2	0	2
	Other	0	4	0	4
Conditions		6	9	8	12
	Training	2	6	2	6
	Standardization	3	2	3	2
	Animal distress	2	1	2	1
	Other	1	3	1	3
Publication		4	11	4	13
	Transparency	1	5	1	5
	Publication bias	1	5	1	5
	Publication pressure	1	2	1	2
	Influence of journals	1	1	1	1
Methodology		5	8	5	9
	Biostatistics	2	4	2	4
	Poor quality studies	0	2	0	2
	Data interpretation	1	1	1	1
	Other	2	2	2	2
Reproducibility		3	9	3	10
Funding		3	7	3	8
	Costs	2	1	2	1
	Funding structure	1	3	1	4
	Other	0	3	0	3
Perception		2	2	2	3
Incentives		0	2	0	2
Researchers' attitudes		2	1	2	1
Other		3	14	3	18
No comment		0	2	0	2
TOTAL NUMBER		16	46	47	129

Themes and subthemes identified in the open question about the biggest challenges to improve the conduct of animal studies for translational research. Respondents = number of respondents reporting on this (sub) theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes.

APPENDIX 10

Subgroup analysis - Benefits of preregistration

Table 1. Subgroup analysis Australian versus Dutch researchers.

Theme	Subthemes	Resp AUS	Resp NL	Quotes AUS	Quotes NL
Study design		**13**	**10**	**14**	**11**
	More thought on design	7	2	7	2
	Access to examples	2	3	2	3
	Better review of design	2	1	2	1
	Other	3	5	3	5
Reduce waste of time/animals		**3**	**9**	**4**	**10**
	Reduce number of animals	3	8	3	8
	Reduce time	1	2	1	2
Collaboration		2	9	3	10
	Optimize design	**2**	**3**	**2**	**3**
	Identify collaborators	0	4	0	4
	Other	1	3	1	3
Reproducibility		**3**	**1**	**3**	**1**
Accountability		**4**	**3**	**4**	**3**
Transparency		**4**	**7**	**4**	**7**
Data interpretation		**3**	**2**	**3**	**2**
Other		**4**	**2**	**5**	**2**
Minimal or none		**5**	**5**	**5**	**5**
Negative		**2**	**1**	**2**	**1**
TOTAL		**28**	**34**	**47**	**52**

Themes and subthemes identified in the open question about the benefits of preregistration. Respondents = number of respondents reporting on this (sub)theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes.

Table 2. Subgroup analysis researchers with < 5 years of experience versus ≥ 5 years of experience.

Theme	Subthemes	Resp <5	Resp ≥5	Quotes <5	Quotes ≥5
Study design		**8**	**15**	**8**	**17**
	More thought on design	3	6	3	6
	Access to examples	1	4	1	4
	Better review of design	2	1	2	1
	Other	2	6	2	6
Reduce waste of time/animals		**2**	**10**	**2**	**12**
	Reduce number of animals	2	9	2	9
	Reduce time	0	3	0	3
Collaboration		**4**	**7**	**6**	**7**
	Optimize design	4	1	4	1
	Identify collaborators	0	4	0	4
	Other	2	2	2	2

Reproducibility	**2**	**2**	**2**	**2**
Accountability	**3**	**4**	**3**	**4**
Transparency	**1**	**10**	**1**	**10**
Data interpretation	**2**	**3**	**2**	**3**
Other	**2**	**4**	**3**	**4**
Minimal or none	**2**	**8**	**2**	**8**
Negative	**0**	**3**	**0**	**3**
TOTAL	**16**	**46**	**29**	**70**

Themes and subthemes identified in the open question about the benefits of preregistration. Respondents = number of respondents reporting on this (sub)theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes

APPENDIX 11

Subgroup analysis - Concerns of preregistration

Table 1. Subgroup analysis Australian versus Dutch researchers.

Theme	Subthemes	Resp AUS	Resp NL	Quotes AUS	Quotes NL
Theft of ideas/IP		**6**	**14**	**7**	**14**
	Stealing of ideas	4	5	4	5
	Risk to IP	3	6	3	6
	Other	0	3	0	3
Time		**10**	**7**	**10**	**7**
Lack of flexibility		**10**	**5**	**12**	**5**
	Protocol adjustment	3	2	4	2
	Post-hoc analysis	4	0	5	0
Administrative burden / bureaucracy	Other	3	3	3	3
		6	**7**	**6**	**7**
	Bureaucracy	3	3	3	3
	Administrative burden	3	4	3	4
Resources		**7**	**3**	**7**	**3**
Pilot studies		**3**	**1**	**3**	**1**
Animal activists		**0**	**2**	**0**	**2**
Other		**6**	**6**	**6**	**8**
No risk		**3**	**4**	**3**	**4**
TOTAL		**28**	**34**	**54**	**51**

Themes and subthemes identified in the open question about the challenges in preregistration. Respondents = number of respondents reporting on this (sub)theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes.

Table 2. Subgroup analysis researchers with < 5 years of experience versus ≥ 5 years of experience.

Theme	Subthemes	Resp <5	Resp ≥5	Quotes <5	Quotes ≥5
Theft of ideas/IP		**7**	**13**	**8**	**13**
	Stealing of ideas	4	5	4	5
	Risk to IP	4	5	4	5
	Other	0	3	0	3
Time		**3**	**14**	**3**	**14**
Lack of flexibility		**3**	**12**	**3**	**14**
	Protocol adjustment	1	4	1	5
	Post-hoc analysis	2	2	2	3
	Other	0	6	0	6
Administrative burden / bureaucracy		**0**	**13**	**0**	**13**
	Bureaucracy	0	6	0	6
	Administrative burden	0	7	0	7
Resources		**1**	**9**	**1**	**9**
Pilot studies		**0**	**4**	**0**	**4**
Animal activists		**0**	**2**	**0**	**2**
Other		**2**	**10**	**2**	**12**
No risk		**4**	**3**	**4**	**3**
TOTAL		**16**	**46**	**21**	**84**

Themes and subthemes identified in the open question about the challenges in preregistration. Respondents = number of respondents reporting on this (sub)theme. Quote = number of quotes identified in this (sub)theme. Note that some respondents provided multiple quotes per theme and/or subtheme and some respondents provided quotes on multiple (sub)themes.

PART II

Optimizing delivery techniques for cardiac repair

CHAPTER 7

Stem cell therapy for the damaged heart

Adapted from
Nederlands Tijdschrift voor Hematologie, 2016
https://www.ntvh.nl/journal-article/stamceltherapie-voor-het-beschadigde-hart/

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**shared first author*

ABSTRACT

Current therapies for ischemic heart failure are not sufficient. Regenerative therapy is a promising strategy that has rapidly developed into a clinical phase. Although the exact mechanism of action is not entirely understood, clinical results show a significant effect of stem cells in ischemic heart diseases. At this moment there is no consensus on the clinical relevance of stem cell therapy. In this review the rationale for stem cell therapy is discussed. We will focus on autologous bone marrow cells in clinical setting. Finally, we will give an overview of new developments that will optimize future cell therapy.

INTRODUCTION

Ischemic heart disease is one of the main causes of mortality in the Netherlands. More than 5.000 people suffered from a fatal acute myocardial infarction (AMI) in the Netherlands in 2014 and another 3500 patients died of other coronary vessel diseases, such as ischemic heart failure (IHF) and angina pectoris (AP). Mortality of AMI has decreased the last decades[1]. This trend is seen internationally and can be explained by improved prevention and treatment, partly due to the introduction of primary coronary interventions (PCI) and stents[2]. However, the heart failure population is growing progressively[3]. The only curative treatment for end stage heart failure is heart transplantation, but the demand of donor hearts is exceeding the availability. Therefore, the development of novel therapies is essential. Stimulating cardiac regeneration is a potential strategy. In this review we discuss the rationale of cell therapy for the damaged heart and clinical experience, focused on bone marrow-derived cells.

Rationale of stem cell therapy

In the 1990s differentiative plasticity of specialized cells has been shown. These studies mainly concern bone marrow cells. Initially, the capacity of bone marrow cells to migrate to the brain and remain in the parenchyma was demonstrated[4]. Circulating extrahepatic stem cells, probably derived from bone marrow, were also found to differentiate to de novo hepatocytes and cholangiocytes[5]. Moreover, bone marrow-derived progenitor cells were shown to migrate to degenerated muscle and produce fully differentiated muscle fibers[6]. Hereafter, it was hypothesized that bone marrow cells might migrate to the myocardium and differentiate to new cardiomyocytes. To examine this hypothesis, Lin^- $c\text{-}kit^+$- bone marrow cells of transgenic mice were injected in the border of infarcted myocardium. The study demonstrated 50% newly formed myocardium in 40% of mice. This result was explained by transdifferentiation of bone marrow cells into de novo myocardium[7]. However, other research groups have not been able to demonstrate transdifferentiation of transplanted bone marrow cells in an ischemic mouse model[8,9].

In the human heart, proliferation of cardiomyocytes was considered impossible, since the heart was assumed to be a postmitotic organ. This concept was recently questioned by intruiging research. In the zebrafish heart, total regeneration based on increased cardiomyocyte proliferation is seen (from 3% in the healthy heart to maximum 34% after injury) after 20% resection of the ventricle[10]. In another study, regeneration in the heart was demonstrated by determining the age of cardiomyocytes. The basis of this analysis was ^{14}C integration in DNA, since the atmospherical ^{14}C concentration suddenly rose and exponentially fell due to nuclear atom bomb tests during the Cold War. Cell turnover of cardiomyocytes was demonstrated, annualy 1% at the age of 25 and 0.45% at the age of 75[11]. It is not known whether the cell renewal

was caused by proliferation of cardiomyocytes or differentiation of stem cells into cardiomyocytes.

An alternative mechanism of action of stem cells is the paracrine hypothesis. Stem cells produce cytokines and growth factors, that can lead to neovascularisation, decreased apoptosis and inflammation, improved metabolism, increased contractility and reduced remodeling. This hypothesis is supported by similar effects of conditioned medium of stem cells compared to cell therapy[12].

Translational studies

The promising hypothesis of cardiac regeneration led to a rapid translation to the clinic. Many developments in the preclinical setting were directly translated to clinical studies. In 2001 formation of de novo cardiomycytes was described in a mouse model and in 2002 the first clinical trial with mononucleair bone marrow cells was published[7,13]. Meanwhile, various cell types have been tested preclinically for their capacity of repairing and regenerating the damaged heart. The majority of large animal studies has been performed in pigs due to the large anatomical similarity to the human coronary arteries. A significant effect of cell therapy is seen in large animals (n=1.415)[14]. Left ventricular ejection fraction (LVEF) (the amount of blood pumped out per beat) increases with 8.3%. This is a functional parameter for the pump function of the heart, that is use in clinical care with an important prognostic value.

Clinical trials

More than 50 clinical studies have been performed in which over 2.600 patients have been treated. Many studies did not have enough “power” to demonstrate a reduction in mortality as an endpoint. Meta-analyses do not show an important mortality reduction[15]. A more frequently used endpoint is LVEF. The most recent systematic review and meta-analysis of randomized clinical trials with bone marrow cells for ischemic heart disease shows an increase in ejection fraction (2.92%), smaller infarct size (2.25%) and decreased left ventricular end-systolic volume (LVESV) (6.37ml) compared to standard therapy[16]. Although the reported differences are statistically significant, clinical relevance varies. Some studies show a relevant improvement in exercise tolerance and quality of life, while other studies do not confirm this. Consequently, there is no consensus on the value of stem cell therapy.

The effect size of primary studies varies a lot. This finding cannot be explained by the diverse patient populations (AMI, chronic IHF and AP). A possible explanation is the variation in cell type, cel treatment protocol and administration route. No consensus exists on the best strategy. An overview of clinical trials in the Netherlands is summarized in Table 1.

Table 1. Clinical trials in the Netherlands.

Study	Cell	Patient	Administration	Praimary endpoint	Status
Registry	BM-MNC	AP	IM	Perfusion	Enrolling
AMICI	Allogeneic MSC	AMI	IC	Safety & feasibility	Enrolling
BAMI	BM-MNC	AMI	IC	Mortality	Starts 2016
REPEAT	BM-MNC	IHF	IC	Mortality	Starts 2016
SCIENCE	Allogeneic MSC	IHF	IM	LV systolic volume	Expected 2016

BM-MNC= bone marrow mononuclear cell; MSC=mesenchymal stem cell; AMI = acute myocardial infarction; HF = heart failure, IHF = ischemic heart failure; AP = angina pectoris; IC = intracoronary infusion; IM = intramyocardial injections; LF = left ventricle

Cell type

Bone marrow

The most frequently used cells in clinical setting are the undifferentiated mononuclear bone marrow cells (BM-MNCs), amongst others consisting of hematopoietic, mesenchymal and endothelial stem- and progenitor cells. Other cell types that have been used for clinical studies are flow cytometry selected $CD34^+$ (hematopoietic) and $CD133^+$ (hematopoietic and endothelial) cell populations(17). Mesenchymal stromal cells (MSCs), selected (by attachment to plastic) from the mononuclear fraction and then cultured, have been examined to a lesser degree in clinical setting[17,18]. MSCs are of interested due to their strong paracrine effects[19]. A 6.2% increase in LVEF is reported in a clinical study with heart failure patients[18]. G-CSF ('granulocyte colony stimulating factor') is a routine therapy to mobilize stem cells for transplantation from peripheral blood. No significant functional improvement is seen after G-CSF-treatment in AMI patients[20].

Adipose tissue

Stem cells can also be isolated from other tissues. Limited results are available concerning adiposed tissue-derived cells. Improvements in wall motions, but not in LVEF, have been reported[21].

Heart

The discovery of endogenous cardiac stem cells, that have the potential to differentiate into cardiomyocytes, gave rise to a novel therapeutic aim: stimulating regeneration through already existing cardiac stem cells[22]. These $c\text{-}kit^+$ endogenous stem cells were isolated from the right atrial appendage and administered as autologous therapy after expansion in the SCIPIO trial[23]. Cardiosphere-derived cells (CDCs) were used in the CADUCEUS trial[24]. Both studies were phase 1/2a trials showing feasibility and safety.

Autologous versus allogeneic

Quality of stem cells is hypothesized to be influenced by cardiovascular risk factors and chronic disease. Allogeneic stem cells might benefit treatment. In addition, allogeneic cells can be used 'off-the-shelf' in an acute situation. MSCs can be used as an allogeneic product without a immune reaction. The difference between autologous and allogeneic cells has not been clinically investigated properly[25]. No difference in efficacy is seen in large animals[14].

Cell treatment

Various methods of cell treatment have been applied in clinical trials. Different density gradient media, Ficoll or Lymphoprep, are used for cell isolation. Furthermore, washing media, e.g. NaCl-heparin-plasma or phosphate buffer, vary. A couple of research groups have applied different protocols in the same cell population. It is unknown which protocol leads to the most optimal results[26].

Administration method

Multiple techniques to transplant cells into the heart exist. In this paragraph we will focus on the most clinically relevant methods (Figure 1).

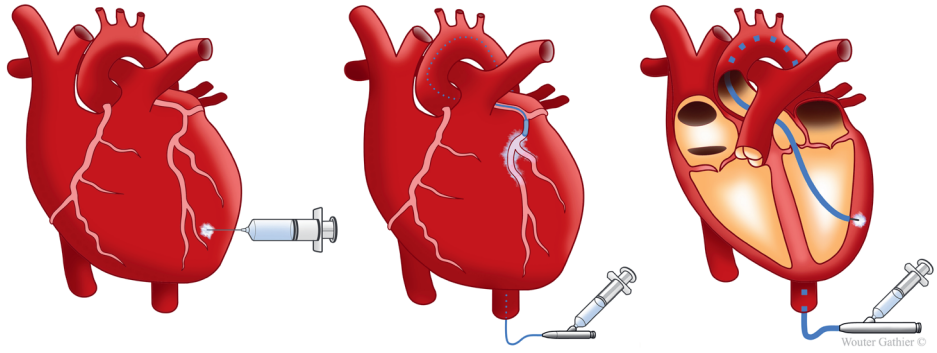

Figure 1. Administration methods

Epicardial injection

The epicardial technique (Figure 1A) is the gold standard for cell application since injections in the target area take place under direct visualization. This approach is only used in combination with another cardiac surgery in clinical setting as an open chest is necessary for this route.

Intracoronary infusion

During a heart catheterization cells can be infused in a coronary artery through the central lumen of a balloon catheter (Figure 1B). This method is especially suitable for cell therapy after an AMI, and can be performed right after the percutaneous coronary intervention (PCI). Another advantage of this approach is that is concerns a well-known technique for interventional cardiologists. The main disadvantage of this method is that is not applicable in case of/for occluded coronary arteries[27].

Intramyocardial injection

For this percutaneous injection method (Figure 1C) an electromechanical mapping system (NOGA, Biosense Webster) is most frequently used. This means both electrical and mechanical activity of the left ventricle is measured in the endocardium with a dedicated catheter and a 3D-model of the endocard is made. With this system the target area for injections can be defined accurately and can thereafter be used to navigate an injection catheter to the target area and finally injection. This application route can also be used in occluded coronary arteries. However, this procedure is time consuming, expensive and demands specific expertise[27].

Clues/suggestions for the future

Potential cells

As described earlier, multipotent cells (BM- and cardiac derived) have not been able to truly regenerate the human heart. For real regeneration pluripotent cells theoretically propose a better possibility than the so far used multipotent stem cells (see Table 2). Pluripotent stem cells have the property of differentiating to cells from all germ layers (ecto-, meso- and endoderm). With that that have the potential to regenerate the heart, but also to potentially form teratomas. Moreover, functional properties of newly formed cardiomyocytes are unknown[28]. A new strategy of improving the effect of cell therapy is to combine different cell types (NCT02501881, NCT02503280). There is also research performed on repeated cell administrations (NCT01693042).

Cell-less therapy

In combination with the paracrine hypothesis, various experimental regenerative strategies with only cytokines are possible; “cell-less therapy”. Examples are growth factors (IGF-1/HGF) and exosomes. Gene therapy that influences cardiogenic activity, like micro-RNA and follistatin-like 1 (FSTL-1), is studied in striving true regeneration. These therapies are promising in preclinical research, but not yet clinically applicable due to unknown systemic effect[29,30].

Optimizing cell retention

Although the epicardial injection method is the reference for cell transplantation, efficiency with this method varies considerably. The intramyocardial and intracoronary

method are comparable with regard to cell retention [31]. Less than 4% of cells is found back in the heart after an hour of administration [32]. Hence, the success of cardiac cell therapy (and other regenerative therapies) is importantly limited by insufficient retention and survival of cells. The use of biomaterials, especially hydrogels, is an approach to stimulate cell retention. While translation to clinical setting is ongoing, results in small and large animals with several hydrogels (fibrin, gelatin, hyaluronic acid, poly ethylene glycol (PEG)) are promising [33,34]. For example, in a large animal model of chronic myocardial infarction a better functional effect was seen after treatment with growth factors in a (UPy) PEG-gel compared to only growth factors [34]. In addition to an increase in cell retention and survival, biomaterials can also be used to support thinned myocardial wall (after infarction or in dilated cardiomyopathy), of even better to prevent remodeling post-infarction. This was recently shown in a mice study, where a 3D-printed patch of hyaluronic acid/gelatin and human cardiac progenitor cells (hCMPCs) was transplanted after a myocardial infarction [33].

Conclusion

Indications for proliferation and transdifferentiation of stem cells to cardiomyocytes are present. This theory has led to rapid development of cardiac stem cell therapy resulting in years of clinical experience, proving safety of the therapy. Until now, true regeneration has not been shown successfully in clinical setting. A significant but modest functional improvement is seen, likely based on paracrine effects. Promising results in animal models have not yet been translated to a clinically relevant effect. To enhance the results of cell therapy innovations in the basal and technical field are essential. Not only different cell types and cell products, but also biomaterials might play an important role. In addition, optimizing current administration methods is crucial. The ultimate goal, true regeneration, is searched in combining cell(-less) therapies. It is a dynamic research field in a large patient population that craves novel therapies.

REFERENCES

1. Centraal Bureau voor de Statistiek.
2. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction : a quantitative review of 23 randomised trials. 2003;361.
3. Koopman C, Van Dis I, Bots M, Vaartjes I. *Feiten En Cijfers Hartfalen*. Vol Juni.; 2012.
4. Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci U S A*. 1997;94(8):4080-4085. doi:10.1073/pnas.94.8.4080
5. Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. *Hepatology*. 2000;32(1):11-16. doi:10.1053/jhep.2000.9124
6. Ferrari G, Angelis GC De, Coletta M, et al. Muscle Regeneration by Bone Marrow – Derived Myogenic Progenitors. :1-4.
7. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Bone marrow stem cells regenerate infarcted myocardium. *Nature*. 2001;410:701-705.
8. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*. 2004;428:668-673. doi:10.1038/nature02446.Published
9. Murry CE, Soonpa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac mycocytes in myocardial infarcts. *Nature*. 2004;428(6983):664-668. doi:10.1038/nature02421.1.
10. Poss KD, Wilson LG, Keating MT. Heart Regeneration in Zebrafish. *Science (80-)*. 2002;298(5601):2188-2190. doi:10.1126/science.1077857
11. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science (80-)*. 2009;324(5923):98-102. doi:10.1126/science.1164680
12. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res*. 2008;103(11):1204-1219. doi:10.1161/CIRCRESAHA.108.176826
13. Strauer BE. Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans. *Circulation*. 2002;106(15):1913-1918. doi:10.1161/01.CIR.0000034046.87607.1C
14. Jansen Of Lorkeers SJ, Eding JEC, Vesterinen HM, et al. Similar effect of autologous and allogeneic cell therapy for ischemic heart disease: systematic review and meta-analysis of large animal studies. *Circ Res*. 2015;116(1):80-86. doi:10.1161/CIRCRESAHA.116.304872
15. Martin-Rendon E, Brunskill S, Dorée C, et al. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev*. 2008;(4):CD006536. doi:10.1002/14651858.CD006536.pub2
16. Afzal MR, Samanta A, Shah ZI, et al. Adult bone marrow cell therapy for ischemic heart disease: Evidence and insights from randomized controlled trials. *Circ Res*. 2015. doi:10.1161/CIRCRESAHA.114.304792
17. Chavakis E, Koyanagi M, Dimmeler S. Enhancing the outcome of cell therapy for cardiac repair: Progress from bench to bedside and back. *Circulation*. 2010;121(2):325-335. doi:10.1161/CIRCULATIONAHA.109.901405
18. Mathiasen AB, Qayyum AA, Jørgensen E, et al. Bone-marrow derived mesenchymal stromal cell treatment in patients with severe ischemic heart failure : a randomised placebo-controlled trial (MSC-HF trial). *Eur Heart J*. 2015;36:1744-1753.
19. Hatzistergos KE, Quevedo H, Oskouei BN, et al. Bone Marrow Mesenchymal Stem Cells Stimulate Cardiac Stem Cell Proliferation and Differentiation. *Circ Res*. 2010;107:913-922. doi:10.1161/CIRCRESAHA.110.222703
20. Abdel-Latif A, Bolli R, Zuba-Surma EK, Tleyjeh IM, Hornung CA, Dawn B. Granulocyte colony-stimulating factor therapy for cardiac repair after acute myocardial infarction: A systematic review and meta-analysis of randomized controlled trials. *Am Heart J*. 2008;156(2):218-226.e9. doi:10.1016/j.ahj.2008.03.024
21. Perin EC, Sanz-Ruiz R, Sánchez PL, et al. Adipose-derived regenerative cells in patients with ischemic cardiomyopathy: The PRECISE Trial. *Am Heart J*. 2014;168(1):88-95.e2. doi:10.1016/j.ahj.2014.03.022
22. Senyo S, Wang M, Wu T, Lechene CP. Mammalian heart renewal by preexisting cardiomyocytes. *Nature*. 2013;493(7432):433-436. doi:10.1038/nature11682.Mammalian
23. Chugh AR, Beache GM, Loughran JH, et al. Administration of cardiac stem cells in patients with ischemic cardiomyopathy: the SCIPIO trial: surgical aspects and interim analysis of myocardial function and viability by magnetic resonance. *Circulation*. 2012;126(11 Suppl 1):S54-64. doi:10.1161/CIRCULATIONAHA.112.092627
24. Malliaras K, Makkar RR, Smith RR, et al. Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction). *J Am Coll Cardiol*. 2014;63(2):110-122. doi:10.1016/j.jacc.2013.08.724
25. Hare JM, Fishman JE, Gerstenblith G, et al. Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopa-

thy: the POSEIDON randomized trial. *Jama*. 2012;308(22):2369-2379. doi:10.1001/jama.2012.25321
26. Yeo C, Saunders N, Locca D, et al. Ficoll-Paque™ versus Lymphoprep™: a comparative study of two density gradient media for therapeutic bone marrow mononuclear cell preparations. *Regen Med*. 2009;4(5):689-696.
27. van der Spoel TIG, Lee JC-T, Vrijsen K, et al. Non-surgical stem cell delivery strategies and in vivo cell tracking to injured myocardium. *Int J Cardiovasc Imaging*. 2011;27(3):367-383. doi:10.1007/s10554-010-9658-4
28. du Pré BC, Doevendans P a, van Laake LW. Stem cells for cardiac repair: an introduction. *J Geriatr Cardiol*. 2013;10(2):186-197. doi:10.3969/j.issn.1671-5411.2013.02.003
29. van Rooij E, Olson EN. MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. *Nat Rev Drug Discov*. 2012;11(11):860-872. doi:10.1038/nrd3864
30. Wei K, Serpooshan V, Hurtado C, et al. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. *Nature*. 2015. doi:10.1038/nature15372
31. van der Spoel TIG, Vrijsen KR, Koudstaal S, et al. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischaemic cardiomyopathy: a study on delivery efficiency. *J Cell Mol Med*. 2012;16(11):2768-2776. doi:10.1111/j.1582-4934.2012.01594.x
32. Hou D, Youssef EA-S, Brinton TJ, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation*. 2005;112(9 Suppl):I150-6. doi:10.1161/CIRCULATIONAHA.104.526749
33. Gaetani R, Feyen D a. M, Verhage V, et al. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. *Biomaterials*. 2015;61:339-348. doi:10.1016/j.biomaterials.2015.05.005
34. Koudstaal S, Bastings MMC, Feyen DA, et al. Sustained delivery of insulin-like growth factor-1/hepatocyte growth factor stimulates endogenous cardiac repair in the chronic infarcted pig heart. *J Cardiovasc Transl Res*. 2014;7(2):232-241. doi:10.1007/s12265-013-9518-4

CHAPTER 8

Stem cell treatment for acute myocardial infarction

Submitted to
Cochrane Database of Systematic Reviews

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ABSTRACT

Background Cell transplantation offers a potential therapeutic approach to the repair and regeneration of damaged vascular and cardiac tissue after acute myocardial infarction (AMI). This has resulted in multiple randomised controlled trials (RCTs) across the world.

Objectives To determine the safety and efficacy of autologous adult bone marrow-derived cells as a treatment for AMI, focusing on clinical outcomes.

Search methods This Cochrane review is an update of a previous version (published in 2015). We searched the Cochrane Central Register of Controlled Trials (CENTRAL 2022, Issue 2 of 12), MEDLINE (1950 to February 2022), EMBASE (1974 to February 2022, CINAHL (1982 to February 2022) and the Transfusion Evidence Library (1980 to February 2022. In addition, we searched several international and ongoing trial databases in February 2022, handsearched relevant conference proceedings to January 2011 and searched relevant recent reviews and meta-analyses.

Selection criteria RCTs which compared autologous bone marrow-derived cells to no cells (either placebo or optimal standard of care) in patients diagnosed with AMI were eligible.

Data collection and analysis Two review authors independently screened all references, assessed the risk of bias in the included trials and extracted data. We conducted meta-analyses using random-effects models throughout. We analysed the outcomes at short-term (less than 12 months) and long-term (12 months or more) follow-up. Dichotomous outcomes are reported as risk ratio (RR) and continuous outcomes are reported as mean difference (MD) or standardised MD (SMD). We performed sensitivity analyses to evaluate the results in the context of the risk of selection, performance and attrition bias. Exploratory subgroup analysis investigated the effects of baseline cardiac function (left ventricular ejection fraction; LVEF), cell dose, cell type and timing of administration, as well as the use of heparin in the final cell solution.

Main results Fifty-three RCTs that recruited 4159 participants (2297 cell therapy, 1862 controls) were eligible for inclusion. Cell treatment was not associated with any change in the risk of all-cause mortality at short term follow up (24/1145 versus 18/779; RR 0.79, 95% CI 0.44 - 1.40; 1950 participants; 21 studies; moderate quality evidence) or long term follow up (49/998 versus 51/912; RR 0.88, 95% CI 0.60 - 1.31; 1910 participants; 22 studies; moderate quality evidence). Cell treatment was not associated with any

change in the risk of cardiovascular mortality at short term follow up (8/348 versus 9/329; RR 0.73, 95% CI 0.31 - 1.71; 677 participants; 9 studies; moderate quality evidence) or long term follow up (29/595 versus 29/563; RR 0.91, 95% CI 0.55 - 1.53; 1158 participants; 13 studies; moderate quality evidence). Cell treatment was not associated with any change in the risk of the composite measure of mortality, reinfarction and re-hospitalisation for heart failure at short term follow up (5/198 versus 12/181; RR 0.36, 95% CI 0.12 - 1.14; 379 participants; 3 studies; moderate quality evidence) or long term follow up (24/262 versus 33/235; RR 0.63, 95% CI 0.36 - 1.10; 497 participants; 6 studies; moderate quality evidence). Statistical heterogeneity was low (I2 = 0% to 12%). Serious periprocedural adverse events were rare and were generally unlikely to be related to cell therapy. Additionally, cell therapy had no effect on morbidity or quality of life/performance. In the combined analysis, LVEF as measured by magnetic resonance imaging demonstrated a significant improvement at long term follow of +1.84% (p = 0.04, 95% CI 0.12 - 3.57; 968 participants; 12 studies; moderate quality evidence) but there was no difference at short term follow up. In subgroup analyses, baseline LVEF <45% on MRI was a predictor for LVEF improvement on MRI, but not for overall mortality. There remains a significant improvement in LVEF as measured by echocardiography and SPECT at both the short and long term timepoints. Results were robust to the risk of selection, performance and attrition bias from individual studies.

Authors' conclusions There remains no evidence for a reduction in mortality and morbidity when autologous bone marrow-derived cells are administered to patients who have undergone primary angioplasty following AMI.

Plain language summary

Review question: Are cells taken from a patient's bone marrow and delivered to their heart a safe and effective treatment following a heart attack?

Background: Heart attacks are caused by a blockage in an artery supplying blood to the heart muscle. Currently, the standard treatment for people who suffer a heart attack is the re-opening of the blocked artery with a tiny balloon in a procedure called primary angioplasty, and an introduction of a small tube (called a stent) into the artery to keep it open. Over the last two decades, bone marrow-derived cells have been investigated as an additional treatment for heart attacks based on their ability to repair damaged heart muscle.

Study characteristics: This review includes clinical trials that randomised patients diagnosed with a heart attack to either cell treatment, a placebo or to continue on optimal medical therapy alone. In order to identify these trials, we searched databases

to February 2022. This review was supported by the National Institute of Health Research (NIHR) through its Cochrane Incentive Award programme.

Key results: In this review we analysed data from 53 trials which recruited 4159 patients. Our analysis suggests that cell treatment does not lead to an improvement in outcomes (such as death, improved heart function and hospital readmissions) when compared to standard treatment in the short or long term.

Quality of evidence for primary outcomes: The evidence in this review is of moderate quality due to the small number of events.

BACKGROUND

Description of the condition

Worldwide, ischaemic heart disease is the most common cause of death; it now accounts for 1.8 million annual deaths (or 20%) of deaths in Europe (Townsend 2016). Ischaemic heart disease presents acutely with myocardial infarction. Acute myocardial infarction (AMI) occurs when myocardial ischaemia (due to a decreased supply of blood flow to the epicardial coronary arteries) reaches a critical threshold and myocardial necrosis occurs (Reed 2017). This disruption in the blood supply is most commonly caused by the rupture of an atherosclerotic plaque in the coronary artery which can cause thrombosis and subsequent occlusion (Falk 1995). Consequently, both the infarcted and unaffected myocardium undergo adverse remodelling involving the ventricular wall which can lead to heart failure. The first changes occur almost immediately after coronary occlusion and lead to a loss of contractility, followed by the growth of the necrotic areas in the following days. The infarcted region rebuilds in the following two to three months, leaving a scar (a fibrotic, non-contracting region) in the ventricular wall, thereby compromising cardiac function (ESC/ACC 2000). Over the last three decades there has been a profound increase in survival rates. This is mainly due to primary angioplasty which revolutionised the treatment of AMI. Concurrent improvements in medical therapy (e.g. antiplatelets and anticoagulants, alongside secondary prevention strategies such as statins) have also contributed to the improved outcomes seen today (Reed 2017). However, these improved AMI treatments, and the subsequent substantial increase in survival rates, has led to a growing population of patients with impaired cardiac function and consequent heart failure. Furthermore, due to population growth, ageing and the increasing prevalence of comorbidities, hospital admissions for heart failure are expected to increase considerably, perhaps by up to 50% in the next 25 years (Savarese 2017) (Al-Mohammad 2010). Therefore, the search for new treatment strategies that prevent heart failure remains an important research area in cardiology.

Description of the intervention

The concept of regenerative cell therapy is to either take a patient's own cells (autologous) or an off-the-shelf cell product (allogeneic) and deliver them to the site of myocardial injury. Two main cell delivery routes have been explored: intramyocardial (both transepicardial and transendocardial) and intracoronary. Other delivery routes, such as intravenous injection and retrograde coronary sinus injection, have been also been tested; but there is limited evidence of efficacy and safety.

The intramyocardial route directly injects the cells into the myocardium, usually into the area bordering the myocardial infarct. The transepicardial route benefits from the direct visualisation of injection sites and an accurate delivery of cells to the peri-infarct area (although some areas like the septum may not be accessible). As this method is highly invasive and requires exposure of the heart via a sternotomy or a left thoracotomy, it is really only suitable for patients undergoing concomitant open-heart surgery such as coronary artery bypass grafting or left ventricular assist device implantation. The transendocardial injection, however, is performed percutaneously in conscious patients. Catheters are passed from peripheral vessels into the left ventricular cavity and specialist injection systems are used to deliver the cellular product. For the intracoronary route, cells are infused into the coronary circulation using recognised angioplasty techniques that most interventional cardiologists are familiar with. Importantly, cells are delivered into a static pool of blood within the coronary artery which is achieved using over-the-wire balloon delivery systems. The vast majority of cell therapy trials for acute myocardial infarction have used the intracoronary route.

This review has solely focussed on trials that have used autologous bone marrow-derived cells; these trials (from pilot through to Phase III) represent the vast majority in this field. They have used either fractionated or unfractionated bone marrow, different cell isolation and preparation protocols, different dosages (dose size and repeat dosing) and different timings of the infusion procedure post-infarction.

Bone marrow is harvested under local anaesthesia from the iliac crest or other bone tissue. After it has been purified, it is either left unfractionated (bone marrow monounuclear cells) or it can be fractionated into different cell types (e.g. CD34+, CD133+, mesenchymal stem cells). The enriched or cultured cell populations are infused into the recipient's heart either using an angioplasty technique (intracoronary) or direct injection (transepicardial and transendocardial) using needle-like catheters as described above.

How the intervention might work

Since our last Cochrane review, the current level of evidence exploring cell therapy's potential mechanisms of action remains similar. Regardless of the intensive preclinical and clinical research over the past two decades, the mode of action of cell therapies remains unclear and is probably multifactorial.

Although transplanted cells are thought to benefit heart function through direct mechanisms, such as homing to the site of injury and differentiating into neighbouring cardiac tissues (Leri 2009), there is growing evidence to suggest that their benefit is more likely to be indirect. There is a strong likelihood that cell-based therapies primarily have a paracrine effect (Bartunek 2010; Behfar 2014) which is mediated by the release of cytokines from the transplanted cells. These stimulatory cytokines likely have multiple mechanisms of action including: increasing vascularity and collateral growth, reparative action on damaged cardiomyocytes and potentially the promotion of cardiomyocyte proliferation(Bartunek 2010; Behfar 2014; Cheng). There is also evidence to suggest that transplanted cells may confer benefit through an immunomodulatory process (Atoui 2012). These multiple effects may all contribute to an improvement in cardiac function and a reduction in scar size.

Why it is important to do this review

The first version of this review evaluated the clinical evidence from 13 randomized controlled trials (RCTs), the majority of which had short-term follow-up (e.g. less than six months follow-up)(Martin-Rendon 2008a; Martin-Rendon 2008b) . These first-generation clinical trials were not powered to assess the effect of cell therapies on clinical outcomes such as mortality. The main aim of these trials was to assess the safety of the intervention and the benefit of the treatment, measuring left ventricular ejection fraction (LVEF) as a surrogate outcome. We defined safety as the absence of adverse events (e.g. increased mortality and morbidity, increased risk of secondary infarction, restenosis and arrhythmias, development of heart failure) and efficacy as an improvement in cardiac function associated with cell therapy.

The second version of this review (Clifford 2012), evaluated 33 RCTs and long-term follow-up data had started to emerge (Cao 2009; Grajek 2010; Jin 2008; Meluzin 2008; Penicka 2007; Piepoli 2010; Yao 2009; Zhukova 2009). In that update we included 20 new studies. Unlike other systematic reviews with broader inclusion criteria (Jeevanantham 2012), our systematic review was the first to determine that there was no evidence of a difference in mortality rates between treated participants and controls (Clifford 2012).

The third version of this review (Fisher 2015b) evaluated 41 RCTs with a total of 2732 participants. Cell therapy was not associated with any changes in all-cause mortality, cardiovascular mortality, reinfarction or readmission for heart failure at long-term follow up. Additionally, cell therapy had no meaningful effect on morbidity, quality of life or left ventricular ejection fraction (as measured by magnetic resonance imaging). However, as most of the evidence came from small trials, we concluded that further adequately powered trials were required to definitively address cell therapy's efficacy. At the time of publication, a Phase III trial with a primary endpoint of all-cause mortality (BAMI) had started.

This fourth, and probably final, review is important as we can now evaluate 20 years of research in this area. This review incorporates 53 RCTs and 4201 participants, and now includes the only 2 published Phase III clinical trials (Mathur 2020 and Nair 2015). We extracted, analysed and conducted a risk of bias assessment on the data collected from the newly identified studies using the same methodology described in the previous reviews (Fisher 2015b; Clifford 2012; Martin-Rendon 2007; Martin-Rendon 2008a; Martin-Rendon 2008b). This version of the systematic review concludes that cell therapies for AMI are safe but have no beneficial effect on mortality or morbidity compared to the current standard of care. Given the logistical issues (time, cost and regulations) surrounding the performance of clinical trials in this area, alongside the efficacy of current treatments for AMI, further, larger Phase III trials are unlikely to be initiated.

Objectives

To determine the safety and efficacy of autologous adult bone marrow stem cells as a treatment for acute myocardial infarction (AMI), focusing on clinical outcomes.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials.

Types of participants

Any participants with a clinical diagnosis of AMI with no restriction on age.

Types of interventions

Studies involving the administration of autologous adult bone marrow-derived cells following successful revascularisation by angioplasty or cardiac surgery. Participants in the comparator treatment arm of the trial would have had either no intervention or placebo (e.g. medium where the stem cells are suspended, or plasma). Trials where surgery (e.g. coronary artery bypass graft (CABG)) or percutaneous angioplasty (e.g. PCI) have been administered were eligible.

In summary:

- any autologous human adult bone marrow stem cells;
- any method of stem/progenitor cell isolation or enrichment;
- any route of administration; any co-intervention (e.g. surgery or angioplasty); and
- any single dose or multiple doses of intervention.

Types of outcome measures

We assessed all outcomes at short-term (less than 12 months) and long-term (12 months or more) follow-up. In this version of the review, we have continued to focus on clinical outcomes. However, the surrogate endpoint of LVEF remains a standard, widely reported surrogate for cardiac function and has been retained as a reference point with other trials and systematic reviews in AMI and the cell therapy field. Surrogate outcomes other than LVEF reported in previous versions of this review, namely engraftment and survival of the infused stem cells, left ventricular end-systolic volume, left ventricular end-diastolic volume, wall motion score, stroke volume index and infarct size, are not included as outcomes.

Primary outcomes

- All-cause mortality
- Cardiovascular mortality
- Composite measures of major adverse cardiac events (MACE)
- Periprocedural adverse events

Secondary outcomes

- Morbidity including reinfarction, incidence of arrhythmias, incidence of restenosis, target vessel revascularisation and rehospitalisation for heart failure
- Quality of life and performance status (if measured separately from a quality of life measurement)
- Left ventricular ejection fraction (LVEF)

Search methods for identification of studies

We updated the searches, originally run in August 2007 (Appendix 1), in January 2011 (Appendix 2), in March 2015 (Appendix 3) and then again in February 2022 (Appendix 4). We identified relevant studies from searching the following:

Electronic searches

- Cochrane Central Register of Controlled Trials (CENTRAL 2022, Issue 2 of 12);
- MEDLINE (OvidSP, 1946 to 2 February 2022);
- EMBASE (OvidSP, 1974 to 2 February 2022);
- CINAHL (EBSCOhost, 1982 to 2 February 2022);
- PubMed (for e-publications only, 15 March 2015, search used in previous meta-analysis);
- LILACS (1982 to 2 February 2022);
- KoreaMed (1997 to 15 March 2015, search used in previous meta-analysis);
- IndMed (1986 to 15 March 2015, search used in previous meta-analysis);
- PakMediNet (1995 to15 March 2015, search used in previous meta-analysis);
- Web of Science: Conference Proceedings Citation Index - Science (CPCI-S) (1990 to 2 February 2022).

Searching other resources

In addition, we carried out the following.

- Handsearching of conference abstracts from relevant heart and/or stem cell conferences, e.g. the American Heart Association, International Society of Stem Cell Research (from 2005 to January 2011). Handsearching was not continued post-January 2011, as these conference abstracts are now included within EMBASE.
- Searches of three databases of ongoing trials, all performed on 2 February 2022:
- ClinicalTrials.gov (https://clinicaltrials.gov/);
- ISRCTN Register (http://www.isrctn.com/);
- World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (http://apps,who.int/trialsearch/).
- Searches of the reference lists of all identified eligible papers and relevant systematic and/or narrative reviews.
- Checking all trials listed as ongoing in the previous review.

We applied no language or date restrictions.

Data collection and analysis

The Cochrane information specialist (Farhad Shokraneh) conducted the final electronic search on 2 February 2022 for potentially relevant papers and removed references that were duplicates, clearly irrelevant and/or included in previous search results.

Selection of studies

Two review authors (PPZ, MvdN for this update) independently screened all titles and abstracts of references identified by the review search strategy for relevancy to the review question. We exclude studies that clearly did not meet the eligibility criteria at this stage. Two review authors (PPZ, MvdN) independently assessed all other studies on the basis of their full text for inclusion/exclusion using the criteria indicated above (type of studies, participants, interventions and outcome measures). disagreements were resolved through discussion and if needed discussed with a third investigator (AM).

Data extraction and management

Two review authors (PPZ, MvdN for this update) extracted data and undertook data extraction for all eligible studies independently. Aside from details relating to the quality of included studies, we extracted the following two groups of data:

- Trial characteristics: place of publication, date of publication, population characteristics, setting, detailed nature of intervention, detailed nature of comparator, detailed nature of outcomes. A key purpose of these data was to explain clinical heterogeneity between included studies independently from analysis of the results.
- Results of included studies for each of the main outcomes indicated in the review question. For dichotomous outcomes, we recorded the numbers of outcomes in the

treatment and control groups. For continuous outcomes, we recorded the mean and standard deviation. Where standard deviations of mean change from baseline values were not explicitly reported, where possible we calculated the standard deviation based on reported confidence intervals or P values as described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011), and we used these values in the analysis. In the 2015 version of the review the authors identified a systematic error in the previous versions of the review in the calculation of standard deviations for mean change from baseline values. This issue has now been corrected and these updated values are being used in the 2022 version; the discrepancies between the correct and previously reported values were small in all cases. In some studies it was not possible to calculate the value of the standard deviation and imputation techniques were deemed unsuitable due to the relatively high proportion of studies with missing standard deviations in some analyses (Higgins 2011). These studies, previously analysed as mean change from baseline values, are now incorporated in combined analyses using the mean endpoint value.

We resolved data extraction disagreements by consensus between the review authors. When disagreements regarding any of the above could not be resolved through discussion, we attempted to contact authors of the original trials to provide further details (see Dealing with missing data below). We then transcribed the data into the systematic review computer software Review Manager 5.3 (Review Manager 2014).

In light of the number of studies included in the previous version of this review that have had additional publications since, we checked all previous data and updated numbers from new publications if necessary. Where possible, we used the latest published.

Assessment of risk of bias in included studies

Two review authors (PPZ, DJ for this update), undertaking the data extraction independently, assessed the risk of bias for each trial using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011). For comparability with previous results, we deliberately used the 2011 version and not the updated Risk of Bias-tool in the Handbook. We assessed the design, conduct and analysis of the trial using a three-point scale: low, high or unclear risk of bias. To assess risks of bias, the authors used the following questions for each included trial:

- Was the allocation sequence adequately generated?
- Was allocation adequately concealed?
- Was knowledge of the allocated intervention adequately prevented (i.e. blinded) throughout the trial?
- Were incomplete outcome data adequately addressed for every outcome?
- Were reports of the trial free of selective outcome reporting?

• Was the trial apparently free of other problems that could put it at risk of bias?
We resolved disagreements through discussion with a third review author.

A study of trials published in Chinese medical journals that were described as randomised found that a high proportion of these trials did not adhere to accepted methodology for randomisation and hence could not be deemed authentic RCTs (Wu 2009). It is now widely accepted that trials carried out in China may lack appropriate randomisation, therefore we deemed any Chinese studies for which methods of randomisation were not described and could not be clarified with trial authors to have a high risk of selection bias; we evaluated sensitivity to these trials through sensitivity analyses (see Sensitivity analysis section below).

Measures of treatment effects
Several choices were made for dealing with certain type of analyses and missing data.

Unit of analysis issues
In the analysis of quality of life outcomes, we converted Minnesota Living with Heart Failure (MLHF) scores to negative values in order to include these in a meta-analysis with other measures on different scales using the standardised mean difference.

Dealing with missing data
We sought clarification of the extent of possible participant overlap between potentially related studies from nine trial authors by email contact. Eight authors responded during past reviews and we reached the following conclusions through email correspondence:
- Twenty treatment arm participants and 10 control arm participants were included in two trials published separately (Plewka 2009). Due to the extensive participant overlap and the shared protocol design of these two studies, we extracted and combined data as a single trial.
- In a large trial of 200 participants (Tendera 2009), 12 patients were also included in a separate trial (Grajek 2010). In view of the small degree of overlap, we have extracted data from these trials separately and included as them independent studies in this review.
- A 2014 publication by Ryabov et al was a long-term follow-up of an earlier trial already included in an early version of this review (Karpov 2005).
- A 2012 conference abstract published by Turan et al described long-term follow-up of an earlier trial reported in full (Turan 2012).

The following issues are still awaiting resolution:
- The extent of possible participant overlap between two conference abstracts (Huans 2007b; Huang 2008), and four separate studies from the same research group (Ge

2006; Huang 2006; Huang 2007; Yao 2006), could not be confirmed as email contact with the authors was unsuccessful. As a result, we have listed both Huang 2007b and Huang 2008 as studies awaiting classification.

We contacted a further four authors of trials published in abstract form only at the time of study selection to establish whether these trials were expected to be published in full. Two of these trials have now been published in full (Hirsch 2011; Roncalli 2010), and we have since excluded one trial (Perez-Oteyza 2006). No further publications have been identified for the fourth trial (Fernandez-Pereira 2006); this trial is therefore included in studies awaiting classification. We contacted one trial author to clarify the publication of further follow-up data (Roncalli 2010).

We made attempts to contact the authors of 24 included studies by email requesting additional information on the trial design and methodology, clarification regarding data discrepancies, further detail about patient demographics and/or additional data (Cao 2009; Colombo 2011; Chen 2004; Huang 2006; Huang 2007; Janssens 2006; Jazi 2012; Jin 2008; Kim 2018; Lunde 2006; Nair 2015; Naseri 2018; Nogueira 2009; Piepoli 2010; Roncalli 2010; Ruan 2005; Schachinger 2006; Sürder 2013; Tendera 2009; Turan 2012; Wang 2014; Wohrle 2010; Xiao 2012; Yao 2006). Authors of seven trials kindly responded as follows; key data provided by authors included the following:

- Kim 2018: clarification on cumulative counting of arrhytmias.
- Lunde 2006: mean change from baseline echocardiography, MRI and SPECT data were confirmed.
- Nair 2015: clarification of all serious adverse events and which ones happened in therapy or control group.
- Piepoli 2010: the number of participants included in the analyses and details of withdrawals and exclusions were clarified; mean and standard deviation values for echocardiography data were provided.
- Schachinger 2006: surrogate endpoint data from MRI at 24-month follow-up were provided.
- Tendera 2009: mean and standard deviation values for MRI data were provided.
- Turan 2012: details of the number of withdrawals and exclusions with reasons were provided, together with clarification of patient demographics.

Assessment of heterogeneity

Heterogeneity was assessed by multiple sensitivity analyses (see also Sensitivity analysis); baseline LVEF (< or ≥45%), cell type (mononuclear cells vs mesenchymal stem cells vs hematopoietic progenitor cells), dose of stem cells (≤108 vs >108 and ≤109 vs >109), timing of cell administration (≤10 days since AMI vs >10 days since AMI) and the use of heparine in the therapeutic cell solution (heparin vs no-heparin). Due to broad

consensus on the existence of imporatnt heterogeneity in cell therapy trials, random effects meta-analysis was performed.

Assessment of reporting biases

Although we believe that we made every effort to identify unpublished studies, we assessed publication bias for the primary outcome of mortality using a funnel plot and with a formal test for publication bias using Egger's test for asymmetry (Egger 1997), implemented with the statistical software programme R v2.14.1 (R Core Team 2013) and the meta package (Schwwarzer 2016). If needed, trim and fill analysis will be performed.

Data synthesis

We undertook meta-analyses using Review Manager Web Version 4.3.0 (Review Manager 2014), using random-effects models throughout due to the anticipated heterogeneity arising from differences in participant characteristics, interventions and duration of follow-up. Random-effects meta-analyses on this subject has been implemented since the 2015 version. Previous versions of the review used fixed-effect models. Although quantitative synthesis was the main method of analysis, we incorporated insights from a qualitative evaluation of studies for an overall interpretation of the data. We based conclusions on patterns of results identified across clearly tabulated results of included studies as well as summary measures, taking both direction and magnitude of any mean effect sizes from random-effects models into account. We included all studies in the main analyses irrespective of risk of bias; we performed sensitivity analyses for risk of selection, performance and attrition bias as described in the Sensitivity analysis subsection below. We summarised periprocedural adverse events for each trial in tabular form and evaluated them descriptively.

Within each included trial, all participants were analysed in the treatment groups to which they had been randomised. We have undertaken an available case analysis, including all participants who were randomised to treatment and were included in the analysis, irrespective of whether or not they received their randomised treatment. If multiple manuscripts on the same trial were present describing long-term data (12 months or more), we included or updated the numbers to the results from the latest publication.

We carried out separate analyses according to the duration of follow-up after treatment: short-term (less than 12 months) and longterm (12 months or more). We expressed dichotomous data for each arm in a particular trial as a proportion or risk and the treatment effect as a risk ratio (RR) with 95% confidence intervals (CIs). Trials that did not record any incidence of a recorded outcome measure in both groups, was left out of the analysis, as done in the previous versions of this review.

We expressed continuous data for each arm in a particular trial as a mean and

standard deviation, and the mean treatment effect as the mean difference (MD) if outcomes were measured in the same way across trials. For outcomes measured using different scales (physical capacity and quality of life measures), we combined the treatment effect data and analysed them using the standardised mean difference (SMD).

Although we intended to analyse continuous outcomes as mean change from baseline, several studies only reported baseline and endpoint data. Where possible, we calculated the standard deviation of the mean change from baseline based on reported confidence intervals or P values as described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011), and we used these values in the analysis. However, for several studies, insufficient information was reported to calculate the standard deviation. The mean difference based on the change from baseline can be assumed to address the same underlying intervention effects as an analysis based on final measures (i.e. the differences in mean final values will on average be the same as the differensces in mean change scores). Therefore we combined studies reporting mean change from baseline values with those reporting endpoint values (using preferentially mean change values where both were reported), but presented mean change and endpoint values separately as well as in combined analyses for clarity, as suggested in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011). We did not conduct this pooling of studies by method of reporting of continuous measures for analyses of quality of life or physical capacity, since the assumption of consistent underlying effects does not hold for standardised mean differences.

Ten trials reported multiple intervention groups. In order to avoid double-counting of controls, in the main analyses we pooled data from active intervention arms across different doses (high dose/low dose (Meluzin 2008) or high/medium/low dose (Quyyumi 2011)), delivery routes (arterial or venous) (Nogueira 2009), timing of cell delivery (early or late) (Sürder 2013), type of cells (selected or unselected (Tendera 2009), BMMNC or CD133 cells (Naseri 2018), number of cell doses (Yao 2009), product (Wollert 2017) or regular/intensified atorvastatin treatment (Yang 2020). One trial used two interventions (BMMNC therapy and GCSF stimulated BMMNC therapy) and had two appropriate control groups alongside of it (San Roman 2015). For this reason, this trial is included in the results twice, with reporting of separate results for the BMMNC therapy and the GCSF-stimulated BMMNC therapy, with separate control gorups. Of note, in previous versions of this review trials were excluded for not having a GCSF control group when using GCSF-stimulation before cell harvesting in the therapy group.

When combining groups, we calculated the new standard devation and mean based on reported standard deviations and means per individual group, as described in the Cochrane Handbook for Systematic Reviews of Interventions, Chapter 7.7.3 (Higgins 2011).

We produced a 'Summary of findings' table for the primary outcomes of all-cause

mortality, cardiovascular mortality and the composite measure of major adverse clinical cardiac events at both short-term and long-term follow-up, using the GRADEpro GDT software (GRADEpro GDT 2014). We calculated risk ratios excluding trials with important risks of different biases (selection bias, attrition bias and performance bias), assuming a potential risk from the observed data from these included trials.

For the 2022 update, a mortality figure was created with year of publication on the x-axis and a bubble plot for visualization of the number of included patients per trial for the control group (ranging n=3-190). The control groups were deliberately chosen, as they should follow the natural course of the disease, compared to a potential different course in therapy groups with an effective treatment. A line was fitted for this dataset, following the means of the mortality rates, with a variance estimate of $1/\sqrt{n}$ (with no standard error available for these mortality numbers) and with 3 cubic splines. This figure was created using statistical software programme R v2.14.1 (R Core Team 2013) and the ggplot2 package (Wickham 2016).

Subgroup analysis and investigation of heterogeneity

A range of different methods were used to measure LVEF across studies (magnetic resonance imaging (MRI), left ventricular angiography (LVA), single photon emission computed tomography (SPECT), echocardiography and radionuclide ventriculography (RNV)), with several studies reporting LVEF as an outcome using more than one method of measurement. The limitations of some of these methods are well known (Arnesen 2007). Consistent with the previous version of this review, we subgrouped analyses of LVEF according to the measurement method used.

We grouped trials according to baseline cardiac function (defined by mean baseline LVEF < 45% or ≥ 45%), mean cell dose (≤ 108 , > 108 and ≤ 109 , > 109), timing of stem cell administration (≤10 days or >10 days after AMI) and use of heparinised cell solution. Planned subgroup analysis of the type/route of cell delivery was not possible as all but two trials (Nogueira 2009, Nair 2015) administered cells into the coronary artery. For one study (Yang 2020) no cell dose was mentioned, for which the assumption was made that it would fall in the $<10^8$ cells group as the isolation (form iliac crest, 80-100ml) and administration of the aspirates performed on the same day.

We performed an a priori subgroup analyses for the primary outcome of mortality. For other outcomes with substantial observed heterogeneity (I2 ≥ 50%) (Higgins 2003), and a minimum of two studies in each subgroup, we investigated potential sources of heterogeneity by performing the subgroup analyses described above as exploratory analyses, and by visual inspection of forest plots with consideration of individual trial characteristics.

For trials with multiple active intervention arms, in subgroup analyses where the intervention arms were stratified across the subgrouping strata, we used the single control group as the comparator in each subgroup.

Sensitivity analysis

We assessed the robustness of results for the primary outcomes of all-cause mortality, cardiovascular mortality and composite measures of MACE for sensitivity to risk of selection bias (excluding studies with a high risk of bias from random sequence generation) and attrition bias (excluding studies with a high or unclear risk of attrition bias). We also assessed the primary clinical outcomes for sensitivity to risk of performance bias (excluding those studies with a known lack of blinding of participants and clinicians).

We also assessed the primary outcome of mortality and any additional outcomes that showed evidence of a difference between trial arms for sensitivity to differences in the route of cell delivery, by excluding one trial that administered cells into the coronary artery (Nogueira 2009). This trial did not report the primary outcomes of cardiovascular mortality and composite measures of MACE.

Differences in methods of reporting for continuous outcomes across trials led us to combine mean change from baseline and endpoint data for LVEF (see Data synthesis above). We have presented the results separately as well as in combination for clarity and to assess the sensitivity of the results to the method of reporting.

Summary of findings and assessment of the certainty of the evidence

A summary of findings table was created for all primary outcomes (Summary of findings table 1), including a grading on the certainty of evidence, based on the GRADE Working group grades of evidence(https://gdt.gradepro.org).

RESULTS

Description of studies

Below are the results of our search and a breakdown of included and excluded studies.

Results of the search

Given that a wide variety of products and terms have been used in the comparator arms of the included trials, for ease of reference we will use the term 'control' throughout this review to refer to the comparator treatment arm.

We identified a total of 5889 records (4954 records after deduplication) from electronic searches of the CENTRAL, MEDLINE, EMBASE, SRI Transfusion Evidence Library, ClinicalTrials.gov, CDSR, DARE, CINAHL and Current Controlled Trials databases from March 2015 till February 2022 (including previous versions, 11388 records were screened). We identified four further references from reference lists of reviews identified in the database search and ongoing trials, to give a total of 5893 citations.

Additionally, handsearching of the American Heart Association Scientific Sessions, European Society of Cardiology Congress and World Congress of Cardiology annual

conference proceedings from 2005 to January 2011 identified an additional 96 references in previous versions, and de-duplication and removal of all previously screened references by the SRI Information Specialist (CD) excluded 1753 references.

Screening of the remaining 4958 records by two review authors (PPZ & MvdN) independently resulted in exclusion of 4768 records (4156 references and 612 trial records), which were clearly irrelevant. Detailed assessment of the remaining 107 references and 48 trial records identified a total of 70 records, comprised of 40 full papers and 30 (partially ongoing) trial records, which described a total of 12 new trials and multiple updated trials included in this review (see study flow diagram in Figure 1). The total number of included trials is 53.

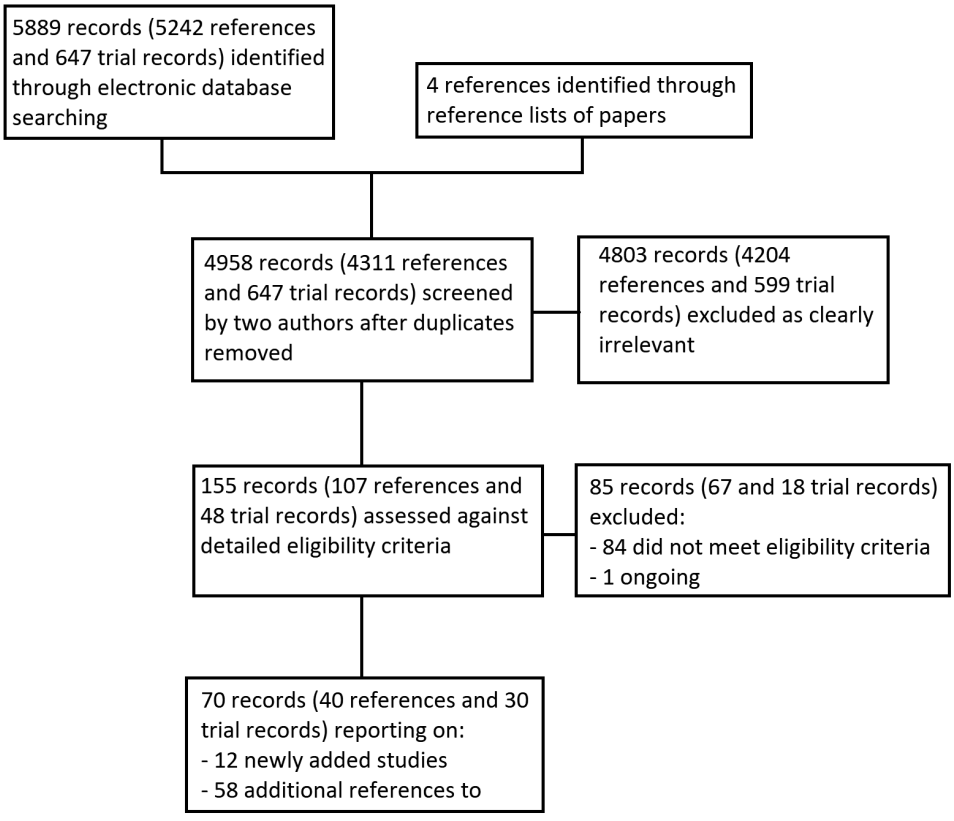


Figure 1. Flowchart for search (adapted from flowchart in previous version)

Trials included in the review

We translated six trials from Chinese (Mandarin) to English (Huang 2006; Huang 2007; Jin 2008; Yao 2006; You 2008; Xiao 2012), and two from Russian to English (Karpov

2005; Zhukova 2009), prior to inclusion in this review, including one report of long-term follow-up, which we translated using Google Translate (https://translate.google.com/) for this update. An English version of a seventh Chinese paper was identified (Ruan 2005). Following careful cross-checking between the Chinese and English versions of the paper, which confirmed that both papers reported the same data from one trial, we used the English version of the paper within this review.

One trial included in the previous version of the review was previously referred to as Meyer 2006. This study is now referred to as Wollert 2004 in accordance with the first publication that reported results from this trial. Three trials included in the previous version of the review are now not included: two trials that used G-CSF to mobilise stem cells in the cell therapy arm did not give G-CSF to the control group and in view of the lack of this co-intervention in the control arm, these studies are now excluded (Kang 2006; Li 2006), and one trial published in abstract form only has been reclassified as awaiting classification as there were insufficient data provided for inclusion in any analyses (Fernandez-Pereira 2006).

Six trials had three-arm comparisons (Meluzin 2008; Nogueira 2009;Naseri 2018; Sürder 2013; Tendera 2009; Yao 2009), and three trials had a four-arm comparison (Quyyumi 2011;San Roman 2015; Yang 2020). In Meluzin 2008, the two treatment arms compared different doses (low dose or high dose) of stem/progenitor cells administered. Likewise, in Quyyumi 2011, the three treatment arms compared low, moderate and high-dose administrations of selected CD34+ cells. The two treatment arms in Yao 2009 compared a single dose (SD arm) of stem/progenitor cells at three to seven days post-AMI to a repeated dose (DD arm) - i.e. administration of stem/progenitor cells at both three to seven days and three months post-AMI. The two treatment arms in Nogueira 2009 compared intracoronary artery (arterial group – AG) delivery of stem/progenitor cells against intracoronary venous (venous group – VG) delivery of stem/progenitor cells. In Tendera 2009, the two treatment arms compared selected CD34+ CXCR4+ (selected –S) stem/progenitor cell administration versus non-selected (unselected – U) mononuclear cell administration. Sürder 2013 included two intervention groups comparing either five to seven days (early - E) or three to four weeks (late - L) cell administration. Naseri 2018 included two intervention groups comparing mononuclear cells and CD-133+ positive cells to a placebo group. San Roman 2015 compared both a direct bone marrow isolation and a G-CSF stimulation before bone marrow isolation. The trial included both a regular control group and a control group receiving only the G-CSF, but no marrow isolation. Yang 2020 investigated the use of low and high doses of atorvastatin next to mononuclear cell therapy versus a control group in a 2x2 design, leading to four groups.

As stated in the Methods section, we pooled active intervention arms for the main analyses and compared this with the single control group. If multiple control groups were present, we pooled these as well. Only for San Roman 2015 we deliberately

created two comparisons (one with and one without G-CSF), as G-CSF stimulation is thought to potentially influence both regenerative capacity and mobilization of the bone marrow cells.

We included a total of 53 trials; the number of participants included in each trial ranged from 11 to 375, and a total of 4159 participants (1427 added this update), from 2297 cell therapy patients and 1862 controls (733 and 694 added this update) were included in the 54 comparisons of the rev The mean age of participants across all included trials ranged from 46.6 years (Jazi 2012) to 65.2 years (Piepoli 2010), with the mean age of participants between 50 and 60 years in all but eight trials (Table 1). All trials included predominantly male participants, with the per cent male ranging from 60.6% (Wang 2014) to 100% (Colombo 2011; Kim 2018; Zhukova 2009); four trials reported female participants in only one arm of the trial only (Gao 2013; Ge 2006; Penicka 2007 Ruan 2005) (Table 1). Ethnicity data were not available.

The trials included in the review were conducted in 22 countries, which included Belgium (Janssens 2006), Brazil (Angeli 2012; Nogueira 2009), Canada (Haddad 2020), China (Cao 2009; Chen 2004; Gao 2013; Ge 2006; Huang 2006; Huang 2007; Jin 2008; Ruan 2005; Wang 2014; Xiao 2012; Yang 2020; Yao 2006; You 2008; Zhang 2021), Czech Republic (Meluzin 2008; Penicka 2007), Finland (Huikuri 2008), France (Roncalli 2010), Germany (Turan 2012; Wohrle 2010; Wollert 2004), India (Nair 2015), Iran (Jazi 2012; Naseri 2018), Italy (Colombo 2011; Piepoli 2010; Yao 2009), Korea (Kim 2018), the Netherlands (Hirsch 2011), Norway (Lunde 2006), Poland (Grajek 2010; Plewka 2009; Tendera 2009), Russia (Karpov 2005; Zhukova 2009; Kirgizova 2015), South Korea (Lee 2014), Spain (Suarez de Lezo 2007; San Roman 2015), Switzerland (Sürder 2013), the United Kingdom (Choudry 2016) and the USA (Quyyumi 2011; Quyyumi 2017 Traverse 2010; Traverse 2011; Traverse 2018), one trial was carried out in Germany and Switzerland (Schachinger 2006), one in Germany and Norway (Wollert 2017) and one trial was conducted throughout Europe in the United Kingdom, Belgium, Czech Republic, Denmark, Finland, France, Germany, Italy, the Netherlands, Poland and Spain (Mathur 2020). Twenty-eight trials compared the active intervention (autologous bone marrow stem/progenitor cells) with no intervention and 25 trials compared the active intervention with placebo (Table 2). The majority of trials used PCI as the primary treatment for AMI. Thrombolytic therapy without PCI was used as the primary treatment in all patients in two trials (Huikuri 2008; You 2008), and some patients in two trials (Lee 2014; Zhukova 2009). Five trials used PCI in combination with thrombolytic therapy either in all patients (Jin 2008; Karpov 2005; Nogueira 2009; Sürder 2013), or in some patients (Wollert 2004; Mathur 2020; San Roman 2015; Wollert 2017) (Table 1). One trial performed a catheterization and most of the times (but not always) PCI one to three weeks after AMI (Yang 2020). One trial included only patients suitable for elective CABG after AMI, who did not need emergency PCI or thrombolysis (Naseri 2018). All trials maintained the patients with a standard set of drugs, including aspirin, clopidogrel,

heparin, β-blockers, statins, angiotensin converting enzyme (ACE) inhibitors, nitrates and/or diuretics. We have analysed outcome data separately in this review; we have incorporated the maximum short-term or long-term time point from each trial into the analyses.

Trial design characteristics – interventions

Details of the individual trial interventions are given in the Characteristics of included studies tables and are summarised in Table 2.

Almost all trials isolated the stem/progenitor cells by bone marrow aspiration and separated the mononuclear cell fraction by gradient centrifugation. Three trials failed to report the method of cell isolation or processing (Angeli 2012; Ge 2006; Ruan 2005).

Forty trials administered unfractionated bone marrow-derived mononuclear cells intracoronally via an inflated balloon catheter. This mononuclear cell population contains stem/progenitor cells and other blood cells (Angeli 2012; Cao 2009; Chen 2004; Choudry 2016; Ge 2006; Grajek 2010; Hirsch 2011; Huang 2006; Huang 2007; Huikuri 2008; Janssens 2006; Jazi 2012; Jin 2008; Karpov 2005; Lunde 2006; Meluzin 2008;Mathur 2020; Nair 2015; Nogueira 2009; Penicka 2007; Piepoli 2010; Plewka 2009; Roncalli 2010; Ruan 2005; San Roman 2015; Schachinger 2006 ; Suarez de Lezo 2007; Sürder 2013; Tendera 2009; Traverse 2010; Traverse 2011; Traverse 2018; Turan 2012; Wohrle 2010; Wollert 2004; Wollert 2017; Yang 2020; Yao 2006; Yao 2009; Zhukova 2009). Five trials processed the mononuclear cell fraction using immunomagnetic selection to isolate and administer a suspension containing a selected CD133+ cell population (Colombo 2011; Haddad 2020; Kirgizova 2015; Quyyumi 2011; Quyyumi 2017), or in one trial this was part of one of three treatment arms (Naseri 2018) or in one intervention arm of a three-arm trial, CD34+ /CXCR4+ cells (Tendera 2009). Seven trials cultured cells to isolate mesenchymal stem cells (BM-MSC) (Gao 2013; Kim 2018; Lee 2014; Wang 2014; Xiao 2012; You 2008; Zhang 2021).

One three-arm trial also administered unfractionated mononuclear cells intravenously to the coronary vein corresponding to the culprit coronary artery via a multipurpose guiding catheter (Nogueira 2009). Simultaneous total occlusion of the coronary vein was achieved via an inflated balloon catheter in the culprit coronary artery. Another trial delivered it's cell product after CABG through intramyocardial injections (Naseri 2018).

Cells were suspended in heparinised saline (Cao 2009; Chen 2004; Gao 2013; Haddad 2020; Huang 2006; Huang 2007; Jin 2008; Kirgizova 2015; Plewka 2009;San Roman 2015; Suarez de Lezo 2007; Wang 2014; Wollert 2004; Wollert 2017; Yang 2020), heparinised saline with human serum albumin (Hirsch 2011), or heparinised saline with autologous serum (Huikuri 2008; Janssens 2006; Kim 2018), heparinised plasma (Lunde 2006; Yao 2009), saline solution with autologous serum (Naseri 2018), saline solution and human serum albumin (Colombo 2011; Nogueira 2009; Traverse 2010;

Traverse 2011; Traverse 2018), with 0.1% autologous erythrocytes (Wohrle 2010), heparinised phosphase buffered saline, autologous serum and human serum albumin (Quyyumi 2011; Quyyumi 2017), human serum albumin solution (Roncalli 2010), diluted autologous serum (Ruan 2005; Sürder 2013), autologous serum (Zhukova 2009), X-vivo medium and autologous serum (Choudry 2016; Mathur 2020; Schachinger 2006), or autologous plasma (Grajek 2010), M199 medium (Jazi 2012), phosphate buffered saline (Tendera 2009) with human serum albumin (Piepoli 2010), lymphocyte isolation medium (Yao 2006) or saline (Zhang 2021).

Ten trials did not report details of the cell suspension (Angeli 2012; Ge 2006; Karpov 2005; Lee 2014; Meluzin 2008; Nair 2015; Penicka 2007; Turan 2012; Xiao 2012; You 2008).

Timing of stem cell administration post-AMI

Twenty-one trials delivered cells within seven days of AMI: seven trials within the first 24 to 48 hours (Choudry 2016; Gao 2013; Ge 2006; Huang 2006; Huang 2007; Janssens 2006; Ruan 2005), and 13 trials at up to seven days after AMI (Cao 2009; Grajek 2010; Haddad 2020; Huikuri 2008; Nogueira 2009; Piepoli 2010; San Roman 2015; Schachinger 2006; Sürder 2013; Traverse 2018; Turan 2012; Wohrle 2010; Wollert 2004; Yao 2009), including two trials with patients randomised to receive cells at either three days or seven days (Traverse 2018), or at five to seven days or three to four weeks (Sürder 2013) after AMI, and one trial in which some patients were randomised to receive a second dose at three months (Yao 2009).

In 10 trials cells were administered within seven days in some patients although other patients received cells at up to eight days (Hirsch 2011; Lunde 2006; Mathur 2020), nine days (Angeli 2012; Meluzin 2008), 10 days (Traverse 2010), 11 days (Penicka 2007; Plewka 2009;), and 12 days (Suarez de Lezo 2007; Tendera 2009) after AMI.
Twenty-two trials administered cells at more than seven days after AMI (Chen 2004; Colombo 2011; Jazi 2012; Jin 2008; Karpov 2005; Kim 2018; Kirgizova 2015; Lee 2014; Nair 2015; Naseri 2018; Quyyumi 2011; Quyyumi 2017; Roncalli 2010; Sürder 2013; Traverse 2011; Wang 2014; Wollert 2017; Xiao 2012; Yang 2020; You 2008; Zhang 2021; Zhukova 2009)

Comparator arm

Twenty-five trials administered a placebo intervention to the control group (Angeli 2012; Choudry 2016 Cao 2009; Chen 2004; Ge 2006; Haddad 2020; Huang 2006; Huang 2007; Huikuri 2008; Janssens 2006; Kirgizova 2015; Ruan 2005; Schachinger 2006; Suarez de Lezo 2007; Traverse 2010; Traverse 2011; Traverse 2018; Wang 2014; Wohrle 2010; Xiao 2012; Yao 2009). In two trials the placebo medium was not reported (Angeli 2012; Ge 2006). Of the remaining 23 trials, all but one, Xiao 2012, used the same media used to re-suspend cells in the corresponding treatment arm to patients in the

comparator arm (no cells). Xiao 2012 administered heparinised saline to the control group but did not report the re-suspension medium used in the cell therapy group. Twenty-eight trials did not use a placebo intervention (Colombo 2011; Gao 2013; Grajek 2010; Hirsch 2011; Jazi 2012; Jin 2008; Karpov 2005; Kim 2018; Lee 2014; Lunde 2006; Mathur 2020; Meluzin 2008; Nair 2015; Nogueira 2009; Penicka 2007; Piepoli 2010; Plewka 2009; Quyyumi 2011; Roncalli 2010; Sürder 2013; Tendera 2009; Turan 2012; Wollert 2004; Yao 2006; You 2008; Zhukova 2009); no other interventions were reported other than optimal medical therapy.

Dose of stem/progenitor cells administered

The dose of cells administered varied considerably between trials; for simplicity we have grouped trials according to the mean dose: 10^6 cells; 10^7 cells; 10^8 cells; 10^9 cells and 10^{10} cells.

Three trials administered magnetically selected cells at a dose of 106 CD133+ cells (Colombo 2011), (up to) 107 CD133+ cells (Haddad 2020; Naseri 2018), 106 CD34+ CXCR4+ cells (Tendera 2009), mean of 1.5*107 CD34+ cells (Quyyumi 2017) and 106 or 107 CD34+ cells (three randomised cell dose groups) (Quyyumi 2011). In five trials that administered mesenchymal stem cells, cells were administered at a dose of 106 (Gao 2013), up to 107 (Lee 2014; Wang 2014; You 2008; Zhang 2021), and 108 (Xiao 2012).

Bone marrow mononuclear cells were administered to patients at a dose of up to 10^7 (Choudry 2016; Ge 2006; Jin 2008; Karpov 2005; Kirgizova 2015; Lunde 2006; Nogueira 2009; Roncalli 2010; Traverse 2010; Zhukova 2009), 108 (Angeli 2012; Cao 2009; Grajek 2010; Hirsch 2011; Huang 2006; Huang 2007; Huikuri 2008; Janssens 2006; Kim 2018; Piepoli 2010; Plewka 2009; Schachinger 2006; Suarez de Lezo 2007; Sürder 2013; Tendera 2009; Traverse 2011; Traverse 2018; Wohrle 2010; Yao 2006; Yao 2009), 109 (Jazi 2012; Mathur 2020; Nair 2015; Naseri 2018; Penicka 2007; Wollert 2004), and 1010 (Chen 2004). Two trial compared two doses of BMMNC: 106 or 108 (Meluzin 2008) and up to 108 or 109 (Wollert 2017). One trial compared regular isolation and isolation after G-CSF stimulation, resulting in up to 108 and up to 109 cells respectively (San Roman 2015). Only three trials did not give details of the cell dose administered to patients (Ruan 2005; Turan 2012; Yang 2020)

Trials excluded from the review

After our current search, we excluded 85 records from the review following full-text eligibility assessment. In summary, the reasons for exclusion were as follows: 19 studies were not classified as AMI, seven studies were non-randomised controlled trials or trials without a proper control arm, 12 studies did not use autologous bone marrow stem cells, 26 studies were systematic reviews or metaanalyses, 14 studies were commentaries or summaries, seven studies were translational/experimental animal studies (see Characteristics of excluded studies). One study was added to ongoing

trials, with no reported results yet. We deliberately kept the excluded studies from previous versions in for completeness (see Excluded studies).

Trials awaiting assessment and ongoing trials

Nine trials described in 10 references appeared to meet the eligibility criteria for this review but reported insufficient information for the trials to be included (see Characteristics of studies awaiting classification). We await further publications on these trials. We identified 14 eligible ongoing trials described in 3 references and 12 ongoing trial database records (see Characteristics of ongoing studies). Current ongoing/registered trials intend to recruit 1029 patients.

Risk of bias in included studies

A description of the risk of bias for individual studies is given in the Characteristics of included studies tables. A summary of the risk of selection bias, performance and detection bias, attrition bias, reporting bias and other potential sources of bias including baseline imbalances between trial arms, publication bias and study funding is given below and in Figure 2.

Allocation

Twenty six trials provided details as to the generation of the randomisation sequence (Cao 2009; Choudry 2016; Colombo 2011; Gao 2013; Ge 2006; Grajek 2010; Hirsch 2011; Huikuri 2008; Janssens 2006; Kirgizova 2015; Lunde 2006; Mathur 2020; Nair 2015; Naser 2018; Nogueira 2009; Roncalli 2010; Schachinger 2006; Sürder 2013; Traverse 2010; Traverse 2011; Wollert 2004; Wollert 2017; Yang 2020; Yao 2009; You 2008; Zhang 2021. These methods included: sequential numbers (Gao 2013; Ge 2006; Wollert 2004), "uneven vs. even numbers" (Piepoli 2010), a randomisation table (You 2008), a randomisation list generated in permuted blocks of 10, stratified according to centre (Lunde 2006), a randomisation list generated in permuted blocks of six (Grajek 2010), a randomisation list generated in permuted blocks of undefined size (Colombo 2011), a randomisation list generated in permuted blocks with variable block sizes (Huikuri 2008), a randomisation list generated according to infarct size (Nogueira 2009), a permuted-block randomisation list stratified according to centre, diabetes status and time to PCI after the onset of AMI (Roncalli 2010), a randomization list by a computer programme using permuted blocks of variable length by central data coordinator off-site (Nair 2015), a computer generated randomization sequence (aNaseri 2018), a randomization block stratified by site, prepared by Prometris (Wollert 2017), an interactive web-based randomisation session using randomly selected block sizes of six or nine, stratified by centre (Traverse 2011), a permutedblock randomisation list stratified according to site (Hirsch 2011), computer-generated random lists (Cao 2009; Janssens 2006; Schachinger 2006; Yao 2009; Traverse 2018; Yang 2020), a randomisation

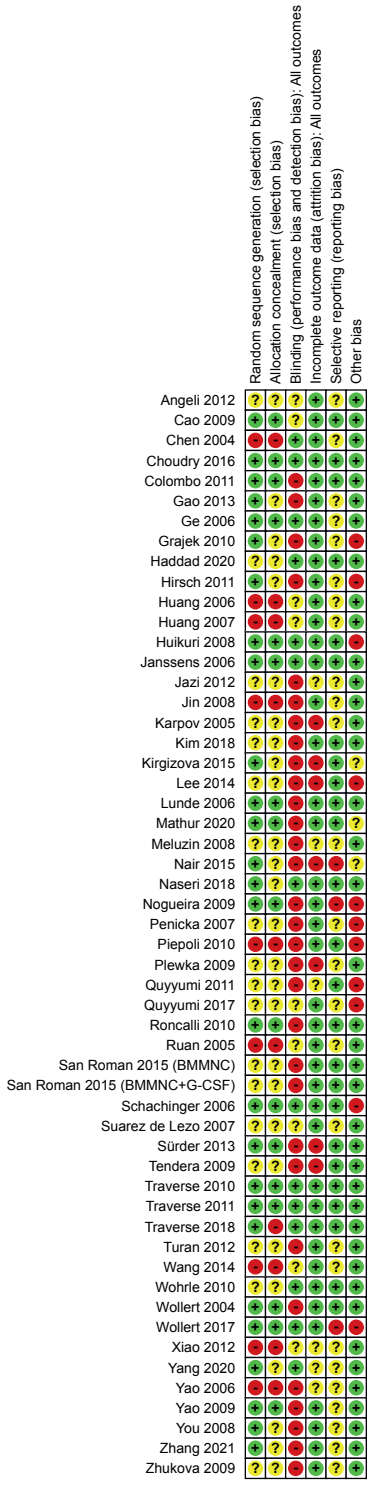


Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.

algorithm developed by a biostatistician (Traverse 2010), randomization performed by dedicated clinical trial software IHD CLINICAL (Choudry 2016), randomization stratified per country through IVRS (Mathur 2020), random number generation through technical services of CCVD per individual patient (Zhang 2021). Four trials reported using sealed envelopes (Ge 2006; Kirgizova 2015; Nogueira 2009; Sürder 2013; Wollert 2004), and two trials generated randomisation lists at a site external to the trial site (Schachinger 2006; Wollert 2004). We defined 26 trials as having a low risk of selection bias due to random sequence generation; we considered one trial that allocated treatment using even versus uneven numbers to have a high risk of selection bias (Piepoli 2010); we also deemed this trial to have a high risk of selection bias due to insufficient allocation concealment. We also deemed 16 trials to have used an appropriate method of allocation concealment (Cao 2009; Colombo 2011; Ge 2006; Huikuri 2008; Janssens 2006; Lunde 2006; Mathur 2020; Nogueira 2009; Roncalli 2010; Schachinger 2006; Sürder 2013; Traverse 2010; Traverse 2011; Wollert 2004; Wollert 2017; Yao 2009). One trial reported that the randomisation scheme was not blinded and we therefore considered it to have a high risk of selection bias due to lack of allocation concealment (Traverse 2018). Allocation concealment was unclear in the remaining 10 trials (Choudry 2016; Gao 2013; Grajek 2010; Hirsch 2011; Kirgizova 2015; Nair 2015; Naseri 2018; Yang 2020; You 2008; Zhang 2021).

We defined the generation of the randomisation sequence as unclear in the 'Risk of bias' tables in 16 trials in which no description was given as to what methods were used to generate the random sequence (Angeli 2012; Haddad 2020; Jazi 2012; Karpov 2005; Kim 2018; Lee 2014; Meluzin 2008; Penicka 2007; Plewka 2009; Quyyumi 2011; Quyyumi 2017; Suarez de Lezo 2007; Tendera 2009; Turan 2012; Wohrle 2010; Zhukova 2009). The method of generation of randomisation sequence was also not reported in eight Chinese trials, which we deemed to have a high risk of bias (Chen 2004; Huang 2006; Huang 2007; Jin 2008; Ruan 2005; Wang 2014; Xiao 2012; Yao 2006).

Blinding

In trials, the control group underwent bone marrow aspiration and were given a placebo injection. These trials also reported blinding of outcome assessors or described the trial as "double-blind" and we therefore considered them to have a low risk of performance and detection bias (Chen 2004; Choudry 2016; Ge 2006; Haddad 2020; Huikuri 2008; Janssens 2006; Naseri 2018; Schachinger 2006; Traverse 2010; Traverse 2011; Traverse 2018; Wohrle 2010; Wollert 2017; Yang 2020). In a further eight trials a placebo injection was also administered (Angeli 2012; Cao 2009; Huang 2006; Huang 2007; Ruan 2005; Suarez de Lezo 2007; Wang 2014; Xiao 2012), in which bone marrow aspiration in the control group was either not undertaken (Cao 2009; Suarez de Lezo 2007; Xiao 2012), or was not reported (Angeli 2012; Huang 2006; Huang 2007; Ruan 2005; Wang 2014); in these eight trials the risk of performance bias was unclear. Only

four of these trials reported blinding of outcome assessors (Cao 2009; Ruan 2005; Suarez de Lezo 2007; Xiao 2012); blinding of outcome assessors was otherwise not reported (Angeli 2012; Huang 2006; Huang 2007; Wang 2014). In one other trial, although the control group received a placebo injection, only the active intervention groups underwent bone marrow aspiration (Yao 2009). Furthermore, the active treatment groups were recalled for a second infusion of cells or placebo whereas the control group was not, and we therefore deemed these trials to have a high risk of performance bias. In one other trial, both groups underwent bone marrow aspiration and catheterization, but although reported as double-blind, there is no mentioning of blinding of the outcome assessors. This trial was also deemed as unclear risk of bias (Quyyumi 2017). Participants were not blinded to treatment in 30 trials in which no placebo infusion was administered Colombo 2011; Gao 2013; Grajek 2010; Hirsch 2011; Jazi 2012; Jin 2008; Karpov 2005; Kim 2018; Kirgizova 2015; Lee 2014; Lunde 2006; Mathur 2020; Meluzin 2008; Nair 2015; Nogueira 2009; Penicka 2007; Piepoli 2010; Plewka 2009; Quyyumi 2011; Roncalli 2010; San Roman 2015; Sürder 2013; Tendera 2009; Turan 2012; Wollert 2004; Yao 2006; Yao 2009; You 2008; Zhang 2021; Zhukova 2009, which we considered to have a high risk of performance bias. Outcome assessors were reported to be blinded in all trials except six: one trial stated that study processes were not blinded (Hirsch 2011), and in five trials blinding of outcome assessors was not reported (Jazi 2012; Karpov 2005; Kirgizova 2015; Yao 2006; You 2008).

Incomplete outcome data

Twenty-two trials had a low risk of attrition bias as either all randomised participants were included in the analysis of all outcome data or all participant withdrawals were due to death or other major clinical adverse events (Angeli 2012; Cao 2009; Chen 2004; Colombo 2011; Ge 2006; Grajek 2010; Huang 2006; Huang 2007; Jin 2008; Kim 2018; Mathur 2020; Nogueira 2009; Penicka 2007; Piepoli 2010; Ruan 2005;San Roman 2015Suarez de Lezo 2007; Traverse 2010; Turan 2012; Yang 2020; You 2008; Zhukova 2009). We also deemed a further 20 trials to have a low risk of attrition bias as withdrawals were low and balanced between treatment arms (Choudry 2016; Gao 2013;Haddad 2020; Hirsch 2011; Huikuri 2008; Kirgizova 2015; Janssens 2006; Lunde 2006;Naseri 2018; Quyyumi 2017; Roncalli 2010; Schachinger 2006; Traverse 2011; Traverse 2018; Wang 2014; Wohrle 2010; Wollert 2004; Wollert 2017; Yao 2009; Zhang 2021).

In two trials the risk of attrition bias was unclear as the number of participants randomised to each treatment arm was not reported (Jazi 2012; Meluzin 2008). The number of withdrawals was unbalanced in a further three trials (Quyyumi 2011; Xiao 2012; Yao 2006), although reasons for participant withdrawal were reported; these trials were considered to have an unclear risk of bias. Six trials had a high risk of attrition bias. In three trials the number of withdrawals was high or unbalanced

between treatment arms (Lee 2014; Sürder 2013; Tendera 2009), in two trials there was incomplete participant overlap across multiple trial reports (Karpov 2005; Plewka 2009) and in one trial the withdrawal and exclusion rate was imbalanced and high (Nair 2015).

In the analysis of clinical outcomes, 28 trials included all randomised participants and 15 included over 90% of randomised participants. Seven trials included between 80% and 90% (Grajek 2010; Kirgizova 2015; Meluzin 2008;Naseri 2018Sürder 2013; Wollert 2017 ; Yao 2009). All seven trials explained the reasons for participant withdrawal or exclusion although in one trial these did not fully account for discrepancies in the number of participants included in individual analyses (Sürder 2013). One trial only included 72.5% of randomised participants in the analysis of clinical outcomes (Lee 2014); reasons included protocol violation, loss to follow-up and the opinion of the investigator. In one trial it was unclear how many participants were randomised to treatment (Jazi 2012). Another trial analyzed 75.2% of the randomized patients and performed a nested matched cohort analysis on 56.8% of the randomised patients a a primary analysis(Nair 2015).

In the analysis of LVEF, all trials that reported LVEF measured by echocardiography, SPECT, left ventricular angiography or radionuclide ventriculography included over 80% of randomised participants in the analysis of this outcome, with the exception of three trials, which analysed 75.2% (Nair 2015)), 72.5% (Lee 2014) and 60% (Plewka 2009) of randomised participants. A higher rate of withdrawals was observed in the analysis of LVEF measured by MRI in which five trials analysed less than 80% of randomised participants: 79.2% (Traverse 2018), 67.7% (Quyyumi 2011), 763.6% (Zhukova 2009), 58.5% (Tendera 2009) and 28.9% (Schachinger 2006), although it should be noted that not all participants are willing or able to undergo an MRI scan, potentially leading to an expected reduction in the number of patients analysed.

One trial was terminated prematurely after enrolment of the first 27 participants (Penicka 2007). The trial was reported as being terminated early “due to the unexpected occurrence of serious complications in the BMSC group and no incremental functional effects of BMSC as compared with control patients”. Fourteen of the 17 participants randomised to the BMSC arm provided scientific outcome data at four and 12-month follow-up assessments. All participants in the control arm were included in the final analysis in this trial.

Selective reporting

Out of 53 trials (with 4159 participants) only 21 trials (2080 participants) reported a published protocol (see Characteristics of included studies) and in this sub-sample there was no evidence of selective reporting. However, given that the majority of trials did not report details of their protocol it is difficult to ascertain whether these trials are at low risk of selective reporting. We considered three trial to have a high risk of

reporting bias. One study in which all prespecified adverse events are only mentioned together, but not with specific numbers per adverse event (Nair 2015), one study in which the authors failed to report quality of life and cost-effectiveness despite these outcomes being described in their trial protocol (Nogueira 2009) and one study in which the prespecified MRI-analysis (at 18 months) quality of life measurements and cardiopulmonary exercise tests where described in the protocol but not reported (Wollert 2017)

We identified no obvious asymmetry from a funnel plot for mortality (using the maximum duration of follow-up for all trials that reported mortality) (Figure 3). In a regression test for asymmetry (Egger's test, using the log(RR) and it's respective standard error), there was no significance at both short-term and long-term follow-up (P value = 0.15 and 0.42 respectively), suggesting no evidence of publication bias.

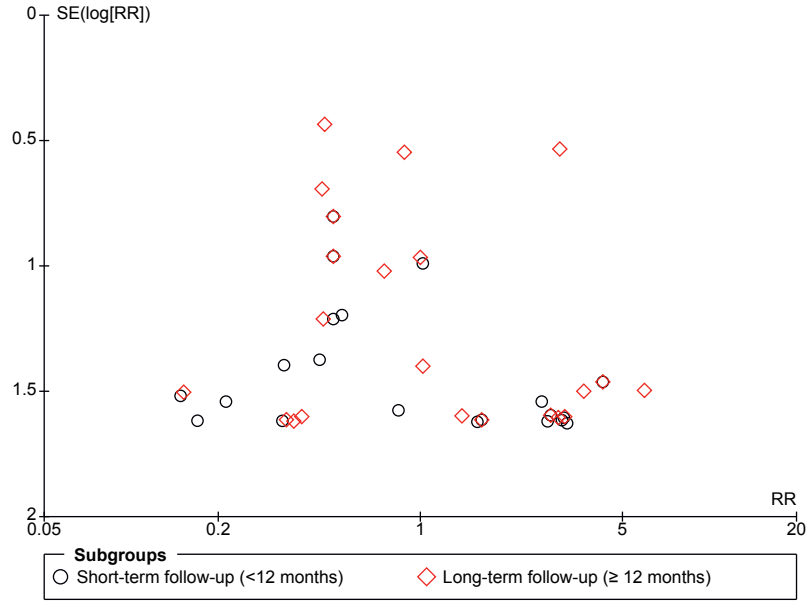


Figure 3. Funnel plot for mortality.

Other potential sources of bias

Six trials reported statistically significant baseline differences in participant characteristics between trial arms: Naseri 2018 reported an LVEDD and LVESD at baseline that was different between the two treatment groups (higher in the CD133 group compared to the mononuclear cell group). Quyyumi 2017. reported a longer ischemic time before primary PCI in the cell therpay group compared to the control group. Sürder 2013 reported a lower percentage of smokers in the late treatment arm than controls (40.3%

versus 62.7%; P value = 0.01) and a lower median baseline LVEF (median 35.6% versus 39.6%, P value = 0.03) in the cell therapy group compared with controls; Traverse 2011 reported a higher mean heart rate on initial presentation to the emergency department in the placebo group than the cell therapy group (90.3% versus 77.5%, P value = 0.01); Traverse 2018 observed high peak creatine kinase and troponin levels in the bone marrow cell (BMC) group randomised to day seven and a lack of diabetes in the placebo group randomised to day seven (P values not reported); and in Wohrle 2010 there was a significant baseline imbalance in the proportion of males (62% in the placebo group compared with 90% in the cell therapy group, P value = 0.04). These baseline differences are more likely to be a source of diversity than study bias.

Ten trials did not report the source of funding (Angeli 2012; Chen 2004; Huang 2006; Jazi 2012; Karpov 2005; Ruan 2005; Suarez de Lezo 2007; Wang 2014; Wohrle 2010; Zhukova 2009). Of 43 trials that reported funding and support, all but four trials (Haddad 2020, Lee 2014, Quyyumi 2017and Schachinger 2006), received research grant funding from universities, charities or governmental agencies (see Characteristics of included studies).Haddad 2020 received support through a research grant from Miltenyi Biotec, Schachinger 2006 received a research grant from Guidant (Guidant Corporation, part of Boston Scientific, which designs and manufactures cardiovascular medical products), as well as support from Eli Lilly (Eli Lilly is a global pharmaceutical company). Quyyumi 2017 was funded and conducted by Caladrius Biosciences and Lee 2014 was funded by PCB-Pharmicell Company Limited, Seongnam, South Korea (a biotechnology company focusing on the development and commercialisation of stem cell therapeutics). Five trials were commercially funded in part: Huikuri 2008 received a research grant from Boston Scientific Sverige AB (a global pharmaceutical company); Grajek 2010 received a research grant from Servier Polska (a global pharmaceutical company); Hirsch 2011 received "unrestricted grants" from Biotronik (Biotronik designs and manufactures cardiovascular medical products), Boston Scientific, Guerbet (Guerbet designs and manufactures medical imaging products including contrast agents), Medtronic (Medtronic designs and manufactures cardiovascular medical products), Novartis, Pfizer and Sanofi-Aventis (all global pharmaceutical companies). Mathur 2020 multiple authors have reported fees from pharmaceutical companies and one author is co-founder of t2cure, a manufacturer of cellular products. Quyyumi 2011 was funded by Amorcyte Inc (Amorcyte Inc. develops cell therapy products to treat cardiovascular disease); and in Nogueira 2009 cell preparation and characterisation was carried out by Exellion Biomedical Services S/A. Wollert 2017 Two authors have applied for a patient regarding the therapeutic potential of cell-secreted growth factors.

A total of 76 patients from 12 trials randomised to cell therapy did not receive treatment as randomised but were included in the analysis (Hirsch 2011; Lunde 2006; Meluzin 2008;Mathur 2020; Nair 2015; Nogueira 2009;Quyyumi 2017; Penicka 2007; Roncalli 2010; Traverse 2011; Wollert 2017Yao 2009), as well as 16 patients randomised

to a placebo arm who did not receive the placebo medium (Schachinger 2006; Quyyumi 2017; Wollert 2017); in most cases this was due to adverse clinical events, which precluded cell or placebo administration. In other studies (Mathur 2020;Nair 2015; Quyyumi 2017) patients refused, the quality or yield of cells was poor, or the procedure wasn't possible (either due to technical or timing issues).

Effects of interventions

An overview of results for the primary outcomes of all-cause mortality, cardiovascular mortality and composite measures of major adverse cardiac events (MACE) are given in Summary of findings table 1. An overview off all recorded outcome measures per trial and the total amount of patients analyzed per trial can be found in Table 3.

Primary outcomes

*All-cause mortality*Twenty-two trials reported incidences of mortality in the short-term follow-up period of less than 12 months from cell therapy (Gao 2013; Huikuri 2008; Janssens 2006; Kim 2018; Nair 2015;Naseri 2018Nogueira 2009; Penicka 2007; Piepoli 2010; Plewka 2009; Quyyumi 2011;Quyyumi 2017; Roncalli 2010; Schachinger 2006; Sürder 2013; Tendera 2009; Traverse 2011; Traverse 2018; Wang 2014; Wohrle 2010;Wollert 2017; Zhukova 2009). All incidences of mortality in the short-term follow-up period occurred within 12 months of cell therapy. Some trials reported no events for short-term mortality (see Table 3).

In trials that reported long-term follow-up, 22 reported incidences of mortality (Cao 2009; Choudry 2016; Gao 2013; Grajek 2010; Haddad 2020; Hirsch 2011; Karpov 2005; Kirgizova 2015; Lunde 2006; Mathur 2020; Naseri 2018; Penicka 2007; Piepoli 2010; Plewka 2009; Quyyumi 2011; Quyyumi 2017;San Roman 2015; Schachinger 2006; Traverse 2018; Wollert 2004; Zhang 2021; Zhukova 2009), with several other trials reporting no deaths during long-term follow-up (see Table 3). The duration of long-term followup ranged from 12 months (Grajek 2010; Piepoli 2010; Quyyumi 2011; Quyyumi 2017; San Roman 2015; Zhang 2021), 18 months (Naseri 2018), 24 months (Gao 2013; Mathur 2020; Penicka 2007; Plewka 2009; Traverse 2018), 36 months (Lunde 2006; Zhukova 2009) and 48 months (Cao 2009), to 60 months (Hirsch 2011; cChoudry 2016Schachinger 2006; Wollert 2004). Three trials stood out with a mean follow-up of 7.7 years (Kirgizova 2015), 8.2 years (Karpov 2005) and a median follow-up of 8.5 years (Haddad 2020, no mean follow-up reported).

The mortality incidence rate was low in all trials. Overall, there was no evidence for a difference in the risk of mortality between patients who received cell therapy and those who received no cells at short-term (24/1145 versus 18/779; risk ratio (RR) 0.79, 95% confidence interval (CI) 0.44 to 1.40; 1924 participants; 21 studies) or long-term follow-up (49/998 versus 51/912; RR 0.88, 95% CI 0.60 to 1.31; 1910 participants; 22 studies) with no evidence of heterogeneity (I2 = 0% in both analyses) (Analysis 1.1).

Sensitivity analyses did not affect the results for mortality. Exclusion of the trials that did not administer cells via the coronary artery (Naseri 2018; Nogueira 2009), did not affect short-term mortality (22/1076 versus 18/749; RR 0.77, 95% CI 0.42 to 1.39; 1825 participants; 18 studies) or long-term mortality (48/953 versus 51/888; RR 0.87, 95% CI 0.59-1.30; 1841 participants; 21 studies) (Analysis 2.1). Only one trial included in the analysis of short-term follow-up had a high risk of selection bias due to lack of appropriate randomisation sequence generation (Wang 2014); the difference in risk of mortality between groups when we excluded this trial was negligible (21/1098 versus 12/730; RR 0.87, 95% CI 0.46 to 1.65; 1828 participants; 19 studies) (Analysis 3.1). No trials reporting longterm follow-up had a high risk of selection bias due to randomisation methods. When we excluded trials with a high or unclear risk of attrition bias, there remained no evidence for a difference in all-cause mortality at either short-term (16/705 versus 15/527; RR 0.72, 95% CI 0.37 to 1.41; 1232 participants; 16 studies) or long-term follow-up (35/904 versus 38/27; RR 0.75, 95% CI 0.47 to 1.20; 1731 participants; 18 studies) (Analysis 4.1). Similarly, exclusion of trials with a high risk of performance bias due to lack of blinding revealed no evidence for differences in the risk of mortality at either short-term (8/568 versus 10/423; RR 0.63, 95% CI 0.26 to 1.50; 991 participants; 10 studies) or long-term follow-up (16/522 versus 21/450; RR 0.64, 95% CI 0.33 to 1.24; 972 participants; 10 studies) (Analysis 5.1).

Subgroup analysis of mortality measured at both short-term and long-term follow-up revealed no differences between trials grouped according to baseline left ventricular ejection fraction (LVEF, < or ≥45%) as measured by magnetic resonance imaging (MRI) (Analysis 6.1; Analysis 6.2), cell type (Analysis 7.1; Analysis 7.2), cell dose (Analysis 8.1; Analysis 8.2), timing of cell infusion (Analysis 9.1; Analysis 9.2), or use of heparinised cell solution (Analysis 10.1; Analysis 10.2).

Cardiovascular mortality

Incidence of cardiovascular mortality was reported in nine trials at short-term follow-up (Gao 2013; Huikuri 2008; Nair 2015; Penicka 2007; Piepoli 2010; Plewka 2009; Quyyumi 2011; Quyyumi 2017; Zhukova 2009), and 13 trials at long-term follow-up (Gao 2013; Karpov 2005; Kirgizova 2015; Mathur 2020; Naseri 2018; Penicka 2007; Piepoli 2010; Plewka 2009; Quyyumi 2011; Quyyumi 2017; Schachinger 2006; Wollert 2004; Zhukova 2009). There was no evidence for a difference in the risk of cardiovascular mortality at either short-term (8/348 versus 9/329; RR 0.73, 95% CI 0.31 to 1.71; 777 participants; nine studies) or at long-term follow-up (29/595 versus 29/563; RR 0.91, 95% CI 0.55 to 1.53; 1158 participants; 13 studies) (Analysis 1.2).

None of the trials that reported cardiovascular mortality had a high risk of selection bias. The lack of evidence for a difference in the risk of cardiovascular mortality remained when we excluded trials with a high or unclear risk of attrition bias at both short-term (4/183 versus 7/177; RR 0.59, 95% CI 0.20 to 1.72; 360 participants; four

studies) and long-term follow-up (16/503 versus 21/480; RR 0.70, 95% CI 0.37 to 1.31; 983 participants; nine studies) (Analysis 4.2). The sensitivity analysis for high risk of performance bias did not detect any important differences between the groups at short-term (1/227 versus 3/240; RR 0.59, 95% CI 0.10 to 3.56; 467 participants; three studies) or long-term follow-up (6/227 versus 12/240; RR 0.54, 95% CI 0.21 to 1.38; 467 participants; three studies) (Analysis 5.2).

Composite measures of major adverse cardiac events (MACE)

Composite measures of MACE were reported in 17 trials (Choudry 2016; Gao 2013; Haddad 2020; Hirsch 2011;Kim 2018; Mathur 2020; Naseri 2018; Penicka 2007; Plewka 2009; Quyyumi 2017; San Roman 2015; Schachinger 2006; Sürder 2013; Traverse 2018; Wohrle 2010; Wollert 2004; Xiao 2012). Six trials defined composite MACE as death, reinfarction or re-hospitalisation for heart failure (Gao 2013; Hirsch 2011; Penicka 2007; Schachinger 2006; Wohrle 2010; Wollert 2004). Other definitions of composite MACE were as follows: death, reinfarction or target vessel revascularisation (Hirsch 2011; Schachinger 2006), death, reinfarction, re-hospitalisation for heart failure or revascularisation (Plewka 2009; Sürder 2013), death, reinfarction, revascularisation, ICD-implantation (Choudry 2016), death, reinfarction, re-hospitalisation for heart failure, stroke or arrhythmia (Gao 2013), death, reinfarction, re-hospitalisation for heart failure, revascularisation or stroke (Haddad 2020), death, reinfarction, re-hospitalisation for heart failure, revascularisation or arrhythmia, although this trial failed to mention the exact numbers for this outcome (Kim 2018), death, reinfarction, ICD-implantation, infection or arrhythmia (Naseri 2018), cardiovascular death or rehospitalization for heart failure (Mathur 2020) rehospitalization for reinfarction, heart failure, revascularisation, implantable cardioverter-defibrillator (ICD) implantation or stroke (Mathur 2020) , cardiovacsular death, heart failure, reinfarction or revascularization (Quyyumi 2017), death, reinfarction, heart failure, rehospitalisation, revascularisation, ventricular arrhytmia or stroke (San Roman 2015), death, reinfarction, re-hospitalisation for heart failure, revascularisation, implantable cardioverter-defibrillator (ICD) implantation or stroke (Traverse 2018). One trial did not define the composite measure of MACE (Xiao 2012). Analysis was restricted to composite death, reinfarction or re-hospitalisation for heart failure due to the lack of data from alternative measures. Of note, one study with mortality data reported at five-year follow-up only reported two-year follow-up data for composite MACE, the incidence of which is lower than the five-year mortality rate (Schachinger 2006).

There was no evidence for a reduction in the risk of composite death, reinfarction or re-hospitalisation for heart failure associated with cell therapy at either short-term (5/198 versus 12/181; RR 0.36, 95% CI 0.12 to 1.14; 379 participants; three studies) or long-term follow-up (24/262 versus 33/235; RR 0.63, 95% CI 0.36 to 1.10; 497 participants; six studies) with low or negligible heterogeneity in both analyses (I2 = 0%;

I2 = 12% respectively) (Analysis 1.3). The limited number of trials that reported other composite measures of MACE at short-term or long-term follow-up prevented formal analysis of these outcomes.

We did not perform sensitivity analysis as no trials that reported composite measures of MACE had a high risk of selection bias or a high or unclear risk of attrition bias, and the number of appropriately blinded trials precluded sensitivity analysis for performance bias.

Periprocedural adverse events

Thirty-four trials reported periprocedural adverse events as an outcome, eight of which reported no periprocedural adverse events (Colombo 2011; Ge 2006; Karpov 2005; Kim 2018; Naseri 2018; Traverse 2010; Turan 2012; Wollert 2004) (see Table 4 for details). Adverse events associated with bone marrow aspiration were rare; one trial reported a serious adverse event at the time of bone marrow harvest (one patient experienced a stent thrombosis with reinfarction which occurred immediately after the procedure) (Penicka 2007); a second trial reported three patients with mild self limiting vasovagal reactions during bone marrow aspiration (Huikuri 2008). A third trial reported eight serious adverse events from time of BM harvest up to start of cell infusion, although these serious adverse events were not otherwise specified (Quyyumi 2017). No other adverse events associated with bone marrow harvest were reported. Three deaths were reported in patients randomised to cell therapy prior to cell infusion (one patient died due to subarachnoid haemorrhage (Traverse 2018) and in two patients the cause of death was not reported (Sürder 2013)), and three patients died soon after cell therapy was administered (one at three days after cell therapy due to suspected acute in-stent thrombosis (Gao 2013), one patiente experienced ventricular fibrillation attributed to recurrent myocardial infarction from stent thrombosis preceding cell infusion (Quyyumi 2011), and one with cause of death not reported (Schachinger 2006)). Five patients had an adverse event during cell infusion in another trial, (one dissection, one stent trombosis, two flow reductions after cell infusion and one major groin hematoma for which transfusion was needed)(Wollert 2017). Two patients had ventricular fibrillation between the time of bone marrow harvest and cell infusion, which was adequately defibrillated (Choudry 2016). Other serious periprocedural adverse events observed in patients who received cell therapy included one transient acute heart failure (Cao 2009), one acute coronary occlusion during cell injection (Gao 2013), one patient with a small thrombus in the infarct-related artery diagnosed immediately after cell transplantation (Meluzin 2008), one patient with sub-acute stent thrombosis (Huikuri 2008), six patients with periprocedural myocardial infarction (Lee 2014; San Roman 2015Schachinger 2006), six patients with acute or subacute stent thrombosis, including one death (vs four patients in the control group) and one transient ischaemic attack in the control group (Nair 2015) one transient

ischaemic attack (Roncalli 2010), and one post-procedural arteriovenous fistula of the femoral artery (Tendera 2009).

In summary, serious periprocedural adverse events were rare and unlikely to be associated with treatment.

Secondary outcomes

Reinfarction

Twentyone trials reported incidences of reinfarction in the short-term follow-up period of less than 12 months from stem cell therapy (Gao 2013; Grajek 2010; Hirsch 2011; Huikuri 2008; Karpov 2005;Kim 2018; Lee 2014; Lunde 2006; Mathur 2020; Meluzin 2008; Naseri 2018; Penicka 2007; Plewka 2009; Sürder 2013; Tendera 2009; Traverse 2011; Traverse 2018; Wollert 2004; Yao 2009). A further five trials reported that no incidences of reinfarction occurred during short-term follow-up (Cao 2009; Huang 2006; Jazi 2012; Suarez de Lezo 2007; Wohrle 2010).

Incidences of reinfarction occurred in 24 trials at long-term follow-up (Choudry 2016; Gao 2013; Haddad 2020; Hirsch 2011; Karpov 2005; Kim 2018; Kirgizova 2015; Lunde 2006; Mathur 2020; Meluzin 2008; Naseri 2018; Penicka 2007; Plewka 2009; Quyyumi 2017;San Roman 2015Schachinger 2006; Traverse 2010; Traverse 2018; Wollert 2004; Yang 2020; Yao 2006; Yao 2009; Zhang 2021; Zhukova 2009; one further trial reported no incidences of reinfarction (Cao 2009).

There was no evidence for a difference in the risk of reinfarction between treatment groups at either short-term (21/1157 versus 25/808; RR 0.63, 95% CI 0.36 to 1.12; 2094 participants; 19 studies) or long-term follow-up (42/1066 versus 47/927; RR 0.87, 95% CI 0.57 to 1.33; 1993 participants; 21 studies) with no evidence of heterogeneity (I2 = 0% for both analyses) (Analysis 1.4).

Arrhythmias

Twenty-eight trials reported arrhythmia as an outcome, although two trials reported summary results only (Piepoli 2010; Yao 2009), and in 13 of these trials arrhythmias were not observed during follow-up (see Table 3) and in one trial it was mentioned to be measured as an outcome but the incidence was not reported in the results (Nair 2015). In 14 trials that reported incidences of arrhythmias, arrhythmia was defined as incidences of supraventricular arrhythmia (Janssens 2006), supraventricular tachycardia (Zhukova 2009), atrial fibrillation and ventricular tachycardia (Mathur 2020), documented ventricular arrhythmia (Schachinger 2006), ventricular fibrillation (Hirsch 2011), sustained ventricular arrhythmia (Lunde 2006), repetitive ventricular arrhythmia (Colombo 2011), sustained ventricular tachycardia, ventricular fibrillation or torsades de pointes (Haddad 2020), ventricular arrhythmia or syncope (San Roman 2015), malignant ventricular arrhythmias such as ventricular tachycardia, flutter or fibrillation (Yang 2020), clinically significant arrhythmia (Kirgizova 2015) malignant arrhythmia

(Xiao 2012), malignant arrhythmia/syncope (Zhang 2021) and arrhythmia (unspecified) (Naseri 2018; Roncalli 2010).

Six trials reported incidences of arrhythmias at short-term follow-up (Hirsch 2011; Janssens 2006; Kim 2018; Roncalli 2010; Schachinger 2006; Xiao 2012). There was no evidence for a difference in the risk of arrhythmias at short-term follow-up between patients who received cell therapy and those who did not (16/278 versus 15/273; RR 1.05, 95% CI 0.54 to 2.03; 551 participants; six studies). Similarly, in 12 trials that reported incidences of arrhythmia at long-term follow-up (Cao 2009; Colombo 2011; Hirsch 2011; Karpov 2005; Kim 2018; Lunde 2006; Mathur 2020; San Roman 2015; Schachinger 2006; Yang 2020; Zhang 2021), there was no difference in the risk of arrhythmias between treatment arms (34/517 versus 41/523; RR 0.85, 95% CI 0.55 to 1.32; 1040 participants; nine studies) (Analysis 1.6).

Restenosis

Fifteen trials reported incidences of restenosis during follow-up (Cao 2009; Grajek 2010; Huikuri 2008; Janssens 2006; Jazi 2012; Lunde 2006; Meluzin 2008; Nogueira 2009; Penicka 2007; Piepoli 2010; Quyyumi 2011; Roncalli 2010;Suarez de Lezo 2007; Traverse 2010; Wohrle 2010; Wollert 2004; Yao 2006). However, one trial did not report restenosis as an outcome in the control arm of the trial (Nogueira 2009), and one trial reported results descriptively (Huikuri 2008). One trial with long-term follow-up data did not report individual group sample sizes (Meluzin 2008). Two trials reported no incidences of restenosis during follow-up (Jazi 2012; Suarez de Lezo 2007).

Restenosis at short-term follow-up was reported in eight trials (Grajek 2010; Janssens 2006; Lunde 2006; Meluzin 2008; Roncalli 2010; Wohrle 2010; Wollert 2004; Yao 2006). The rate of restenosis at short-term follow-up was similar in patients who received cell therapy and in the control group (42/353 versus 34/288; RR 0.95, 95% CI 0.63 to 1.43; 641 participants; eight studies). There was also no evidence for a difference in the risk of restenosis at long-term follow-up in five trials (Cao 2009; Penicka 2007; Piepoli 2010; Traverse 2010; Yao 2006) (10/213 versus 14/182; RR 0.58, 95% CI 0.27 to 1.25; 395 participants; six studies) (Analysis 1.7). The 2022 update did not provide any new data to this analysis.

Target vessel revascularisation

The requirement for percutaneous coronary intervention in the infarct-related vessel during follow-up and after the therapy procedure was determined as target vessel revascularisation. Nineteen trials reported incidences of target vessel revascularization in one or both trial arms (Cao 2009; Choudry 2016; Grajek 2010; Hirsch 2011; Janssens 2006; Kim 2018; Lee 2014; Lunde 2006; Quyyumi 2011; Schachinger 2006; Suarez de Lezo 2007; Tendera 2009; Traverse 2010; Traverse 2011; Traverse 2018; Wohrle 2010; Wollert 2004; Yang 2020; Zhang 2021). Seven trials reported no events of target vessel

revascularisation during follow-up (Janssens 2006;Kim 2018; Lee 2014; Suarez de Lezo 2007; Wohrle 2010; Yang 2020; Zhang 2021). One trial reported target vessel revascularization as a potential outcome in methods, but did not report an incidence in the results (San Roman 2015).

At short-term follow-up, there was no evidence for a difference in the risk of target vessel revascularisation between patients who received cell therapy and those who did not (50/497 versus 40/292; RR 0.70, 95% CI 0.47 to 1.06; 789 participants; six studies). There was also no difference in the risk of target vessel revascularisation between treatment arms at long-term follow-up (71/454 versus 65/389; RR 1.02, 95% CI 0.74 to 1.40; 843 participants; nine studies) (Analysis 1.8).

Of note, the incidence of restenosis seems to be lower than the incidence of target vessel revascularisation, and this may look like a discrepancy as the latter is a consequence of the former. However, the trials included in these two meta-analyses differ, as not all trials reported both outcomes. Three trials reported both restenosis and target vessel revascularisation (Cao 2009; Quyyumi 2011; Traverse 2010), and the numbers were the same for both outcomes.

Re-hospitalisation for heart failure

Incidences of hospital readmission for heart failure were reported in 15 trials at short-term follow-up (Colombo 2011; Hirsch 2011; Huikuri 2008; Kim 2018Lunde 2006; Meluzin 2008; Penicka 2007; Roncalli 2010; Schachinger 2006; Sürder 2013; Traverse 2011; Traverse 2018; Wohrle 2010; Wollert 2004; Wollert 2017), and 17 trials at long-term follow-up (Choudry 2016; Colombo 2011; Gao 2013; Haddad 2020; Hirsch 2011; Kim 2018; Lunde 2006; Mathur 2020;Meluzin 2008; Penicka 2007; Plewka 2009; Quyyumi 2011; Quyyumi 2017; Schachinger 2006; Traverse 2018; Wollert 2004; Zhang 2021). However, in one trial reporting discrepancies between publications could not be resolved with the study authors and therefore we omitted this study from the analysis at long-term follow-up (Colombo 2011).

At short-term follow-up there was no evidence for a difference in the risk of re-hospitalisation for heart failure between patients who received cell therapy and those who did not (18/769 versus 16/548; RR 0.77, 95% CI 0.39 to 1.51; 1317 participants; 15 studies). However, at long-term follow-up of 12 months or longer, there was marginally significant evidence for a difference between treatment groups in favour of cell therapy (37/817 versus 54/729; RR 0.65, 95% CI 0.42 to 1.00; 1546 participants; 17 studies) (Analysis 1.5).

Quality of life and performance status

Quality of life measures were reported in seven trials (Choudry 2016; Jin 2008; Karpov 2005; Lunde 2006; Penicka 2007; Roncalli 2010; You 2008). Three trials used the Minnesota Living with Heart Failure Questionnaire (MLHFQ) (Jin 2008; Karpov 2005;

Roncalli 2010), two trials used the Short Form 36 Health Survey (Lunde 2006; Penicka 2007) and one trial used the European Quality of Life-5 Dimensions and Visual Analog Scale (VAS) at follow-up for which the mean change of the VAS was used in the analysis (Choudry 2016); in one trial the quality of life measure was undefined (You 2008) (see Table 5). Three trials only reported summary results and therefore could not be included in the meta-analysis (Penicka 2007; Roncalli 2010; You 2008). At short-term follow-up there was no difference in quality of life score between treatment groups (standardised mean difference (SMD) 0.38, 95% CI -0.43 to 1.19; 209 participants; four studies). Similarly, at long term follow-up there was no difference in quality of life scores between treatment groups (SMD 1.46, 95% CI -1.91 to 4.84; 81 participants; two studies).

Eleven trials measured New York Heart Association (NYHA) class as a measure of performance status at follow-up (Choudry 2016; Hirsch 2011; Jazi 2012; Jin 2008; Lunde 2006;Naseri 2018; Penicka 2007; Sürder 2013; Turan 2012; You 2008; Zhang 2021), although one trial only reported the percentage of patients in NYHA class I (Choudry 2016), another trial reported only percentages of patients in class ≥II or higher (Kirgizova 2015) and another trial reported summary results only (You 2008). Functional classification of heart failure was also measured in one further trial but it was unclear whether this was NYHA class (Karpov 2005). At short-term follow-up, in five trials there was no difference in NYHA class at the time of follow-up between patients who received cell therapy and those who did not (mean difference (MD) -0.07, 95% CI -0.24 to 0.09; 398 participants; five studies). Similarly, at long-term follow-up there was no difference in NYHA class (MD -0.15, 95% CI -0.34 to 0.04; 342 participants; six studies) (Analysis 1.2), with considerable heterogeneity between studies (I2 = 68%).

The use of exercise tests to measure performance was reported in seven trials (Colombo 2011; Grajek 2010; Huikuri 2008; Karpov 2005;Kirgizova 2015; Lunde 2006; Piepoli 2010). Exercise performance was evaluated using a treadmill test (Grajek 2010; Piepoli 2010), a six minute walk test (Karpov 2005; Kirgizova 2015), an electrically braked bicycle ergometer (Lunde 2006), and a symptomlimited maximal exercise test (Huikuri 2008). The method of measuring exercise tolerance was not reported in one trial (Colombo 2011) (see Table 5); we excluded this trial from meta-analyses of exercise tolerance as median rather than mean values were reported. Meta-analysis of the remaining trials showed no difference in exercise tolerance at short-term follow-up between patients who received cell therapy and those who did not (SMD 0.19, 95% CI -0.06 to 0.43; 267 participants; five studies) or long-term followup (MD 0.08, 95% CI -0.41 to 0.58;71 participants; two studies) (Analysis 1.2).

Similarly there were no differences in maximum VO2 (MD 1.15 mL/kg/min, 95% CI -0.77 to 3.07; 175 participants; three studies) (Analysis 1.2), VE/VCO2 slope (MD 0.28, 95% CI -1.02 to 1.57; 174 participants; three studies) (Analysis 1.3) or peak heart rate (MD 0.55 bpm, 95% CI -6.79 to 7.89; 198 participants; three studies) (Analysis 1.3). Two trials reported exercise tolerance at long-term follow-up (Grajek 2010; Piepoli 2010);

although the latter trial did not report endpoint values. In the remaining trial there was no difference between treatment groups (SMD -0.05, 95% CI -0.68 to 0.58; 45 participants; one study) (Analysis 1.2).

Left ventricular ejection fraction (LVEF)

In order to limit possible heterogeneity, we have subgrouped trials reporting LVEF by the method of measurement. Results are shown in forest plots for the combined analyses of mean change from baseline and endpoint values as well as separately, as described in the Methods section.

Eighteen trials used multiple methods to measure left ventricular function (Angeli 2012; Cao 2009; Choudry 2016; Grajek 2010; Huang 2006; Huikuri 2008;Kim 2018; Lee 2014; Lunde 2006;Naseri 2018; Nogueira 2009; Piepoli 2010; Plewka 2009; Quyyumi 2017; Roncalli 2010; Schachinger 2006; Yang 2020; Zhang 2021). Three trials measured these outcomes by three methods: MRI, echocardiography and LV angiography (Choudry 2016), MRI, echocardiography and single photon emission computed tomography (SPECT) (Lunde 2006), or MRI, echocardiography and radionuclide ventriculography (RNV) (Roncalli 2010). The 15 remaining trials each measured these outcomes using two methods: seven used echocardiography and SPECT (Angeli 2012; Cao 2009;Kim 2018; Lee 2014;Naseri 2018; Piepoli 2010; Plewka 2009; Zhang 2021), two used MRI and left ventricular angiography (Huang 2006; Schachinger 2006), two used echocardiography and RNV (Grajek 2010; Nogueira 2009), one used MRI and echocardiography (Yang 2020), one used MRI and SPECT (Quyyumi 2017) and one used left ventricular angiography and echocardiography (Huikuri 2008). Baseline LVEF values for each trial are given in Table 6 for each method of measurement. In the studies that used multiple modalities, all outcome measures were included in the analyses.

(i) Magnetic resonance imaging (MRI)

Five trials measured baseline LVEF by MRI after cell administration, at one to three days after cells (Tendera 2009), at three to five days after cells (Janssens 2006), between four days prior to six days after cells (Schachinger 2006), after one week (Huang 2006), and after two to three weeks (Lunde 2006); these trials have been pooled alongside the outcome data for all other trials.

Eightteen trials reported LVEF measured by MRI at short-term follow-up (Choudry 2016;Hirsch 2011; Huang 2006; Janssens 2006; Lunde 2006; Quyyumi 2011; Quyyumi 2017; Roncalli 2010; Schachinger 2006; Sürder 2013; Tendera 2009; Traverse 2010; Traverse 2011; Traverse 2018; Wohrle 2010; Wollert 2004; Wollert 2017; Yao 2009), with all but two trials, Huang 2006 and Yao 2009, reporting mean change from baseline values. In the combined analysis of mean change from baseline and endpoint values, there was no evidence for a difference in mean LVEF between treatment arms (MD 1.07, 95% CI -0.31 to 2.44; 1476 participants; 18 studies); we observed substantial

heterogeneity across studies (I2 = 60%) (Analysis 1.15).

At long-term follow-up, mean change from baseline values were reported in eight trials (Choudry 2016; Hirsch 2011; Janssens 2006; San Roman 2015; Sürder 2013; Traverse 2018; Wohrle 2010; Wollert 2004); a further four trials reported endpoint values only (Lunde 2006; Schachinger 2006; Yao 2009; Zhukova 2009), although in one trial LVEF was only reported for two patients (Zhukova 2009); we therefore excluded this trial from the meta-analysis. In the combined analysis of mean change from baseline and endpoint values (MD 1.84, 95% CI 0.12 to 3.57; 988 participants; 12 studies). There was evidence of substantial heterogeneity across studies (I2 = 60%) (Analysis 1.16).

We observed substantial heterogeneity at both short-term (I2 = 60%) and long-term follow-up (I2 = 60%).

We carried out exploratory subgroup analyses to investigate potential sources of heterogeneity as described in the Methods section. .There was a significant improvement on short-term LVEF measured by MRI when baseline LVEF was 108 to 109 cells did not show a significant improvement (MD 1.58, 95% CI -0.92-4.08; 512 participants, eight studies) with the test for subgroup differences gave a Chi2 of 1.02, p=0.31 (Analysis 8.4). For other subgroup analyses, both timing of cell administration (Analysis 9.3; Analysis 9.4) or use of heparinised cell solution (Analysis 10.3; Analysis 10.4) at either short-term or long-term follow-up showed no clear difference on LVEF measured by MRI. There were insufficient trials using cells other than mononuclear cells to perform subgroup analysis for cell type.

(ii) Echocardiography

LVEF measured by echocardiography at short-term follow-up was reported in 21 trials (Angeli 2012; Cao 2009; Colombo 2011; Gao 2013; Ge 2006; Grajek 2010; Huang 2007; Huikuri 2008; Jin 2008; Karpov 2005; Kim 2018; Lee 2014; Lunde 2006; Nogueira 2009; Penicka 2007; Piepoli 2010; Plewka 2009; Roncalli 2010; Ruan 2005; Xiao 2012; You 2008). Of these 21 trials, all reported endpoint LVEF values but only seven reported mean change from baseline values (Gao 2013; Huang 2007; Huikuri 2008;Kim 2018; Lee 2014; Lunde 2006; Plewka 2009). Meta-analysis of these seven trials showed evidence for a difference in mean change from baseline LVEF in favour of cell therapy (MD 2.83, 95% CI 1.76 to 3.89; 398 participants; seven studies). This improvement in LVEF associated with cell therapy was also seen in the combined analysis of all 21 trials (MD 2.41, 95% CI 1.44 to 3.37; 888 participants; 21 studies) (Analysis 1.2). The observed difference was robust to sensitivity analysis excluding the trial that administered cells via the coronary artery (Nogueira 2009).

At long-term follow-up, only five trials reported mean change in LVEF from baseline (Gao 2013;Kim 2018; Kirgizova 2015; Piepoli 2010; Plewka 2009). Meta-analysis of these five trials showed no evidence for a difference in mean change from baseline values

between trial arms (MD 2.09, 95% CI -0.29 to 4.48; 179 participants; five studies). However, in 13 trials that reported LVEF values at the time of follow-up (Angeli 2012; Cao 2009; Colombo 2011; Gao 2013; Grajek 2010; Jin 2008;Kim 2018; Kirgizova 2015; Lunde 2006; Penicka 2007; Piepoli 2010; Yang 2020; Zhang 2021), LVEF values at follow-up were higher in patients who received cell therapy than those who did not (MD 2.43, 95% CI 1.27 to 3.58; 542 participants; 13 studies). Evidence for an improvement in LVEF associated with cell therapy was also seen in the combined analysis (MD 1.89, 95% CI 0.89 to 2.90; 598 participants; 14 studies) (Analysis 1.2).
The observed heterogeneity was moderate (I2 = 35%) at short-term follow-up and low at long-term follow-up (I2 = 0%) and therefore we performed no exploratory subgroup analyses for LVEF measured by echocardiography.

(iii) SPECT
Eight trials reported LVEF measured by SPECT at short-term follow-up (Angeli 2012; Cao 2009;Kim 2018; Lee 2014; Lunde 2006; Meluzin 2008; Piepoli 2010; Plewka 2009), of which six trials reported mean change from baseline values (Kim 2018; Lee 2014; Lunde 2006; Meluzin 2008; Piepoli 2010; Plewka 2009). In one trial, endpoint values (but not mean change values) reflect an expanded cohort (Meluzin 2008). Meta-analysis showed a greater mean change from baseline values in patients who received cell therapy compared with those who did not (MD 3.14, 95% CI 1.32 to 4.97; 312 participants; six studies). This effect was also demonstrated in seven trials that reported LVEF values measured by SPECT at follow-up (MD 2.57, 95% CI 1.36 to 3.77; 401 participants; seven studies) and in the combined analysis of mean change from baseline and endpoint values (MD 2.93, 95% CI 1.44 to 4.43; 420 participants; eight studies) (Analysis 1.3).

An improvement in LVEF measured by SPECT associated with cell therapy was also found at long-term follow-up in five trials (Angeli 2012; Cao 2009; Meluzin 2008;Naseri 2018; Piepoli 2010) (MD 5.24, 95% CI 3.48 to 7.00; 269 participants; five studies); this improvement was observed in both trials that reported mean change from baseline (MD 5.63, 95% CI 1.77 to 9.49; 92 participants; two studies) and trials that only reported endpoint values (MD 4.58, 95% CI 2.33 to 6.83; 250 participants; four studies) (Analysis 1.3).

There was only moderate evidence for heterogeneity at borh long-term follow-up (I2 = 34%) and short-term follow-up (I2 = 36%). We therefore did not perform subgroup analyses.

(iv) Left ventricular angiography
Ten trials reported LVEF measured by left ventricular angiography at short-term follow-up (Chen 2004; Choudry 2016Huang 2006; Huikuri 2008; Jazi 2012; Schachinger 2006; Suarez de Lezo 2007; Turan 2012; Wang 2014; Yao 2006). All trials reported endpoint

LVEF values but only four reported mean change from baseline values (Choudry 2016; Huikuri 2008; Schachinger 2006; Suarez de Lezo 2007). Meta-analysis of these four trials showed a evidence for a difference in mean change from baseline LVEF in favour of cell therapy (MD 5.06, 95% CI 0.96 to 9.16; 366 participants; four studies). In the combined analysis of all nine trials, this effect remained (MD 4.81, 95% CI 0.92 to 8.69; 798 participants; ten studies) with considerable heterogeneity across studies (I2 = 95%) (Analysis 1.3). Two trials reported long-term follow-up of LVEF measured by left ventricular angiography (Turan 2012; San Roman 2015); The two trials combined did not find a significantly higher mean LVEF at follow-up in patients who received cell therapy compared with those who did not (MD 5.0, 95% CI 4-1.23 to 11.28; 160 participants; two studies) (Analysis 1.4). We observed considerable heterogeneity at short-term follow-up (I2 = 95%). Visual inspection of the forest plot revealed two potential outliers (Chen 2004; Yao 2006), although considerable heterogeneity remained when we excluded these two studies from the analysis. Exploratory subgroup analyses revealed that when trials were subgrouped according to cell dose, meta-analysis of two trials that used > 109 cells showed a significant difference when compared to six trials that used > 108 and ≤ 109 cells (test for subgroup differences, P value = 0.0008) (Analysis 8.5), although substantial heterogeneity remained in both subgroups. We found no subgroup differences when we subgrouped trials by either timing of cell administration (P value = 0.29) (Analysis 9.5) or use of heparinised cell solution (P value = 0.22) (Analysis 10.5). The limited number of trials within groups precluded subgroup analysis by baseline LVEF or type of cells.

(v) Radionuclide ventriculography (RNV)

Four trials reported LVEF measured by radionuclide ventriculography (Grajek 2010;Nair 2015; Nogueira 2009; Roncalli 2010). There were no differences between treatment groups in analyses of mean change in LVEF from baseline (MD 0.24, 95% CI -1.92 to 2.41; 344 participants; three studies), mean LVEF at endpoint (MD 1.08, 95% CI -4.88 to 7.04; 157 participants; three studies), or in the combined analysis (MD 0.81, 95% CI -1.57 to 3.19; 383 participants; four studies) (Analysis 1.3). Only one trial reported LVEF measured by radionuclide ventriculography at long-term follow-up (Grajek 2010); this trial found no evidence for a difference between treatment groups in LVEF measured at long-term follow-up (MD 6.30, 95% CI -1.03 to 13.63; 39 participants; one study) (Analysis 1.2).

DISCUSSION

Over the last 20 years, randomised controlled trials (RCTs) have evaluated cell therapy in patients who have suffered an acute myocardial infarction (AMI). This present study

is an update of the Cochrane systematic review published previously (Fisher 2015b).

Fifty-three RCTs with 4159 participants were eligible for inclusion in this updated review (an additional 12 studies and 1427 patients were added since the last review). The characteristics of the interventions are summarised in Table 2. Participants recruited to these trials had suffered a recent AMI and received either cell treatment, a placebo or continued on optimal medical therapy following a successful revascularisation of the infarct-related coronary artery.

There was substantial clinical heterogeneity and diversity within and between the trials: the characteristics of the participants, the type and size of infarct and the baseline outcome values (e.g. left ventricular ejection fraction) at admission all differed. The cell type, dose, delivery route and time of administration, as well as the media in which cells were re-suspended, also differed. Whilst the vast majority of the studies administered the cell-based treatment via the intracoronary route, one trial injected the cells intramyocardially during CABG (Naseri 2018). All trials included in this review delivered cells of bone marrow origin, with bone marrow mononuclear cells being the most widely investigated cell type. The rest of the trials investigated more specific cells such as mesenchymal stem cells, CD34+ or CD133+ cells. The trials also differed in their design (e.g. blinded versus open-label), the length of follow-up (short and long-term) and the methodology used to measure surrogate outcome data (e.g. magnetic resonance imaging, echocardiography, single photon emission computed tomography).

Meta-analyses in cell therapy can help to show the safety of the approach and generate hypotheses, but due to the extent of the heterogeneity of the biologically active product, any analysis of efficacy must be marked with a great caveat. Unlike traditional drugs used in cardiology, autologous cell therapies are experimental interventions with much more complex and individualised properties; therefore, they do not adhere to established pharmacokinetics. There is no standard definition of an 'active' cell product at present as the number of administered cells cannot be equated to active dose, and the number of cells retained in the target region might be affected by disease and patient-related factors.

Main findings

There are 12 new trials included in this update of the Cochrane review; by pooling the data together, we can conclude the following

- There remains no evidence for a difference in the risk of all-cause mortality, cardiovascular mortality, incidence of rehospitalisation for heart failure, re-infarction, arrhythmias, restenosis or target vessel revascularisation in cell-treated patients compared to controls.
- Accordingly, there remains no evidence for a difference in the composite measure of major adverse cardiac events (MACE; as defined by death, re-infarction and re-hospitalisation for heart failure) between treated patients and the control group.

- Cell therapy has a comparable safety profile to conventional interventional therapies - there were no major differences in periprocedural adverse events associated with cell treatment.
- In this latest analysis, in the combined analysis of left ventricular ejection fraction (LVEF), cell treatment was associated with an improvement at ≥ 12 months as measured by MRI. There remains a significant improvement in LVEF as measured by echocardiography and SPECT at both the short and long term timepoints.
- We observed no differences between treatment groups in New York Heart Association class, quality of life measures and exercise/performance measures. There were too few new trials to draw any meaningful conclusions.
- Taken together, the results suggest that bone marrow-derived cell therapy has no substantial beneficial effect for patients who have suffered an AMI.

Despite including two attempts at a Phase III trial, including over 1400 new patients and doubling the number of patients for the longterm primary outcome measures, this meta-analysis remains underpowered to draw definitive conclusions on effects on mortality.

Left ventricular ejection fraction

As LVEF is one of the most reported measurements, we grouped LVEF data according to the method of measurement. Although each technique has its limitations, it is widely accepted that MRI is the gold standard method to measure surrogate outcomes such as LVEF. Due to the differences in LVEF reporting, we presented forest plots for mean change from baseline, mean value at endpoint and a combination of the two for clarity and transparency. In this latest review, in the combined analysis, there is now evidence for an improvement in LVEF measured by MRI at longer term follow-up (≥ 12 months; p = 0.04); however, there was no improvement in LVEF as measured by MRI at shorter term follow-up (< 12 months). There are also indications of an improvement in LVEF in cell-treated patients when measured by other imaging modalities, such as echocardiography and SPECT at both the short and long term endpoints. However, these modalities are less robust due to a proven lack of reproducibility in combination with high interoperator variability.

Heterogeneity & Sub-group Analyses

Previous versions of this Cochrane review have shown a considerable degree of heterogeneity among trials which has been extensively explored (Fisher 2015b; Clifford 2012; Fisher 2012; Martin-Rendon 2008a; Martin-Rendon 2008b). The clinical heterogeneity justifies the random-effects model in the meta-analyses conducted. We have attempted to address some of the issues of heterogeneity by conducting exploratory subgroup analyses for baseline LVEF as measured by MRI, cell dose, cell

type, timing of cell administration and whether heparin was used in the cell solution. Baseline LVEF has been previously reported to have an effect on outcome (Beitnes 2009; Schachinger 2009; Delewi 2014; Zwetsloot 2016). In this review, patients with a baseline LVEF

With regards to the timing of cell delivery, we have distinguished between the 'early' and 'late' administration of cells (defined here as< 10 days and ≥10 days) as the level of inflammation and remodelling process of the damaged tissue is very different at these timepoints. Although cell administration after 10 days is arguably no longer within the acute window, we have included them in this analysis as the studies reported their results as a treatment for AMI. This subgroup analysis, however, did not demonstrate any difference in the effect on all-cause mortality or LVEF.

Regarding cell dose, one may expect more of an effect with an increased dose if conventional pharmacokinetics was relevant. This analysis stratified the cell dose to: ≤ 10(8), 10(8) - ≤ 10(9) and >10(9). Generally, there does not appear to be any differences associated with dose response; however, LVEF measured by MRI at ≥ 12 months, did demonstrate a benefit when cells were administered at a ≤ 10(8) dose. This contrasts with the previous review (Fisher 2015b) which demonstrate a reduction in long-term mortality in favour of the 10(8) - ≤ 10(9) cell dose. Caution is required here, as the sample sizes are far too small for robust conclusions.

The subgroup analysis for cell type did not show any difference; but, again, the sample sizes for the more specific cell type groups were too small to draw meaningful conclusions.

The use of heparin in the final solution suggested a beneficial effect on LVEF measured by left ventricular angiography at <12 months ($p = 0.04$); these results again should be interpreted with caution, as the sample size precludes any definitive conclusion.

Study limitations

There are a number of limitations to the strength of any conclusion that can be drawn from this meta-analysis due to the sample sizes of the individual trials, their statistical power, the clinical heterogeneity and the risk of bias.

In general, the sample sizes were small in all trials included, perhaps with the exception of six trials that included at least 200 participants (Schachinger 2006; Sürder 2013; Tendera 2009; Nair 2015; Haddad 2020; Mathur 2020). These larger trials include the 2 attempts at a definitive Phase III trial. However, although BAMI (Mathur 2020) was powered around a clinical endpoint, it only recruited 375 out of the needed 3000 patients; and Nair 2015 (n=250) used a surrogate primary endpoint. Therefore, systematic reviews and meta-analysis are required to compensate for the lack of statistical power in individual trials and generate hypotheses.

In summary, this review finds that the results from the meta-analysis are of

moderate quality for the primary outcomes (see Summary of findings table 1) due to the information size criterion not being met.

This systematic review is based on a comprehensive search strategy; but, despite this, the dataset can still be incomplete due to certain types of bias. The risk of bias present in the included trials is summarised in Figure 3. All trials stated that they randomised the participants, but only 50% (n = 27) of the included trials documented adequate methods for the generation of randomised sequences and only 32% (n = 17) documented adequate methods for the concealment of treatment allocation. Blinding (performance and detection bias) was reported in 26% (n = 14) of the included trials, whilst the remaining 40 trials were described as either not blinded (n = 31) or blinding was unclear (n = 9). Attrition bias was low in 78% (n = 42) of the included trials and was unclear or high in the remaining trials. Finally, selective reporting bias was low in 46% (n = 25) of the included trials. Sensitivity analyses conducted for the major outcome of all-cause mortality showed that excluding those trials with high risk of selection, attrition or performance bias had a negligible effect on all-cause mortality. There was no evidence of publication bias in funnel plots and egger's regression.

Nowadays, large trials in cardiac disease are powered on combined clinical endpoints. Previously, universal combined outcome measures were proposed for better comparability of cell therapy trials (Bartunek 2006). In this final meta-analysis 15 years later, 15 different combined endpoints were used and only 17 trials reported any form of a combined endpoint. The previously determined combined outcome measure for this meta-analysis (death, recurrent infarction, heart failure hospitalization) was reported in 6/53 trials. Using combined endpoints seems to most useful in either very large clinical trials or in individual patient-data meta-analyses, but has not been helpful in identifying a positive signal in this meta-analysis.

Agreements and disagreements with other studies or reviews

In this review, we have focused on clinical outcomes such as death, cardiovascular death, reinfarction (MI), arrhythmias, restenosis, target vessel revascularisation, re-hospitalisation for heart failure and major adverse cardiac events. Our results suggest that cell therapy does not have a beneficial effect in patients who have suffered an AMI. This is in agreement with the last version of this review (Fisher 2015b) and with other systematic reviews and meta-analysis on the subject (de Jong 2014; Delewi 2014; Gyöngyösi 2015). de Jong 2014 reported a meta-analysis of 22 cell-based therapy RCTs (2037 participants) and found that cell therapy had no effect on major adverse clinical cardiac events including all-cause mortality. In an individual patient data meta-analysis, which included 12 trials (1252 participants), Gyöngyösi 2015 also confirmed there were no significant differences in all-cause mortality or major adverse cardiac events. In concurrence with this review, these previous meta-analyses also demonstrated low periprocedural adverse event rates and a low incidence of clinical endpoints.

The picture is somewhat less consistent when measuring surrogate outcomes such as LVEF. When measured by MRI, this Cochrane review demonstrated an improvement in LVEF at longer term (≥12 months) follow up (p = 0.04) and, when looking at patients with a baseline LVEF <45%, at shorter term and longer term follow up (<12 months; p = 0.02, ≥12 months; p = 0.01). de Jong 2014 observed a significant improvement in LVEF during short-term follow-up (in 1513 participants) which was not sustained long-term and was explained by a gradual increase in LV volumes during the first year after AMI in reperfused patients (Engblom 2009). Another metaanalysis of 16 studies including 1641 patients with STEMI showed a modest, but significant, improvement in LVEF of 2.55% and indices of LV remodeling at 3-6 months after intracoronary bone marrow-derived administration (Delewi 2014). Yet, Gyöngyösi 2015 observed no significant difference in 1252 participants when analysing any of these parameters.

In combination with our review, these results reflect the challenges inherent in using surrogate endpoints as markers of clinical efficacy, and the discrepancies involved in the use of different measuring techniques.

SUMMARY

This review suggests that cell-based therapies do not lead to a reduction in hard clinical outcomes such as all-cause mortality, cardiovascular mortality, rehospitalisation for heart failure, target vessel re-vascularisation or composite measures of MACE in patients who have suffered an acute myocardial infarction, which confirms earlier analyses. As a surrogate measure of heart function, an improvement in LVEF as measured by MRI was seen at the longer term follow up. The findings of this review remain largely consistent with the previous version Fisher 2015b) and with other published individual patient data analysis (Gyöngyösi 2015). Although our results are robust to sensitivity analyses, this systematic Cochrane review is likely underpowered.

Since the last review in 2015, there has been a substantial decrease in the number of clinical trials addressing the role of cell transplantation in the treatment of acute myocardial infarction. The previous review occurred 3 years after its predecessor and included an additional 8 trials; yet this review, occurring 7 years after the last, only added 12. Of note, the number of included patients for long-term primary outcome measures more than doubled in this final review. Two Phase III clinical trials have been performed (Mathur 2020; Nair 2015) which recruited a total of 630 patients. Although BAMI used a clinical primary endpoint of all-cause mortality, it failed to recruit anywhere near the 3000 patients that it was designed around. It was also powered around a historical mortality and MACE rate (12% mortality in 2 years), which over the duration of the trial, was found to be substantially reduced due to the successes of primary angioplasty following AMI. As Bolli has noted, all major cell therapy trials conducted in

STEMI patients over the past decade have reported very low rates of mortality (averaging 1.2% at 1 year - range 0–3%) and MACE (e.g. average rate of heart failure admissions at 1 year, 3%; range 0–7%) even in high-risk patients with moderate to severe LV dysfunction (Bolli 2020). Figure 4 shows that, despite more recent studies including only larger infarcts, there is a downward trend in long-term mortality rates since the start of the first cell therapy trials.

Thus, given the very low event rates that now occur following primary angioplasty; future trials of bone marrow-derived cell therapy for acute myocardial infarction will require overwhelmingly large sample sizes to detect an effect on mortality (based on BAMI's results, over 10,000 patients would be needed) or on combined endpoints. Given the clear effectiveness of current guideline directed medical therapy weighed up against the cost and logistics of these trials, it is highly unlikely that a large enough trial will take place. This Cochrane review, therefore, will probably remain contemporaneous for some time.

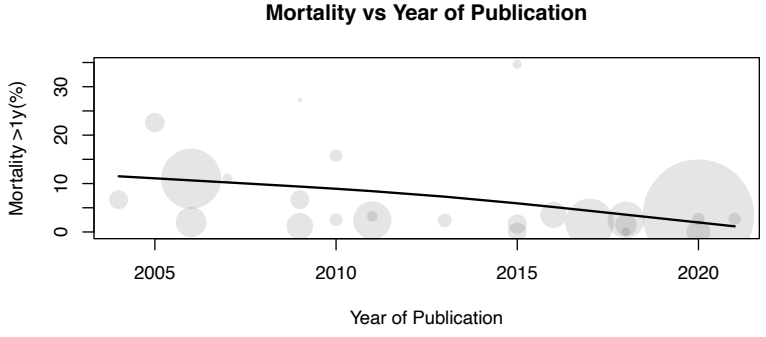


Figure 4 Mortality was plotted against year of publication. All control groups of studies used in the long-term all-cause mortality analyses were used. The bubbles resemble the size of the groups, ranging from n=3-190. A downward trends is visible for the fitted line since the publication of the earlier cell therapy trials.

AUTHORS'CONCLUSIONS

Implications for practice

This analysis indicates that autologous bone marrow-cell therapy is safe but does not reduce mortality and morbidity beyond standard therapy in this group of patients. This review shows that currently there is no evidence for a reduction in mortality and morbidity when bone marrow-derived cell treatment is administered to patients who had undergone primary angioplasty following AMI. As we don't expect this to change, new clinical trials in this field may wish to focus on other strategies. Yet, the current improved results from guideline directed medical therapy with subsequent lowering of mortality rates will provide a challenge for appropriate clinical trial design for any new therapy.

Implications for research

Results obtained in systematic reviews and meta-analysis looking at the effect of cell-based therapy in other cardiovascular diseases, such as heart failure (Afzal 2015; Fisher 2014; Fisher 2015; Gyöngyösi 2015; Poglajen 2014) and refractory angina (Jones 2019; Henry 2018), have shown more positive results in terms of clinical and surrogate endpoints. It may be that these patient populations should be the target of future cell-based therapy trials.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The outcomes from the previous version of this review have been maintained in this update, focusing on clinical outcomes. The previously defined outcomes are: primary outcomes as (i) all-cause mortality, (ii) cardiovascular mortality, (iii) composite measures of major adverse cardiac events (MACE), and (iv) periprocedural adverse events. Secondary outcomes include morbidity, LVEF and quality of life and performance measures.

Due to the many potential sources of heterogeneity across trials, we maintained the use of random-effects models throughout (as done in the previous review), instead of the earlier proposed fixed-effects models.

The trial sequental analysis (TSA) from the 2015 version has been left out and was not updated, as this technique is abandoned by the Cochrane methodology.

A new figure (Figure 4) was generated to show the trend in mortality in cell therapy trials.

Tables 4 from the 2015 version have been left out, as the current authors did not deem it informative enough.

Summary of findings 1. Cells compared to no cells for acute myocardial infarction

[1]Imprecision: information size criterion not met. Small size effect.

Cells compared to no cells for acute myocardial infarction (AMI)

Patient or population: patients with AMI

Settings: Hospitalised patients

Intervention: cells

Comparison: no cells

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	No cells	Cells				
All-cause mortality - short-term follow-up (< 12 months)	Study population		RR 0.79 (0.44 to 1.40)	1924 (21 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Further research may change the estimate
	23 per 1000	21 per 1000				
All-cause mortality - long-term follow-up (≥ 12 months)	Study population		RR 0.88 (0.60 to 1.31)	1910 (22 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Further research may change the estimate
	56 per 1000	49 per 1000				
Cardiovascular mortality - short-term follow-up (< 12 months)	Study population		RR 0.73 (0.31 to 1.71)	677 (9 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Further research may change the estimate
	27 per 1000	23 per 1000				
Cardiovascular mortality - long-term follow-up (≥ 12 months)	Study population		RR 0.94 (0.55 to 1.53)	1158 (13 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Further research may change the estimate
	52 per 1000	49 per 1000				
Composite death, reinfarction and hospitalisation for heart failure - short-term follow-up (< 12 months)	Study population		RR 0.36 (0.12 to 1.14)	379 (3 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Further research may change the estimate
	66 per 1000	24 per 1000 (8 to 76)				
Composite death, reinfarction and hospitalisation for heart failure - long-term follow-up (≥ 12 months)	Study population		RR 0.63 (0.36 to 1.10)	497 (6 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Further research may change the estimate
	140 per 1000	88 per 1000 (51 to 154)				

*The **assumed risk** is based on the observed incidence across the pooled control groups. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio

GRADE Working Group grades of evidence. **High quality:** Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

Comparison 1. Cells compared to no cells

Outcome or subgroup title	No of studies	No of participants	Statistical method	Effect size
1.1 All-cause mortality	34		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.1.1 Short-term follow-up (<12 months)	21	1924	Risk Ratio (MH, Random, 95% CI)	0.79 [0.44, 1.40]
1.1.2 Long-term follow-up (≥ 12 months)	23	1910	Risk Ratio (MH, Random, 95% CI)	0.88 [0.60, 1.31]
Cardiovascular mortality	15		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.2.1 Short-term follow-up (<12 months)	9	677	Risk Ratio (MH, Random, 95% CI)	0.73 [0.31, 1.71]
1.2.2 Long-term follow-up (≥12 months)	13	1158	Risk Ratio (MH, Random, 95% CI)	0.91 [0.55, 1.53]
1.3 Composite measure of death, reinfarction, re-hospitalisation for heart failure	6		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.3.1 Short-term follow-up (<12 months)	3	379	Risk Ratio (MH, Random, 95% CI)	0.36 [0.12, 1.14]
1.3.2 Long-term follow-up (≥12 months)	6	497	Risk Ratio (MH, Random, 95% CI)	0.63 [0.36, 1.10]
1.4 Incidence of reinfarction	28		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.4.1 Short-term follow-up (<12 months)	19	1965	Risk Ratio (MH, Random, 95% CI)	0.63 [0.36, 1,12]
1.4.2 Long-term follow-up (≥12 months)	22	1993	Risk Ratio (MH, Random, 95% CI)	087 [0.57, 1.33]
1.5 Incidence of re-hospitalisation for heart failure	23		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.5.1 Short-term follow-up (<12 months)	14	1291	Risk Ratio (MH, Random, 95% CI)	0.77 [0.39, 1.51]
1.5.2 Long-term follow-up (≥12 months)	16	1546	Risk Ratio (MH, Random, 95% CI)	0.65 [0.42, 1.00]
1.6 Incidence of arrhythmias	13		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.6.1 Short-term follow-up (<12 months)	6	551	Risk Ratio (MH, Random, 95% CI)	1.05 [0.54, 2.03]
1.6.2 Long-term follow-up (≥12 months)	10	1040	Risk Ratio (MH, Random, 95% CI)	0.85 [0.55, 1.32]
1.7 Incidence of restenosis	13		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.7.1 Short-term follow-up (<12 months)	8	641	Risk Ratio (MH, Random, 95% CI)	0.95 [0.63, 1.43]
1.7.2 Long-term follow-up (≥12 months)	6	395	Risk Ratio (MH, Random, 95% CI)	0.58 [0.27, 1.25]
1.8 Incidence of target vessel revascularisation	12		Risk Ratio (MH, Random, 95% CI)	Subtotals only
1.8.1 Short-term follow-up (<12 months)	6	789	Risk Ratio (MH, Random, 95% CI)	0.70 [0.47, 1.06]
1.8.2 Long-term follow-up (≥12 months)	9	843	Risk Ratio (MH, Random, 95% CI)	1.02 [0.74, 1.40]
1.9 Quality of life measures	4		Std. Mean Difference (IV, Random, 95% CI)	Subtotals only
1.9.1 Short-term follow-up (<12 months)	4	209	Std. Mean Difference (IV, Random, 95% CI)	0.38 [-0.43, 1.19]
1.9.2 Long-term follow-up (≥12 months)	2	81	Std. Mean Difference (IV, Random, 95% CI)	1.46 [-1.91, 4.84]
1.10 NYHA classification	9		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.10.1 Short-term follow-up (<12 months)	5	398	Mean Difference (IV, Random, 95% CI)	-0.07 [-0.24, 0.09]
1.10.2 Long-term follow-up (≥12 months)	6	342	Mean Difference (IV, Random, 95% CI)	-0.15 [-0.34, 0.04]
1.11 Exercise tolerance	6		Std. Mean Difference (IV, Random, 95% CI)	Subtotals only
1.11.1 Short-term follow-up (<12 months)	5	267	Std. Mean Difference (IV, Random, 95% CI)	0.19 [-0.06, 0.43]
1.11.2 Long-term follow-up (≥12 months)	2	71	Std. Mean Difference (IV, Random, 95% CI)	0.08 [-0.41, 0.58]
1.12 Maximum VO_2 (mL/kg/min)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.12.1 Short-term follow-up (<12 months)	3	175	Mean Difference (IV, Random, 95% CI)	1.15 [-0.77, 3.07]
1.12.2 Long-term follow-up (≥12 months)	1	45	Mean Difference (IV, Random, 95% CI)	0.40 [-3.76, 4.56]
1.13 VE/VCO_2 slope	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.13.1 Short-term follow-up (<12 months)	3	174	Mean Difference (IV, Random, 95% CI)	0.28 [-1.02, 1.57]
1.13.2 Long-term follow-up (≥12 months)	1	45	Mean Difference (IV, Random, 95% CI)	0.00 [3.07, 3.07]
1.14 Peak heart rate (bpm)	3		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.14.1 Short-term follow-up (<12 months)	3	198	Mean Difference (IV, Random, 95% CI)	0.55 [6.79, 7.89]
1.14.2 Long-term follow-up (≥12 months)	1	45	Mean Difference (IV, Random, 95% CI)	-9.10 [-20.59, 2.39]
1.15 LVEF measured by MRI (<12 months)	18		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.15.1 Mean change from baseline	16	1398	Mean Difference (IV, Random, 95% CI)	0.55 [-0.77, 1.88]
1.15.2 Mean value at endpoint	17	1315	Mean Difference (IV, Random, 95% CI)	0.80 [-0.66, 2.26]
1.15.3 Combined	18	1476	Mean Difference (IV, Random, 95% CI)	1.07 [-0.31, 2.44]
1.16 LVEF measured by MRI (≥ 12 months)	13		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.16.1 Mean change from baseline	9	707	Mean Difference (IV, Random, 95% CI)	0.85 [-0.38, 2.09]
1.16.2 Mean value at endpoint	12	801	Mean Difference (IV, Random, 95% CI)	0.98 [-1.70, 3.67]
1.16.3 Combined	13	968	Mean Difference (IV, Random, 95% CI)	1.84 [0.12, 3.57]
1.17 LVEF measured by echocardiography (<12 months)	21		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.17.1 Mean change from baseline	7	398	Mean Difference (IV, Random, 95% CI)	2.83 [1.76, 3.89]
1.17.2 Mean value at endpoint	21	888	Mean Difference (IV, Random, 95% CI)	2.13 [0.96, 3.30]
1.17.3 Combined	21	888	Mean Difference (IV, Random, 95% CI)	2.41 [1.44, 3.37]
1.18 LVEF measured by echocardiography (≥ 12 months)	14		Mean Difference (IV, Random, 95% CI)	Subtotals only

Comparison 1. Continued

Outcome or subgroup title	No of studies	No of participants	Statistical method	Effect size
1.18.1 Mean change from baseline	5	179	Mean Difference (IV, Random, 95% CI)	2.09 [-0.29, 4.48]
1.18.2 Mean value at endpoint	13	542	Mean Difference (IV, Random, 95% CI)	2.43 [1.27, 3.58]
1.18.3 Combined	14	598	Mean Difference (IV, Random, 95% CI)	1.89 [0.89, 2.90]
1.19 LVEF Measured by SPECT (<12 months)	8		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.19.1 Mean change from baseline	6	312	Mean Difference (IV, Random, 95% CI)	3.14 [1.32, 4.97]
1.19.2 Mean value at endpoint	7	401	Mean Difference (IV, Random, 95% CI)	2.57 [1.36, 3.77]
1.19.3 Combined	8	420	Mean Difference (IV, Random, 95% CI)	2.93 [1.44, 4.43]
1.20 LVEF measured by SPECT (≥ 12 months)	5		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.20.1 Mean change from baseline	2	92	Mean Difference (IV, Random, 95% CI)	5.63 [1.77, 9.49]
1.20.2 Mean value at endpoint	4	250	Mean Difference (IV, Random, 95% CI)	4.58 [2.33, 6.83]
1.20.3 Combined	5	269	Mean Difference (IV, Random, 95% CI)	5.24 [3.48, 7.00]
1.21 LVEF measured by left ventricular angiography (< 12 months)	10		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.21.1 Mean change from baseline	4	366	Mean Difference (IV, Random, 95% CI)	5.06 [0.96, 9.16]
1.21.2 Mean value at endpoint	10	798	Mean Difference (IV, Random, 95% CI)	4.39 [0.24, 8.54]
1.21.3 Combined	10	798	Mean Difference (IV, Random, 95% CI)	4.81 [0.92, 8.69]
1.22 LVEF measured by left ventricular angiography (≥ 12 months)	2		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.22.1 Mean change from baseline	2	160	Mean Difference (IV, Random, 95% CI)	3.71 [-5.10, 12.52]
1.22.2 Mean value at endpoint	1	98	Mean Difference (IV, Random, 95% CI)	1.60 [-3.41, 6.61]
1.22.3 Combined	2	160	Mean Difference (IV, Random, 95% CI)	5.03 [-1.23, 11.28]
1.23 LVEF measured by radionuclide ventriculography (RNV) (< 12 months)	4		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.23.1 Mean change from baseline	3	344	Mean Difference (IV, Random, 95% CI)	0.24 [-1.92, 2.41]
1.23.2 Mean value at endpoint	3	157	Mean Difference (IV, Random, 95% CI)	1.08 [-4.88, 7.04]
1.23.3 Combined	4	383	Mean Difference (IV, Random, 95% CI)	0.81 [-1.57, 3.19]
1.24 LVEF measured by radionuclide ventriculography (≥ 12 months)	1		Mean Difference (IV, Random, 95% CI)	Subtotals only
1.24.1 Mean value at endpoint	1	39		6.30 [-1.03, 13.63]

Comparison 2. Sensitivity analysis – route of cell delivery

Outcome or subgroup title	No. of studies	No. of participants	Statistcial method	Effect size
2.1 All-cause mortality	32		Risk Ratio (MH, Random, 95% CI)	Subtotals only
2.1.1 Short-term follow-up (<12 months)	19	1825	Risk Ratio (MH, Random, 95% CI)	0.77 [0.42, 1.39]
2.1.2 Long-term follow-up (≥ 12 months)	22	1841	Risk Ratio (MH, Random, 95% CI)	0.87 [0.59, 1.30]

Comparison 3. Sensitivity analysis – selection bias

Outcome or subgroup title	No. of studies	No. of participants	Statistcial method	Effect size
3.1 All-cause mortality	19		Risk Ratio (MH, Random, 95% CI)	Subtotals only
3.1.1 Short-term follow-up (<12 months)	19	1828	Risk Ratio (MH, Random, 95% CI)	0.87 [0.46, 1.65]

Comparison 4. Sensitivity analysis – attrition bias

Outcome or subgroup title	No. of studies	No. of participants	Statistcial method	Effect size
4.1 All-cause mortality	27		Risk Ratio (MH, Random, 95% CI)	Subtotals only
4.1.1 Short-term follow-up (<12 months)	16	1232	Risk Ratio (MH, Random, 95% CI)	0.72 [0.37, 1.41]
4.1.2 Long-term follow-up (≥ 12 months)	19	1731	Risk Ratio (MH, Random, 95% CI)	0.75 [0.47, 1.20]
4.2 Cardiovascular mortality	10		Risk Ratio (MH, Random, 95% CI)	Subtotals only
4.2.1 Short-term follow-up (<12 months)	6	360	Risk Ratio (MH, Random, 95% CI)	0.59 [0.20, 1.72]
4.2.2 Long-term follow-up (≥ 12 months)	9	983	Risk Ratio (MH, Random, 95% CI)	0.70 [0.37, 1.31]

Comparison 5. Sensitivity analysis – performance bias

Outcome or subgroup title	No. of studies	No. of participants	Statistcial method	Effect size
5.1 All-cause mortality	16		Risk Ratio (MH, Random, 95% CI)	Subtotals only
5.1.1 Short-term follow-up (<12 months)	10	991	Risk Ratio (MH, Random, 95% CI)	0.63 [0.26, 1.50]
5.1.2 Long-term follow-up (≥ 12 months)	10	972	Risk Ratio (MH, Random, 95% CI)	0.64 [0.33, 1.24]
5.2 Cardiovascular mortality	5		Risk Ratio (MH, Random, 95% CI)	Subtotals only
5.2.1 Short-term follow-up (<12 months)	3	467	Risk Ratio (MH, Random, 95% CI)	0.59 [0.10, 3.56]
5.2.2 Long-term follow-up (≥ 12 months)	3	430	Risk Ratio (MH, Random, 95% CI)	0.54 [0.21, 1.38]

Comparison 6. Subgroup analysis – baseline LVEF measured by MRI

Outcome or subgroup title	No. of studies	No. of participants	Statistcial method	Effect size
6.1 All-cause mortality (<12 months)	11		Risk Ratio (MH, Random, 95% CI)	Subtotals only
6.1.1 Baseline LVEF <45 %	4	478	Risk Ratio (MH, Random, 95% CI)	0.77 [0.19, 3.16]
6.1.2 Baseline LVEF ≥45%	7	648	Risk Ratio (MH, Random, 95% CI)	0.85 [0.30, 2.39]
6.2 All-cause mortality (≥ 12 months)	10		Risk Ratio (MH, Random, 95% CI)	Subtotals only
6.2.1 Baseline LVEF <45 %	2	136	Risk Ratio (MH, Random, 95% CI)	0.61 [0.13, 2.83]
6.2.2 Baseline LVEF ≥45%	8	715	Risk Ratio (MH, Random, 95% CI)	0.73 [0.38, 1.44]
6.3 LVEF measured by MRI (< 12 months)	17		Mean Difference (IV, Random, 95% CI)	Subtotals only
6.3.1 Baseline LVEF <45 %	6	579	Mean Difference (IV, Random, 95% CI)	2.28 [0.43, 4.13]
6.3.2 Baseline LVEF ≥45%	11	746	Mean Difference (IV, Random, 95% CI)	0.38 [-1.58, 2.34]
6.4 LVEF measured by MRI (≥ 12 months)	13		Mean Difference (IV, Random, 95% CI)	Subtotals only
6.4.1 Baseline LVEF <45 %	5	402	Mean Difference (IV, Random, 95% CI)	4.41 [1.01, 7.81]
6.4.2 Baseline LVEF ≥45%	8	516	Mean Difference (IV, Random, 95% CI)	1.02 [-0.46, 2.50]

Comparison 7. Subgroup analysis – cell type

Outcome or subgroup title	No. of studies	No. of participants	Statistcial method	Effect size
7.1 All-cause mortality (<12 months)	21		Risk Ratio (MH, Random, 95% CI)	Subtotals only
7.1.1 Mononuclear cells	17	1525	Risk Ratio (MH, Random, 95% CI)	0.80 [0.42, 1.50]
7.1.2 Mesenchymal stem cells	2	101	Risk Ratio (MH, Random, 95% CI)	1.01 [0.15, 6.60]
7.1.3 Haematopoietic progenitor cells	4	357	Risk Ratio (MH, Random, 95% CI)	0.64 [0.12, 3.50]
7.2 All-cause mortality (≥12 months)	22		Risk Ratio (MH, Random, 95% CI)	Subtotals only
7.2.1 Mononuclear cells	17	1554	Risk Ratio (MH, Random, 95% CI)	0.93 [0.60, 1.43]
7.2.2 Mesenchymal stem cells	2	79	Risk Ratio (MH, Random, 95% CI)	3.08 [0.33, 28.39]
7.2.3 Haematopoietic progenitor cells	3	229	Risk Ratio (MH, Random, 95% CI)	0.52 [0.09, 3.07]

Comparison 8. Subgroup analysis – dose of stem cells

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
8.1 All-cause mortality (<12 months)	20		Risk Ratio (MH, Random, 95% CI)	Subtotals only
8.1.1 $\leq 10^8$ cells	6	458	Risk Ratio (MH, Random, 95% CI)	0.79 [0.23, 2.71]
8.1.2 $>10^8$ and $\leq 10^9$ cells	15	1420	Risk Ratio (MH, Random, 95% CI)	0.72 [0.37, 1.39]
8.1.3 $>10^9$ cells	1	59	Risk Ratio (MH, Random, 95% CI)	0.79 [0.05, 12.01]
8.2 All-cause mortality (≥ 12 months)	23		Risk Ratio (MH, Random, 95% CI)	Subtotals only
8.2.1 $\leq 10^8$ cells	11	650	Risk Ratio (MH, Random, 95% CI)	1.17 [0.59, 2.30]
8.2.2 $>10^8$ and $\leq 10^9$ cells	10	1151	Risk Ratio (MH, Random, 95% CI)	0.64 [0.38, 1.09]
8.2.3 $>10^9$ cells	2	87	Risk Ratio (MH, Random, 95% CI)	1.56 [0.32, 7.55]
8.3 LVEF measured by MRI (< 12 months)	17		Mean Difference (IV, Random, 95% CI)	Subtotals only
8.3.1 $\leq 10^8$ cells	6	514	Mean Difference (IV, Random, 95% CI)	0.42 [-2.06, 2.89]
8.3.2 $>10^8$ and $\leq 10^9$ cells	12	889	Mean Difference (IV, Random, 95% CI)	1.06 [-0.42, 2.54]

Comparison 8. Continued

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
8.3.3 >10^9 cells	1	59	Mean Difference (IV, Random, 95% CI)	1.00 [-2.66, 4.66]
8.4 LVEF measured by MRI (≥ 12 months)	13		Mean Difference (IV, Random, 95% CI)	Subtotals only
8.4.1 ≤10^8 cells	5	316	Mean Difference (IV, Random, 95% CI)	3.40 [0.91, 5.89]
8.4.2 >10^8 and ≤ 10^9 cells	8	612	Mean Difference (IV, Random, 95% CI)	1.58 [-0.92, 4.08]
8.5 LVEF measured by left ventricular angiography (< 12 months)	9		Mean Difference (IV, Random, 95% CI)	Subtotals only
8.5.1 ≤10^8 cells	1	87	Mean Difference (IV, Random, 95% CI)	2.10 [-3.43, 7.63]
8.5.2 >10^8 and ≤ 10^9 cells	6	548	Mean Difference (IV, Random, 95% CI)	2.26 [-0.71, 5.23]
8.5.3 >10^9 cells	2	101	Mean Difference (IV, Random, 95% CI)	11.64 [7.52, 15.75]

Comparison 9. Subgroup analysis – timing of cell administration

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
9.1 All-cause mortality (<12 months)	17		Risk Ratio (MH, Random, 95% CI)	Subtotals only
9.1.1 ≤10 days since AMI	12	1097	Risk Ratio (MH, Random, 95% CI)	0.86 [0.40, 1.82]
9.1.2 >10 days since AMI	5	451	Risk Ratio (MH, Random, 95% CI)	0.56 [0.15, 2.06]
9.2 All-cause mortality (≥ 12 months)	19		Risk Ratio (MH, Random, 95% CI)	Subtotals only
9.2.1 ≤10 days since AMI	15	1587	Risk Ratio (MH, Random, 95% CI)	0.68 [0.41, 1.11]
9.2.2 >10 days since AMI	4	168	Risk Ratio (MH, Random, 95% CI)	1.20 [0.41, 3.51]
9.3 LVEF measured by MRI (< 12 months)	16		Mean Difference (IV, Random, 95% CI)	Subtotals only
9.3.1 ≤10 days since AMI	15	1208	Mean Difference (IV, Random, 95% CI)	1.15 [-0.34, 2.65]
9.3.2 >10 days since AMI	2	190	Mean Difference (IV, Random, 95% CI)	-0.71 [-4.90, 3.48]
9.4 LVEF measured by MRI (≥ 12 months)	13		Mean Difference (IV, Random, 95% CI)	Subtotals only
9.4.1 ≤10 days since AMI	12	843	Mean Difference (IV, Random, 95% CI)	1.47 [-0.27, 3.21]
9.4.2 >10 days since AMI	2	185	Mean Difference (IV, Random, 95% CI)	3.78 [-1.44, 9.00]
9.5 LVEF measured by left ventricular angiography (< 12 months)	9		Mean Difference (IV, Random, 95% CI)	Subtotals only
9.5.1 ≤10 days since AMI	6	662	Mean Difference (IV, Random, 95% CI)	2.16 [-1.14, 5.47]
9.5.2 >10 days since AMI	3	156	Mean Difference (IV, Random, 95% CI)	7.42 [-1.83, 16.66]

Comparison 10. Subgroup analysis – heparinised cell solution

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
10.1 All-cause mortality (<12 months)	18		Risk Ratio (MH, Random, 95% CI)	Subtotals only
10.1.1 Heparin	8	597	Risk Ratio (MH, Random, 95% CI)	0.71 [0.27, 1.82]
10.1.2 No heparin	10	999	Risk Ratio (MH, Random, 95% CI)	0.66 [0.30, 1.45]
10.2 All-cause mortality (≥ 12 months)	19		Risk Ratio (MH, Random, 95% CI)	Subtotals only
10.2.1 Heparin	13	909	Risk Ratio (MH, Random, 95% CI)	0.99 [0.55, 1.77]
10.2.2 No heparin	6	493	Risk Ratio (MH, Random, 95% CI)	0.65 [0.34, 1.25]
10.3 LVEF measured by MRI (< 12 months)	18		Mean Difference (IV, Random, 95% CI)	Subtotals only
10.3.1 Heparin	9	682	Mean Difference (IV, Random, 95% CI)	1.48 [-0.57, 3.53]
10.3.2 No heparin	9	794	Mean Difference (IV, Random, 95% CI)	0.64 [-1.22, 2.50]
10.4 LVEF measured by MRI (≥ 12 months)	12		Mean Difference (IV, Random, 95% CI)	Subtotals only
10.4.1 Heparin	7	525	Mean Difference (IV, Random, 95% CI)	2.51 [-0.11, 5.13]
10.4.2 No heparin	5	443	Mean Difference (IV, Random, 95% CI)	0.85 [-1.41, 3.11]
10.5 LVEF measured by left ventricular angiography (< 12 months)	9		Mean Difference (IV, Random, 95% CI)	Subtotals only
10.5.1 Heparin	5	256	Mean Difference (IV, Random, 95% CI)	6.82 [0.25, 13.39]
10.5.2 No heparin	4	480	Mean Difference (IV, Random, 95% CI)	1.89 [-2.58, 6.36]

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: short report Source of funding: not reported Country of origin: Brazil Number of centres: 1 Dates of trial enrolment: not reported Length of follow-up: 12 months Number (N) of participants randomised to each arm: 11 in the treatment arm, 11 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 11 in the treatment arm, 11 in the control arm
Participants	Population: AMI successfully treated with PCI and with LVEF < 45% Age, mean (SD) each arm: not reported Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 5 to 9 days post-symptoms Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: methods of cell isolation not reported Dose of stem cells: a single dose of 2.6 (± 1.6) x 108 /mL mononuclear cells Timing of stem cell procedure: cells infused 5 to 9 days following the onset of symptoms and 4 hours following harvest. Intracoronary infusion of cells in the infarct-related artery Comparator arm: not reported
Outcomes	Primary outcomes: not reported Secondary outcomes: LVEF, LV perfusion defect, adverse events Outcome assessment points: 4 and 12 months Method(s): echocardiography, SPECT
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The trial was described as "double blind" and a placebo was used. It was unclear whether the control group underwent bone marrow aspiration. Blinding of outcome assessors was not reported
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Angeli 2012

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Shanxi Scientific and Technical Key Project, Xijing Research Boosting Program on Stem Cell Research (No. XJZT08Z04), Xijing Research Boosting Program on Cardiac Microvascular Formation Research (No. XJZT07Z05) and National Basic Research Program of China Country of origin: China Number of centres: 1 Dates of trial enrolment: 07/03 to 03/04 Length of follow-up: 48 months Number (N) of participants randomised to each arm: 41 in treatment arm/45 in control arm Number (N) of participants analysed (primary outcome) in each arm: 41 in treatment arm/45 in control arm
Participants	Population: AMI, within 12 hours. PCI within 12 hours Age, mean (SD) each arm: 50.7 (SEM 1.1) years in treatment arm, 51.0 (SEM 1.0) years in control arm Sex, % male in each arm: 95.1% in treatment arm, 93.3% in control arm Number of diseased vessels: 1 Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 6.5 (0.3) hours (mean ± SEM) before PCI in treatment arm, 6.8 (0.3) (mean ± SEM) hours before PCI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 40 mL bone marrow aspirated 7 days after PCI. Density gradient centrifugation (Ficoll) used to isolate BMMNC. Mononuclear cell layer harvested, washed 3 times and re-suspended in 10 mL heparinised saline. Intracoronary infusion using PCI technique, over-the-wire balloon catheter advanced to the proximal part of the stented culprit lesion, inflated with 4 to 5 Atm pressure for 1 minute to occlude blood flow. At the same time MNC suspension injected into the IRA. Procedure repeated 4 times Dose of stem cells: 4 doses of 2.5 mL cell suspension containing ~1.25 x 108 MNC for a total of ~ 5.00 x 108 MNC Timing of stem cell procedure: primary PCI performed within 12 hours of onset of symptoms, cell infusion performed 7 days after primary PCI Comparator arm: patients received a 10 mL placebo intracoronary saline injection
Outcomes	Primary outcomes: ESV, EDV, LVEF, WMSI, infarct size, coronary artery restenosis Secondary outcomes: none Outcome assessment points: baseline, 1, 3, 6, 12 and 48 months Method(s): echocardiography, ECG-gated 99m Technetium SPECT, quantitative coronary angiography
Notes	Baseline values taken at day 0 (day of AMI and primary angioplasty) and at day 7 (day of BMMNC treatment or sham procedure), day 7 values entered. SPECT was also used to measure infarct size LVEF, ESV and EDV but results were not published

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random numbers between 0 and 1 were generated and a median value was calculated. Random numbers greater than the median value were allocated to the BMMNC group
Allocation concealment (selection bias)	Low risk	Randomisation details provided in consecutively numbered, sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group did not undergo bone marrow aspiration although they received an injection of heparinised saline and therefore it is unclear whether participants and clinicians were sufficiently blinded to treatment. Outcome assessors were blinded to treatment allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	1 patient in the BMMNC group (1/41) had transient acute HF seven days after transplant. 1 patient in the control group (1/45) had instent restenosis and was subjected to repeat PCI at 1-year follow-up. It is unclear whether these patients were included at follow-up. One additional control had died at 1-year follow-up
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00626145) were reported
Other bias	Low risk	None reported or identified

Cao 2009

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: China Number of centres: 1 Dates of trial enrolment: 11/02 to 05/03 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 34 in treatment arm/35 in control arm Number (N) of participants analysed (primary outcome) in each arm: 34 in treatment arm, 35 in control arm
Participants	Population: AMI, within 12 hours Age, mean (SD) each arm: 58 (7.0) years in treatment arm, 57 (5.0) years in control arm Sex, % male in each arm: 94% in treatment arm, 97% in control arm Number of diseased vessels: 1.6 (0.5) in treatment arm, 1.7 (0.4) in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 8.3 (3.8) hours from AMI to PCI in treatment arm; 8.5 (3.9) hours from AMI to PCI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 60 mL of autologous bone marrow was aspirated under local anaesthesia from the ilea of all 69 patients in the morning 8 days after PCI and cultured for 10 days. Cells were harvested and washed 3 to 4 times with heparinised saline, and the cell suspension was mixed with heparin, filtrated and prepared for implantation 2 hours before implantation. 6 mL of the cell suspension was injected directly into the target coronary artery through an inflated over-the-wire balloon catheter in the central lumen with high pressure (10 atm). The balloon remained inflated for 2 or more minutes to occlude anterior blood flow just before beginning the BMMNC injection Dose of stem cells: 6 mL containing 8 to 10 x 109 cells/mL Timing of stem cell procedure: 18.4 (0.5) days after PCI Comparator arm: 6 mL standard saline via PCI method
Outcomes	Primary outcomes: cardiac death Secondary outcomes: "Left ventricular haemodynamics": functional defect (%), infarcted area movement velocity, LVEF. "Cardiac functional indexes": LVESV, LVEDV, circumferential shortening, Psyst/ESV, perfusion defect by PET. Measured by echocardiography and PET Outcome assessment points: baseline, 3 and 6 months Method(s): PET, echocardiography, NOGA, left ventriculography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Low risk	The control group underwent bone marrow aspiration and received an injection of saline by the same method as the BMSC group. Blinding of clinicians was not reported. Outcome assessors were blinded to treatment allocation. 3 independent statisticians who had no knowledge of the study collected and analysed outcome data
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	Incomplete data for LVEDV and LVESV were provided in the results although these outcomes are not included in this review. It would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Chen 2004

Study characteristics	
Methods	Type of study: parallel RCT Source of funding: UK Stem Cell Foundation, Heart Cells Foundation and Barts and the London Charity Country of origin: UK, Switzerland, Denmark Number of centres: 5 Enrolment: total 100 (1:1 randomisation, 45 cell therapy, 55 placebo). Analysis in 86 patients.
Participants	Population: AMI Age, mean (SD) each arm: cell therapy group 56.7 (10.7) placebo group 56.4 (10.4) Sex, % male in each arm: cell therapy 91%. placebo 84%. Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: cell therapy 193min. placebo 233min. Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow mononuclear cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate and gradient centrifugation. Following the method set up by Schachinger 2006 Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: placebo (saline)
Outcomes	Primary outcomes: changes in LVEF from baseline to 12 months (by MRI) Secondary outcomes: changes in LVEF at 6 months (by echocardiography and LV angiography), major adverse clinical cardiac events Outcome assessment points: baseline, 6 and 12 months Method(s): MRI, echocardiography and LV angiography
Notes	www.clinicaltrials.gov: NCT00765453

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Study design paper: The randomisation process will be performed by dedicated trial software IHD CLINICAL. This is a webbased password secured and encrypted data management system designed specifically for clinical trials in this area. uneven randomisation numbers were used
Allocation concealment (selection bias)	Low risk	due to the use of uneven randomisation numbers and double-blinding, allocation concealment was preserved in this trial.
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants, investigators, and treating clinicians remained blinded to group assignment
Incomplete outcome data (attrition bias) All outcomes	Low risk	no imcomplete data seen in outcomes. numbers match.
Selective reporting (reporting bias)	Low risk	described all outcome measures in paper (as noted on clinicaltrials.gov). no selective reporting seen.
Other bias	Low risk	no other risk

Choudry 2016

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: supported by grants from the Italian Ministry of Health (Progetto Ricerca Finalizzata 2002 and 2005, Progetto ex art. 56 2007); the Italian Ministry of University and Research, and the 6FP EU Project - THERCORD. Materials for CD133+ cell separations were kindly provided by Miltenyi Biotec Country of origin: Italy Number of centres: 2 Dates of trial enrolment: 10/03 to 10/06 Length of follow-up: 12 months Number (N) of participants randomised to each arm: 5 in the treatment arm, 5 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 5 in the treatment arm, 4 in the control arm
Participants	Population: STEMI with PCI within 6 hours of symptom onset Age, mean (SD) each arm: median 54 (range 47 to 60) years in treatment arm, median 56 (range 44 to 58) years in control arm Sex, % male in each arm: 100% in both trial arms Number of diseased vessels: 1 Number of stunned hyperkinetic, etc segments: mean 4.2 (1.6) in treatment therapy arm, mean 3.8 (1.3) in control arm Time from symptom onset to initial treatment: median 265 hours from symptoms onset to PCI; cell therapy on day 9 to 16 after PCI Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: CD133+ Type of stem cells: CD133 selected bone marrow-derived stem cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspiration followed by immunomagnetic selection with specific monoclonal antibody using the CliniMacs System. Re-suspended in 10 mL (± 2) of normal saline solution (0.9% NaCl) with 10% human serum albumin. Delivery via intracoronary infusion by PCI over the wire balloon catheter technique Dose of stem cells: median 5.9 x 106 (range 4.9 +/- 13.5) CD133+ cells Timing of stem cell procedure: cell infusion was done 9 to 13 days following STEMI and successful PCI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: 1. any adverse event during hospital stay, 2. PET-derived changes in myocardial perfusion and infarct size at 12 months, and 3. variations in LVDV, LVEF and WMSI at 12 months by echocardiography Secondary outcomes: all-cause death, cardiac death, symptomatic heart failure and coronary symptoms requiring hospitalisation and target vessel revascularisation Outcome assessment points: 3, 6, 12 months Method(s): echocardiography, gated PET
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was undertaken using a permuted block randomisation system and numbered containers
Allocation concealment (selection bias)	Low risk	Randomisation, patient enrolment and assignment to study group was done by a blinded co-ordinator
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration; no placebo was administered to controls. After randomisation, study processes were blinded to the researchers involved in echocardiography and PET evaluation
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and scientific outcomes at 6 months. 1 patient in the control group underwent heart transplantation 6 months after STEMI and was not included in 12-month evaluation
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00400959) were reported
Other bias	Low risk	None reported or identified

Colombo 2011

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: grant of the National Advanced Technology Development Plan of China Country of origin: China Number of centres: 4 Dates of trial enrolment: 05/08 to 11/09 Length of follow-up: 24 months Number (N) of participants randomised to each arm: 21 in the treatment arm, 22 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 19 in the treatment arm, 20 in the control arm
Participants	Population: acute STEMI reperfused within 12 hours by PCI Age, mean (SD) each arm: 55 (SEM 1.6) years in treatment arm, 58.6 (SEM 2.5) years in control arm Sex, % male in each arm: 100% in treatment arm, 86.4% in control arm Number of diseased vessels: 1 (42.9%), 2 (19.0%), 3(38.1%) in treatment arm, 1 (50%), 2 (18.2%), 3 (31.8%) in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 17.1 (SEM 0.6) days from reperfusion to infusion of cells Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BM-MSC Type of stem cells: bone marrow-derived mesenchymal stromal cells (MSC) Summary of how stem cells were isolated and type and route of delivery: bone marrow (80 mL in 2000 IU of heparin) was harvested from each patient in the treatment group from the posterior iliac crest under local anaesthesia by a haematologist 2 to 3 days after primary PCI. The bone marrow aspirate was shipped at room temperature to the central cell-processing laboratory. The mononuclear cell fraction was isolated using a density gradient with Lymphocyte Separation Medium (Biowhittaker) and then the low-density cells were washed and viable cells were counted. The BM-MCs were seeded into 75 cm^2 tissue culture flasks in MSCs medium consisting of Dulbecco's modified Eagle's medium containing 4.5% glucose (DMEM-4.5, HyClone), supplemented with 10% fetal bovine serum (GIBCO) and 1% antibiotic-antimycotic solution (Lift Technologies). The cell suspension was removed after 72 hours and the adherent cells were cultured in at 37 °C with 5% CO2. The culture medium was changed every 3 to 4 days until colonies were formed. After 14.6 ± 0.7 days of culture, passage 2 (P2) cells were harvested by trypsin treatment. Cells were washed, and viability was tested by trypan blue exclusion. Cell counts were performed, and the cells at 4 °C were delivered to the catheterisation laboratory. Cell were re-suspended in heparinised saline Dose of stem cells: 3.08 (± 0.52) x 106 cells Timing of stem cell procedure: 16 to 17 days after PCI. Time from reperfusion to infusion of study therapy = 17.1 (SEM 0.6) days Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: absolute changes in myocardial viability and perfusion in the infarcted region measured by F-18-FDGi SPECT at 6 months, and in global LVEF measured by 2D echocardiogram at 6, 12 and 24 months after cell infusion Secondary outcomes: incidence of cardiovascular events, total mortality and adverse events at 12 and 24 months follow-up Outcome assessment points:6, 12, 24 months Method(s): echocardiography, F-18-FDG SPECT
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Participants were randomised 1:1 to treatment or control using sequential numbers
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as "open label". Controls did not undergo bone marrow aspiration; no placebo was administered to controls. Echocardiography data were analysed independently by 2 experienced observers who were unaware of patients' treatment assignment
Incomplete outcome data (attrition bias) All outcomes	Low risk	1 participant (1/22) in the control arm was lost to follow-up at 6 months and 1 patient (1/21) in the BMSC arm had died at 6 months follow-up; all other randomised participants were included in the analysis of clinical and scientific outcomes at 6 months. 2 further participants (1 in each treatment group) were lost to follow-up at 12 and 24 months' follow-up
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Gao 2013

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Shanghai Scientific Research Fund Country of origin: China Number of centres: 1 Dates of trial enrolment: not reported Length of follow-up: 6 months Number (N) of participants randomised to each arm: 10 in treatment arm/10 in control arm Number (N) of participants analysed (primary outcome) in each arm: 10 in treatment arm/10 in control arm
Participants	Population: AMI, within 24 hours. PCI within 24 hours. Cell transplantation after successful PCI Age, mean (SD) each arm: 58 (11) years in treatment arm, 59 (8) years in control arm Sex, % male in each arm: 80% in treatment arm, 100% in control arm Number of diseased vessels: 1:7, 2:2, 3:1 in treatment arm; 1:7, 2:3, 3:0 in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 7.9 (3.8) hour in treatment arm/7.1(3.1) hour in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate (40 mL). The method of cell separation was not reported. Cells were infused after successful PCI Dose of stem cells: a single dose of 4 x 107 /mL mononuclear cells Timing of stem cell procedure: cells infused within 15 hours of onset of AMI Comparator arm: 15 mL injection of bone marrow supernatant
Outcomes	Primary outcomes: LVEF, LVEDD, myocardial perfusion defect Secondary outcomes: not listed Outcome assessment points: baseline, 1 week and 6 months Method(s): echocardiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomised in a 1:1 ratio with the use of sequentially numbered, sealed envelopes
Allocation concealment (selection bias)	Low risk	Sequentially numbered, sealed envelopes were used
Blinding (performance bias and detection bias) All outcomes	Low risk	Controls underwent bone marrow aspiration and received an injection of BM supernatant. The study states that clinical data were acquired and analysed in a 'blinded fashion' by clinicians who were blinded to the groups' identities
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Ge 2006

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Polish Cardiac Society, Servier Polska and the Polish Committee for Scientific Research (Komitet Badan Naukowych) PBZ-KBN099/P05/03 Country of origin: Poland Number of centres: 1 Dates of trial enrolment: 06/03 to 06/06 Length of follow-up: 12 months Number (N) of participants randomised to each arm: 31 in treatment arm/14 in control arm Number (N) of participants analysed (primary outcome) in each arm: 31 at 3 and 6 months, 27 at 12 months in treatment arm/14 at 3 and 6 months, 12 at 12 months in control arm
Participants	Population: AMI, within 12 hours. Age, mean (SD) each arm: 49.9 (8.4) years in treatment arm, 50.9 (9.3) years in control arm Sex, % male in each arm: 87% in treatment arm, 86% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 290 (234) minutes from AMI to PCI in treatment arm/190 (212) minutes from AMI to PCI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 80 (±30) mL (range 50 to 150 mL) bone marrow was collected from the pelvic bones into phosphate-buffered saline (PBS) with heparin (50 U/mL) under local anaesthesia. Diluted 1:2 with PBS and centrifuged in Ficoll gradient. MNC collected, washed in PBS with heparin, re-suspended in a few mL of X-vivo 15 medium with 2% heat-inactivated autologous plasma, placed in Teflon bags and overnight cultivated. Cells harvested and washed 3 times with heparinised PBS the next day. BMSC administered via IRA to the infarcted zone with a stop-flow technique through an over-the wire-balloon catheter Dose of stem cells: 0.410 ± 0.18 x 109 BMMNC (12.25 ± 2.05 mL) divided into 3 to 4 portions containing 3 to 4 mL cell suspension each Timing of stem cell procedure: 4 to 5 days after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: left ventricle perfusion, LVEF Secondary outcomes: LVESV, LVEDV, WMSI, cardiopulmonary exercise testing results, MACE (death, AMI, and need for revascularisation) Outcome assessment points: baseline, 3, 6 and 12 months Method(s): echo, SPECT, RNV, cardiopulmonary exercise treadmill test, coronary angiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Patients were assigned to the BMSC or control group by means of restricted randomisation (permuted blocks randomisation). The block size was 6 and the number of block was chosen using a computer random number generator. Patients having numbers 1 to 4 were allocated to the treatment group, whereas patients having numbers 5 or 6 were allocated to the control group (2:1 ratio)
Allocation concealment (selection bias)	Unclear risk	Prepared envelopes with treatment assignment were used; it is unclear whether these were sealed or opaque
Blinding (performance bias and detection bias) All outcomes	High risk	The study was "not blinded for the patients"; controls did not undergo bone marrow aspiration and no placebo was administered. Investigators assessing outcome measures were blinded to the group assignment
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical and scientific outcomes at 6 months. At 12 months, there were 4/31 withdrawals in the BMSC arm (1 sudden death at 7 months, 3 patients revascularised between 6 and 12 months) and 2/14 withdrawals in the control arm (2 patients revascularised between 6 and 12 months)
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	High risk	Supported in part by commercial funding

Grajek 2010

Study characteristics	
Methods	Type of study: randomized, double-blind placebo-controlled phase II study. Source of funding: SamerMansour and Nicolas Noiseux received financial support from Fonds de la recherche en sant duQu bec,Miltenyi Biotec, Inc. and Boston Scientific in Canada. Funding sources have no involvement in the collection, analysis or interpretation of data. Country of origin: Canada Number of centres: single center study Intended enrolment: not mentioned
Participants	Population: first AMI Age, median (IQR) each arm: Cell therapy = 41.0 [43.0-60.5], placebo =50.5 [48.3-63.3]. Sex, % male in each arm: cell therapy = 82,4%, placebo = 95%. Number of diseased vessels: not reported Used cutoff for Number of stunned hyperkinetic, etc segments: not reported. Time from symptom onset to initial treatment: Cell therapy = 247min [146-380], placebo = 224 [129-677]. Statistically significant baseline imbalances between the groups?: no.
Interventions	Intervention arm: autologous CD-133+ BMSC. Type of stem cells: BMSC Summary of how stem cells were isolated and type and route of delivery: BM aspiration and separation of mononuclear cells using gradient centrifugation. CD133-positive cells were immunomagnetically separated using the Clinimacs (Miltenyi) Dose of stem cells: 10 million cells. Timing of stem cell procedure: 3 to 7 days after initial PCI. Comparator arm: saline and 10% autologous plasma
Outcomes	Primary outcomes: safety and efficacy and functional effect of the treatment. LVEF. Death, MACE, reinfarction, stroke, repeat vascularization, hospitalization for heart failure, ICD shocks. Secondary outcomes: not reported. Outcome assessment points: baseline, 4 months and 12 months Method(s): echocardiography
Notes	Previously registered under ongoing studies (Mansour 2011)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	there is randomization, but unclear how it was performed.
Allocation concealment (selection bias)	Unclear risk	it is unclear how randomization was performed.
Blinding (performance bias and detection bias) All outcomes	Low risk	neither the patients nor treating physicians nor data managers have access to the randomization code for the duration of the study All invasive and noninvasive analyses will be performed by operators blinded to all clinical and other functional data
Incomplete outcome data (attrition bias) All outcomes	Low risk	38 patients were enrolled between November 2007 and July 2012, but one patient subsequently withdrew consent. PS. intended enrollment = 2x20 based on publication at one year? not mentioned anywhere in the paper
Selective reporting (reporting bias)	Low risk	No signs of selective reporting.
Other bias	Low risk	No other forms of biases.

Haddad 2020

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Interuniversity Cardiology Institute of The Netherlands (ICIN), the Netherlands Heart Foundation (grant 2005T101, 2003B126), Biotronik, Boston Scientific, Guerbet, Guidant, Medtronic, Novartis, Pfizer, Sanofi-Aventis Country of origin: the Netherlands Number of centres: 8 Dates of trial enrolment: 08/05 to 04/08 Length of follow-up: 5 years Number (N) of participants randomised to each arm: 69 in treatment arm/65 in control arm Number (N) of participants analysed (primary outcome) in each arm: 67 in treatment arm/60 in control arm
Participants	Population: first STEMI. PCI with stent within 12 hours Age, mean (SD) each arm: 56 (9) years in treatment arm, 55 (10) years in control arm Sex, % male in each arm: 84% in treatment arm, 86% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: 53.3 (19.6)% dysfunctional segments in treatment arm/56.2 (24.7)% dysfunctional segments in control arm Time from symptom onset to initial treatment: median 3.5 (IQR 2.4 to 5.1) hours in treatment arm/median 3.4 (IQR 2.3 to 4.2) hours in control arm Statistically significant baseline imbalances between the groups?: none reported
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 60 mL BM aspirated from iliac crest under local anaesthesia, collected in a sterile container with heparin, sent to 1 of 6 cell-processing labs. MNC isolated by density gradient centrifugation using LymphoprepTM, washed twice and re-suspended in 15 to 20 mL saline with 4% human serum albumin and 20 IU/mL sodium heparin. Cells were infused into the infarctrelated artery through the central lumen of an over-the-wire balloon catheter in 3 sessions of 3 minutes of coronary occlusion, interrupted by 3 minutes of coronary flow Dose of stem cells: total 296 (164) x 106 BMMNC Timing of stem cell procedure: cells infused 3 to 8 days after primary PCI (median 6 days) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: "The change in regional myocardial function in dysfunctional segments at baseline defined as the percentage of dysfunctional segments with improved segmental wall thickening at 4 months" Secondary outcomes: "changes in absolute segmental wall thickening in dysfunctional segments, changes in global LVEF, volumes, mass, and infarct size, and changes in regional myocardial function stratified by transmural extent of infarction." Outcome assessment points: baseline, 4 months, 2 years, 5 years Method(s): MRI, angiogram
Notes	3 patients did not receive cell therapy as randomised: 1 withdrew consent, 1 aspiration was unsuccessful and 1 patient experienced an occluded infarct-related artery

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Permuted block randomisation was performed with stratification according to site, with the use of a computerised voice-response system
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered. "After randomisation, study processes were not blinded"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes at 4 months, with the exception of 1 patient in the BMSC group who withdrew consent. In the analysis of MRI data at 4 months, 1 further patient in the BMSC group (total 2/69) and 5 patients in the control group (5/65) withdrew or were excluded due to poor quality MRI (1 BMSC patient and 3 controls), 1 control patient who received and implanted ICD, and 1 control patient who refused follow-up. At 2 years follow-up, a total of 10/69 BMSC patients and 13/65 control patients were withdrawn or excluded from MRI analysis; reasons were given. In the analysis of clinical outcomes at 5 years, 9 patients (BMSC: 4/69 versus controls: 5/65) were lost to follow-up
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the study design protocol are reported apart from exercise tolerance, which was included as a secondary outcome
Other bias	High risk	Supported in part by commercial funding

Hirsch 2011

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: China Number of centres: 1 (assumed) Dates of trial enrolment: 05/04 to 05/05 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 20 in treatment arm/20 in control arm Number (N) of participants analysed (primary outcome) in each arm: 20 in treatment arm/20 in control arm
Participants	Population: AMI, within 24 hours. PCI within 24 hours. Cell transplantation within 2 hours of successful PCI Age, mean (SD) each arm: 57.3 (10.1) years in treatment arm, 56.7 (9.2) years in control arm Sex, % male in each arm: 65% in treatment arm, 70% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 6.3 (4.2) hours in treatment arm/6.3 (3.9) hours in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate (80 to 140 mL). Cells separated by gradient centrifugation. Cells re-suspended in heparinised saline (with 0.9% NaCl) prior to transplantation. Intracoronary infusion using a microcatheter (Judkins method) Dose of stem cells: a single dose of 1.8 (4.2) x108/mL cells Timing of stem cell procedure: cells infused within 2 hours of successful PCI Comparator arm: 15 mL of heparinised saline (with 0.9% NaCl)
Outcomes	Primary outcomes: not reported Secondary outcomes: LVEF, LVEDV and infarct size measured by CMR imaging and LV arteriography Outcome assessment points: baseline, 1 week and 6 months Method(s): CMR imaging
Notes	Translated from Chinese (Mandarin)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group received a placebo but it was unclear whether they underwent bone marrow aspiration and therefore it was unclear whether they were appropriately blinded. Blinding of clinicians and outcome assessors was not reported
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Huang 2006

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: National Technology Excellence Programme (2004BA714B05-2) Country of origin: China Number of centres: 1 Dates of trial enrolment: 08/05 to 12/05 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 20 in treatment arm/20 in control arm Number (N) of participants analysed (primary outcome) in each arm: 20 in treatment arm/20 in control arm
Participants	Population: AMI within 24 hours. PCI within 24 hours Age, mean (SD) each arm: 54.8 (5.8) years in treatment arm, 55.4 (7.1) years in control arm Sex, % male in each arm: 85% in treatment arm, 90% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI within 6.9 (2.7) hours of AMI in treatment arm/PCI within 6.5 (2.4) hours of AMI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 80 to 140 mL of bone marrow aspirated from the hip bone under local anaesthetic. BMMNC isolated by gradient centrifugation. Intracoronary transplantation of BMMNC via a micro-infusion catheter immediately after PCI Dose of stem cells: single dose of (1.2 ± 6.5) x 108 BMMNC Timing of stem cell procedure: PCI performed within 24 hours of symptom onset, BMSC transplantation performed within 2 hours of PCI Comparator arm: intracoronary transplantation of heparinised saline via a micro-infusion catheter immediately after PCI
Outcomes	Primary outcomes: none
Notes	Translated from Chinese (Mandarin)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group received an injection of heparinised saline although it is not reported whether they underwent bone marrow aspiration. It is therefore unclear whether participants and clinicians were sufficiently blinded to treatment. It was not reported whether outcome assessors were blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of scientific outcomes. No clinical outcomes were reported
Selective reporting (reporting bias)	Unclear risk	LVESV and LVEDV were assessed but data were not provided although these outcomes are not included in this review. All other outcomes mentioned in the methods are reported in the results
Other bias	Low risk	None reported or identified

Huang 2007

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Medical Council of the Academy of Finland, the Finnish Foundation for Cardiovascular Research & the Foundation for the Northern Health Support, Boston Scientific Sverige AB, Stockholm, Sweden Country of origin: Finland Number of centres: 2 Dates of trial enrolment: 10/04 to 02/07 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 40 in treatment arm/40 in control arm Number (N) of participants analysed (primary outcome) in each arm: 36 for LV angiography, 39 for 2-D echocardiography, 28 for IVUS in treatment arm/36 for LV angiography, 38 for 2-D echocardiography, 30 for IVUS in control arm
Participants	Population: AMI, within 12 hours. Thrombolysis within 12 hours. PCI within 2 to 3 days Age, mean (SD) each arm: 60 (10) years in treatment arm, 59 (10) years in control arm Sex, % male in each arm: 90% in treatment arm, 85% in control arm Number of diseased vessels: 19 (48%) had 1 vessel disease, 15 (37%) had 2, 6 (15%) had 3 in treatment arm, 25 (62%) had 1 vessel disease, 13 (33%) had 2, 2 (5%) had 3 in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 2.8 (2.3) hours from AMI to thrombolysis, 48 (12) hours from thrombolysis to PCI in BMSC arm; 3.1 (3.9) hours from AMI to thrombolysis, 44 (13) hours from thrombolysis to PCI in treatment arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 80 mL bone marrow was aspirated into heparin-treated syringes from the posterior iliac crest under local anaesthesia. Mononuclear cells were isolated from aspirate using density gradient centrifugation on Ficoll-Hypaque, washed twice with heparinised physiological saline and re-suspended in 10 mL of medium containing 5 mL of the patient's own serum and heparinised physiological saline. BMC suspension then was filtered through 100 micrometre nylon mesh. Medium containing the BMCs was injected intracoronally through over the wire balloon by using intermittent balloon inflation in the stent at the time of injection Dose of stem cells: mean 402 (196) x 106 mononuclear cells injected (median = 360 x 106) of which a mean of 2.6 (1.6) x 106 Timing of stem cell procedure: the time interval between the AMI and cell transfer was 70 (36) hours (median 60 hours) in BMMNC arm Comparator arm: placebo medium containing the same solution as cell medium without the cells
Outcomes	Primary outcomes: (1) Absolute change in global LVEF from baseline to 6 months. (2) Absolute changes in the measures obtained by IVUS. (3) Changes in arrhythmia risk variables from baseline to 6 months Secondary outcomes: exercise stress test Outcome assessment points: baseline and 6 months Method(s): 2-D echocardiography, LV angiography, IVUS, ECG
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation codes for each patient were generated by a laboratory nurse in Ouluusing using "a computer-generated randompermuted block design with variable block sizes and selected on the basis of whether a suspension containing BMCs or placebo medium was given to each patient". The laboratory nurse in Turku was informed by a telephone call from Oulu about the randomisation and type of treatment
Allocation concealment (selection bias)	Low risk	The laboratory nurse in Turku was informed by a telephone call from Oulu about the randomisation and type of treatment. The lab nurses who prepared the treatment or placebo solution according to patient allocation did not take part in any other parts of the research protocol
Blinding (performance bias and detection bias) All outcomes	Low risk	All patients had bone marrow aspiration and control group patients were given an intracoronary injection of placebo medium. The treatment and control media were externally prepared by laboratory nurses. Blinded outcome assessors not involved in randomisation quantitatively analysed angiograms, echocardiograms and intravascular ultrasounds in a central core laboratory. Consecutively numbered, sealed envelopes were provided and stored in the Clinical Research Laboratory of the University of Oulu and were opened after all baseline and 6-month data were analysed from all patients
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. In the analysis of scientific outcomes by echocardiography at 6-month follow-up, the number of withdrawals was low in both trial arms (1 patient in each treatment arm due to refusal from repeat testing and 1 death in the placebo arm). Further withdrawals from LV angiography were low and balanced between treatment groups (BMSC: 4/40 versus placebo: 4:40). Analysis by IVUS incurred a higher number of withdrawals but these were balanced between treatment arms (BMSC: 28/40 versus placebo: 30/40)
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00363324) were reported
Other bias	High risk	Supported in part by commercial funding

Huikuri 2008

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Medical Council of the Academy of Finland, the Finnish Foundation for Cardiovascular Research & the Foundation for the Northern Health Support, Boston Scientific Sverige AB, Stockholm, Sweden Country of origin: Finland Number of centres: 2 Dates of trial enrolment: 10/04 to 02/07 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 40 in treatment arm/40 in control arm Number (N) of participants analysed (primary outcome) in each arm: 36 for LV angiography, 39 for 2-D echocardiography, 28 for IVUS in treatment arm/36 for LV angiography, 38 for 2-D echocardiography, 30 for IVUS in control arm
Participants	Population: AMI, within 12 hours. Thrombolysis within 12 hours. PCI within 2 to 3 days Age, mean (SD) each arm: 60 (10) years in treatment arm, 59 (10) years in control arm Sex, % male in each arm: 90% in treatment arm, 85% in control arm Number of diseased vessels: 19 (48%) had 1 vessel disease, 15 (37%) had 2, 6 (15%) had 3 in treatment arm, 25 (62%) had 1 vessel disease, 13 (33%) had 2, 2 (5%) had 3 in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 2.8 (2.3) hours from AMI to thrombolysis, 48 (12) hours from thrombolysis to PCI in BMSC arm; 3.1 (3.9) hours from AMI to thrombolysis, 44 (13) hours from thrombolysis to PCI in treatment arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 80 mL bone marrow was aspirated into heparin-treated syringes from the posterior iliac crest under local anaesthesia. Mononuclear cells were isolated from aspirate using density gradient centrifugation on Ficoll-Hypaque, washed twice with heparinised physiological saline and re-suspended in 10 mL of medium containing 5 mL of the patient's own serum and heparinised physiological saline. BMC suspension then was filtered through 100 micrometre nylon mesh. Medium containing the BMCs was injected intracoronally through over the wire balloon by using intermittent balloon inflation in the stent at the time of injection Dose of stem cells: mean 402 (196) x 106 mononuclear cells injected (median = 360 x 106) of which a mean of 2.6 (1.6) x 106 Timing of stem cell procedure: the time interval between the AMI and cell transfer was 70 (36) hours (median 60 hours) in BMMNC arm Comparator arm: placebo medium containing the same solution as cell medium without the cells
Outcomes	Primary outcomes: (1) Absolute change in global LVEF from baseline to 6 months. (2) Absolute changes in the measures obtained by IVUS. (3) Changes in arrhythmia risk variables from baseline to 6 months Secondary outcomes: exercise stress test Outcome assessment points: baseline and 6 months Method(s): 2-D echocardiography, LV angiography, IVUS, ECG
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation codes for each patient were generated by a laboratory nurse in Ouluusing using "a computer-generated randompermuted block design with variable block sizes and selected on the basis of whether a suspension containing BMCs or placebo medium was given to each patient". The laboratory nurse in Turku was informed by a telephone call from Oulu about the randomisation and type of treatment
Allocation concealment (selection bias)	Low risk	The laboratory nurse in Turku was informed by a telephone call from Oulu about the randomisation and type of treatment. The lab nurses who prepared the treatment or placebo solution according to patient allocation did not take part in any other parts of the research protocol
Blinding (performance bias and detection bias) All outcomes	Low risk	All patients had bone marrow aspiration and control group patients were given an intracoronary injection of placebo medium. The treatment and control media were externally prepared by laboratory nurses. Blinded outcome assessors not involved in randomisation quantitatively analysed angiograms, echocardiograms and intravascular ultrasounds in a central core laboratory. Consecutively numbered, sealed envelopes were provided and stored in the Clinical Research Laboratory of the University of Oulu and were opened after all baseline and 6-month data were analysed from all patients
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. In the analysis of scientific outcomes by echocardiography at 6-month follow-up, the number of withdrawals was low in both trial arms (1 patient in each treatment arm due to refusal from repeat testing and 1 death in the placebo arm). Further withdrawals from LV angiography were low and balanced between treatment groups (BMSC: 4/40 versus placebo: 4:40). Analysis by IVUS incurred a higher number of withdrawals but these were balanced between treatment arms (BMSC: 28/40 versus placebo: 30/40)
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00363324) were reported
Other bias	High risk	Supported in part by commercial funding

Huikuri 2008

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Fund of Scientific Research Flanders Country of origin: Belgium Number of centres: 1 Dates of trial enrolment: 05/03 to 11/04 Length of follow-up: 4 months Number (N) of participants randomised to each arm: 33 in treatment arm/34 in control arm Number (N) of participants analysed (primary outcome) in each arm: 33 in treatment arm/34 in control arm
Participants	Population: AMI, within 24 to 48 hours Age, mean (SD) each arm: 55.8 (11) years in treatment arm, 57.9 (10) years in control arm Sex, % male in each arm: 82% in treatment arm, 82% in control arm Number of diseased vessels: 1 in treatment arm (36% right artery/64% left artery)/1 in control arm (38% right artery/62% left artery) Number of stunned hyperkinetic, etc segments: 3 or more contiguous segments out of total 17 Time from symptom onset to initial treatment: 3.7 hours (median) before PCI in treatment arm/4.1 hours (median) before PCI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirated, cells separated using gradient centrifugation. 4 to 6 hours after harvest, cells were washed and re-suspended in 10 mL of saline containing 0.9% NaCl and 5% autologous serum. Intracoronary infusion using an inflated balloon catheter. 3 fractions of cells were infused over 2 to 3-minute periods separated by 3-minute reperfusion Dose of stem cells: 10 mL of cell suspension, a total dose of 3.0 (1.28) x 108 nucleated cells containing 1.72 (0.72) x 108 MNC Timing of stem cell procedure: PCI was performed about 4 hours after onset of symptoms. Cell treatment was conducted within 1 day of PCI Comparator arm: placebo consisting of 10 mL of saline containing 0.9% NaCl and 5% autologous serum
Outcomes	Primary outcomes: changes in LVEF at 4 months Secondary outcomes: changes in: 1. infarct size 2. LV function Outcome assessment points: baseline, 4 and 12 months. Method(s): MRI
Notes	This trial includes some patients with previous AMI, but data analysis without these patients did not significantly change the final results

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	A computerised randomisation list was used
Allocation concealment (selection bias)	Low risk	Sequentially numbered, sealed envelopes were used
Blinding (performance bias and detection bias) All outcomes	Low risk	The trial was described as "double blind". All patients underwent bone marrow aspiration and control group patients were given an intracoronary injection of placebo medium. Outcome assessors were blinded to treatment
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. In the analysis of scientific outcomes measured by MRI at 4 and 12 months, the number of withdrawals was low and balanced between trial arms (BMSC: 3/33 versus control: 4/34). Reasons for withdrawal were 1 x technical failure, 2 x claustrophobia to MRI, 2 x patient refusal, 1 x intracochlear implant and 1 death in the BMSC arm due to haemorrhagic shock)
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00264316) were reported
Other bias	Low risk	None reported or identified

Janssens 2006

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: Iran Number of centres: 1 Dates of trial enrolment: 06/02 to 01/04 Length of follow-up: 6 months Number (N) of participants randomised to each arm: not reported Number (N) of participants analysed (primary outcome) in each arm: 16 in the treatment arm, 16 in the control arm
Participants	Population: AMI within 1 month with a history of anterior MI and LVEF < 35% Age, mean (SD) each arm: 48.0 (SEM 2.5) years in treatment arm, 45.2 (SEM 3.2) years in control arm Sex, % male in each arm: 66% in treatment arm, 90% in control arm Number of diseased vessels: 1 Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: up to 1 month Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirates were obtained under local anaesthesia with a standard Jamshidi needle with heparin (50 U/mL) from posterior iliac crests. Bone marrow-derived mononuclear cells (BMCs) were isolated by layering on a Ficoll-Paque gradient. Cell populations included hematopoietic progenitor cells. A haemocytometer was used to estimate the number of nucleated cells in the final preparation of bone marrow cells. Nucleated cell viability was assessed by trypan blue exclusion. Nucleated cells were cultured in an M199 medium, 10% human serum supplemented with 50 ng/mL vascular endothelial growth factor (VEGF), 1 ng/mL basic fibroblast growth factor (bFGF), and 2 ng/mL insulin-like growth factor-1 (IGF-1). The cells were incubated overnight at 37 ºC in a fully humidified atmosphere with 5% CO2. Then, cells were washed twice and re-suspended in 5 mL human serum Dose of stem cells: (24.6 ± SEM 8.4) × 108 cells Timing of stem cell procedure: within 1 month of AMI, at the time of PCI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: not reported Secondary outcomes: perfusion defects, regional wall motion of LV and LVEF, adverse events Outcome assessment points: 6 months Method(s): SPECT, echocardiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. Blinding of outcome assessors was not reported
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	The number of participants randomised to each treatment arm was unclear; the study states that 20 participants met the inclusion criteria but the analysis includes 16 participants in each group. It is therefore unclear how many patients were randomised to each treatment group. No details of patient withdrawal were reported
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although echocardiography measurements taken at 1 month were not reported. It would be difficult to rule out other selective reporting
Other bias	Low risk	None reported or identified

Jazi 2012

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: the Scientific Research Program of Shanghai Health Bureau, No. 054065 Country of origin: China Number of centres: 1 Dates of trial enrolment: 05/05 to 09/06 Length of follow-up: 12 months Number (N) of participants randomised to each arm: 14 in treatment arm/12 in control arm Number (N) of participants analysed (primary outcome) in each arm: 14 in treatment arm/12 in control arm
Participants	Population: AMI, within 24 hours. Thrombolysis within 24 hours Age, mean (SD) each arm: 62.3 (7.68) years in treatment arm, 60.6 (6.46) years in control arm Sex, % male in each arm: 71.4% in treatment arm, 75% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI within 7 to 10 days of AMI symptom onset Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 40 mL BM aspirated under local anaesthesia from the left posterior superior iliac spine. Suspended in 160 mL solution of heparinised normal saline, filtered twice, centrifuged to isolate MNC, washed twice, resuspended in heparinised normal saline. PCI to IRA with an over-the-wire balloon catheter delivering BMMNC to the proximal end of the LAD in one dose within 2 to 3 minutes Dose of stem cells: 1 dose of 15 ± 2 mL BMMNC suspension containing 6.27 ± 1.75 x 107 BMMNC and 0.36 ± 0.11% CD133+, 0.69 ± 0.13% CD34+ cells Timing of stem cell procedure: 7 to 10 days after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: none Secondary outcomes: LVEF, parameters of cardiac geometric pattern, serum NT-proBNP, Minnesota heart failure questionnaire before and after treatment Outcome assessment points: baseline, 6 and 12 months Method(s): echocardiography, Minnesota heart failure questionnaire, blood biochemistry tests
Notes	Translated from Chinese (Mandarin)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. Echocardiogram images were analysed by experienced independent echocardiographers unaware of patient allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Jin 2008

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: Russia Number of centres: 1 (assumed) Dates of trial enrolment: not reported Length of follow-up: mean 8.23 (0.72) years Number (N) of participants randomised to each arm: 22 in treatment arm/22 in control arm. 8-year follow-up: 28 in the treatment arm and 34 in the
Participants	Population: AMI, within 7 to 21 days Age, mean (SD) each arm: 55.2 (8.6) years in treatment arm, 52.1 (3.2) years in control arm Sex, % male in each arm: 90% in treatment arm, 73% in control arm Number of diseased vessels: 1:1; 2:14; 3:4 in treatment arm/1:8; 2:6; 3:3 in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI within 4 hours of onset of symptoms Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM aspirates and cells separated by density gradient centrifugation. Cells re-suspended in heparinised solution prior to transplantation. Route of delivery not reported in the study Dose of stem cells: a single dose of 88.5 (49.2) x 106 MNC Timing of stem cell procedure: within 7 to 21 days after PCI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: not reported Secondary outcomes: not reported, but give data on mortality, morbidity, quality of life, exercise tolerance and engraftment of infused cells Outcome assessment points: baseline, 3 months and 6 months, mean 8.23 (0.72) years (clinical outcomes) Method(s): 6-minute walking test, QoL scores, % radioactivity/no. of cells
Notes	Secondary 2006 and 2014 papers translated from Russian used 2017 paper to update mortality numbers. SD from echo data could not be derived because of an obvious typo in the 95%CI of the control group. this data was not updated.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. Blinding of outcome assessors was not reported
Incomplete outcome data (attrition bias) All outcomes	High risk	In an early publication, 3 patients in the BMSC group (4/22) and 3 patients in the control group (3/22) were excluded due to "repeated AMI, restenosis or the infarction-related artery, and microcoronary angiography" (no breakdown between groups was reported). However, in a subsequent study of a larger cohort reporting long-term follow-up, a lower number of withdrawals or exclusions was reported (BMSC: 2/28 versus controls: 2/34); reasons for withdrawals were not given. It is unclear to what extent these 2 publications overlap
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Karpov 2005

Study characteristics	
Methods	Type of study: RCT Source of funding: none Country of origin:Korea Number of centres: single center Intended enrolment: none specified
Participants	Population: first AMI Age, mean (SD) each arm: not reported (> 30 years) Sex, % male in each arm: 100% vs 100% Number of diseased vessels (ctrl vs MSC): 1-vessel disease 8/12 vs 11/14, 2-vessel disease 3/12 vs 2/14, 3-vesseld disease 1/12 vs 1/14 (but culprit always in LAD) Used cutoff for Number of stunned hyperkinetic, etc segments: none (LVEF <40% Time from symptom onset to initial treatment: <24h Statistically significant baseline imbalances between the groups?: no
Interventions	Intervention arm: Type of stem cells: BM-MSCs. Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate. delivery through intracoronary infusion Dose of stem cells: 7.2 (SD 0.9) *10^7 cells. Timing of stem cell procedure: Harvest of BM after 3.0 (SD 1.5) days. Infusion of BM 25.0 (SD 2.4) days after harvest. Comparator arm: regular care (no sham BM-asp or cath)
Outcomes	Primary outcomes: changes in LVEF from baseline to 4 months Secondary outcomes: changes in LVEF at 12 months, LVEDV, LVESV, adverse events (death, aryhtmias, revascularisation) Outcome assessment points: baseline, 4 and 12 months Method(s): SPECT and echocardiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Twenty-six out of 30 patients were randomly allocated to each treatment group. no mentioning of how randomization was performed
Allocation concealment (selection bias)	Unclear risk	no mentioning of allocation concealment.
Blinding (performance bias and detection bias) All outcomes	High risk	For performance bias it is not stated that physicians and patients were blinded. Control subjects did not get a BM aspiration or cath. Here there is almost certain no adequate blinding for at least the patients. For detection bias, echo's were assessed by blinded investigators. so here there is a low risk of bias.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No missing data.
Selective reporting (reporting bias)	Low risk	Reported all outcomes mentioned.
Other bias	Low risk	No other risks.

Kim 2018

Study characteristics	
Methods	Type of study: RCT Source of funding: This Research is supported by Tomsk State University Competitiveness Improvement Program. Work was conducted with the application of the Tomsk regional common use center technical equipment acquired thanks to a grant of the Russian Ministry of the Agreement No.14.594.21.0001 (RFMEFI59414X0001). Country of origin: Russia Number of centres: 1
Participants	Population: first AMI (cell treated vs controls) Age, mean (SD) each arm: 60.3 (12.2) vs 58.4 (10.4) Sex, % male in each arm: 60% vs 81% Number of diseased vessels: 3/5/1 vs 0/6/4 Used cutoff for Number of stunned hyperkinetic, etc segments: not mentioned. Time from symptom onset to initial treatment: 5.25 (0.7) vs 4.9 (0.6). Statistically significant baseline imbalances between the groups?: No
Interventions	Intervention arm: Type of stem cells: CD133+ BM cells. Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate, centrifugation. CD133 Microbead system.resuspension in heparinized solution. administration intracoronary. Dose of stem cells: 5-10*10^6 Timing of stem cell procedure: 16(6) days after AMI. ±5h after BM-harvest. Comparator arm: usual care.
Outcomes	Primary outcomes: mortality Secondary outcomes: cardiovascular mortality, reperated MI, Repeated PCI, Pacemaker implantation, changes in LVEDV/LVESV/ LVEF at 7.7y Outcome assessment points: baseline, 7.7y Method(s): echocardiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	randomization per envelop method
Allocation concealment (selection bias)	Unclear risk	envelop method, but no mentioning of allocation concealment.
Blinding (performance bias and detection bias) All outcomes	High risk	no placebo therapy or sham procedures.
Incomplete outcome data (attrition bias) All outcomes	High risk	there is talk of 23 patiens with known status after 7y, but Table 2 has 10+16=26 patients in it for outcomes after 7y.
Selective reporting (reporting bias)	Low risk	all outcomes on clinicaltrials.gov mentioned in the article
Other bias	Unclear risk	from the text, it is not always clear how all procedures were performed. furthermore, unclear if and where blinding was performed, resulting in risk of multiple biases.

Kirgizova 2015

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: funded by PCB-Pharmicell Company Limited (Seongnam, Korea) Country of origin: South Korea Number of centres: 3 Dates of trial enrolment: 03/07 to 09/10 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 40 in the treatment arm, 40 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 30 in the treatment arm, 28 in the control arm
Participants	Population: AMI within 96 hours Age, mean (SD) each arm: 53.9 (10.5) years in treatment arm, 54.2 (7.7) years in control arm Sex, % male in each arm: 90.0% in treatment arm, 89.3% in control arm Number of diseased vessels: 1 (n = 16), 2 (n = 11), 3 (n = 3) in treatment arm, 1 (n = 16), 2 (n = 8), 3 (n = 4) in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 350.8 (325.4) minutes in treatment arm, 115.3 (35.5) minutes in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BM-MSC Type of stem cells: bone marrow-derived mesenchymal stromal cells Summary of how stem cells were isolated and type and route of delivery: 20 to 25 mL (mean ± SD: 23.1 ±1 1.5 mL) of BM aspirates were obtained under local anaesthesia from the posterior iliac crest in the treatment group on 3.8 ± 1.5 days after admission. All manufacturing and product testing procedures for the generation of clinical-grade autologous MSCs were carried out under good manufacturing practice (FCB-Pharmicell Company Limited, Seongnam, Korea). Mononuclear cells were separated from the BM by density gradient centrifugation (HISTOPAQUE-1077; Sigma-Aldrich, St. Louis, MO, USA) and washed with phosphate-buffered saline (PBS). Cells were re-suspended in Dulbecco's modified Eagle's medium-low glucose (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco), 100 U/mL penicillin/100 μg/mL and streptomycin (Gibco). They were plated at 2 to 3 × 10^5 cells/cm2 into 75 cm^2 flasks. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO2. After 5 to 7 days, non-adherent cells were removed by replacing the medium; adherent cells were cultured for another 2 to 3 days. When the cultures were near confluence (70% to 80%), adherent cells were detached by using trypsin containing ethylene diamine tetra-acetic acid (EDTA; Gibco) and replated at 4 to 5 × 10^3 cells/cm^2 in 175 cm^2 flasks. Cells were serially subcultured up to passage 4 or passage 5 for infusion (mean ±SD: 4.4 ± 0.5 passages). On the day of administration, MSCs were harvested using trypsin and EDTA, washed twice with PBS and once with salinesolution, and re-suspended to a final concentration of 1 × 10^6 cells/kg. The criteria for the release of MSCs for clinical use included viability > 80%, absence of microbial contamination (bacteria, fungus, virus and mycoplasma) if undertaken 3 to 4 days before administration, and expression of CD73 and CD105 by > 90% of cells and absence of CD14, CD34 and CD45 by < 3% of cells as assessed by flow cytometry Dose of stem cells: a single dose of 7.2 (± 0.90) × 10^7 cells Timing of stem cell procedure: 25 (± 2.4) days following BM aspiration Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: absolute changes in global LVEF from baseline to 6 months Secondary outcomes: changes in LVEDV, LVESV, WMSI, major adverse cardiac events Outcome assessment points: 6 months Method(s): SPECT, echocardiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as "open label". Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. The analysis of SPECT images was performed by blinded independent investigators at each participating centre; off-line assessment of all echocardiographic images was performed by one blinded independent investigator
Incomplete outcome data (attrition bias) All outcomes	High risk	The number of withdrawals and exclusions was high (BMSC: 10/40 versus controls: 12/40). Although reasons were given, frequency differences were observed between groups including exclusions due to protocol violation, loss to follow-up and the "opinion of the investigator"
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT01392105) were reported
Other bias	High risk	This is a commercially funded trial

Lee 2014

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: supported by research fellowships from the Norwegian Council on Cardiovascular Diseases and Medinnova and by grants from Inger and John Fredriksen's Heart Foundation Country of origin: Norway Number of centres: 2 Dates of trial enrolment: 09/03 to 05/05 Length of follow-up: 36 months Number (N) of participants randomised to each arm: 50 in treatment arm/51 in control arm Number (N) of participants analysed (primary outcome) in each arm: 50 in treatment arm/51 in control arm
Participants	Population: AMI, within 2 to 12 hours Age, mean (SD) each arm: 58.1 (8.5) years in treatment arm, 56.7 (9.6) years in control arm Sex, % male in each arm: 84% in treatment arm, 84% in control arm Number of diseased vessels: 1:42; 2:6; 3:2 in treatment arm/1:36; 2:12; 3:2 in control arm Number of stunned hyperkinetic, etc segments: > 3 in both arms Time from symptom onset to initial treatment: median 210 minutes (range 180 to 330 minutes) in treatment arm/median 230 minutes (180 to 330 minutes) in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM aspirates 6 days (median, range 5 to 6 days) after PCI were separated by Ficoll gradient centrifugation and re-suspended in heparinised plasma prior to transplantation. Intracoronary infusion using an inflated balloon catheter. Dose of stem cells: a single dose of 0.68 x 108 MNC (median, range 0.54 to 1.3 x 10^8 MNC) containing 0.7 x 10^6 CD34+ cells (median, range 0.4 to 1.6 x 10^6 CD34+ cells) Timing of stem cell procedure: 4 to 8 days after primary PCI. Median 6 days (interquartile range 5 to 6) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in LVEF (%) measured by SPECT, echocardiography and MRI Secondary outcomes: changes in LVEDV (mL) and infarcted size. Also reported: NYHA class, quality of life, exercise tolerance Outcome assessment points: baseline, 3, 6, 12, 36 months Method(s): echocardiography, SPECT and MRI, SF-36, electrically braked bicycle ergometer
Notes	Three patients did not receive cell therapy as randomised: 1 patient had low cell viability and 2 patients had stent thrombosis in the acute phase

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was generated by permuted blocks stratified according to centre
Allocation concealment (selection bias)	Low risk	Randomisation details were provided in consecutively numbered, sealed envelopes
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. Echocardiograms and angiograms were analysed by investigators blinded to treatment allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	In the analysis of clinical outcomes and scientific outcomes measured by echocardiography and SPECT, all randomised patients were included with the exception of 1 patient in the control group who received a heart transplant at day 30. The number of withdrawals from MRI analysis was low and balanced between treatment arms (BMSC: 4/50 versus control: 4/51). Reasons were described as "contraindications or logistics" or in one case, due to incomplete MRI data
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00199823) were reported
Other bias	Low risk	None reported or identified

Lunde 2006

Study characteristics	
Methods	Type of study: parallel RCT Source of funding: European Union FP7 programme Country of origin: Belgium, Czech Republic, Denmark, Finland, France, Germany, Italy, Poland, Spain, UK Number of centres: 37 Intended enrolment: 3000
Participants	Population: AMI, PCI in <24 and LVEF<45% 2-6d after PCI on echo. (cells vs controls) Age, mean (SD) each arm: 59±SD11 vs 60±SD11 Sex, % male in each arm: 84% vs 77%. Number of diseased vessels: not mentioned. Used cutoff for Number of stunned hyperkinetic, etc segments: LVEF <45% 2-6d after PCI on echo. Time from symptom onset to initial treatment: median 3.6 (Q1 2.2; Q3 7.2) vs 3.8 (Q12.3 vs Q3 7.6) Statistically significant baseline imbalances between the groups?: no
Interventions	Intervention arm: Type of stem cells: BMMNC Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate (isolation through Ficoll and t2cure method), intracoronary delivery in culprit coronary artery. Dose of stem cells: 25-500 * 10^6 cells Timing of stem cell procedure: 2-8 days after primary PCI Comparator arm: regular care
Outcomes	Primary outcomes: all-cause mortality Secondary outcomes: cardiac mortality, the composite of cardiovascular death or heart failure re-hospitalization, and the composite of re-hospitalization for repeat myocardial infarction, revascularization, heart failure, implantable cardioverter-defibrillator (ICD), or stroke. Safety endpoints included: adverse events (collected up to 6months) and serious adverse events, syncope, arrhythmia, neoplastic disease, and bleeds at 2 years. Outcome assessment points: baseline and 36 months.
Notes	Contact information Principal Investigator: Professor Anthony Mathur, MB BChir, FRCP, PhD; Queen Mary University of London, UK infused cells range from 0.25-5*10^8 cells. Mean number of cells >10^8, so added to subanalysis of >10^8.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Patients will be randomised to treatment or control group in a 1:1 ratio. Randomisation will be stratified according to country Randomisation will be completed via IVRS (in study protocol)
Allocation concealment (selection bias)	Low risk	Endpoints were reported throughout the follow-up period and were adjudicated by an independent Clinical Event Committee (CEC, Supplementary material online, Appendix S6) blinded to the patient treatment allocation.
Blinding (performance bias and detection bias) All outcomes	High risk	Endpoint committee was blinded and echocardiography analysis at core lab was blinded. However, due to the open-label nature of the study, patients and caretakers were not blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	no incomplete outcome data seen. Patients were added to the analysis, even though patients in the cell therapy group did not always receive the allocated therapy.
Selective reporting (reporting bias)	Low risk	all outcomes reported, that were mentioned on clinicaltrials.gov and in their study rationale paper.
Other bias	Unclear risk	In >10% of cell therapy group, the patients after randomization did not receive allocated intervention. multiple authors have reported fees from pharmaceutical companies and one author is co-founder of t2cure, a manufacturer of cellular products.

Mathur 2020

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Ministry of Health, Czech Republic Country of origin: Czech Republic Number of centres: 1 Dates of trial enrolment: 11/03 to 08/05 Length of follow-up: 12 months Number (N) of participants randomised to each arm: not reported (73 in total across both intervention arms and the control group) Number (N) of participants analysed (primary outcome) in each arm: 20 treatment/20 control. Extended study of high-dose cell therapy versus controls: 37 in the treatment group and 36 in the control group
Participants	Population: AMI, within 24 hours Age, mean (SD) each arm: 54 (SEM 2) years in the high cell dose group, 54 (SEM 2) years in the low cell dose group, and 55 (SEM 2) years in control Sex, % male in each arm: 90% in the high cell dose group, 95% in the low dose group, and 90% in controls Number of diseased vessels: 1:14, 2:6, 3:0 (high dose); 1:11, 2:8, 3:1 (low dose); 1:14, 2:6, 3:0 in control Number of stunned hyperkinetic, etc segments: 0.4 (0.2) (high dose), 0.5 (0.2) (low dose), 0.4 (0.2) (controls). Irreversibly damaged segments: 6.2 (SEM 0.6) (high dose), 5.9 (SEM 0.5) (low dose), 6.1 (SEM 0.5) (controls) Time from symptom onset to initial treatment: 444 minutes (SEM 163 minutes) (high dose), 401 minutes (SEM 133 minutes) (low dose), 552 minutes (SEM 204 minutes) (controls) Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM aspirates after PCI. Cells were separated by density centrifugation. Cells cultivated overnight and re-suspended in 22 mL prior to transplantation. Intracoronary infusion using an inflated balloon catheter. 7 balloon inflations for 3 minutes each, separated by 3-minute intervals of balloon deflation. 3 mL BM cell suspension injected at each balloon deflation Dose of stem cells: 1×10^8 MNC (range 0.9 to 2×10^8 cells) (high dose) or 1×10^7 MNC (range 0.9 to 2×10^7 cells) (low dose) Timing of stem cell procedure: PCI within 24 hour of AMI symptoms, 3 to 7 days for randomisation, 5 to 9 days BM aspiration and infusion. Time from onset to cell transplantation: 6.8 (0.3) days (high dose) and 6.9 (0.3) days (low dose) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: change in regional systolic function of the infarcted wall Secondary outcomes: changes in 1. LVEF, 2. LV volumes, 3. Perfusion defect size Outcome assessment points: baseline and 3, 6 and 12 months Method(s): SPECT and Echo
Notes	Data from the 2 active intervention arms of the trial are pooled in this review. 2 patients had fever and 1 patient had brachycardia, all within 20 hours prior to cells; these 3 patients were randomised to cell therapy (unclear whether high or low dose) but they did not receive cell therapy as randomised

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of participants and clinicians was not reported although controls did not undergo bone marrow aspiration and no placebo was administered. Echocardiographers were blinded to treatment assignment
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	From a total of 73 patients randomised to 1 of 3 treatment arms, 7 withdrew or were excluded from the analysis of all outcomes: 1 control patient was excluded because PET did not confirm the irreversibility of the myocardial damage and 2 controls underwent repeat MI 2 days after the hospital discharge due to in-stent thrombosis. 3 patients randomised to BMSC were not transplanted because of complications within 20 hours before the procedure and a 4th patient was excluded because of an inadequate amount of implanted MBM cells; it was unclear whether these patients were randomised to high or low-dose BMSC. 4 patients (cells: 2/22 versus no cells: 2/22) were missing from SPECT analysis at 3 and 12 months follow-up; reasons for missing data were not reported. In separate publications, an expanded cohort of up to 73 patients (37 high dose cells and 36 controls) were included in SPECT analysis at 3, 6 and 12 months; the number of randomised patients was unclear
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Meluzin 2008

Study characteristics	
Methods	Type of study: parallel RCT Source of funding: Department of Biotechnology, New Delhi Country of origin: India Number of centres: 5 Final recruitment: 250
Participants	Population: patients with AMI Age, mean (SD) each arm: not reported (aged 30 to 65 years) Sex, % male in each arm: not reported Number of diseased vessels: proximal and/or mid left anterior descending artery involvement by angiography Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: > 2 hours to PCI Statistically significant baseline imbalances between the groups? It was noted that the participants from trial deviate group were older (n=38, 50.26 ±9.16 yr) compared to non trial deviate group (n=71, 46.22 ± 9.44 yr) (P<0.05). Similarly, there were greater number of hypertensives in trial deviate group (P<0.05) compared to non trial deviate group.
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells Summary of how stem cells were isolated and type and route of delivery: not reported Dose of stem cells: 5 to 10 x 108 stem cells Timing of stem cell procedure: not reported Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in LVEF from baseline to 6 months Secondary outcomes: mortality, rehospitalisation for chest pain, heart failure or arrhythmias, and safety of the intervention to 6 months Outcome assessment points: baseline and 6 months Method (s): multi-gated acquisition (MUGA) scan
Notes	Contact info: nairvelu2000@yahoo.com SAE: no overview. in the text there is mentioning of one patient dying in the cell therapy group because of LV failure. assumed that there is 1 death in the total population here. "AEs and SAEs recorded during six months follow up were equally distributed in both the groups with no significant difference. The AEs reported were hospitalization, chest pain, dyspnoea and other symptoms. There were 15 AEs in stem cell group and 11 in non stem cell group. Overall, 14 SAEs were reported, of which nine were in the stem cell group and f ive in the non stem cell group." --> no definite conclusions for our secondary outcomes here. Emailed the authors for clarification. Median time to therapy was 15d. assumed that cell therapy was (on average) >10d after AMI.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	The randomization list and numbered packing of the intervention, allocating patient in 1:1 ratio to either SCT or non SCT groups, were prepared off site by central data coordinator, for all centres. The random numbers were generated by a computer programme using permuted blocks of variable length.
Allocation concealment (selection bias)	Unclear risk	no mentioning of allocation concealment
Blinding (performance bias and detection bias) All outcomes	High risk	The nuclear medicine specialists in all centers and the independent external observer were blinded to each other and patient assignment. However, due to the open-label nature of the study (control patients did not undergo sham procedures, but received standard care) there was no blinding for patient and caretaker.
Incomplete outcome data (attrition bias) All outcomes	High risk	large withdrawal rate in cell therapy group, many patients not included in analysis due to low cell yield and primary analysis on nested cases (short term mortality rates are mentioned and all patients were followed, so in our primary analysis used all randomized patients for short-term data on mortality).
Selective reporting (reporting bias)	High risk	there is selective reporting of the adverse events, not mentioning exact numbers for all events and not
Other bias	Unclear risk	the analysis plan was not prespecified.

Nair 2015

Study characteristics	
Methods	Type of study: RCT Source of funding: his project was financially supported in part by a grant from Royan Institute, the Iran Industrial Development and Renovation Organization (IDRO), and the Small Business Development Center (SBDC). Country of origin: Iran Number of centres: 5 (all in Tehran) Intended enrolment: 90 Actual enrolment: 77
Participants	Population: First anterior STEMI, eligible for elective CABG, LVEF <45% Age, mean (SD) each arm: Cell therapy MNC 51/5 (7.5) Cell therapy CD-133+ (53.1 (8.6) Placebo 55.5 (8.5) Sex, % male in each arm: Cell therapy MNC 90% Cell therapy CD-133+ 90.5% Placebo 88.5% Number of diseased vessels (1/2/3-vessel) Cell therapy MNC 5/11/14 Cell therapy CD-133 0/6/15 Placebo 2/4/20 Time from symptom onset to initial treatment: 10-30 days. Statistically significant baseline imbalances between the groups?: yes. LVEDD and LVESD at baseline slightly differed (higher in CD133 group compared to MNC group)
Interventions	Intervention arm: Type of stem cells: BMMNC or CD-133+ cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate from iliac crest. Delivery through 10x0.2ml injection Dose of stem cells: MNC group 564.63×10^{6} (± 69.35) MNC cells per recipient. CD-133 group: 8.19×10^{6} (± 4.26) CD133+ cells per recipient. Timing of stem cell procedure: 10-30 days after STEMI Comparator arm: sham operation and injection of normal saline supplemented with 2% autologous serum
Outcomes	Primary outcomes: changes in LVEF from baseline to 6 and 18 months Secondary outcomes: Adverse cardiac events that included death, reinfarction, implantable cardioverter defibrillator (ICD) placement, infection, and arrhythmia, ii. Changes in wall motion score (WMS), decreased systolic wall thickening (Dec. Thickening) of the myocardium, non-viable (NV) segments, and perfusion defect score (PDS) assessed by gated SPECT, and iii. NYHA classification. We compared and analyzed endpoint data amongst the three groups during the 18 months of follow up. Outcome assessment points: baseline, 6 and 18 months Method(s): SPECT and stress echocardiography
Notes	Starting date: Januari 2008 Contact Info: Nasser.aghdami@royaninstitute.org Not clear if authors used heparin for their cell solution. Not clear how many patients have undergone SPECT at 6m. Assumed that all patients that were not lost to follow-up have undergone SPECT at 18m.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	randomized eligible patients via a computer-generated randomization sequence in a 1:1:1 ratio between the CD133+, the MNC, and placebo groups.
Allocation concealment (selection bias)	Unclear risk	it is not clear who performed the randomization procedure and if this person was involved with the trial later on.
Blinding (performance bias and detection bias) All outcomes	Low risk	Patients underwent bone marrow or sham aspirations. Patients and investigators not affiliated with the cell-processing laboratory were blinded to preparation and administration of the study product.
Incomplete outcome data (attrition bias) All outcomes	Low risk	good explanation for reaching 77 patients instead of the proposed 90. no signs of attrition bias in this paper.
Selective reporting (reporting bias)	Low risk	there is mentioning of adverse events in the text, which was not reported as initial outcomes in the clicaltrialsn.gov registratioh. however, no outcomes are left out. We assumed that there was no case of selective reporting here, as the authors report more (and completely) their adverse events.
Other bias	Low risk	slight baseline differences between trial arms, however most likely due to chance

Naseri 2018

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: supported by Pro-Cardiaco Hospital - in charge of patients' care - and by Exellion Biomedical Services S/A - in charge of cell preparation and characterisation Country of origin: Brazil Number of centres: 2 Dates of trial enrolment: 01/05 to 01/06 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 14 in intracoronary artery route (AG) arm, 0 in intracoronary venous route (VG) arm, 6 in control arm Number (N) of participants analysed (primary outcome) in each arm: 14 in AG arm, 8 in VG arm, 6 in control arm
Participants	Population: AMI, within 24 hours. Thrombolysis and/or PCI within 24 hours Age, mean (SD) each arm: 59.7 (14.3) years in AG arm, 53.6 (8.3) years in VG arm, 57.2 (10.8) years in control arm Sex, % male in each arm: 71% in AG arm, 70% in VG arm, 67% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: AG group: 29% < 12 hours, 21% > 12 hours, 50% > 6 hours and after thrombolysis (all within 24 hours) VG group: 20% < 12 hours, 20% > 12 hours, 60% > 6 hours and after thrombolysis (all within 24 hours) Control group: 50% > 12 hours, 33% > 6 hours and after thrombolysis (all within 24 hours) Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC (coronary artery route, AG or coronary venous route, VG) Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: approx. 80 mL bone marrow aspirated from the posterior iliac crest under sedation, analgesia and local anaesthesia. MNC were isolated and centrifuged in a Ficoll-Pacque Plus and handled under aseptic conditions. The cells were washed and suspended in saline solution with 5% human serum albumin, re-suspended and filtered to remove cell aggregates prior to transplantation. Arterial delivery via over-the-wire balloon catheter PCI. Venous delivery via an additional over-the-wire balloon catheter positioned side-by-side with the balloon in the artery where the stent was located Dose of stem cells: 10 mL of solution containing 100 x 10^6 MNC Timing of stem cell procedure: the time interval between the AMI and cell transfer was 5.5 (1.28) days (AG) and 6.1 (1.37) days (VG) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: LVEF, WMSI, EDV, ESV Secondary outcomes: radiolabeled cells retention and washout in the heart tissue Outcome assessment points: baseline, 3 and 6 months Method(s): echocardiography, RNV
Notes	Data from the 2 active intervention arms of the trial are pooled in this review

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random assignment was made in blocks according to the AMI size (≤ 25% or < 25%), by means of sealed envelopes. Random allocation was stratified according to infarct size in 3 blocks of different size, for each stratum, with the use of sealed envelopes
Allocation concealment (selection bias)	Low risk	Randomisation details were provided in sealed envelopes
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered. Outcome assessors were blinded. Blinding of participants and clinicians not reported. The trial was described as "open-label in relation to the clinical analysis and blind in relation to the echocardiographic analysis"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants in the control group were included in the analysis of clinical outcomes and scientific outcomes. 2 patients in the intravenous cell group were missing from echocardiographic analysis at 3 and 6 months follow-up (1 sudden death 1 month after cell therapy, 1 tortuous anterior interventricular vein complicating BMSC transfer)
Selective reporting (reporting bias)	High risk	The secondary outcomes of QoL, Seattle Angina Questionnaire and cost-effectiveness described in the trial protocol (www.clinicaltrials.gov: NCT00350766) were not reported
Other bias	High risk	Supported in part by commercial funding

Nogueira 2009

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: supported by Azienda USL di Piacenza and Fondazione Piacenza & Vigevano Country of origin: Italy Number of centres: 1 Dates of trial enrolment: 07/05 to 06/07 Length of follow-up: 24 months Number (N) of participants randomised to each arm: 19 in treatment arm/19 in control arm Number (N) of participants analysed (primary outcome) in each arm: 17 in treatment arm, 15 in control arm
Participants	Population: AMI, within 6 hours. PCI within 2 to 6 hours of onset of symptoms Age, mean (SD) each arm: 63.1 (SEM 2.7) years in treatment arm, 67.2 (SEM 2.4) years in control arm Sex, % male in each arm: 68.4% in treatment arm, 68.4% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 248 (SEM 68.7) minutes from AMI to PCI in treatment arm; 265 (SEM 34.4) minutes from AMI to PCI in treatment arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 100 mL of autologous bone marrow was aspirated under local the posterior-superior iliac crest by multiple aspirations into heparinised syringes. The cells were suspended in 7 mL of PBS-EDTA buffer containing 3mL of human albumin 5% W/V. Mononuclear cell fraction was concentrated into a final volume of 25 to 30 mL. Balloon catheter was positioned at the site of the former infarct-vessel occlusion and PCI performed 4 to 5 times, for 2 minutes each time. During this time intracoronary cell transplantation via the balloon catheter was performed, using 4 to 5 fractional high-pressure infusions of 2 to 3 mL of the cell suspension Dose of stem cells: mononuclear cells: mean 248.78 x 10^6 were infused (minimum 75.4 x 10^6; maximum 570.0 x 10^6) Timing of stem cell procedure: 4 to 7 days after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: LVEF, LVEDV, LVESV Secondary outcomes: heart rate variability, baroreflex sensitivity, arrhythmias, exercise tolerance Outcome assessment points: baseline, 6, 12, 24 months Method(s): ECG, echocardiography, rest and stress perfusion scintigraphy G-SPECT, cardiopulmonary exercise testing (CPET)
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	Random assignment was made by uneven versus even numbers in a 1:1 fashion into 2 parallel groups
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of participants and clinicians was not reported although controls did not undergo bone marrow aspiration and no placebo was administered. 2 independent investigators who had no knowledge of the study collected and analysed outcome data
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. 6 patients were missing from SPECT/echocardiography analysis at follow-up: 2/19 in the BMSC arm (1 sudden death after 2 months, 1 death due to refractory heart failure at 3 months) and 4/19 in the control arm (1 sudden death after 3 months, 2 deaths due to refractory heart failure after 1 month, 1 accidental death at 2 months)
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00437710) were reported
Other bias	High risk	Supported in part by commercial funding

Piepoli 2010

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Amorcyte Inc., New Jersey Commission of Science and Technology (06-2042-014-77) Country of origin: USA Number of centres: not reported (multicentre) Dates of trial enrolment: not reported Length of follow-up: 12 months Number (N) of participants randomised to each arm: 6 (high dose, HD), 5 (moderate dose, MD), 5 (low dose, LD), 15 (controls) Number (N) of participants analysed (primary outcome) in each arm: 2 (high dose), 4 (moderate dose), 5 (low dose), 10 (controls)
Participants	Population: acute STEMI. PCI with stent within 3 days Age, mean (SD) each arm: median 50.5 (IQR 45.0 to 53.0) years (HD), 63.0 (IQR 57.0 to 66.0) years (MD), 52.0 (IQR 51.0 to 52.0) years (LD), 52.0 (IQR 47.0 to 57.0) years (controls) Sex, % male in each arm: 100% (HD), 80% (MD), 80% (LD), 87% (controls) Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: median 3.5 (IQR 2.8 to 5.1) hours (HD), 1.3 (IQR 6.2 to 22.1) hours (MD), 21.0 (IQR 7.1 to 41.3) hours (LD), 6.7 (IQR 3.9 to 23.8) hours (controls) Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: CD34+, high dose (HD), moderate dose (MD) or low dose (LD) Type of stem cells: bone marrow-derived CD34+ cells Summary of how stem cells were isolated and type and route of delivery: 320 mL (median 402 (17) mL including heparin) BM harvested under conscious sedation and local anaesthesia. CD34+ cells selected using the anti-CD34 Mab and Dynabeads on the Isolex 300i system. CD34+ cell product re-suspended in 6 mL of PBS, 4 mL (40%) of autologous human serum containing 1% human serum albumin and 25 USP U/mL of heparin sodium. Cell suspension infused via an over-the-wire balloon catheter positioned in the stented segment of the IRA Dose of stem cells: 14.3 (1.6) x 10^6 CD34+ cells (HD), 9.9 (0.7) x 106 CD34+ cells (MD), 4.8(0.4) x 10^6 CD34+ cells (LD) Timing of stem cell procedure: cells infused median 207.3 (IQR 191 to 215) hours (HD), 210 (IQR 194 to 210) hours (MD), 191.4 (IQR 167 to 201) hours (LD) after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: none stated Secondary outcomes: 1. Quantitative rest hypoperfusion score measured by SPECT, 2. LVEF, LVEDV, LVESV, infarct size by MRI, 3. Clinical adverse events (arrhythmia, chest pain, musculoskeletal pain, upper respiratory tract infection, rash, dyspnoea, fever, acute stent thrombosis, death MI, rehospitalisation for heart failure, cerebral infarction, ventricular arrhythmia or syncope, chronic myeloid leukaemia, revascularisation, septic thrombophlebitis) Outcome assessment points: baseline, 3, 6 and 12 months Method(s): gadolinium-enhanced cardiac MRI, SPECT, echocardiography, ECG
Notes	Data from the 3 active intervention arms of the trial are pooled in this review

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as "open label". Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. However, "all studies were analysed by operators blinded to the patient treatment designation"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	1 patient in the high-dose arm was excluded due to acute stent thrombosis soon after cell infusion. All other randomised patients were included in the analysis of clinical outcomes. For MRI assessment at 3 and 6 months, 1 patient had died due to ventricular fibrillation soon after cell infusion. 2 further patients in the high-dose BMSC group (total 4/6), 1 patient in the medium-dose BMSC arm (1/5) and 5 patients in the control group (5/15) were missing from MRI assessment. There were no withdrawals or exclusions (0/5) in the low-dose BMSC group. The reasons for patient drop-out were given as "death, refused, defibrillators, stent thrombosis, and poor image quality", however the number of patients falling into each category was not reported
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00313339) were reported
Other bias	High risk	This is a commercially funded trial

Quyyumi 2011

Study characteristics	
Methods	Type of study: RCT Source of funding: This work was supported by Caladrius Biosciences, Inc.. Neostem, Inc. Country of origin: United States Number of centres: 60 Enrolment: 195
Participants	Population: AMI and succesfull PCI. Age, mean (SD) each arm: Cell therapy: 57.1 (10.1) Placebo: 56.4 (10.1). Sex, % male in each arm: Cell therapy: 85%. Placebo: 20%. Number of diseased vessels: not reported Used cutoff for Number of stunned hyperkinetic, etc segments: >2/3 of left ventricular anteroseptal, lateral or inferior wall Time from symptom onset to initial treatment: Cell therapy: 931(1277) minutes. Placebo: 569(864) minutes Statistically significant baseline imbalances between the groups?: longer ischemic time in cell therapy group compared to the control group.
Interventions	Intervention arm: Type of stem cells: CD34+ cells Summary of how stem cells were isolated and type and route of delivery: iliac bone marrow aspirate, intracoronary delivery Dose of stem cells: 40.2(25.9) million CD34+ cells isolated, 14.9 (8) million cells in final cell product. Timing of stem cell procedure: within 72 hours after BM isolation and within 11d after stent placement. Comparator arm: also BM biopsy. coronary infusion with 10ml PBS with autologous serum and albumin.
Outcomes	Primary outcomes: AEs, SAEs, and MACE, and the assessment of myocardial perfusion by quantitative gated SPECT MPI looking at RTSS. Secondary outcomes: 1.) Change in LVEF from baseline to 6 months post randomization as measured by CMR. For subjects unable to complete paired CMR imaging, baseline and 6 month post stenting LVEF was characterized using gated SPECT MPI; 2.) Preservation of LVEF at 6 months post randomization. Subjects having an absolute decrease in LVEF greater than 2% were characterized as having a decrease (non-preservation) in LVEF measured by CMR. All other subjects were characterized as having a preserved LVEF; 3.) Time to individual and cumulative MACE events defined as cardiac mortality, hospitalization for worsening heart failure, or recurrent AMI) with a minimum of 6 months of follow-up and subsequently, with a maximum of 3 years follow-up. Outcome assessment points: baseline, 6 and at least 12 months follow-up for AE, SAE and MACE. Method(s): MRI and SPECT
Notes	Baseline LVEF measured by MRI <48% (so not included for baseline measurements < or> 45% subgroup analyses) For the mortality analyses the tables mention only cardiac mortality, but in the text total mortality (= similar to cardiac mortality) is mentioned. Filled in the modified intention-to-treat analysis for all outcomes (patients left after bone marrow harvest and infusion). These numbers are 0 deaths for cell therapy group, 3 deaths in the control group.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as randomized, but method not described.
Allocation concealment (selection bias)	Unclear risk	No description of any allocation concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Both therapy group and control group underwent BM aspiration and subsequent catheterization. Is described as double-blind, but there is no mentioning of blinding of the assessors of the imaging data.
Incomplete outcome data (attrition bias) All outcomes	Low risk	no signs of incomplete outcome data in the paper.
Selective reporting (reporting bias)	Unclear risk	mentioned all outcomes that were previously reported on clinicaltrials.gov. mentioning of modified intention to treat analysis (patients who were actually infused with BM) as primary analysis makes the judgement on reporting bias 'unclear risk of bias'.
Other bias	High risk	Funded by industry (Caladrius Biosciences)

Quyyumi 2017

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: French Department of Health - Programme Hospitalier de Recherche Clinique (PHRC), the Association Francaise contre les Myopathies, the Fondation de France Country of origin: France Number of centres: 6 Dates of trial enrolment: 12/04 to 01/07 Length of follow-up: 12 months Number (N) of participants randomised to each arm: 52 in treatment arm/49 in control arm Number (N) of participants analysed (primary outcome) in each arm: 48 in BMSC arm/44 in control arm
Participants	Population: acute STEMI, PCI with stent within 24 hours Age, mean (SD) each arm: 56 (12) years in treatment arm, 55 (11) years in control arm Sex, % male in each arm: 80.8% in treatment arm, 89.8% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: "within 24h after the onset of chest pain"; < 12 hours in 75% of BMSC arm/75.5% of control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 50 mL of bone marrow was aspirated into heparinised syringes under local anaesthesia from the iliac crest. Lymphocyte preparation medium centrifugation procedures were used to isolate and enrich progenitor cells. A heterogeneous cell suspension population was obtained that consisted of haematopoietic, endothelial and other progenitor cells, as well as mononuclear cells. A single syringe of 100 x 10^6 BMCs was prepared in 10 mL 4% human albumin. Intracoronary infusion using over-the-wire balloon catheter technique positioned within the stented segment Dose of stem cells: 100 x 10^6 autologous BMMNC Timing of stem cell procedure: infusion performed 9.3 ± 1.7 days after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: improvement of myocardial viability - "a gain of at least 2/17 viable segments 3 months after STEMI, assessed by resting 4 h thallium-201-gated-SPECT." Secondary outcomes: 1. changes in LVEF evaluated by RNA, MRI, and echocardiography, 2. changes in LVEDV and LVESV, 3. infarct size by MRI, 4. binary restenosis by coronary angiography, 5. segment-by-segment improvement of myocardial viability. Also measured: QOL Outcome assessment points: baseline, 1 month, 3 months, 12 months Method(s): radionuclide angiography (RNA), echocardiography, MRI, T201-SPECT, MLHFQ
Notes	1 patient did not receive BM aspirate due to thrombopenia but was included as randomised

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Patients were randomly assigned in a 1:1 ratio to either the control group or BMC group using permuted-block randomisation stratified according to centre, diabetes status and time to PCI after the onset of AMI (≤ 12 or > 12 hours)
Allocation concealment (selection bias)	Low risk	Consecutively numbered, sealed envelopes were provided to all participant centres
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as "open label"; controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. 3 independent core imaging laboratories, blinded to treatment assignment, performed all cardiac imaging measurements
Incomplete outcome data (attrition bias) All outcomes	Low risk	In the analysis of clinical outcomes, there were 9 withdrawals or exclusions: 4/52 in the BMSC arm (2 withdrawals due to adverse clinical events, 1 withdrawal due to randomisation error and 1 refusal to complete follow-up) and 5/49 in the control arm (1 patient had steroid therapy for angioneurotic oedema, 1 had post-MI ventricular septal defect and 3 patients refused follow-up). In the analysis of scientific outcomes at 3 months, 1 further patient in the BMSC arm had died and 1 additional patient in the control arm was missing, the reason for which was not reported
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00200707) were reported
Other bias	Low risk	None reported or identified

Roncalli 2010

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: China Number of centres: 1 Dates of trial enrolment: 07/03 to 08/04 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 9 in the BMSC arm/11 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 9 in the BMSC arm/11 in the control arm
Participants	Population: AMI, within 24 hours Age, mean (SD) each arm: 61 (8) years in treatment arm, 58 (6) years in control arm Sex, % male in each arm: 88.9% in treatment arm, 100% in control arm Number of diseased vessels: range 1 to 3 but no more details stated Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 12.7 (12.6) hours in treatment arm/12.3 (13.4) hours in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: the study does not state how the cells were isolated or processed. Except that cells were suspended in diluted serum prior to transplantation. Cells were infused by percutaneous transmural coronary angioplasty (PTCA) Dose of stem cells: not reported Timing of stem cell procedure: within 2 hours of successful PTCA Comparator arm: diluted serum
Outcomes	Primary outcomes: the study does not state clearly a primary outcome. The aim is to assess changes in LV segmental function by Doppler imaging Secondary outcomes: changes in 1. LV global function and volume, 2. LVEDV (mL), 3. LVESV (mL), 4. LVEF (%) Outcome assessment points: baseline, 3 months and 6 months Method(s): Doppler imaging and echocardiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised; patients were selected "prospectively and consecutively"
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group received an injection of heparinised saline although it is not reported whether they underwent bone marrow aspiration. It is therefore unclear whether participants and clinicians were sufficiently blinded to treatment. Outcome assessors were blinded to clinical and angiographic information
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Ruan 2005

Study characteristics	
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Spain Number of centres: 1 Intended enrolment: 120
Participants	Population: AMI Age, mean (SD) each arm: not reported (18 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: < 24 hours Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow mononuclear cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirated, mononuclear cells isolated by Ficoll technique, delivery via intracoronary injection Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: change in LVEF and LVESV Secondary outcomes: change in LVEDV, segment contractility, wall thickness and intravascular ultrasound re-endothelialisation, safety Outcome assessment points: baseline, 9 months and 12 months Method(s): MRI, ultrasound
Notes	Estimated completion date: November 2009. This trial includes 2 additional randomised groups: G-CSF plus bone marrow mononuclear cells and progenitor cells mobilised through GCSF. This Cochrane review has previously excluded G-CSF studies due to the lack of a G-CSF control group. Since in this study, this control group is implemented, we have added this comparison as a separate entry. There are two study IDs. San Roman (BMMNC) and San Roman (BMMNC+G-CSF)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Mentioning of randomization, but could not find the method of randomization that is used.
Allocation concealment (selection bias)	Unclear risk	Mentioning of randomization, but could not find the method of randomization that is used.
Blinding (performance bias and detection bias) All outcomes	High risk	single-blinded trial. Clinical outcome was adjudicated by an independent clinical events committee, blinded to study group assignment. All CMR, coronary angiograms, and LV angiographies were analyzed at an independent central imaging core laboratory (ICICORELAB, Valladolid, Spain) blinded to patient treatment assignment. Due to the open-label nature of the trial, both patients and caretakers where aware of at least part of the grouping information. therefore, high risk of bias.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No discrepancies in data. Good explanation on reduced number of patients in paired LV function assessments.
Selective reporting (reporting bias)	Low risk	all outcome measurements desribed on clinicaltrials.gov were reported.
Other bias	Low risk	This study is an academic clinical trial. The studysponsors had no role in the study design, data collection, data analysis, data interpretation, orwriting of this report. The corresponding authors had full access to all study data and were responsible for the decision to submit for publication.

San Roman 2015 (BMMNC+G-CSF)

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: research grant from Guidant and support from Eli Lilly Country of origin: Germany and Switzerland Number of centres: 17 (16 in Germany + 1 in Switzerland) Dates of trial enrolment: 04/04 to 04/05 Length of follow-up: 5 years Number (N) of participants randomised to each arm: 101 in the treatment arm/103 in control arm Number (N) of participants analysed (primary outcome) in each arm: 95 in treatment arm/92 in control arm
Participants	Population: AMI, within 5 days Age, mean (SD) each arm: 55 (11) years in treatment arm, 57 (11) years in control arm Sex, % male in each arm: 82% in treatment arm, 82% in control arm Number of diseased vessels: 1:61; 2:24; 3:16 in treatment arm/1:60; 2:32; 3:11 in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 7.5 (8.0) hours to PCI in treatment arm/7.0(6.5) hours to PCI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM aspirates 3 to 6 days after PCI, cells were separated by Ficoll gradient centrifugation and re-suspended in 10 mL of X-VIVO medium containing 20% autologous serum. Intracoronary infusion using an inflated balloon catheter. 3 portions of 3.3 mL cell suspension were infused in 3-minute occlusion time for each portion and 3-minute intervals Dose of stem cells: 10 mL of a single dose containing 2.36 (1.74) x 10^8 mononuclear cells Timing of stem cell procedure: PCI within 12 hrs of AMI symptoms, harvest 3 to 6 days after PCI, randomisation and transport prior to infusion 3 to 6 days Comparator arm: placebo consisting of 10 mL X-VIVO medium with 20% autologous serum
Outcomes	Primary outcomes: changes in LVEF Secondary outcomes: 1. Improvement of global LVEF, 2. Reduction of LVESV, 3. Improvement of regional wall motion and myocardial contractility, 4. Assessment of major adverse events, such as revascularisation, death and hospitalisation due to heart failure Outcome assessment points: baseline, 4, 12, 24 months, 5 years Method(s): LV angiography
Notes	3 patients randomised to the placebo arm did not receive placebo medium but were included in the analysis: 1 patient in placebo group had angiographic evidence of a thrombus in a non-infarct-related artery, 1 patient had an air embolism during initial angiography before the guidewire could be advanced and in 1 patient the guidewire could not be advanced into the infarct-related artery

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was carried out using computer-generated randomised lists maintained at a site external to the trial
Allocation concealment (selection bias)	Low risk	Bone marrow aspirates were sent to the cell processing centre (centralisation)
Blinding (performance bias and detection bias) All outcomes	Low risk	All patients underwent bone marrow aspiration and control group patients were given an intracoronary injection of placebo medium. Bone marrow aspirates were then sent to a central cell processing centre; participants and clinicians were therefore blinded to treatment. LV angiography was performed by an experienced investigator in a central core laboratory who was unaware of the patient's treatment assignment until after analysis of 4-month data was complete. Study centres and investigators and those entering the data into databases remained blinded until 12-month follow-up was complete.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised patients were included in the analysis of clinical outcomes at 4 months follow-up; 3 and 2 patients in the control group were lost to follow-up at 12 months and 2 years respectively. In the analysis of scientific outcomes, 6/101 in the BMSC group and 11/103 in the placebo group were missing from LV angiography analysis at 4 months (2 had poor quality results on angiography, 4 deaths before 4 months, 5 declined and 6 did not undergo angiography). A subset of 59 patients were included in a sub-study of MRI 2 years
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00279175) were reported, with the exception of NYHA class, although all other pre-specified morbidity outcomes were reported
Other bias	Low risk	This is a commercially funded trial

Schachinger 2006

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: Spain Number of centres: 1 (assumed) Dates of trial enrolment: from 01/05, end not reported Length of follow-up: 3 months Number (N) of participants randomised to each arm: 10 in the treatment arm/10 in control arm Number (N) of participants analysed (primary outcome) in each arm: 10 in treatment arm/10 in control arm
Participants	Population: AMI, within 12 days Age, mean (SD) each arm: 52 (12) years in treatment arm, 55 (11) years in control arm Sex, % male in each arm: 80% in treatment arm, 70% in control arm Number of diseased vessels: at least 1, left anterior descendent (LAD) artery in treatment arm/at least 1 (LAD) in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI was carried out 3 to 5 days post AMI, treatment intervention took place 7 (2) days after PCI Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM aspirates (80 to 100 mL), cells were separated by Ficoll gradient centrifugation and re-suspended in 10 mL of 0.9% sodium chloride (saline) and 0.1% heparin. Intracoronary infusion using an inflated balloon catheter during 2 to 4 minutes Dose of stem cells: 10 mL of a single dose containing 9 x 10^8 mononuclear cells, corresponding to 17 (13) x 10^6 CD34+ cells. Timing of stem cell procedure: PCI within 3 to 5 days of AMI symptoms, bone marrow harvest and infusion 7 (2) days post PCI Comparator arm: placebo consisting of 0.9% sodium chloride (saline) and 0.1% heparin
Outcomes	Primary outcomes: changes in LVEF Secondary outcomes: 1. LVEF, 2. LVESV, 3. LVEDV, 4. Wall motion Outcome assessment points: baseline, 3 months Method(s): LV angiography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"Randomisation by telephone was performed" but the sequence generation procedure was not described
Allocation concealment (selection bias)	Unclear risk	"Randomisation by telephone was performed"
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group did not undergo bone marrow aspiration although they received an injection of heparinised saline and therefore it is unclear whether participants and clinicians were sufficiently blinded to treatment. 2 angiographers were unaware of patient group assignment
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Suarez de Lezo 2007

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: funded by Fondazione Cardiocentro Ticino, Lugano, Switzerland; Zurich Heart House-Foundation for Cardiovascular Research, Zurich, Switzerland; Bern University Hospital, Bern, Switzerland; Cardiovascular Research Foundation, Zurich, Switzerland, and an unrestricted grant from Abbott Vascular Country of origin: Switzerland Number of centres: 4 Dates of trial enrolment: 10/06 to 01/12 Length of follow-up: 4 months Number (N) of participants randomised to each arm: 66 in the early cell therapy arm, 67 in the late cell therapy arm, 67 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 58 in the early cell therapy arm, 49 in the late cell therapy arm, 60 in the control arm
Participants	Population: STEMI with PCI in 24 hours and EF ≤ 45% Age, mean (SD) each arm: median 55 (IQR 15) years (early cells), 62 (IQR 15) years (late cells), 56 (IQR 14.5) years (controls) Sex, % male in each arm: 86.2% (early cells), 82.5% (late cells), 83.6% (controls) Number of diseased vessels: 1 (54%), 2 (32%), 3 (14%) (early cells), (57%), 2 (27%), 3 (16%) (late cells), 1 (64%), 2 (21%), 3 (15%) (controls) Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 6 (2) days (early cells) or 24 (7) days (late cells) after AMI Statistically significant baseline imbalances between the groups? Higher age in the late treatment group compared with controls (median 62 years versus 56 years; P value = 0.06); lower percentage of smokers in the late treatment group compared with controls (40.3% versus 62.7%; P value = 0.01); higher baseline LVEF in the control group compared with the treatment group (median 39.6% versus 35.6%, P value = 0.03)
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspiration was performed 5 to 7 days after AMI. Between 60 and 80 mL of bone marrow was collected from the iliac crest under local anaesthesia. Then 1 mL of a solution containing 1000 IU heparin was added to each 10 mL of bone marrow aspirate to prevent clotting. Then the aspirate and 20 mL of the patient's serum were sent at room temperature by courier to the cell-processing centre. The BM-MNC cell suspension was shipped back to the participating hospital within 24 hours. Briefly, with the use of density gradient centrifugation, the mononuclear cell fraction was re-suspended in 10 mL of serum-free medium with 20% of autologous serum added without any additional heparin. An aliquot of cell suspension was utilised for fluorescence-activated cell sorting analysis with the use of fluorochrome conjugated antibodies against anti-human CD34 and CD133; cell viability was assessed by 7-AAD cell uptake, and sterility was assessed by the Bact/Alert rapid method. Release criteria of the BMMNC were product sterility, a cell count between 5×10^7 and 5×10^8, and cell viability of ≥ 80% Dose of stem cells: 1.59 (± 1.25) x 10^8 cells (early cells); 1.39 (± 1.20) x 10^8 cells (late cells) Timing of stem cell procedure: 5 to 7 days post-AMI (early cells); 3 to 4 weeks post-AMI (late cells) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: absolute change in global LVEF from baseline to 4 months Secondary outcomes: change in LVEF, LVESV, LVEDV infarct size proportion of scar mass to total LV mass, global and regional myocardial thickening, major adverse events Outcome assessment points: 4 and 12 months Method(s): MRI
Notes	Data from the 2 active intervention arms of the trial are pooled in this review. There is a discrepancy between the absolute change LVEF values and baseline/endpoint values reported. The authors were contacted to request clarification on this discrepancy but none was forthcoming

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was performed using closed envelopes in a 1:1:1 pattern
Allocation concealment (selection bias)	Low risk	Closed envelopes were used
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as "open label"; controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. However, it is reported that "the entire analysis was performed in a CMR core laboratory, blinded to the treatment assignment of the patients enrolled."
Incomplete outcome data (attrition bias) All outcomes	High risk	In the analysis of clinical outcomes, the number of withdrawals and exclusions was unbalanced between groups (early cells: 11/66 versus late cells: 15/67 versus control: 7/67). Although reasons for withdrawals were given (withdrawal of informed consent or death in all missing patients), these do not fully explain the sample sizes described in individual analyses. In the analysis of scientific outcomes by MRI analysis at 4 months, 8 additional patients were missing in the BMSC arm due to the lack of paired MRI data
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00355186) were reported
Other bias	Low risk	None reported or identified

Sürder 2013

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Polish Ministry of Science and Higher Education (grants number PBZ-KBN-099/P05/2003, 0651/P01/2007/32, 2422/P01/2007/32) Country of origin: Poland Number of centres: 5 Dates of trial enrolment: 03/05 to 09/07 Length of follow-up: 6 years Number (N) of participants randomised to each arm: 80 (selected cells), 80 (unselected cells), 40 (controls) Number (N) of participants analysed (primary outcome) in each arm: 51 (selected cells), 46 (unselected cells), 20 (controls)
Participants	Population: AMI, within 12 hours. PCI within 12 hours Age, mean (SD) each arm: median 58 years (selected cells), 55 years (unselected cells), 59 years (controls) Sex, % male in each arm: 63.7% (selected cells), 70.6% (unselected cells), 75% (controls) Number of diseased vessels: 1 in all trial arms Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: from AMI to PCI: median 303 minutes (101 to 1100) (selected cells), 309 minutes (117 to 1000) (unselected cells), 300 minutes (120 to 1080) (controls) Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: CD34+CXCR4+ or BMMNC Type of stem cells: selected cells: CD34+CXCR4+ selected bone marrow-derived stem cells; unselected cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 100 to 120 mL bone marrow aspirated from the posterior superior iliac spine into heparinised syringes under general anaesthesia Selected cells: Ficoll density gradient centrifugation to isolate mononuclear cells, CD34+CXCR4+ cell population was isolated using two-step immunomagnetic selection with monoclonal antibodies coupled with magnetic beads and MidiMACS System. Re-suspended in phosphatebuffered saline (final volume 10 mL). Delivery via intracoronary infusion by PCI over the wire balloon catheter technique Unselected cells: Ficoll density gradient centrifugation to isolate mononuclear cells. Delivery via intracoronary infusion by PCI over the wire balloon catheter technique Dose of stem cells: 3 infusions delivering a median of 1.9 x 10^6 CD34+CXCR4+ cells in total (selected cells); median of 1.78 x 10^8 MNCs (unselected cells) Timing of stem cell procedure: BM aspiration and BMSC infusion was done 7 (3 to 12) (median (range)) days after primary PCI. Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: LVEF by MRI Secondary outcomes: LVEF by LV angiography, LVESV, LVEDV, MACE (death, re-infarction, stroke and target vessel revascularisation (TVR)) Outcome assessment points: baseline, 6 months, 6 years Method(s): echocardiogram, LV angiography, MRI
Notes	Data from the 2 active intervention arms of the trial are pooled in this review. Table 1 footnote says values expressed as medians with quartiles, whereas text describes means and ranges - unclear whether values throughout paper for medians are whole ranges or interquartile ranges

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"Eligible patients were randomised by centre in 2:2:1 fashion into three parallel groups" but the sequence generation procedure was not described
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as "open label"; controls did not undergo bone marrow aspiration and no placebo was administered. Investigators assessing cMRI and LV angiography outcome measures were blinded to the group assignment
Incomplete outcome data (attrition bias) All outcomes	High risk	All randomised participants were included in the analysis of clinical outcomes. For MRI assessment at 6 months follow-up, there was 29/80 missing in selected BMSC arm (1 death, 28 unexplained), 34/80 missing in unselected BMSC arm (1 death, 33 unexplained), and 20/40 missing in control arm (1 death, 19 unexplained)
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00316381) were reported, although LVEF and LV volumes were measured by MRI and LV angiography rather than echocardiography
Other bias	Low risk	None reported or identified

Tendera 2009

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: the Jon Holden DeHaan Foundation, The Production Assistance for Cellular Therapies, N01-HB-37164 Country of origin: USA Number of centres: 1 Dates of trial enrolment: "beginning in 12/05" Length of follow-up: 6 months Number (N) of participants randomised to each arm: 30 in treatment arm/10 in control arm Number (N) of participants analysed (primary outcome) in each arm: 30 in treatment arm/10 in control arm
Participants	Population: first anterior STEMI, PCI with stent implantation Age, mean (SD) each arm: median 52.5 years (IQR = 43, 64) in treatment arm, median 57.5 years (IQR = 54, 59) in control arm Sex, % male in each arm: 83.33% in treatment arm, 60% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: median 4.6 hours (IQR = 2, 12 hours) in treatment arm/median 2.9 hours (IQR = 2.8, 10.6 hours) in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: patients lightly sedated, 50 to 70 mL bone marrow aspirated from posterior iliac crest. Aspirate heparinised and transported within 1 hour to cell therapy laboratory. BMMNC isolated by Ficoll density centrifugation at 450 g, cells counted with an automated cell counter and the cell suspension volume was adjusted to reach a final product of 100 million BMCs with 5% human serum albumin in 20 mL. Administered via intracoronary perfusion Dose of stem cells: 10^8 BMSC Timing of stem cell procedure: median 4.5 days (IQR = 4,7 days) after PCI, within 8 hours of BM aspiration Comparator arm: solution of 0.9% isotonic sodium chloride solution and 5% human serum albumin in an identical volume
Outcomes	Primary outcomes: "To investigate the effects of BMC administration in patients following STEMI on recovery of LV function using cardiac MRI" Secondary outcomes: LV volumes by MRI, safety as assessed by MACE (death, repeated target vessel revascularisation, recurrent MI, hospitalisation for chronic heart failure, and internal cardia defibrillator (ICD) placement) Outcome assessment points: baseline and 6 months Method(s): MRI
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was based on an algorithm developed by a biostatistician
Allocation concealment (selection bias)	Low risk	Randomisation was performed at the cell processing facility following preparation of the bone marrow cells
Blinding (performance bias and detection bias) All outcomes	Low risk	The trial was described as "double blind"; all patients underwent bone marrow aspiration and control group patients were given an intracoronary injection of placebo medium. Blinding of clinicians was not reported. Outcome measurements were assessed by MRI readers blinded to treatment allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical and scientific outcomes
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00268307) were reported, with the exception of infarct size which was included as a secondary outcome
Other bias	Low risk	None reported or identified

Traverse 2010

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: supported by the National Heart, Lung, and Blood Institute Country of origin: USA Number of centres: 5 Dates of trial enrolment: 07/08 to 02/11 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 59 in the treatment arm, 29 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 55 in the treatment arm, 26 in the control arm
Participants	Population: AMI within 2 to 3 weeks after PCI Age, mean (SD) each arm:57.6 (11) in the treatment arm, 54.6 (11) in the control arm Sex, % male in each arm: 79% in the treatment arm, 90% in the control arm Number of diseased vessels: 1 or 2 or 3 Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: median 3.4 (IQR 2.3 to 14.3) hours from onset to PCI; median 17.4 (IQR 15.5 to 20.0) days from PCI to infusion Statistically significant baseline imbalances between the groups? Baseline heart rate at initial presentation was higher in the placebo group than the treatment group (90.3% versus 77.5%; P value = 0.01)
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells (MNC) Summary of how stem cells were isolated and type and route of delivery: approximately 80 to 90 mL of bone marrow was aspirated from the iliac crest using standard techniques. The aspirate was processed at all sites with a closed, automated cell processing system (Sepax, Biosafe SA) to ensure a uniform cellular product. After BMC enrichment, cells were washed 3 times and suspended in 5% human serum albumin/saline solution. The composition of CD34 and CD133 cells was determined by fluorescent activated cell sorting Dose of stem cells: 1.47 (± 1.7) x 10^8 cells Timing of stem cell procedure: median (IQR) 17.4 (15.5 to 20.0) days after PCI Comparator arm: placebo (0.9% saline and 5% human serum albumin)
Outcomes	Primary outcomes: 1. change in global LV function, 2. change in regional function by wall motion in the infarct and border zones Secondary outcomes: composite measure of major adverse clinical events, LV mass, LVEDV, LVESV, infarct size Outcome assessment points: 6 months Method(s): cardiac MRI
Notes	1 patient in the BMSC group did not receive treatment due to a new 90% stenosis in the left main artery before cell infusion but was included in the analysis as randomised

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Patients were randomly assigned to one to the selected treatment strategies using an interactive web-based randomisation session in a 2:1 ratio using randomly selected block sizes of 6 or 9 and stratified by centre
Allocation concealment (selection bias)	Low risk	Randomisation was performed by the data co-ordinating centre. Treatment assignment was masked to all but one designated cell processing team member at each of the 5 centres who was not involved in patient care
Blinding (performance bias and detection bias) All outcomes	Low risk	All patients underwent bone marrow aspiration and control group patients were given an intracoronary injection of placebo medium. Patients and research staff, including the CCTRN physicians and interventional cardiologists, were blinded to treatment assignment. The MRI core laboratory was blinded to study group assignment
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. 6 patients (BMSC: 3/58 versus placebo: 3/29) were not included in MRI analysis at 6 months. In the placebo group, 1 patient experienced acute pancreatitis at 3 months and in 2 patients, MRI was contraindicated due to a new ICD. In the BMSC group, 1 patient did not receive cells due to severe LMS stenosis and 2 patients did not attend the 6-month follow-up visit
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00684060) were reported
Other bias	Low risk	None reported or identified

Traverse 2011

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: National Heart, Lung, and Blood Institute under co-operative agreement 5 UO1 HL087318-04. Support for cell processing (Sepax) was provided by Biosafe SA Inc. Angioplasty catheters were provided by Boston Scientific Corporation Country of origin: USA Number of centres: 5 Dates of trial enrolment: 07/08 to 01/11 Length of follow-up: 12 months Number (N) of participants randomised to each arm: 79 (day 3/day 7: 43/36) in the treatment arm, 41 (day 3/day 7: 24/17) in the control arm Number (N) of participants analysed (primary outcome) in each arm: 75 (day 3/day 7: 41/34) in the treatment arm, 37 (day 3/day 7: 22/15) in the control arm
Participants	Population: STEMI within 7 days Age, mean (SD) each arm: 55.6 (10.8) years (day 3) and 58.2 (11.3) years in the treatment arm, 57.0 (12.4) years (day 3) and 57.0 (8.0) years (day 7) in the control arm Sex, % male in each arm: 88.4% (day 3) and 86.1% (day 7) in the treatment arm, 87.5% (day 3) and 88.3% (day 7) in the control arm Number of diseased vessels: 1 or 2 Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI to infusion: median 3.3 (IQR 2.8 to 3.8) days or median 7.4 (IQR 7.0 to 7.9) days in BMSC arm, median 3.2 (IQR 2.5 to 4.1) days or median 7.6 (IQR 7.0 to 8.3) days in the control arm. Statistically significant baseline imbalances between the groups? Higher peak creatine kinase and troponin levels among patients randomised to day 7 treatment group and lack of diabetes among patients randomised to day 7 placebo
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells (MNC) Summary of how stem cells were isolated and type and route of delivery: patients underwent bone marrow aspiration on the morning of their treatment day, and BMCs were isolated using a closed, automated Ficoll cell processing system (Sepax, Biosafe) to ensure a uniform cellular product across centres Dose of stem cells: 1.50 x 10^8 cells Timing of stem cell procedure: 3 or 7 days post AMI Comparator arm: placebo (0.9% saline and 5% human serum albumin)
Outcomes	Primary outcomes: change in global LVEF and regional LV function (infarct and border zone) (day 7) and whether these changes were dependent on day of cell administration (day 3 versus day 7) Secondary outcomes: major adverse cardiovascular events, LV volumes, infarct size Outcome assessment points: 6 and 12 months Method(s): cardiac MRI
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	A computer-generated scheme randomly allocated eligible patients to an intervention time group (3 or 7 days post-PCI), with subsequent randomisation after BM aspiration to BMC or placebo group by a computer-generated scheme
Allocation concealment (selection bias)	High risk	The computer-generated randomisation scheme was not blinded
Blinding (performance bias and detection bias) All outcomes	Low risk	All patients underwent bone marrow aspiration and control group patients were given an intracoronary injection of 5% human serum albumin in an identical volume of saline with a 100 µL of blood matching the appearance of an active cell preparation and thereby blinding the identity of the infusate being delivered. Blinding of outcome assessors was not reported although the trial was described as "double-blind"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. 8 patients (BMSC: 4/79 versus placebo: 4/41) were not included in MRI analysis at 6 months. 1 patient in the BMSC group died due to subarachnoid haemorrhage after randomisation but before cell delivery, MRI was contraindicated in 2 BMSC patients and 1 control patient, and MRI was not performed (reason not reported) in 1 BMSC patient and 3 control patients
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00684021) were reported
Other bias	Low risk	None reported or identified

Traverse 2018

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: funded by the Division of Cardiology, Dept of Internal Medicine, University Hospital Rostock, Germany Country of origin: Germany Number of centres: not reported Dates of trial enrolment: not reported Length of follow-up: 12 months Number (N) of participants randomised to each arm: 42 in the treatment arm, 20 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 42 in the treatment arm, 20 in the control arm
Participants	Population: acute STEMI with successful revascularisation Age, mean (SD) each arm: 61 (15) years in the treatment arm, 60 (11) years in the control arm Sex, % male in each arm: 67% in the treatment arm, 70% in the control arm Number of diseased vessels: 1 (n = 30), 2 (n = 12) in the treatment arm, 1 (n = 14), 2 (n = 6) in the control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 7 days Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells (MNC) Summary of how stem cells were isolated and type and route of delivery: 7 days after AMI, a total of 120 mL bone marrow was taken from the iliac crest after local anaesthesia and mononuclear cells were isolated freshly by use of point of care system (with using of Harvest Technologies GmbH, Munich, Germany) and identified including CD34+ and CD133+. The cell suspension consisted of a heterogeneous cell population including haematopoietic, mesenchymal and other progenitor cells Dose of stem cells: not reported Timing of stem cell procedure: 7 days post- AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in global EF and infarct size Secondary outcomes: mobilisation of BM-CPCs on days 1, 3, 5, immediately pre- and post day 7, 8 and 3, 6, 12 months after procedure, NYHA classification, brain natriuretic peptide level Outcome assessment points: 3 and 12 months Method(s): left ventriculography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. Outcome data were "obtained by blinded expert readers unaware of patient group assignment"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Turan 2012

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: China Number of centres: 1 Dates of trial enrolment: 07/08 to 10/09 Length of follow-up: 6 months Number (N) of participants randomised to each arm: 30 in the treatment arm, 30 in the control arm Number (N) of participants analysed (primary outcome) in each arm: 27 in the treatment arm, 28 in the control arm
Participants	Population: acute STEMI, primary PCI within 8 hours of onset of symptoms Age, mean (SD) each arm: 58 (10.2) years in the treatment arm, 56.1 (9.8) years in the control arm Sex, % male in each arm: 67.9% in the treatment arm, 53.3% in the control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: 15 (1) days Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BM-MSC Type of stem cells: bone marrow-derived mesenchymal stromal cells (MSC) Summary of how stem cells were isolated and type and route of delivery: approximately 40 mL of human BM was harvested in the morning on the 8th day following PCI. Mononuclear cells were isolated by gradient centrifugation using Ficoll. Cells were then washed, counted and plated in DMEM containing FBS. Media changes every 3 to 4 days. When they were confluent they were split 1:4 and then cultured for 2 weeks before characterisation by FACS analysis. Cells were re-suspended in heparinised saline and adjusted to 5 x 10^7 cells/mL 2 hours before transplantation Dose of stem cells: 1 x 10^8 cells Timing of stem cell procedure: 15 (± 1) days PCI to injection Comparator arm: identical volume of saline
Outcomes	Primary outcomes: not reported Secondary outcomes: LVEF, infarct size, left ventricular diameter, adverse events, rehospitalisation, death Outcome assessment points: 1, 3 and 6 months Method(s): left ventriculography
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group received an injection of saline of identical volume although it is not reported whether they underwent bone marrow aspiration. It is therefore unclear whether participants and clinicians were sufficiently blinded to treatment. All haemodynamic investigations were obtained by 2 independent observers although it was not reported whether they were blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes. 5 patients (BMSC: 3/30 versus placebo: 2/30) were not included in left ventricular angiography analysis at 6 months. 1 patient in the BMSC group and 2 patients in the placebo group died during follow-up; 1 additional patient in each group did not complete left ventricular angiography
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Wang 2014

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: Germany Number of centres: not reported Dates of trial enrolment: not reported Length of follow-up: 36 months Number (N) of participants randomised to each arm: 29 in treatment arm/13 in control arm Number (N) of participants analysed (primary outcome) in each arm: 28 in treatment arm/12 in control arm
Participants	Population: AMI, within 48 hours. PCI within 6 to 48 hours. Treatment transplantation after successful PCI Age, mean (SD) each arm: 61.0 (8.1) years in treatment arm, 61.1 (9.3) years in control arm Sex, % male in each arm: 90% in treatment arm, 62% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: median delay to PCI from symptom onset 14.3 hours (BMC/placebo not distinguished). Placebo: mean 6.6 (SD 1.5), median 6.6 days from symptom onset to infusion of study therapy Statistically significant baseline imbalances between the groups? Difference in male:female ratio, 62% male in control arm versus 90% males in BMSC arm (P value = 0.04)
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM was aspirated from the iliac crest into 20 mL syringes containing 500 IU heparin, 0.04 mg gentamicin and 3000 IU penicillin in 3 mL 0.9% sodium chloride. Mononuclear cells were isolated with Ficoll density gradient centrifugation, washed and re-suspended in 15 mL 0.9% sodium chloride with 2% human albumin. BM aspirated 5 to 7 days post-AMI. PCI stopflow technique through an over-the-wire balloon catheter positioned within the stented segment Dose of stem cells: a single dose of mean 381 x 10^6 (130 x 10^6 SD) MNC Timing of stem cell procedure: cells infused within a median of 6.1 days (interquartile range 5.5 to 7.3) after the onset of AMI and a median of 6.1 hours after BMC aspiration Comparator arm: patients received a placebo consisting of 15 mL 0.9% sodium chloride with 2% human albumin and autologous erythrocytes with a hematocrit of 0.1% without BMC
Outcomes	Primary outcomes: LVEF Secondary outcomes: LVEDVI, LVESVI, infarct size, major adverse cardiac events (death, myocardial infarction recurrence, and rehospitalisation for heart failure) Outcome assessment points: baseline, 1, 3, 6, 12, 24, 36 months Method(s): cardiac MRI
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Paper reported that randomisation was carried out by an external institute in a 2:1 ratio, but the sequence generation procedure was not described
Allocation concealment (selection bias)	Unclear risk	Persons involved in the randomisation had no contact with patients
Blinding (performance bias and detection bias) All outcomes	Low risk	All patients underwent bone marrow aspiration and control group patients were given an intracoronary injection of a visually indistinguishable autologous erythrocyte preparation; both patients and clinicians were blinded. All personnel involved in the measurement of outcome parameters were double-blinded throughout the study
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and in MRI analysis at 3 months follow-up. 1 patient in each treatment arm (BMSC: 1/29 versus placebo: 1/13) was missing from MRI analysis at subsequent follow-up due to death at 121 days and death at 158 days respectively
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00669227) were reported, although LV volumes (included as secondary outcomes) were reported as LV volume indexes
Other bias	Low risk	None reported or identified

Wohrle 2010

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Department of Cardiology, Hannover Medical School, Hannover Country of origin: Germany Number of centres: 1 Dates of trial enrolment: 01/02 to 05/03 Length of follow-up: 60 months Number (N) of participants randomised to each arm: 33 in treatment arm/32 in control arm Number (N) of participants analysed (primary outcome) in each arm: 30 in treatment arm/30 in control arm
Participants	Population: AMI, within 5 days Age, mean (SD) each arm: 53.4 (14.8) years in treatment arm, 59.2 (13.5) years in control arm Sex, % male in each arm: 67% in treatment arm, 73% in control arm Number of diseased vessels: 1 in both arms (23% right artery/77% left artery) Number of stunned hyperkinetic, etc segments: >2/3 LV anteroseptal, lateral or inferior wall in both arms Time from symptom onset to initial treatment: median 9.8 days (range 2 to 22 days) in treatment arm/median 8.0 days (range 3 to 12 days) in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BM aspirate (128 +/- 33 mL) post baseline cardiac MRI Separation of MNC using a 4% gelatin-polysuccinate density gradient, under GMP regulations. Cells re-suspended in saline with 10,000 U/L of heparin. Between 6 and 8 hours after isolation, cells were infused. Intracoronary infusion using a balloon catheter carried out as 4 to 5 coronary occlusions each lasting 2.6 to 4 minutes Dose of stem cells: a single dose of 2.46 +/- 0.94 x 10^9 MNC, of which 9.5 +/- 6.3 x 10^6 CD34+ and 3.6 +/- 3.4 x 10^6 form colonies in CFU assays Timing of stem cell procedure: PCI within 5 days of MI onset. 4.8 +/- 1.3 days after PCI the BMSC were infused G-CSF details: no G-CSF Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in global LVEF Secondary outcomes: changes in: 1. LVEF (%), 2. LVEDV (mL), 3. LVESV (mL), 4. LV mass index (g/m²), 5. Wall thickening: infarct region (%), 6. wall thickening: border zone (%), 7. wall motion: infract region (mm), 8. wall motion: border zone (mm), 9. late contract enhancement volume (LE, mL) Outcome assessment points: baseline, 6, 18, 60 months Method(s): MRI
Notes	-

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Patients were randomised to treatment or control in a 1:1 ratio using sequentially numbered, sealed envelopes provided by an institute external to the trials
Allocation concealment (selection bias)	Low risk	Sequentially numbered, sealed envelopes were provided by another institute
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of participants and clinicians was not reported although controls did not undergo bone marrow aspiration and no placebo was administered. Echocardiography and MRI analyses were performed by 2 investigators blinded to treatment assignments
Incomplete outcome data (attrition bias) All outcomes	Low risk	5 patients (BMSC: 3/33 versus control: 2/32) were withdrawn at the start of the study as "not been able to undergo MRI because of severe obesity or claustrophobia". All other patients were included in analysis of clinical and scientific outcomes
Selective reporting (reporting bias)	Low risk	All outcomes described in the trial protocol (www.clinicaltrials.gov: NCT00224536) were reported
Other bias	Low risk	None reported or identified

Wollert 2004

Study characteristics	
Methods	Type of study: parallel RCT Source of funding: German Research Foundation (Deutsche Forschungsgemeinschaft) Country of origin: Bulgaria, Germany, Norway Number of centres: not reported (multicentre) Intended enrolment: 200
Participants	Population: large STEMI and reduced LVEF on MRI. Age, mean (SD) each arm: loBMC group 53 (11) hiBMC group 57 (10) controls 55 (9) Sex, % male in each arm: loBMC group 87% hiBMC group 85% controls 92% Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: >2/3 of left ventricular anteroseptal, lateral or inferior wall Time from symptom onset to initial treatment, mean (IQR): loBMC group 4.7h (2.9-7.7). hiBMC group 4.1h (3.1-7.3) controls 5.4h (3.1-7.4) Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: high dose and low dose of non-irradiated and irradiated BMSC Type of stem cells: BMSC Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate Dose of stem cells: low and high dose Timing of stem cell procedure: not reported Comparator arm: placebo manufactured from peripheral blood the same way as the cell product was.
Outcomes	Primary outcomes: changes in LVEF from baseline to 6 months Secondary outcomes: LVEDV, LVESV, infarct size, adverse events (death, HF hospitalization, recurrent MI). Outcome assessment points: baseline, 6 months Method(s): MRI
Notes	Combined hiBMC and loBMC group and recalculated means and SDs for the groups (through formulas in Cochrane Handbook). Did not take along the irradiated cell products (the authors of the paper also state that they are not sure if these cell products still have a therapeutic effect). Baseline LVEF 45.0 on average, so included in the group of 45 and above for subgroup analyses.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	We used a block randomisation procedure stratified by study site. The randomisation lists were prepared by Prometris (formerly IST GmbH), Mannheim, Germany. Once a new patient had completed the baseline MRI and was eligible, Prometris was notified by the study site. Prometris randomised the patient in accordance with the stratified randomisation list and sent a fax to team A indicating whether a low dose or a high dose of bone marrow should be collected in that patient. Team B, the patient, and the MRI core lab remained unaware of the treatment code.
Allocation concealment (selection bias)	Low risk	Two independent teams at each study site ensured blinding of the trial. Team A was responsible for cell harvest; team B for patient selection, intracoronary cell infusion, and follow-up. We used a block randomisation procedure stratified by study site. The randomisation lists were prepared by Prometris (formerly IST GmbH), Mannheim, Germany. Once a new patient had completed the baseline MRI and was eligible, Prometris was notified by the study site. Prometris randomised the patient in accordance with the stratified randomisation list and sent a fax to team A indicating whether a low dose or a high dose of bone marrow should be collected in that patient. Team B, the patient, and the MRI core lab remained unaware of the treatment code.
Blinding (performance bias and detection bias) All outcomes	Low risk	Both study team, patient and assessors appropriately blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	no missing patients. good explanation in figure 1 where patients were excluded and why.
Selective reporting (reporting bias)	High risk	there is mentioning of other endpoints like 18m MRI follow-up, quality of life measurements and cardiopulmonary exercise testing in the trial registration on ISRCTN, but these are not reported on or mentioned in the currently included papers
Other bias	High risk	Two authors have a conflict of interest, holding a patent on therapeutic potential of BMC-secreted growth factors.

Wollert 2017

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: funded by the Henan Provincial Public Fund Country of origin: China Number of centres: 1 Dates of trial enrolment: 03/10 to 06/11 Length of follow-up: 3 months Number (N) of participants randomised to each arm: 17 in treatment arm/21 in control arm Number (N) of participants analysed (primary outcome) in each arm: 17 in treatment arm/19 in control arm
Participants	Population: AMI; undergoing elective PCI within 4 weeks of AMI Age, mean (SD) each arm: 60.4 (8.9) years in treatment arm, 58.5 (10.0) years in control arm Sex, % male in each arm: 58.8% in treatment arm, 61.9% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: within 4 weeks of AMI Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BM-MSC Type of stem cells: bone marrow-derived mesenchymal stromal cells (MSC) Summary of how stem cells were isolated and type and route of delivery: 80 to 100 mL bone marrow was aspirated from the iliac crest. Mesenchymal stem cells were isolated from bone marrow and cultured in vitro up to 1 to 10 x 10^8/mL cell suspension. Cells were injected into the infarct related arteries using a guiding catheter Dose of stem cells: 4.8 (± 1.6) x 10^8/mL bone marrow MSC Timing of stem cell procedure: up to 4 weeks after AMI during elective PCI Comparator arm: saline solution
Outcomes	Primary outcomes: not reported Secondary outcomes: death, malignant arrhythmia, and microembolitic events; LVEDD, LVEF and perfusion defect percentage Outcome assessment points: baseline, 1 and 3 months Method(s): echocardiography, SPECT
Notes	Translated from Chinese (Mandarin)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The control group received an injection of heparinised saline although they did not undergo bone marrow aspiration. It is therefore unclear whether participants and clinicians were sufficiently blinded to treatment. The outcome assessors were unaware of grouping details
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	2 patients in the control arm (2/21) were lost to follow-up at 1 and 3 months
Selective reporting (reporting bias)	Unclear risk	Mortality was not explicitly reported; the reported outcome of composite clinical events was not defined. All other outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Xiao 2012

Study characteristics	
Methods	Type of study: parallel RCT Source of funding: Y-JY was supported by grants from CAMS Innovation Fund for Medical Sciences (CIFMS, 2016-12 M-1-009), National Basic Research Program (973 Program) in China (no: 2012CB518602, Ministry of Science and Technology), National High Technology Research and Development Program (863 Program) in China (no: 2013AA020101, Ministry of Science and Technology), National Natural Science Foundation of China (no: 81170129), Research Fund of Capital Medical Development (no: 2007–2018) and China Health and Medical Development Foundation (2008- zhfj2, 2011-zhfj1, 2015-zhfj2). H-YQ was supported by grants from the Clinical and Translational Medicine Research Foundation of Chinese Academy of Medical Sciences (2019XK320061), and National Natural Science Foundation of China (nos: 81000091, 81670337). Country of origin: China Number of centres: 1 Intended enrolment: 100
Participants	Population: STEMI Age, mean (SD) each arm: not reported (30 to 80 years) Sex, % male in each arm: cells = 35/40 = 88% placebo = 33/36 = 92% Number of diseased vessels: 1 vessel disease = cells 24/40. placebo 13/36. 2vessel disease = cells 11/40, placebo 14/36. 3vessel disease = cells 6/40, placebo 8/36. LM lesion = cells 2/40, placebo 4/36. Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups? no
Interventions	Intervention arm: BMSC + Artovastatin (routine or intensive dose) Type of stem cells: BMSC (mononuclear cells) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirated, preparation of media, delivery via intracoronary injection Dose of stem cells: not reported. we assume <10^8 because of immediate isolation and administration. Timing of stem cell procedure: cells = 27.8±19.6 and 28.7±19.1 days. placebo = 24.0±16.7 and 25.6±13.8 days. Comparator arm: atorvastatin (routine or intensive dose)
Outcomes	Primary outcomes: LVEF Secondary outcomes: - change and endpoint values of other functional and morphological parameters and infarct scar sizes as measured by 2DE and MRI, myocardial perfusion and viability by SPECT and PET. - cardiac biomarkers in the blood, including aminoter- minal pro-B-type natriuretic peptide (NT-proBNP), high sensitivity C reactive protein (hs-CRP) and endothelin. - Clinical events were monitored closely, including cardiac death, myocardial reinfarction, repeated revasculari- sation and malignant ventricular arrhythmias, such as ventricular tachycardia, flutter or fibrillation. Outcome assessment points: baseline and 12 months Method(s): ECG, echocardiography, MRI, SPECT and PET.
Notes	Estimated study completion date: January 2012. This study is enrolling participants by invitation only

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	By applying a web-based automated random number generator as previously described a treatment schedule will be prepared by a designated researcher who will have no contact with any participants, and patients at the convalescent stage
Allocation concealment (selection bias)	Unclear risk	By applying a web-based automated random number generator as previously described a treatment schedule will be prepared by a designated researcher who will have no contact with any participants, and patients at the convalescent stage. only patients in the two IA groups received 4 days more of IA then followed by regular atorvastatin as in the two RA groups with clinical followed-up and evaluation for up to 1 year.
Blinding (performance bias and detection bias) All outcomes	Low risk	Patients will have known if they were in the intensive or regular atorvastatin group. However, they will not have known if they were in the cell therapy or placebo group. All the investigators, including clinical and imaging professionals, and the patients were blinded to the information of treatment and grouping.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	They report on the missing patients (24 out of a 100). However, with loosing almost 25% of your study population, it is unclear if there is indeed attrition bias at play. The remaining 24 participants who did not complete the 1-year endpoint evaluations due to geological distance and personal inconvenience were excluded from final analysis.
Selective reporting (reporting bias)	Unclear risk	On clinicaltrials.gov there is only mentioning of a primary outcome measurement LVEF at 12 months. No other (secondary) outcomes mentioned. In the paper they authors give a thorough and complete overview with multiple secondary outcome measurements. however, it is unclear for us if there is any selective reporting at play here.
Other bias	Low risk	no identified risk of other biases

Yang 2020

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: National Key Technologies R & D Program of China Country of origin: China Number of centres: 1 Dates of trial enrolment: 05/03 to 12/05 Length of follow-up: 30 months Number (N) of participants randomised to each arm: 92 in treatment arm/92 in control arm Number (N) of participants analysed (primary outcome) in each arm: 90 in treatment arm/84 in control arm
Participants	Population: AMI within 1 week, PCI within 1 week Age, mean (SD) each arm: 58.3 (9.5) years in treatment arm, 58.1 (9.0) years in control arm Sex, % male in each arm: 89.1% in treatment arm, 88% in control arm Number of diseased vessels: 1 Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI within 1 week of AMI in both arms Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: low temperature density gradient centrifugation of heparinised bone marrow cell suspension in lymphocyte isolation medium. PCI Dose of stem cells: single 2.1(3.7) x 10^8 cells Timing of stem cell procedure: infusion performed 2 hours after revascularisation Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: morbidity, mortality and adverse events Secondary outcomes: LVEF, LVEDD Outcome assessment points: baseline, 6 and 30 months Method(s): echocardiography, LV angiography
Notes	Translated from Chinese (Mandarin)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	High risk	This Chinese trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	High risk	Treatment allocation was not concealed
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor patients were blinded. It was not reported whether outcome assessors were blinded
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	10 randomised participants were withdrawn or excluded from the analysis of all outcomes, 2/92 in the BMSC group (1 emigrated to another country and one could not follow up due to economic change) and 8/92 in the control group (3 had changed address at 12 months, another 3 had changed address at 24 months, and a further 2 non-local participants refused follow-up)
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Yao 2006

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: Shanghai Scientific Research Fund (06DJ14001), Program for Shanghai Outstanding Medical Academic Leader (LJ06008), National Basic Research Program of China (2006CB943704), and Science Foundation for Youth of Shanghai Medical Administrative Bureau (2008Y044) Country of origin: Italy Number of centres: 1 Dates of trial enrolment: 03/04 to 02/06 Length of follow-up: 12 months Number (N) of participants randomised to each arm: 15 in single cell transfer arm (ST), 15 in repeated cell transfer arm (RT) and 15 in control arm Number (N) of participants analysed (primary outcome) in each arm: 12 (ST), 15 (RT), 12 (controls)
Participants	Population: AMI, within 12 hours. Age, mean (SD) each arm: 52.1 (6.3) years in ST arm, 51.3 (7.4) years in RT arm, 52.7 (7.8) years in control arm Sex, % male in each arm: 83.3% in ST arm, 80.0% in RT arm, 91.7% control arm Number of diseased vessels: ST arm: 1 vessel disease = 4/12 (33.33%), 2 vessel disease 5/12 (41.67%), 3 vessel disease 3/12 (25.00%) RT arm: 1 vessel disease = 5/15 (33.33%), 2 vessel disease 6/15 (40.00%), 3 vessel disease 4/15 (26.67%) Controls: 1 vessel disease = 3/12 (25.00%), 2 vessel disease 6/12 (50.00%), 3 vessel disease 3/12 (25.00%) Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: from AMI to PCI: 4.9 (2.9) hours (ST), 4.7(2.9) hours (RT), 6.0 (2.8) hours (controls) Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: single BMMNC dose (SD) or repeated BMMNC dose (DD) Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 90 ± 18 mL bone marrow was aspirated from the posterior superior iliac spine under local anaesthesia. Bone marrow aspirates were diluted with 0.9% NaCl (1:5) and mononuclear cells were isolated by density gradient centrifugation, washed 3 times with PBS and then suspended in 16 mL heparin-treated plasma at a density of $(1.3 \pm 1.0) \times 10^7$ cells/mL at room temperature. Cell transplantation via intracoronary route using an over-the-wire balloon catheter inserted into the stent that was implanted during primary PCI. Procedure repeated at 3 months in repeated cell dose arm Dose of stem cells: mean 1.9 (SE 1.2) x 10^8 BMC (ST), 2.0 (SE 1.4) x 10^8 (RT, first delivery), 2.1 (SE 1.7) x 10^8 (RT, second delivery at 3 months) Timing of stem cell procedure: BMC infusion 3 to 7 days after PCI, and 3 hours after BMC collection, followed by saline infusion (ST group) or second infusion (RT group) 3 months after PCI Comparator arm: saline infusion 3 to 7 days after PCI (no secondary infusion at 3 months)
Outcomes	Primary outcomes: LVEF, LVEDV, LVESV Secondary outcomes: myocardial infarct area, myocardial perfusion defect, survival, re-hospitalisation for congestive heart failure, serious adverse events Outcome assessment points: baseline, 6 and 12 months Method(s): MRI, SPECT, LV angiography
Notes	Data from the 2 active intervention arms of the trial are pooled in this review. 3 patients randomised to single dose BMSC were not transplanted as follows: 1 patient could not undergo MRI due to pacemaker implantation following development of bradycardia, 1 patient developed a fever 12 hours prior to the procedure, and in 1 patient an inadequate amount of cells was acquired

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation was undertaken using a computer-generated random number sequence
Allocation concealment (selection bias)	Low risk	Sequentially numbered, sealed, opaque envelopes were used
Blinding (performance bias and detection bias) All outcomes	High risk	Although the control group received a placebo, only the active treatment groups (single or double dose) underwent BM aspiration. Further, the active treatment groups were recalled for the second infusion of cells or placebo whereas the control group was not recalled for further treatment. Participants were therefore not appropriately blinded. Blinding of clinicians was not reported. MRI and SPECT studies were processed and evaluated at the MRI and scintigraphy core laboratories respectively by experienced operators who were blinded to the assigned therapy
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients in the repeat BMSC arm were included in the analysis of all outcomes. 3 patients in the single BMSC arm and 3 patients in the control arm (3/15) were withdrawn or excluded from the analysis of all outcomes. In the BMSC arm, 1 patient developed a fever 12 hours prior to the procedure, for one patient an inadequate amount of cells was acquired and one patient could not undergo MRI due to pacemaker implantation following development of bradycardia. In the control arm, 1 patient had a reinfarction 5 days after discharge due to in-stent thrombosis, 1 patient was excluded due to diagnosis of liver cancer at 4 months, and 1 patient could not be contacted at 3 months follow-up. One additional patient in the control group was missing from MRI analysis at 12 months follow-up.
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Yao 2009

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: the "135" Major Research Subject for Medical Talent of Jiangsu Province (No. RC2003092); the Social Technical Developing Item of Scientific Bureau of Wuxi City (No. CS040001) Country of origin: Wuxi, Jiangsu Province, China Number of centres: 1 Dates of trial enrolment: 10/03 to 06/05 Length of follow-up: 8 weeks Number (N) of participants randomised to each arm: 7 in treatment arm/16 in control arm Number (N) of participants analysed (primary outcome) in each arm: 7 in treatment arm/16 in control arm
Participants	Population: thrombolysis within 24 hours Age, mean (SD) each arm: 60.5 years in treatment arm, 62.5 years in control arm Sex, % male in each arm: 71.4% in treatment arm, 56.3% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: thrombolysis within 24 hours of AMI symptom onset Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mesenchymal stem cells) Summary of how stem cells were isolated and type and route of delivery: 25 mL bone marrow was aspirated from the superior anterior iliac spine. Aspirate washed and centrifuged to isolate MNC layer. This was cultured in DMEM for a week and passaged 3 times. The cultured cells were harvested and suspended in solution. Infused via the femoral artery PCI route into the left and right coronary arteries Dose of stem cells: 5 mL suspension, 1.5×10^{10} BMSC/L for a total of 7.5×10^{7} cells delivered Timing of stem cell procedure: 14 days after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: none Secondary outcomes: LVEF, CO, infarct area Outcome assessment points: baseline, 2, 4, 6 and 8 weeks Method(s): echocardiography, Sopha PET-CT (radionuclide imaging)
Notes	Translated from Chinese (Mandarin)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random numbers were assigned via a table
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not reported
Blinding (performance bias and detection bias) All outcomes	High risk	The trial was described as a "single-blind" evaluation. Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor clinicians were blinded. The first author designed, carried out, collected data and assessed the results
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical and scientific outcomes
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

You 2008

Study characteristics	
Methods	Type of study: RCT Source of funding: Scientific Research Projects of Sichuan Medical Planning Commission: Heart transplantation effect of stem cells—Study on Paracrine Mechanism (100306) Country of origin: China Number of centres: 6, multicenter. The People's Liberation Army Navy General Hospital, Beijing Armed Police General Hospital, Chinese People's Liberation Army General Hospital, Beijing Huaxin Hospital, Beijing Tongren Hospital, Beijing Chaoyang Hospital West Hospital. Intended enrolment: not mentioned
Participants	Population: first STEMI, onset within 1 month Age: BMC group 59.3 (9), Control group 58.6 (11) Sex, % male in each arm: not reported? BMC 95%, controls 86%. Number of diseased vessels: not reported Used cutoff for Number of stunned hyperkinetic, etc segments: NA Time from symptom onset to initial treatment: divided into two groups. >12h of onset and <12h of onset. Rest of the paper these groups are again combined. Statistically significant baseline imbalances between the groups?: No
Interventions	Intervention arm: Type of stem cells: BM-MSCs Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate Dose of stem cells: 3.3 (1.7)*10^6. Timing of stem cell procedure: 14 (9.5) days after PCI. Comparator arm: conventional treatment
Outcomes	Primary outcomes: changes in myocardial metabolic activity from baseline to 6 months from baseline to 12 months. Secondary outcomes: Incidence of cardiovascular events, overall mortality, and adverse events (death, recurrent MI, heart failure admission, revascularisation, stroke, arrhytmia, tumor, myocardial fibrosis, microvacular embolization, NYHA) 12 months after transplantation of autologous BM-MSCs. Outcome assessment points: baseline, 6 and 12 months Method(s): SPECT and echocardiography
Notes	Starting date: March 2008 Contact: Zhenhong Liu, hzljiayou@126.com

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Participants' random numbers were generated by the network, and technical services were provided by the China Cardiovascular and Cerebrovascular Diseases Pro- fessional Network (CCVD), which was not related to this clinical trial. The participant's information was input into the computer. If the patient met the inclusion criteria, the system would give a random number and grouping to determine the randomization of the patient.
Allocation concealment (selection bias)	Unclear risk	No additional info in methods on allocation concealment
Blinding (performance bias and detection bias) All outcomes	High risk	single-blinded study Because of ethical considerations, we decided not to conduct bone marrow aspiration and left heart catheterization in patients randomized to the control group.
Incomplete outcome data (attrition bias) All outcomes	Low risk	appropriate mentioning of patients lost to follow-up.
Selective reporting (reporting bias)	Unclear risk	there is no study protocol identified that preregistered any primary and secondary outcomes
Other bias	Low risk	no other risk of biases identified.

Zhang 2021

Study characteristics	
Methods	Type of study: parallel RCT Type of publication: full Source of funding: not reported Country of origin: Russia Number of centres: 1 Dates of trial enrolment: not reported Length of follow-up: 36 months Number (N) of participants randomised to each arm: 8 in treatment arm/3 in control arm Number (N) of participants analysed (primary outcome) in each arm: 8 at 1 year, 6 at 3 years in treatment arm/2 at 1 year, 1 at 3 years in control arm
Participants	Population: MI of the front wall and low EF (< 38%). Males with systolic dysfunction who had successful reperfusion therapy (thrombolysis and/or urgent angioplasty) Age, mean (SD) each arm: 48 (7) years in treatment arm, 50 (10) years in control arm Sex, % male in each arm: 100% in treatment arm/100% in control arm Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI within 6.5 (3) hours of AMI in treatment arm/PCI within 6.2 (2) hours of AMI in control arm Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: 50 to 80 mL bone marrow was aspirated and centrifuged to obtain the mononuclear cells. These were re-suspended into autologous patient serum Dose of stem cells: 2 to 5 mL portions for a total of 20 mL; 5 x 10^6 BMMNC Timing of stem cell procedure: 14 to 19 days after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: none Secondary outcomes: mortality, morbidity, QOL, LVEF, LVEDV, LVESV, perfusion defect, myocardial viability Outcome assessment points: baseline, 3, 6, 12, 24 and 36 months Method(s): echocardiography, SPECT, gadolinium-based MRI
Notes	Translated from Russian

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The trial was described as randomised but the method of randomisation was not reported
Allocation concealment (selection bias)	Unclear risk	The use of envelopes was mentioned, but insufficient detail was provided to establish whether appropriate allocation concealment was used
Blinding (performance bias and detection bias) All outcomes	High risk	Controls did not undergo bone marrow aspiration and no placebo was administered; neither participants nor clinicians were blinded. Blinding of outcome assessors was not reported
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised participants were included in the analysis of clinical outcomes and of scientific outcomes at 3 months. In MRI and echocardiographic analysis at 12 months follow-up, 1 control patient had died, and at 3 years follow-up 1 further control and 2 patients in the BMSC group had died
Selective reporting (reporting bias)	Unclear risk	All outcomes mentioned in the methods were reported in the results, although it would be difficult to rule out selective reporting
Other bias	Low risk	None reported or identified

Zhukova 2009

AE, adverse events; AMI, acute myocardial infarction; ASTAMI, Autologous Stem Cell Transplantation in Acute Myocardial Infarction; BM, bone marrow; BMMNC, bone marrow-derived mononuclear cells; BMSC, bone marrow-derived stem cells; CFU, colony forming units; CMR, cardiac magnetic resonance; DMEM, Dulbecco's modified Eagle's medium; DTI, Doppler tissue imaging; ECG, electrocardiogram; Echo, echocardiography; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; GCSF, granulocyte colony stimulating factor; GMP, good manufacturing procedures; HF, heart failure; ICD, internal cardia defibrillator; IQR, interquartile range; IRA, infarct-related artery; IVUS, intravascular ultrasound; LAD, left anterior descending; LSM, lymphocyte separation medium; LV, left ventricle or ventricular; LVDV, left ventricular diastolic volume; LVEDD, left ventricular end diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVESVI, left ventricular end systolic volume index; MBM, creatine kinase-MB mass; MLHFQ, Minnesota Living with Heart Failure Questionnaire; MNC, mononuclear cells; MRI, magnetic resonance imaging; MSC, mesenchymal stromal cells; NNYHA, New York Heart Association; PBS, phosphate buffered saline; PCI, percutaneous coronary intervention; PET, positron emission tomography; PTCA, percutaneous transmural coronary angioplasty; QoL, quality of life; RCT, randomised controlled trial; RNV, radionuclide ventriculography; SD, standard deviation; SEM, standard error of the mean; SPECT, single photon emission computed tomography; STEMI, ST-elevation myocardial infarction; VMC, vasomotor centre; WMSI, wall motion score index.

Characteristics of excluded studies [ordered by ID]

Study	Reason for exclusion
Anastasiadis 2020	Editorial concern, no new data
Ang 2008	A RCT of BMSC in patients with chronic coronary artery disease
Anonymous 2013	Erratum to study (primary study on IHD, no AMI).
Arnesen 2007	A commentary on RCTs of cell therapy in MI
Atsma 2008	An ongoing single-arm trial investigating mesenchymal stem cell therapy after acute MI
Bayes-Genis 2021	Editorial; No AMI
Beeres 2007	A single-arm trial of autologous BMSC in patients with chronic MI
Benedek 2014	A RCT of BMMNC versus placebo in patients with MI. This study was excluded because MI occurred up to 3 months prior to study enrollment and was therefore not classified as AMI
Bolli 2021	No AMI. No BMMNC
Byun 2009	Review
Caramia 2009	Review
Chen 2004a	Stem cells were not removed and then reinfused, rather stem cells were mobilised following G-CSF
Chen 2014	A RCT of G-CSF mobilised peripheral blood stem cells versus placebo in patients with AMI. The control group did not receive G-CSF
Chernyaskiy 2017	No acute myocardial infarction
ChiCtr2018	No published paper, only trial registry
Chin 2021	No AMI
Ctri2009a	use of allegeneic cells
Dash 2020	basic science study
Dib 2008	No AMI
Dolan 2019	basic science paper
Drabik 2021	No BMMNC
Edlinger 2016	basic science study
Engelmann 2006	A RCT of G-CSF mobilised PBSC (no cells administered) compared with placebo in patients with sub-acute MI
Epstein 2019	Editorial
Euctr 2006b	
EUCTR 2010-020497-41-GB	An ongoing trial of allogeneic mesenchymal precursor cells versus placebo in patients with AMI
Fedak 2001	No clinical trial
Fernandez 2004	A comparison of CD34+ cell infusion with a non-randomised control group in patients with AMI
Fernandez Ruiz 2016	Review
Florenzano 2007	Review
Francis 2006	abstract, no clinical original data
Galinanes 2008	study with no AMI (and seems to be peer review rapport and not original study?)
Gao 2015	No BMMNC
Goto 2017	Animal study
Gyongyosi 209	A RCT of BMMNC administration either 2 to 3 weeks or 3 to 4 months post AMI. This study did not include a control group
Haller 2021	meta-analysis
Hare 2007	The trial used allogeneic (not autologous) mesenchymal stem cells, therefore was not eligible for inclusion in the review
Heeger 2012	A non-randomised study of BMMNC compared with a matched control group in patients with AMI
Hendrikx 2006	A RCT of BMSC compared with a control group in patients with chronic ischaemic heart disease undergoing CABG
Henon 2020	Review
Hendrich 2010	Review
Holinski 2011	A non-randomised trial of autologous BM cells in patients with chronic heart failure scheduled for elective CABG compared with a matched control group
Hu 2015	A RCT of normoxia BMMNC versus hypoxia-preconditioned BMMNC in patients with AMI. BMMNC groups were compared with a nonrandomised control group
ISRCTN14054375	No AMI
ISRCTN75217135	No AMI
Jeong 2018	meta-analysis
Jiang 2011	A systematic review of RCTs of BMSC in AMI
JPRNjRCT2053190103	No AMI

Study	Reason for exclusion
Kahn 2006	A summary of stem cell trials in MI presented at the 2nd International Conference on Cell Therapy for Cardiovascular Diseases
Kahn 2016	No clinical/original data
Kang 2004	A commentary on cell therapy trials in MI
Kang 2004b	No BMMNC
Kang 2006	A RCT of infused G-CSF mobilised peripheral blood stem cells versus placebo in patients with AMI. The control group did not receive G-CSF
Kang 2007	A RCT of BMSC infusion compared with G-CSF compared with a control group in patients with AMI or old MI (OMI). Outcome data are not presented separately for the AMI and OMI groups
Kang 2008	A commentary on results from 2 trials of mobilised PBSC in patients with AMI
Kang 2011	A 3-arm trial design protocol of intravenous darbepoetin infusion and intracoronary infusion of G-CSF mobilised PBSC, G-CSF mobilised PBSC alone or standard medical treatment. The control group did not receive G-CSF
Khoei 2020	Review
Kloner 2016	Review
Komok 2019	No AMI
Kwiecien 2020	No BMMNC
Landmesser 2009	Editorial
Li 2006	A RCT of infused G-CSF mobilised PBSC compared with no treatment in patients with AMI. The control group did not receive G-CSF
Li 2008	A RCT of the effect of MSC on vascular endothelial function in AMI patients. The outcomes of this study, published in full, are beyond the scope of this review
Li 2016	Review
Li 2021	Review
Liu 2020	Review
Lu 2012	An experimental animal study comparing MSC and control groups in MI-induced swine
Madeddu 2021	Review
Makkar 2012	A RCT of cardiosphere-derived cells compared with controls in patients with AMI
Marenzi 2007	A comment on the conclusions of the authors of the REPAIR-AMI trial
Menasche 2002	No AMI; No BMMNC
Menasche 2008	No AMI
Messori 2013	A meta-regression analysis of 2 previously published meta-analyses of BMMNC in AMI
Micheu 2015	
Micheu 2017	Review
Milis 2007	An evaluation and commentary on the REPAIR-AMI tria
Musialek 2006	A RCT of 2 active interventions: over-the-wire balloon catheter for bone marrow stem cell delivery and cell infusion via a perfusion catheter with multiple side holes
Musialek 2010	A RCT of 2 active interventions: over-the-wire balloon catheter for bone marrow stem cell delivery and cell infusion via a perfusion catheter with multiple side holes
Nasseri 2013	A RCT of BMMNC versus CD133+ cells versus controls during CABG in patients enrolled 8 to 12 weeks after AMI
NCT00081913	No AMI
NCT00114452	Allogeneic cells, no autologous
NCT00548613	A non-randomised trial cell therapy in patients with AMI, comparing intracoronary infusion with intramyocardial infusion of a cell mixture of BMSC and progenitor cells. This trial did not include a control group
NCT00874354	An ongoing trial investigating 2 different doses of BMSC in patients with AMI. This trial does not include a control group
NCT00877903	A RCT of allogeneic ex vivo cultured adult human MSCs in patients with AMI
NCT00950274	No AMI (chronic ischaemic heart disease)
NCT01768702	No AMI (and no BMMNC cells)
nct03798353	No BMMNC cells; allogeneic cells
nct04011059	No BMMNC; allogeneic cells.
nct04050163	No control group
nct04052191	no AMI
NCT04340609	No BMMNC; allogeneic.
NCT05043610	No BMMNC
Nie 2007	A non-randomised trial of BMMNC compared with a control group in patients with AMI
Obradovic 2009	A non-randomised trial of BMSC compared with a control group in patients with AMI

Study	Reason for exclusion
Osterziel 2007	A comment on the conclusions of the authors of the REPAIR-AMI trial
Ott 2013	A RCT of G-CSF mobilised PBSC (no cell infusion) versus placebo in patients with AMI
PeregudPogorzelska	Non-randomized trial
Peruga 2009	A non-randomised trial of BMSC compared with a control group in patients with AMI
Potapov 2007	No RCT
Qayyum 2017	No BMMNC
Ramireddy 2017	No AMI
Raval 2018	Review
RazeghianJahromi 2021	Review
Reinsch 2018	Review
Ripa 2009	Review
Roberts 2004	Review
Schachinger 2004	A RCT of 2 active interventions: circulating progenitor cells and bone marrow-derived progenitor cells with no control comparator group
Schueller 2007	A non-randomised study of BMSC versus no cells in patients with AMI
Schahid 2016	
Shrimahachota 2011	A RCT of BMSC compared with a control group with patients with AMI which occurred at a mean of 57.2 days and 45.3 days in the BMSC and control groups respectively
Smiseth 2014	conference proceedings, no new data
Soetisna 2020	No AMI
Soetisna 2021	No AMI
Taljaard 2010	An ongoing RCT of autologous endothelial-like culture-modified mononuclear cell infusion (E-CMMs) compared with both an active treatment arm receiving an infusion of autologous E-CMMs transfected with endothelial nitric oxide synthase and a control arm receiving standard therapy. Trial excluded as the mononuclear cells collected from circulating blood are not classified as BMSC
Tatsumi 2006	Review
Tendera 2009b	Editorial
Terrovitis 2011	A RCT of intracoronarily administered G-CSF mobilised peripheral blood stem cells versus placebo in patients with AMI. The control group did not receive G-CSF
Trzos 2009	A RCT of BMSC compared with a control group in patients with AMI. Excluded because this trial, published in full, evaluated heart rate variability which is not covered by the scope of this review
Tyler 2018	Editorial / Commentary
Ulus 2020	No AMI. NO BMMNC
Vanderheyden 2007	A RCT of enriched haematopoietic BMSC therapy in patients with MI randomised to early or late cell therapy. This trial does not include a randomised control group
Vassali 2007	Review
Vrtovec 2019	Commentary
Wang 2006	A non-RCT of BMSC compared with a control group in patients with AMI > 4 weeks before treatment
Warbington 2013	An experimental study of allogeneic cryopreserved purified CD34+ cells to identify potential microRNAs as biomarkers for CD34+ cell SDF-1 driven migration
Welt 2006	Review/Commentary
Wollert 2009	Review
Wollert 2015	Editorial
Wu 2017	Basic study
Yanamandala 2017	Review
Yang 2010	A RCT of BMSC in patients with AMI randomised to delivery via an infarct-related versus non-infarct related artery. This trial does not include a randomised control group
Yoon 2010	basic science/ no human data.
Yu 2005	A single-arm trial of BMMNC in AMI with no control group
Yu 2014	A RCT of G-CSF mobilised peripheral blood stem cells versus no cells in patients with AMI. The control group did not receive the cointervention of G-CSF
Zhang 2021b	Review

AMI, acute myocardial infarction; BMMNC, bone marrow-derived mononuclear cells; BMSC, bone marrow-derived stem cell; CABG, coronary artery bypass graft; CDC, cardiosphere-derived stem cells; E-CMM, endothelial-like culture modified mononuclear cells; G-CSF, granulocyte colony stimulating factor; MI, myocardial infarction; MSC, mesenchymal stromal cells; OMI, old myocardial infarction; PBSC, peripheral blood stem cells; RCT, randomised controlled trial; SDF-1, stromal derived factor, STEMI, ST-segment elevation myocardial infarction

Characteristics of studies awaiting classification [ordered by study ID]

Methods	Type of study: parallel RCT Type of publication: abstract Source of funding: not reported Country of origin: Brazil Number of centres: 1 Dates of trial enrolment: 12/10 to 01/11 Length of follow-up: 5 to 8 years Number (N) of participants randomised to each arm: 10 to control; 10 to ICV and 20 to ICA Number (N) of participants analysed (primary outcome) in each arm: not reported
Participants	Population: patients with ST-elevation MI (STEMI) and LV dysfunction Age, mean (SD) each arm: not reported Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: not reported Summary of how stem cells were isolated and type and route of delivery: administration reported only; intracoronary artery (IC) or intracardiac vein (ICV) Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: not reported
Outcomes	Primary outcomes: death and hospitalisation Secondary outcomes: not reported Outcome assessment points: baseline and 5 to 8 years Method(s): not reported
Notes	-

Alves 2011

Methods	Type of study: parallel RCT We have requested additional data relating to possible patient overlap with Kang 2006
Participants	Population: AMI, within 14 days, successfully treated with drug eluting stent (DES) Age mean (SD) each arm: 56.6 (13.1) years in cell infusion arm/57.1 (11.9) in control arm Sex % male in each arm: 85% in cell infusion arm/80% in control arm Number of diseased vessels: 11/20 (55%) had 1-vessel disease and 9/20 (45%) had 2-vessel disease in cell infusion arm; 11/20 (55%) had 1-vessel disease and 9/20 (45%) had 2-vessel disease in control arm Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: BMSC were mobilised with 10 µg/kg body weight during 3 days. At day 4, the cells were separated using a COBE® Spectra system. Intracoronary infusion using an inflated balloon catheter. SC mobilised and infused after (drug eluting stent) DES Dose of stem cells: a single dose of 1 to 2 x 10^9 MNC that contained a minimum of 7 x 10^6 CD34+ cells Timing of stem cell procedure: not reported (3 days after enrolment?) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: left ventricular synchronous contraction as measured by change in time to peak positive systolic velocity (?Ts-SD) over 6 months Secondary outcomes: LVEF, LVESV, LVEDV, LV stroke volume, Infarct volume, maximal exercise capacity (METs) Outcome assessment points: baseline and 6 months Method(s): echocardiography, cMRI, treadmill testing
Notes	-

Chang 2008

Methods	Type of study: parallel RCT Type of publication: abstract Source of funding: not reported Country of origin: Buenos Aires, Argentina Number of centres: 1 Dates of trial enrolment: 02/04 to 01/06 Length of follow-up: 4 months Number (N) of participants randomised to each arm: not reported Number (N) of participants analysed (primary outcome) in each arm: not reported

Participants	Population: AMI Age mean (SD) each arm: not reported Sex % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups? BMSC group baseline LVEF significantly lower than control group (P value =0.005)
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: not reported Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: LVEF Secondary outcomes: cardiac events (ventricular arrhythmias, restenoses) Outcome assessment points: baseline and 4 months Method(s): angiography
Notes	Total sample size is 30 - BMSC/control group sample sizes not reported 2022: no new data.

Fernandez-Pereira 2006

Methods	Type of study: parallel RCT We have requested additional information relating to possible patient overlap with Huang 2008 abstract
Participants	Population: AMI, within 7 days Age mean (SD) each arm: not reported Sex % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: delivery “via microtubular” Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: saline infusion
Outcomes	Primary outcomes: mortality Secondary outcomes: complications during BMSC infusion, MACE (reinfarction, restenosis, tumour) Outcome assessment points: baseline, 6 months and 12 months Method(s): not reported
Notes	-

Huang 2007b

Methods	Type of study: parallel RCT We have requested additional information relating to possible patient overlap with Huang 2007b abstract
Participants	Population: AMI, with successful PCI with stenting Age mean (SD) each arm: not reported Sex % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: delivery “through micro-catheter” Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: saline infusion
Outcomes	Primary outcomes: not reported Secondary outcomes: safety (cardiovascular events, ventricular arrhythmias, syncope), LVEF Outcome assessment points: baseline and 12 months Method(s): quantitative LV angiography, contrast-enhanced MRI
Notes	— 2022: no new data for this trial.

Huang 2008

Methods	Type of study: parallel RCT Type of publication: abstract Source of funding: not reported Country of origin: China Number of centres: 1 Dates of trial enrolment: not reported Length of follow-up: 6 months Number (N) of participants randomised to each arm: 15 control and 14 BMSC Number (N) of participants analysed (primary outcome) in each arm: not reported
Participants	Population: AMI Age mean (SD) each arm: not reported Sex % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none reported
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells Summary of how stem cells were isolated and type and route of delivery: not reported except the intracoronary delivery of cells Dose of stem cells: not reported Timing of stem cell procedure: 3 hours after successful PCI Comparator arm: not reported
Outcomes	Primary outcomes: changes in LV function and myocardial perfusion Secondary outcomes: not reported Outcome assessment points: 6 months Method(s): echocardiography and LV angiography
Notes	2022: no new data

Lee 2005

Methods	Type of study: parallel RCT Type of publication: abstract Source of funding: not reported Country of origin: Beiging, China Number of centres: 1 Dates of trial enrolment: not reported Length of follow-up: 6 months Number (N) of participants randomised to each arm: not reported Number (N) of participants analysed (primary outcome) in each arm: not reported
Participants	Population: AMI Age mean (SD) each arm: 52.18 (9.98) years Sex % male in each arm: 72% male and 28% female Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: not reported Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: not reported
Outcomes	Primary outcomes: feasibility and safety Secondary outcomes: LVEF, LVEDV, LVESV, cardiac output, cardiac index, cardiac mass Outcome assessment points: 6 months Method(s): MRI
Notes	2022: no new data. From the same group new study included, TEAM-AMI.

Lu 2012b

Methods	Type of study: parallel RCT Type of publication: abstract Source of funding: not reported Country of origin: not reported Number of centres: not reported Dates of trial enrolment: not reported Length of follow-up: 6 months Number (N) of participants randomised to each arm: 26 to control and 28 to treatment Number (N) of participants analysed (primary outcome) in each arm: not reported

Participants	Population: ST elevation MI (STEMI) Age mean (SD) each arm: not reported Sex % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: none reported
Interventions	Intervention arm: mesenchymal stem cells (MSC) Type of stem cells: MSC Summary of how stem cells were isolated and type and route of delivery: not reported, MSC were cultured for 4 weeks Dose of stem cells: 1 x 106 cells Timing of stem cell procedure: not reported Comparator arm: not reported
Outcomes	Primary outcomes: changes in Heart Rate Variability (HRV) Secondary outcomes: arrhythmias, adverse events, LVEF Outcome assessment points: baseline, 1 month and 6 months Method(s): SPECT and transthoracic echocardiography
Notes	-

Park 2011

Methods	Type of study: parallel RCT We are awaiting further information on number of included and followed up patients and full publication details
Participants	Population: patients with AMI. BMSC transplantation after successful PCI Age mean (SD) each arm: not reported Sex % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups? not reported
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow-derived stem cells (mononuclear cells-MNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate (30 to 40 mL). Cells were separated by gradient centrifugation. Cells were infused after successful PCI by intracoronary transfer Dose of stem cells: a single dose of 1.34 (0.65 to 4.0) x 10^8/mL mononuclear cells Timing of stem cell procedure: 1 week after PCI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: LVEF, LVEDV, LVESV Secondary outcomes: not reported Outcome assessment points: baseline and 6 months Method(s): cMRI
Notes	2022: no new data found.

Perez-Oteyza 2006

Methods	Type of study: parallel RCT Type of publication: abstract Source of funding: not reported Country of origin: Portugal Number of centres: 1 Dates of trial enrolment: 01/2011 to 05/2013 Length of follow-up: 12 months Number (N) of participants randomised to each arm: not reported Number (N) of participants analysed (primary outcome) in each arm: not reported
Participants	Population: patients with AMI. BMSC transplantation after successful PCI. PCI within 12 hours of AMI Age mean (SD) each arm: 50.9 (9.5) years Sex % male in each arm: 91% male Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: < 12 hours Statistically significant baseline imbalances between the groups? not reported
Interventions	Intervention arm: bone marrow progenitor cells Type of stem cells: bone marrow progenitor cells Summary of how stem cells were isolated and type and route of delivery: not reported, except for intracoronary delivery of the cells Dose of stem cells: not reported Timing of stem cell procedure: 7 days after AMI Comparator arm: no additional therapy (control)

Outcomes	Primary outcomes: changes in global longitudinal strain (GLS) and LVEF Secondary outcomes: not reported Outcome assessment points: baseline, 6 months and 12 months Method(s): echocardiography
Notes	2022: no new data found.

Silva 2014

18F-FDG, fluorodeoxyglucose; AMI, acute myocardial infarction; BMMNC, bone marrow-derived mononuclear cells; BMSC, bone marrow stem/progenitor cell; BNP, brain natriuretic peptide; cMRI, cardiac magnetic resonance imaging; DES, drug-eluting stent; G-CSF, granulocyte colony stimulating factor; HF, heart failure; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; MACE, major adverse cardiac events; MBF, myocardial blood flow; MHFQ, Minnesota Heart Failure Questionnaire; MNC, mononuclear cell; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; NYHA, New York Heart Association; PET, positron emission tomography; RCT, randomised controlled trial; SPECT, single photon emission computed tomography

Characteristics of ongoing studies [ordered by study ID]

Study name	Effect of intracoronary injection of autologous stem cells on left ventricular ejection fraction and volumes one year after an acute myocardial infarction
Methods	Type of study: parallel RCT Source of funding: Clinica Rotger Country of origin: Spain Number of centres: not reported Intended recruitment: 60
Participants	Population: patients with AMI Age, mean (SD) each arm: not reported (8 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: > 2 segments Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups? not reported
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow mononuclear cells (BMMNC) Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate and gradient centrifugation. Following the method set up by Schachinger 2006. Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: placebo (saline)
Outcomes	Primary outcomes: changes in LVEF, LVEDV, LVESV, perfusion, scar size Secondary outcomes: changes in LVEF at 6 months (by echocardiography and LV angiography), major adverse clinical cardiac events Outcome assessment points: baseline and 12 months Method(s): not reported
Starting date	Not reported
Contact information	Not reported
Notes	-

EUCTR 2006-001772-20-ES

Study name	Open study with blind regulator on the effectiveness of autologous bone marrow mononuclear cells in patients with left ventricular dysfunction after myocardia infarction
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Spain Number of centres: not reported Intended recruitment: 20
Participants	Population: AMI and LVEF < 35% Age, mean (SD) each arm: not reported (18 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups? not reported

Interventions	Intervention arm: BMSC Type of stem cells: bone marrow mononuclear cells (BMMNC) Summary of how stem cells were isolated and type and route of delivery: intracoronary injection. Method of isolation of BMMNC not reported Dose of stem cells: 20 to 30 x 106 cells/mL Timing of stem cell procedure: not reported Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in LVESV Secondary outcomes: NT-proBNP, myocardial perfusion, MACE, hospitalisation within 24 hours Outcome assessment points: baseline and 12 months Method(s): echocardiography
Starting date	Not reported
Contact information	Not reported
Notes	-

EUCTR 2006-005628-17-ES

Study name	Selected bone marrow cell transplantation following MI in patients undergoing coronary surgery
Methods	Type of study: parallel RCT Source of funding: Bristol Royal Infirmary Country of origin: UK Number of centres: 1 Intended enrolment: 60
Participants	Population: recent MI (> 10 days < 3 months) undergoing bypass coronary surgery Age, mean (SD) each arm: not reported Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: > 10 days < 3 months Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: CD133+ bone marrow cells Type of stem cells: bone marrow-derived CD133+ cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirate and selection of CD133+ cells using magnetic immunoaffinity Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: autologous plasma
Outcomes	Primary outcomes: quantitative assessment of myocardium at the site of injection of CD133+ cells Secondary outcomes: not reported Outcome assessment points: not reported Method(s): not reported
Starting date	June 2006
Contact information	Research and Effectiveness Department, Level 1 Old Building, Bristol Royal Infirmary, Marlborough St., Bristol, BS2 8HW
Notes	This trial is marked as completed but no publications have as yet been identified

ISRCTN65630838

Study name	A trial using CD133 enriched bone marrow cells following primary angioplasty for acute myocardial infarction (SELECT-AMI)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Belgium, France, The Netherlands, United Kingdom Number of centres: 4 Intended enrolment: 19
Participants	Population: AMI Age, mean (SD) each arm: not reported (20 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: presence of severe hypokinesia and/or akinesia in >= 2 adjacent segments on echocardiogram at 48 to 72 hours after primary PCI Time from symptom onset to initial treatment: 2 to 24 hours after onset of chest pain Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: CD133+ cells Type of stem cells: bone marrow-derived selected CD133+ cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirated, CD133+ cells selected, intracoronary injection of autologous CD133+ cells Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: buffered normal saline

Outcomes	Primary outcomes: 1) Safety - progression in coronary atherosclerosis burden proximal and distal to the stented segment of the infarct-related artery, 2) Efficacy - changes in myocardial thickening in non-viable akinetic/hypokinetic LV wall segments by cardiac magnetic resonance imaging (cMRI) Secondary outcomes: 1) Safety - development of ventricular arrhythmias including failed sudden cardiac death, development of congestive heart failure 2) Efficacy - LVEF, epicardial resistance and microvascular resistance, the feasibility of the CliniMACS® Reagent System to yield 5 x 10^6 CD133+ cells from 100 to 150 mL of autologous bone marrow Outcome assessment points: baseline and 6 months Method(s): cMRI, echocardiography
Starting date	September 2007
Contact information	Jozef Bartunek, MD (jozef.bartunek@olvz-aalst.be); Jonathan Hill, MD (jonathan.hill@kcl.ac.uk)
Notes	This study has been terminated due to insufficient recruitment

NCT00529932

Study name	Reinfusion of enriched progenitor cells and infarct remodeling in acute coronary syndrome (REPAIR-ACS)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Germany Number of centres: 1 Intended enrolment: 31
Participants	Population: acute non-ST segment elevation myocardial infarction, successful PCI with stent Age, mean (SD) each arm: not reported (18- to 80 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: < 48 hours Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BMSC Type of stem cells: bone marrow stem cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspirated, preparation of media, delivery via intracoronary injection Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: placebo medium
Outcomes	Primary outcomes: improvement of coronary flow reserve in the infarct vessel Secondary outcomes: improvement of relative coronary flow reserve, regional and global LVEF, MACE (death, MI, rehospitalisation for heart failure, revascularisation) Outcome assessment points: baseline, 4 months and 1 year Method(s): intracoronary doppler wire
Starting date	September 2008
Contact information	Andreas M Zeiher, MD (zeiher@em.uni-frankfurt.de); Birgit Assmus, MD (b.assmus@em.uni-frankfurt.de)
Notes	This study has been terminated due to slow recruitment

NCT00711542

Study name	The enhanced angiogenic cell therapy - acute myocardial infarction trial (ENACT-AMI)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Canada Number of centres: 5 Intended enrolment: 100
Participants	Population: AMI Age, mean (SD) each arm: not reported (18 to 80 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: endothelial progenitor cells (EPC) or eNOS transfected EPC Type of stem cells: endothelial progenitor cells (EPC) Summary of how stem cells were isolated and type and route of delivery: not reported Dose of stem cells: 20 x 106 cells in each treatment arm Timing of stem cell procedure: after 5 to 7 days Comparator arm: plasmalyte and 25% autologous plasma
Outcomes	Primary outcome: change in LVEF Secondary outcomes: changes in wall motion, clinical worsening, QoL and safety Outcome assessment points: baseline and 6 months Method(s): MRI
Starting date	July 2013

Contact information	Contact: Dr. Duncan J. Stewart, MD FRCP C, Ottawa Hospital Research Institute
Notes	2022: study active, not recruiting (on clinicaltrials.gov)

NCT00936819

Study name	Bone marrow derived AC 133+ and mono-nuclear cells (MNC) implantation in myocardial infarction (MI) patients
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Iran Number of centres: 1 Intended enrolment: 80
Participants	Population: AMI Age, mean (SD) each arm: not reported (18 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: 1 Number of stunned hyperkinetic, etc segments: more than 2 Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BMSC or CD133+ Type of stem cells: none marrow mononuclear cells (BMMNC) and CD133 cells Summary of how stem cells were isolated and type and route of delivery: not reported Dose of stem cells: not reported Timing of stem cell procedure: within 3 weeks of AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: change in LVEF Secondary outcomes: change in LVEDV, LVESV, segment contractility Outcome assessment points: baseline, 6 months Method(s): echocardiography
Starting date	May 2009
Contact information	Principal Investigator: Masoud Ghassemi, MD; Royan Institute, Tehran, Islamic Republic of Iran
Notes	This trial is marked as completed but no publications have as yet been identified

NCT01187654

Study name	Endocardial mesenchymal stem cells implantation in patients after acute myocardial infarction (ESTIMATION Study)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Russia Number of centres: not reported Intended enrolment: 50
Participants	Population: AMI with successful PCI Age, mean (SD) each arm: not reported (30 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: 1 Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BM-MSC Type of stem cells: bone marrow-derived mesenchymal stem cells Summary of how stem cells were isolated and type and route of delivery: not reported, except for delivery using NOGA mapping Dose of stem cells: not reported Timing of stem cell procedure: 7 to 10 days after PCI Comparator arm: placebo
Outcomes	Primary outcomes: reduction of LVESV by 15% Secondary outcomes: death, Thrombosis, hospitalisation for HF, 6 min-walk, BNP levels Outcome assessment points: baseline, 12 months Method(s): MRI
Starting date	July 2011
Contact information	Principal Investigator: Professor Evgeny Pokushalov, MD; State Research Institute of Circulation Pathology, Novosibirsk, Russian Federation, 630055
Notes	Estimated completion date: November 2012 2022: Recruitment unknown. No publication yet.

NCT01394432

Study name	Rapid delivery of autologous bone marrow derived stem cells in acute myocardial infarction patients (AMIRST)
Methods	Type of study: parallel RCT Source of funding: TotipotentRX Cell Therapy Pvt. Ltd. Country of origin: India Number of centres: not reported Intended enrolment: 30
Participants	Population: AMI, LVEF < 40% Age, mean (SD) each arm: not reported (18 to 75 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: PCI within 24 hours of MI Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells Summary of how stem cells were isolated and type and route of delivery: not reported, intracoronary delivery Dose of stem cells: not reported Timing of stem cell procedure: 3 to 10 days after AMI Comparator arm: placebo
Outcomes	Primary outcomes: AE Secondary outcomes: changes in LVEF, LVEDV, LVESV, infarct size, myocardial perfusion, MACE and QoL Outcome assessment points: baseline and 12 months Method (s): cardiac MRI
Starting date	December 2013
Contact information	Principal Investigators: Sreenivas A Kumar, MD, DM, FACC; CARE Hospitals, Hyderabad, India; Upendra Kaul, MD,DM, FACC; Fortis Flt. Lt. Rajan Dhall Hospital and Ashok Seth, FRCP, FACC; Fortis Escorts Heart Institute and Research Centre, India
Notes	Estimated completion date: January 2015 2022: no publication yet. recruitment status unknown at clinicaltrials.gov

NCT01536106

Study name	Stem cell therapy in patients with myocardial infarction and persistent total occlusion of infarct related artery (COAT)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: India Number of centres: 1 Intended enrolment: 40
Participants	Population: AMI Age, mean (SD) each arm: not reported (18 to 80 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: time to PCI < 24 hours. Time to cell treatment > 24 hours Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells Summary of how stem cells were isolated and type and route of delivery: intracoronary delivery of BMMNC isolated from bone marrow aspirates and gradient centrifugation Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in LVEF Secondary outcomes: changes in functional capacity (NYHA class), 6 minute walking distance, QoL, recurrent MI or death Outcome assessment points: baseline and 3 months Method(s): PET
Starting date	March 2011
Contact information	Principal Investigator: Sandeep Seth, DM; All India Institute of Medical Sciences, New Delhi, India
Notes	Estimated completion date: June 2014 2022: recruitment status unknown. no new publications.

NCT01625949

Study name	A randomised, open labeled, multicenter trial for safety and efficacy of intracoronary adult human mesenchymal stem cells acute myocardial infarction (RELIEF)
Methods	Type of study: parallel RCT Source of funding: Pharmicell Co., Ltd Country of origin: Korea Number of centres: not reported Intended enrolment: 135

Participants	Population: AMI, LVEF < 45% Age, mean (SD) each arm: not reported (20 to 70 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: within 30 days of MI Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BM-MSC Type of stem cells: bone marrow-derived mesenchymal stem cells Summary of how stem cells were isolated and type and route of delivery: intracoronary delivery of MSC, not reported how they are cultured Dose of stem cells: not reported Timing of stem cell procedure: after 30 days (single dose) or after 30 and 60 days (double dose) Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in LVEF Secondary outcomes: not reported Outcome assessment points: baseline and 13 months Method(s): MRI
Starting date	October 2013
Contact information	Principal Investigator: Yang Soo Jang, Ph.D. M.D.; Severance Hospital, Yonsei University College of Medicine; Korea
Notes	Estimated completion date: December 2018 2022: no new publications. recruitment status: recruiting

NCT01652209

Study name	Impact of intracoronary injection of autologous BMMC for LV contractility and remodeling in patients with STEMI (RACE-STEMI)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Poland Number of centres: not reported Intended enrolment: 200
Participants	Population: AMI, LVEF ≤ 45% Age, mean (SD) each arm: not reported (> 18 years) Sex, % male in each arm: not reported Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: not reported Statistically significant baseline imbalances between the groups?: not reported
Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells Summary of how stem cells were isolated and type and route of delivery: intracoronary delivery of BMMNC isolated from BM aspirates and gradient centrifugation Dose of stem cells: not reported Timing of stem cell procedure: not reported Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: changes in LVEF at 12 months Secondary outcomes: LVEDV, LVESV, time to cardiac death, hospitalisation for HF, SAE Outcome assessment points: baseline, 12 months and 36 months Method(s): CT
Starting date	March 2015
Contact information	Principal Investigator: Pawel E Buszman, MD, PhD; American Heart of Poland, Poland
Notes	Estimated completion date: July 2018 2022: Not yet recruiting

NCT02323620

Study name	Intracoronary autologous stem cell transplantation in ST-elevation myocardial infarction (TRACIA STUDY)
Methods	Type of study: parallel RCT Source of funding: not reported Country of origin: Mexico Number of centres: not reported Intended enrolment: not reported
Participants	Population: AMI Age, mean (SD) each arm: 53.25 (5.7) years Sex, % male in each arm: 87.5% Number of diseased vessels: not reported Number of stunned hyperkinetic, etc segments: not reported Time from symptom onset to initial treatment: within 24 hours Statistically significant baseline imbalances between the groups?: not reported

Interventions	Intervention arm: BMMNC Type of stem cells: bone marrow-derived mononuclear cells Summary of how stem cells were isolated and type and route of delivery: bone marrow aspiration and separation of mononuclear cells using a Sepax machine and a gradient centrifugation Dose of stem cells: adjusted for CD34-positive cells 1 to 2 x 10^6 CD34 cells Timing of stem cell procedure: day 5 to 6 after AMI Comparator arm: no additional therapy (control)
Outcomes	Primary outcomes: safety changes in LVEF from baseline to 6 months Secondary outcomes: death, re-infection, restenosis, thrombosis, adverse events, LVEF Outcome assessment points: baseline, 6 months Method(s): MRI and SPECT
Starting date	-
Contact information	Marco Antonio Pena Duque, Juan Badiano No. 1, Col Cession XVI, Llalpan, 14080 Mexico. Email: penmar@cardiologia.org.mx
Notes	2022: no new publications. recruitment status unknown.

Pena-Duque 2011

Study name	TEAM-AMI
Methods	Type of study: RCT Source of funding: The study is supported by the National Major Scientific and Technological Special Project for 'Significant New Drugs Development' during the Twelfth Five-year Plan Period (2014ZX09101042–001), the CAMS Innovation Fund for Medical Sciences (2016-I2M-1-009), the National High Technology Research and Development Program (863 Program) in China (2013AA020101, Ministry of Science and Technology) and the National Natural Science Foundation of China (81874461, 81573957). Country of origin: China Number of centres: not reported? (multicentre?) Intended enrolment: 124
Participants	Population: first AMI Age, mean (SD) each arm: Sex, % male in each arm: Number of diseased vessels: Used cutoff for Number of stunned hyperkinetic, etc segments: Time from symptom onset to initial treatment: e.g. Statistically significant baseline imbalances between the groups?:
Interventions	Intervention arm: Type of stem cells: e.g. BMSC Summary of how stem cells were isolated and type and route of delivery: e.g. bone marrow aspirate Dose of stem cells: low and high dose. Cell count? Timing of stem cell procedure: how many days after MI and/or harvest. Comparator arm: placebo medium vs PBS vs etc
Outcomes	
Starting date	
Contact information	
Notes	

Xu 2019

AE, adverse effect; AMI, acute myocardial infarction; BFU-E, burst-forming unit - erythrocyte, BM, bone marrow; BMMNC, bone marrow-derived mononuclear cells; BMSC, bone marrow-derived stem cells; BM-CPC, bone marrow-derived circulating progenitor cells; BOOST, Benefits of Oxygen Saturation Targeting; CFU-GEMM, colony-forming unit - granulocyte erythrocyte monocyte megakaryocyte; CFU-GM, colony-forming unit - granulocyte monocyte, CK-MB, creatine-kinase muscle and brain; cMRI, cardiac magnetic resonance imaging; CO, FACS, fluorescence-activated cell sorting; G-CSF, granulocyte colony stimulating factor; IVUS, intravascular ultrasound; LAD, left anterior descending; LV, left ventricle or ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MACE, major adverse cardiac events; MI, myocardial infarction; MIBI, methoxyisobutylisonitrile; MNC, mononuclear cells; MRI, magnetic resonance imaging; MUGA, Multi Gated Acquisition Scan; MVO2, myocardial volume oxygen consumption; PCI, percutaneous coronary intervention; PET, positron emission tomography; QoL, quality of life; QLV, quantitative left ventriculography; SAE, serious adverse effect; SC, stem cells; SD, standard deviation; SPECT, single photon emission computed tomography; STEMI, ST-segment elevation myocardial infarction; VEGF, vascular endothelial growth factor; WMSI, wall motion score index

Table 1. Characteristics of study participants.

Study ID	Country of study	Patient population
Angeli 2012	Brazil	STEMI with LVEF < 45%; successful PCI
Cao 2009	China	STEMI; PCI within 12 hours, often with drug-eluting stent implantation
Chen 2004	China	AMI; PCI within 12 hours, mostly with stent implantation
Choudry 2016 (REGENERATE-AMI)	UK	Anterior AMI; significant regional wall motion abnormality at the time of PCI
Colombo 2011	Italy	Large anterior STEMI; PCI with bare metal stent implantation within 12 hours
Gao 2013	China	Acute STEMI; PCI with stent implantation within 12 hours
Ge 2006	China	First STEMI within 24 hours; PCI with stent implantation
Grajek 2010	Poland	First anterior AMI; PCI within 12 hours with bare metal stent implantation
Haddad 2020 (COMPARE AMI)	Canada	STEMI; LVEF 25 -50% within 48h of reperfusion
Hirsch 2011 (HEBE)	The Netherlands	First STEMI; PCI with stent implantation within 12 hours
Huang 2006	China	AMI; PCI within 24 hours
Huang 2007	China	AMI; PCI within 24 hours with bare metal (35%) or drug-eluting (65%) stent implantation
Huikuri 2008 (FINCELL)	Finland	STEMI; thrombolytic drugs initiated within 12 hours
Janssens 2006	Belgium	STEMI; PCI with bare metal stent implantation at median 3.7 hours (IQR 2.5 to 7.6)
Jazi 2012	Iran	Anterior MI within 1 month with a history of anterior MI and LVEF < 35%; PCI
Jin 2008	China	AMI; thrombolytic drugs and PCI
Karpov 2005	Russia	STEMI; PCI with bare metal stent implantation within 6.6 (4.9) hours and thrombolytic drugs
Kim 2018	Korea	Acute ST-segment elevation anterior wall MI; LVEF ≤ 40% <72h after PCI
Kirgizova 2015	Russia	First AMI
Lee 2014 (SEED-MSC)	South Korea	STEMI within 24 hours enrolled < 72 hours after revascularisation by PCI and/or thrombolytic drugs
Lunde 2006 (ASTAMI)	Norway	Anterior STEMI; PCI within 2 to 24 hours
Mathur 2020 (BAMI)	UK; Belgium, Czech Republic, Denmark, Finland, France, Germany, Italy, Poland, Spain	AMI; LVEF <45% 2-6d after PCI

Mean (SD) age of participants (years)	% Male	No. randomised participants receiving intervention	No. randomised participants receiving comparator	Mean duration of followup
n/r	n/r	11	11	12 months
BMMNC: 50.7 (SEM 1.1) Control: 51.1 (SEM 1.0)	BMMNC: 95.1% Control: 93.3%	41	45	48 months
BMMNC: 58 (7.0) Control: 57 (5.0)	BMMNC: 94% Control: 97%	34	35	6 months
BMC: 56.7 (10.7) Control: 56.4 (10.4)	BMC: 91%. Control: 84%.	45	55	12 months
CD133+: median 54 (range 47 to 60) Control: median 56 (range 44 to 58)	CD133+: 100% Control: 100%	5	5	12 months
BM-MSC: 55.0 (SEM 1.6) Control: 58.6 (SEM 2.5)	BM-MSC: 100% Control: 86.4%	21	22	24 months
BMMNC: 58 (11) Control: 59 (8)	BMMNC: 80% Control: 100%	10	10	6 months
BMMNC: 49.9 (8.4) Control: 50.9 (9.3)	BMMNC: 87% Control: 86%	31	14	12 months
Median (IQR) CD133+: 51.0 [43.0-60.5] Control: 50.5 [48.3-63.3]	CD133+: 82.4% Control: 95%.	17	20	120 months
BMMNC: 56 (9) Control: 55 (10)	BMMNC: 84% Control: 86%	69	65	60 months
BMMNC: 57.3 (10.1) Control: 56.7 (9.2)	BMMNC: 65% Control: 70%	20	20	6 months
BMMNC: 54.8 (5.8) Control: 55.4 (7.1)	BMMNC: 85% Control: 90%	20	20	6 months
BMMNC: 60 (10) Control: 59 (10)	BMMNC: 90% Control: 85%	40	40	6 months
BMMNC: 55.8 (11) Control: 57.9 (10)	BMMNC: 82% Control: 82%	33	34	4 months
BMMNC: 48.0 (SEM 2.5) Control: 45.2 (SEM 3.2)	BMMNC: 66% Control: 90%	n/r	n/r	6 months
BMMNC: 62.3 (7.7) Control: 60.6 (6.5)	BMMNC: 71.4% Control: 75.0%	14	12	12 months
BMMNC: 55.2 (8.6) Control: 52.1 (3.2)	BMMNC: 90% Control: 73%	28	34	8.2 (0.72) years
MSC: 55.3 ± 8.6 Control: 57.8 ± 8.9	MSC: 100% Control: 100%	14	12	12 months
CD133+ cells: 60.3 (12.2) controls: 58.4 (10.4)	CD133+cells: 60%. controls: 81%.	10	16	7.7 (0.4) years
BM-MSC: 53.9 (10.5) Control: 54.2 (7.7)	BM-MSC: 90.0% Control: 89.3%	40	40	6 months
BMMNC: 58.1 (8.5) Control: 56.7 (9.6)	BMMNC: 84% Control: 84%	50	51	36 months
BMMNC: 59 (±SD 11) Control: 60 (± SD 11)	BMMNC: 84% Control: 77%	185	190	24 months

Table 1. Continued

Study ID	Country of study	Patient population
Meluzin 2008	Czech Republic	First STEMI; PCI with stent implantation within 12 hours or 3 days
Nair 2015 (MI3)	India	AMI; successful PCI; LVEF 20-50%
Naseri 2018 (COMPARE CPM-RMI)	Iran	First anterior STEMI; eligible for elective CABG; LVEF <45%
Nogueira 2009 (EMRTCC)	Brazil	STEMI; thrombolytic drugs and PCI with stent implantation within 24 hours
Penicka 2007	Czech Republic	First anterior STEMI and LVEF ≤ 50%
Piepoli 2010 (CARDIAC)	Italy	Anterior STEMI; PCI with stent implantation within 2 to 6 hours
Plewka 2009	Poland	First anterior STEMI and LVEF < 40%; PCI within 12 hours
Quyyumi 2011 (ARM-1)	USA	Acute STEMI and LVEF ≤ 50%
Quyyumi 2017 (PRESERVE-AMI)	USA	STEMI; LVEF ≤48% ≥4 days post stent
Roncalli 2010 (BONAMI)	France	Acute STEMI and LVEF ≤ 45%; PCI with bare metal stent implantation within 24 hours
Ruan 2005	China	AMI admitted within mean 12.1 (12.6) hours of onset; PCI
San Roman 2015 (BMMNC+G-CSF) (TECAM)	Spain	AMI successfully reperfused by rapamycin DES implantation
Schachinger 2006 (REPAIR-AMI)	Germany; Switzerland	Acute STEMI and visual estimated LVEF ≤ 45%; PCI with stent implantation at mean 7.5 (8.0) hours
Suarez de Lezo 2007	Spain	Anterior STEMI within 12 hours; PCI (some with stent) or thrombolytics
Sürder 2013 (SWISS-AMI)	Switzerland	Large STEMI with LVEF < 45%; thrombolytics and PCI with stent within 24 hours
Tendera 2009 (REGENT)	Poland	Anterior AMI and LVEF ≤ 40%
Traverse 2010	USA	First anterior STEMI; PCI mostly with drug-eluting stent implantation
Traverse 2011 (LATE-TIME)	USA	STEMI with LVEF ≤ 45%; PCI with stent, mostly drug-eluting, at median 3.4 (IQR 2.3 to 14.3) hours

	Mean (SD) age of participants (years)	% Male	No. randomised participants receiving intervention	No. randomised participants receiving comparator	Mean duration of followup
	BMMNC: 54 (SEM 2) Control: 55 (SEM 2)	BMMNC: 90% (HD), 95% (LD) Control: 90%	n/r [a]	n/r [a]	12 months
	BMSC: 48.07 (9.68) Control: 48.98 (9.76)	BMSC: 88.8% Control: 87.2%	125	125	6 months
	BMMNC :51.5 (7.5) CD133+: 53.1 (8.6) Control: 55.5 (8.5)	BMMNC: 90% CD133+: 90.5% Control: 88.5%	BMMNC: 30 CD133+: 21	26	18 months
	BMMNC: 59.7 (14.3) (AG), 53.6 (8.3) (VG) Control: 57.2 (10.8) (AG), 57.2 (10.8) (VG)	BMMNC: 71% (AG), 70% (VG) Control: 67%	24 (14 AG, 10 VG)	6	6 months
	BMMNC: 61 (14) Control: 54 (10)	BMMNC: 71% Control: 100%	17	10	24 months
	BMMNC: 63.1 (SEM 2.7) Control: 67.2 (SEM 2.4)	BMMNC: 68.4% Control: 68.4%	19	19	24 months
	BMMNC: 59 (9) Control: 56 (8)	BMMNC: 68% Control: 78%	40	20	24 months
	CD34+: median 50.5 (IQR 45 - 53) (HD), 63.0 (IQR 57 - 66) (MD), 52.0 (IQR 51 - 52) (LD) Control: median 52.0 (IQR 47 - 57)	CD34+: 100% (HD), 80% (MD), 80% (LD) Control: 87%	16 (5 LD, 5 MD, 6 HD)	15	12 months
	CD34+: 57.1 (10.1) Control: 56.4 (10.1).	CD34+: 85%. Control: 80%.	100	95	12 months
	BMMNC: 56 (12) Control: 55 (11)	BMMNC: 80.8% Control: 89.8%	52	49	12 months
	BMMNC: 61 (8) Control: 58 (6)	BMMNC: 88.9 Control: 100%	9	11	6 months
	BMMNC: 54 (11) BMMNC + G-CSF: 56 (8) G-CSF: 57 (9) Control: 57 (11)	BMMNC: 97% BMNNC + G-CSF: 86% G-CSF: 83% Control: 90%	BMMNC: 30 BMMNC + G-CSF: 29	Control: 31 G-CSF: 30	12 months
	BMMNC: 55 (11) Control: 57 (11)	BMMNC: 82% Control: 82%	101	103	60 months
	BMMNC: 52 (12) Control: 55 (11)	BMMNC: 80% Control: 70%	10	10	3 months
	BMMNC: median 55 (IQR 15) (E), 62 (IQR 15) (L) Control: median 56 (IQR 14.5)	BMMNC: 86.2% (E), 82.5 (L) Control: 83.6%	133 (66 E, 67 L)	67	12 months
	CD34/CXCR4+: median 58 BMMNC: median 55 Control: median 59	CD34/CXCR4+: 63.7% BMMNC: 70.6% Control: 75.0%	160 (80 CD34/CXCR4+, 80 BMMNC)	40	6 months
	BMMNC: median 52.5 (IQR 43 - 64) Control: median 57.5 (IQR 54 - 59)	BMMNC: 83.3% Control: 60.0%	30	10	15 months
	BMMNC: 57.6 (11) Control: 54.6 (11)	BMMNC: 79% Control: 90%	59	29	6 months

Table 1. Continued

Study ID	Country of study	Patient population
Traverse 2018 (TIME)	USA	Anterior STEMI with LVEF < 45%; PCI with stent, mostly drug-eluting
Turan 2012	Germany	Acute STEMI; PCI with stent implantation
Wang 2014	China	Acute STEMI; PCI predominantly with stent implantation within 8 hours
Wohrle 2010 (SCAMI)	Germany	AMI; PCI with stent, some drug eluting, within 6 to 48 hours
Wollert 2004 (BOOST)	Germany	STEMI within 5 days; PCI with bare metal stent implantation, some with thrombolytic drugs
Wollert 2017 (BOOST-2)	Germany; Norway	STEMI; hypokinesia or akinesia involving more than two thirds of the LV anteroseptal, lateral, and/or inferior wall immediately after PCI
Xiao 2012	China	AMI; undergoing elective PCI within 4 weeks of AMI
Yang 2020	China	STEMI; LVEF ≤45%
Yao 2006	China	STEMI within 1 week; PCI
Yao 2009	China	First anterior STEMI; PCI within 12 hours
You 2008	China	AMI within 24 hours; thombolytic reperfusion
Zhang 2021	China	Acute STEMI; successful PCI
Zhukova 2009	Russia	MI of the front wall; thrombolytic drugs and/or PCI with stent implantation

STEMI, ST-segment elevation myocardial infarction; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; BMMNC, bone marrow mononuclear cells; BM-MSC, bone marrow mesenchymal stem cells; SEM, standard error of the mean; SD, standard deviation; LD, low dose; MD, moderate dose; HD, high dose; AG, arterial group; VG, venous group; E, early cells; L, late cells; S, selected cells; U, unselected cells; SD, single dose; DD, double dose

[(a)]Meluzin 2008: 73 participants were randomised in total - the number randomised to each group was not reported.

	Mean (SD) age of participants (years)	% Male	No. randomised participants receiving intervention	No. randomised participants receiving comparator	Mean duration of followup
	BMMNC: 55.6 (10.8) (day 3)/58.2 (11.3) day 7) Control: 57.0 (12.4) (day 3)/57.0 (8.0) (day 7)	BMMNC: 88.4% (day 3)/86.1% (day 7) Control: 87.5% (day 3)/88.3% (day 7)	43 (day 3) 36 (day 7)	24 (day 3) 17 (day 7)	12 months
	BMMNC: 61 (15) Control: 60 (11)	BMMNC: 67% Control: 70%	42	20	12 months
	BM-MSC: 58 (10.2) Control: 56.1 (9.8)	BM-MSC: 67.9% Control: 53.3%	30	30	6 months
	BMMNC: 61.0 (8.1) Control: 61.1 (9.3)	BMMNC: 90% Control: 62%	29	13	36 months
	BMMNC: 53.4 (14.8) Control: 59.2 (13.5)	BMMNC: 67% Control: 73%	33	32	60 months
	loBMC: 53 (11) hiBMC: 57 (10) Control: 55 (9)	loBMC: 87% hiBMC: 85% Control: 92%	loBMC: 40 hiBMC: 38 (safety analysis, excl irradiated cells)	34 (safety analysis)	6 months
	BM-MSC: 60.4 (8.9) Control: 58.6 (10.0)	BM-MSC: 58.8% Control: 61.9%	17	21	3 months
	BMMNC: 55 (13.3) Control: 51 (10.9)	BMMNC: 88% Control: 92%	50	50	12 months
	BMMNC: 58.3 (9.5) Control: 58.1 (9.0)	BMMNC: 89.1% Control: 88.0%	92	92	30 months
	BMMNC: 52.1 (6.3) (SD), 51.3 (7.4) (DD) Control: 52.7 (7.8)	BMMNC: 83.3& (SD), 80.0% (DD) Control: 91.7%	30 (15 SD, 15 DD)	15	12 months
	BM-MSC: 60.5 Control: 62.5	BM-MSC: 71.4% Control: 56.3%	7	16	8 weeks
	MSC: 59.3 (9) Control: 58.6 (11)	MSC: 95% Control: 86%	21	22	12 months
	BMMNC: 48 (7) Control: 50 (10)	BMMNC: 100% Control: 100%	8	3	36 months

Table 2. Characteristics of study interventions

Study ID	Time of cell administration	Intervention given by:	Route of cell administration	Intervention cell type
Angeli 2012	5 to 9 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Cao 2009	7 days after PCI	Cardiologist	Infusion into IRCA	BMMNC
Chen 2004	Mean 18.4 (0.5) days after PCI	Cardiologist	Infusion into IRCA	BMMNC
Choudry 2016 (REGENERATE-AMI)	<24 h of successful PCI	Cardiologist	Infusion into IRCA	BMMNC
Colombo 2011	Day 9 to 16 after PCI	Cardiologist	Infusion into IRCA	CD133-positive cells
Gao 2013	Mean 17.1 (0.6) hours after PCI	Cardiologist	Infusion into IRCA	BM-MSC
Ge 2006	Within 15 hours of AMI	Cardiologist	Infusion into IRCA	BMMNC
Grajek 2010	5 to 6 days after PCI	Cardiologist	Infusion into IRCA	BMMNC
Haddad 2020 (COMPARE-AMI)	3-7 days post PCI	Cardiologist	Infusion into IRCA	BM CD133+
Hirsch 2011 (HEBE)	3 to 8 days after PCI	Cardiologist	Infusion into IRCA	BMMNC
Huang 2006	Within 2 hours of PCI	Cardiologist	Infusion into IRCA	BMMNC
Huang 2007	Within 2 hours of PCI	Cardiologist	Infusion into IRCA	BMMNC
Huikuri 2008 (FINCELL)	Mean 70 (36) hours after thombolysis	Cardiologist	Infusion into IRCA	BMMNC
Janssens 2006	Within 20 hours of PCI	Cardiologist	Infusion into IRCA	BMMNC
Jazi 2012	Within 1 month of AMI	Cardiologist	Infusion into IRCA	BMMNC
Jin 2008	At least 7 to 10 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Karpov 2005	7 to 21 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Kim 2018	30 ± 1.3 days post PCI	Cardiologist	Infusion into IRCA	BM-MSC
Kirgizova 2015	16 (6) days after AMI	Cardiologist	Infusion into IRCA	BM CD133+
Lee 2014 (SEED-MSC)	25 (2.4) days after BM aspiration at 3.8 (1.5) days after admission	Cardiologist	Infusion into IRCA	BM-MSC

How are cells obtained? (*)	What were they re-suspended in?	Dose administered?	Comparator arm (placebo or control)
n/r	n/r	260 (160) million cells	Placebo (n/r)
BM aspiration (**)	Heparinised saline	500 million cells	Placebo (heparinised saline)
BM aspiration (**)	Heparinised saline	48,000 (60,000) million cells	Placebo (heparinised saline)
BM aspiration (**)	Saline	59.8 (47.1-72.5) million cells	Placebo (10 mL sterile NaCl 0.9% with 33 µL autologous whole bone marrow to colour match BMC product)
BM aspiration (**), immunomagnetic selection to isolate CD133-positive cells	0.9% saline solution and 10% human serum albumin	Median (range): 5.9 (4.9 to 13.5) million cells	No additional therapy (Control)
BM aspiration (**), culture for 14 days to select MSC	Heparinised saline	3.08 (0.52) million cells	No additional therapy (Control)
n/r	n/r	40 million cells	Placebo (n/r)
BM aspiration (**)	X-vivo 15 medium and 2% autologous plasma	410 (180) million cells	No additional therapy (Control)
BM aspiration (**) CD133+ isolated using CliniMACS	Heparinised saline	10 million cells (except 1 patient who received 5.2 million)	Placebo (saline and 10% autologous plasma from which the cells have been eliminated)
BM aspiration (**)	Heparinised saline and 4 % human serum albumin	296 (164) million cells	No additional therapy (Control)
BM aspiration (**)	Heparinised saline	180 (420) million cells	Placebo (heparinised saline)
BM aspiration (**)	Heparinised saline	120 (650) million cells	Placebo (heparinised saline)
BM aspiration (**)	Heparinised saline and 50% autologous serum	402 (196) million cells	Placebo (heparinised saline and 50% autologous serum)
BM aspiration (**)	Heparinised saline and 5% autologous serum solution	172 (72) million cells	Placebo (heparinised saline and 5% autologous serum)
BM aspiration (**)	M199 medium containing VEGF, bFGF, IGF-1 and 10% human serum	2460 (SEM 840) million cells	No additional therapy (Control)
BM aspiration (**)	Heparinised saline	62.7 (17.5) million cells	No additional therapy (Control)
BM aspiration (**)	n/r	88.5 (49.2) million cells	No additional therapy (Control)
BM aspiration (**) MSCs harvested using trypsin and EDTA	Saline	72 (± 9) million cells	No additional therapy (control)
BM aspiration (**), CD133 isolated using Microbead system	Heparinised saline	5-10 million cells	No additional therapy (Control)
BM aspiration (**), culture for 2 to 3 weeks to isolate MSC	n/r	72 (9) million cells	No additional therapy (Control)

Table 2. Continued

Study ID	Time of cell administration	Intervention given by:	Route of cell administration	Intervention cell type
Lunde 2006 (ASTAMI)	4 to 8 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Mathur 2020 (BAMI)	2-8 days post primary PCI	Cardiologist	Infusion into IRCA	BMMNC
Meluzin 2008	5 to 9 days (mean 7 (0.3) days) after AMI	Cardiologist	Infusion into IRCA	BMMNC
Nair 2015 (MI3)	15 (IQR 11-18 days) post PCI	Cardiologist	Infusion into IRCA	BMMNC
Naseri 2018 (COMPARE CPM-RMI)	10-30 days post STEMI	Cardiothoracic Surgeon	Intramyocardial injection during CABG	BMMNCs vs. CD133+
Nogueira 2009 (EMRTCC)	AG: 3 to 6 days (mean 5.5 (1.28) days) after PCI VG: 3 to 6 days (mean 6.1 (1.37) days) after PCI	Cardiologist	Infusion into IRCA (AG) or IRCV (VG)	BMMNC
Penicka 2007	4 to 11 days (median 9 days) after PCI	Cardiologist	Infusion into IRCA	BMMNC
Piepoli 2010 (CARDIAC)	4 to 7 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Plewka 2009	3 to 11 days (mean 7 (2) days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Quyyumi 2011 (ARM-1)	LD: median 191.4 (IQR 167 to 201) hours, MD: 210.0 (IQR 194 to 210) hours, HD: 207.3 (IQR 191 to 215) hours after AMI	Cardiologist	Infusion into IRCA	CD34-positive cells
Quyyumi 2017 (PRESERVE-AMI)	Within 11 days of PCI	Cardiologist	Infusion into IRCA	CD34+
Roncalli 2010 (BONAMI)	At 7 to 10 days (mean 9 (SD 1.7)) days	Cardiologist	Infusion into IRCA	BMMNC
Ruan 2005	Within 2 hours of successful PTCA	Cardiologist	Infusion into IRCA	BMMNC
San Roman 2015 (TECAM)	3-5 days post PCI	Cardiologist	Infusion into IRCA	BMMNC

	How are cells obtained? (*)	What were they re-suspended in?	Dose administered?	Comparator arm (placebo or control)
	BM aspiration (**)	Heparinised plasma	Median (interquartile range): 68 (54 to 130) million cells	No additional therapy (Control)
	Bone marrow aspirate. Isolated using Ficoll density gradient (t2cure method)	X-Vivo 10 medium	25-500 million cells	No additional therapy (control)
	BM aspiration (**)	n/r	LD: 10 million cells (range: 9 to 20 million) HD: 100 million cells (90 to 200 million cells)	No additional therapy (Control)
	BM aspiration	n/r	558 (IQR: 338-2554) million cells	No additional therapy (control)
	BM aspiration. BMMNC isolated by Ficoll density gradient. CD133+ isolated using CliniMACS.	2 ml normal saline supplemented with 2% autologous serum	BMMNC: 564.63 (69.35) million cells CD133+: 8.19 (4.26) million cells	Placebo (2 ml normal saline supplemented with 2% autologous serum)
	BM aspiration (**)	Saline solution and 5% human serum albumin	100 million cells	No additional therapy (Control)
	BM aspiration (**)	n/r	2640 million cells	No additional therapy (Control)
	BM aspiration (**)	Phosphate buffered saline - EDTA and 5% human serum albumin	249 million cells	No additional therapy (Control)
	BM aspiration (**)	Heparinised saline	144 (49) million cells	No additional therapy (Control)
	BM aspiration (**), immunomagnetic selection to isolate CD34-positive cells	Heparinised phosphate buffered saline, 40% autologous serum and 1% human serum albumin	LD: 4.8 (0.4) million cells MD: 9.9 (0.7) million cells HD: 14.3 (1.6) million cells	No additional therapy (Control)
	BM aspiration. CD34+ isolated using CliniMACS	10 mL phosphate-buffered saline supplemented with autologous serum and human serum albumin	14.9(8) million cells (range 8-40 million)	Placebo (10 mL phosphate-buffered saline supplemented with autologous serum and human serum albumin without cells)
	BM aspiration (**)	4% human serum albumin solution	98.3 (8.7) million cells	No additional therapy (Control)
	n/r	Diluted autologous serum	n/r	Placebo (diluted autologous serum)
	BM aspiration. BMMNC isolated by Ficoll technique	Heparinised saline	BMMNC: 83 (60–117) million cells BMMNC + G-CSF: 560 (351–915) million cells	No additional therapy (control)

Table 2. Continued

Study ID	Time of cell administration	Intervention given by:	Route of cell administration	Intervention cell type
Schachinger 2006 (REPAIR-AMI)	Within 5 days (mean 4.3 (1.3) days) of PCI	Cardiologist	Infusion into IRCA	BMMNC
Suarez de Lezo 2007	5 to 12 days (mean 7 (2) days) after AMI	Cardiologist	Infusion into IRCA	BMMNC
Sürder 2013 (SWISS-AMI)	5 to 7 days (E) or 3 to 4 weeks (L) after PCI	Cardiologist	Infusion into IRCA	BMMNC
Tendera 2009 (REGENT)	Median 7 (IQR 3 to 12) days after PCI	Cardiologist	Infusion into IRCA	Selected cells (S): CD34/ CXCR4- positive cells Unselected cells (U): BMMNC
Traverse 2010	3 to 10 days (median 4.5 (IQR 4 to 7) days) after PCI	Cardiologist	Infusion into IRCA	BMMNC
Traverse 2011 (LATE-TIME)	2 to 3 weeks (median 17.5 (IQR 15.5 to 20.0) days) after AMI	Cardiologist	Infusion into IRCA	BMMNC
Traverse 2018 (TIME)	3 days or 7 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Turan 2012	7 days after AMI	Cardiologist	Infusion into IRCA	BMMNC
Wang 2014	15 (1) days after PCI	Cardiologist	Infusion into IRCA	BM-MSC
Wohrle 2010 (SCAMI)	5 to 7 days (median 6.1 (IQR 5.5 to 7.3) days) after AMI	Cardiologist	Infusion into IRCA	BMMNC
Wollert 2004 (BOOST)	4.7 (1.3) days after PCI	Cardiologist	Infusion into IRCA	BMMNC
Wollert 2017 (BOOST-2)	8.1 ± 2.6 days post PCI	Cardiologist	Infusion into IRCA	BMMNC
Xiao 2012	Within 4 weeks of AMI	Cardiologist	Infusion into IRCA	BM-MSC
Yang 2020	28.3 ± 19.4 days post PCI	Cardiologist	Infusion into IRCA	BMMNC
Yao 2006	Within 7 days of AMI	Cardiologist	Infusion into IRCA	BMMNC
Yao 2009	SD: 3 to 7 days after PCI DD 3 to 7 days after PCI; second dose at 3 months	Cardiologist	Infusion into IRCA	BMMNC

	How are cells obtained? (*)	What were they re-suspended in?	Dose administered?	Comparator arm (placebo or control)
	BM aspiration (**)	X-VIVO medium and 20% autologous serum	236 (174) million cells	Placebo (X-VIVO medium and 20% autologous serum)
	BM aspiration (**)	Heparinised saline	900 (300) million	Placebo (heparinised saline)
	BM aspiration (**)	Serum-free medium and 20% of autologous serum	E: 159.7 (125.8) million cells L: 139.5 (120.5) million cells	No additional therapy (Control)
	BM aspiration (**). Selected cells: immunomagnetic selection to isolate CD34/ CXCR4-positive cells	Phosphate-buffered saline	S: 1.9 million cells U: 178 million cells	No additional therapy (Control)
	BM aspiration (**)	0.9% saline solution and 5% human serum albumin	100 million cells	Placebo (0.9% saline solution and 5% human serum albumin)
	BM aspiration (**)	0.9% saline solution and 5% human serum albumin	147 (17) million cells	Placebo (0.9% saline solution and 5% human serum albumin)
	BM aspiration (**)	0.9% saline solution and 5% human serum albumin	150 million cells	Placebo (0.9% saline solution and 5% human serum albumin)
	BM aspiration (**)	n/r	n/r	No additional therapy (control)
	BM aspiration (**) and culture of MSC	Heparinised saline	100 million cells	Placebo (heparinised saline)
	BM aspiration (**)	0.9% saline solution, 2% human serum albumin and 0.1% autologous erythrocytes	381 (130) million cells	Placebo (0.9% saline solution, 2% human serum albumin and 0.1% autologous erythrocytes)
	BM aspiration (**)	Heparinised saline	2460 (940) million cells	No additional therapy (Control)
	BM aspiration. Isolated by gelatine-polysuccinate density gradient sedimentation	Heparinised saline	loBMC: 700 ± 290 million cells hiBMC: 2060 ± 770 million cells loBMCi: 610 ± 260 million cells hiBMCi: 2080 ± 740 million cells	Placebo (pure red blood cell suspension prepared from a peripheral blood sample)
	BM aspiration (**) and culture of MSC	n/r	460 (160) million cells	Placebo (heparinised saline)
	BM aspiration (**)	Heparinised saline	n/r	Placebo (10ml heparinised saline)
	BM aspiration (**)	Lymphocyte isolation medium	210 (370) million cells	No additional therapy (control)
	BM aspiration (**)	Heparinised plasma	SiD: 410 million cells DD: 190 (SE 120) million cells	Placebo (heparinised plasma)

Table 2. Continued

Study ID	Time of cell administration	Intervention given by:	Route of cell administration	Intervention cell type
You 2008	At day 14	Cardiologist	Infusion into IRCA	BM-MSC
Zhang 2021	14.07 ± 9.53 days post PCI	Cardiologist	Infusion into IRCA	BM-MSC
Zhukova 2009	14 to 19 days after AMI	Cardiologist	Infusion into IRCA	BMMNC

AMI - acute myocardial infarction, PCI - percutaneous coronary intervention, BM - bone marrow, PTCA - percutaneous transluminal coronary angioplasty, IRCA - infarct-related coronary artery, IRCV - infarct-related coronary vein, BMMNC - bone marrow mononuclear cells, BM-MSC - mesenchymal stem cells; LD - low dose, MD - moderate dose, HD - high dose, AG - arterial group, VG - venous group, E - early cells, L - late cells, S - selected cells, U - unselected cells, SiD - single dose, DD - double dose
** BM aspiration- bone marrow aspiration and isolation of bone marrow mononuclear cells by gradient centrifugation

	How are cells obtained? (*)	What were they re-suspended in?	Dose administered?	Comparator arm (placebo or control)
	BM aspiration (**), second centrifugation and culture of MSC	n/r	75 million cells	No additional therapy (control)
	BM aspiration (**), cultured in medium and expanded for 72hr before trypsinisation	Saline	3.31 ± 1.70 million cells	No additional therapy (control)
	BM aspiration (**)	Autologous serum	50 million cells	No additional therapy (control)

Table 3. Summary of outcome reporting

	Primary Outcomes				Secondary Outcomes					
Study ID	**All-cause mortality**		**Cardiovascular mortality**		**Composite MACE [a]**		**Reinfarction**		**Hospital readmission for HF**	
	ST	**LT**	**ST**	**LT**	**ST**	**LT**	**ST**	**LT**	**ST**	**LT**
Angeli 2012	PR*	PR*	PR*	PR*	NR	NR	NR	NR	NR	NR
Cao 2009	PR*	FR	NR	NR	NR	NR	PR*	PR*	NR	NR
Chen 2004	PR*	NR	NR	NR	NR	NR	NR	NR	NR	NR
Choudry 2016	NR	FR	NR	NR	NR	NR	NR	FR	NR	PR*
Colombo 2011	PR*	PR*	NR	PR*	NR	NR	NR	NR	FR	PR
Gao 2013	FR	FR	FR	FR	NR	FR	FR	FR	NR	FR
Ge 2006	PR*	NR	NR	NR	NR	NR	NR	NR	NR	NR
Grajek 2010	NR	FR	NR	NR	NR	NR	FR	NR	NR	NR
Haddad 2020	NR	FR	NR	NR	NR	FR	NR	FR	NR	FR
Hirsch 2011	PR*	FR	NR	NR	FR	FR	FR	FR	FR	FR
Huang 2006	PR*	NR	NR	NR	NR	NR	PR*	NR	NR	NR
Huang 2007	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Huikuri 2008	FR	NR	FR	NR	NR	NR	FR	NR	FR	NR
Janssens 2006	FR	NR	PR*	NR	NR	NR	NR	NR	NR	NR
Jazi 2012	PR*	NR	PR*	NR	NR	NR	PR*	NR	NR	NR
Jin 2008	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Karpov 2005	PR*	FR	PR*	FR	NR	NR	FR	FR	NR	NR
Kim 2018	PR*	PR*	NR	NR	NR	NR	PR*	PR*	PR*	PR*
Kirgizova 2015	NR	FR	NR	FR	NR	PR	NR	FR	NR	NR
Lee 2014	PR*	NR	PR*	NR	NR	NR	FR	NR	NR	NR
Lunde 2006	NR	FR	NR	NR	NR	NR	FR	FR	FR	FR
Mathur 2020	NR	FR	NR	FR	NR	FR	NR	FR	NR	FR
Meluzin 2008	PR*	PR*	PR*	PR*	NR	NR	FR	FR	FR	FR
Nair 2015	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Naseri 2018	FR	FR	FR	NR	NR	NR	FR	NR	NR	NR
Nogueira 2009	FR	NR	PR*	NR	NR	NR	NR	NR	NR	NR
Penicka 2007	FR	FR	FR	FR	NR	FR	FR	FR	FR	FR
Piepoli 2010	FR	FR	FR	FR	NR	NR	NR	NR	NR	NR
Plewka 2009	FR	FR	FR	FR	NR	PR	FR	FR	NR	FR
Quyyumi 2011	FR	FR	FR	FR	NR	NR	NR	NR	NR	FR
Quyyumi 2017	FR	FR	FR	FR	NR	PR	NR	FR	NR	FR
Roncalli 2010	FR	PR	NR	NR	NR	NR	NR	NR	FR	NR
Ruan 2005	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
San Roman 2015	NR	FR	NR	NR	NR	NR	NR	FR	NR	NR
Schachinger 2006	FR	FR	NR	FR	FR	FR	FR	FR	FR	FR
Suarez de Lezo 2007	PR*	NR	PR*	NR	NR	NR	PR*	NR	NR	NR
Sürder 2013	FR	PR	NR	NR	PR	PR	FR	NR	FR	NR
Tendera 2009	FR	NR	NR	NR	NR	NR	FR	NR	NR	NR
Traverse 2010	PR*	NR	PR*	NR	NR	NR	NR	FR	NR	NR
Traverse 2011	FR	NR	NR	NR	NR	NR	FR	NR	FR	NR
Traverse 2018	FR	FR	NR	NR	PR	PR	FR	FR	FR	FR

Target vessel revascularisation		Arrhythmias		Restenosis		NYHA class		Quality of life (QoL)		Exercise tolerance		LVEF [b]	
ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR
PR*	FR	NR	NR	PR*	FR	NR	NR	NR	NR	NR	NR	FR	FR
NR	NR	PR*	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	FR	NR	NR	NR	NR	NR	PR	NR	FR	NR	NR	FR	FR
NR	NR	NR	FR	NR	NR	NR	NR	NR	NR	NR	PR	FR	FR
NR	NR	PR*	PR*	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR
NR	NR	PR*	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
FR	NR	NR	NR	FR	NR	NR	NR	NR	NR	FR	FR	FR	FR
NR	PR	NR	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
FR	FR	FR	FR	NR	NR	NR	FR	NR	NR	NR	NR	FR	FR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	PR*	NR	PR	NR	NR	NR	NR	NR	FR	NR	FR	NR
PR*	NR	FR	NR	FR	NR	NR	NR	NR	NR	NR	NR	FR	FR
NR	NR	PR*	NR	PR*	NR	FR	NR	NR	NR	NR	NR	FR	NR
NR	NR	NR	NR	NR	NR	FR	FR	FR	FR	NR	NR	FR	FR
NR	NR	NR	NR	NR	NR	NR	PR	FR	NR	FR	NR	FR	NR
PR*	PR*	FR	FR	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR
NR	PR	NR	FR	NR	NR	NR	NR	NR	NR	NR	FR	NR	FR
PR*	NR	PR*	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	FR	NR	FR	FR	NR	FR	NR	FR	NR	FR	NR	FR	FR
NR	PR	NR	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
NR	NR	PR*	NR	FR	PR	NR	NR	NR	NR	NR	NR	FR	FR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	NR	PR*	NR	NR	FR	FR	NR	NR	NR	NR	FR	FR
NR	NR	NR	NR	PR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	NR	PR*	NR	FR	NR	FR	NR	PR	NR	NR	FR	FR
NR	NR	PR	NR	NR	FR	NR	NR	NR	NR	FR	PR	FR	FR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR
NR	FR	NR	PR*	NR	FR	NR	NR	NR	NR	NR	NR	FR	NR
NR	PR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	FR	NR	FR	NR	NR	NR	PR	PR	NR	NR	FR	PR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	NR	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR
FR	FR	FR	FR	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR
PR*	NR	PR*	NR	PR*	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	NR	NR	NR	NR	FR	NR	NR	NR	NR	NR	FR	FR
FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	FR	NR	NR	NR	FR	NR	NR	NR	NR	NR	NR	FR	NR
FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
FR	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR

Table 3. Continued

Study ID	Primary Outcomes				Secondary Outcomes					
	All-cause mortality		Cardiovascular mortality		Composite MACE [a]		Reinfarction		Hospital readmission for HF	
	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT
Turan 2012	PR*	NR	PR*	NR	NR	NR	NR	NR	NR	NR
Wang 2014	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Wohrle 2010	FR	NR	NR	NR	FR	FR	PR*	NR	FR	NR
Wollert 2004	PR*	FR	NR	FR	NR	FR	FR	FR	FR	FR
Wollert 2017	FR	NR	NR	NR	NR	NR	PR*	NR	FR	NR
Xiao 2012	NR	NR	NR	NR	PR	NR	NR	NR	NR	NR
Yang 2020	NR	PR*	NR	NR	NR	NR	NR	PR*	NR	NR
Yao 2006	NR	PR*	NR	PR*	NR	NR	NR	FR	NR	NR
Yao 2009	PR*	PR*	PR*	PR*	NR	NR	FR	FR	NR	NR
You 2008	PR*	NR	PR*	NR	NR	NR	NR	NR	NR	NR
Zhang 2021	NR	FR	NR	NR	NR	NR	NR	PR*	NR	FR
Zhukova 2009	FR	FR	FR	FR	NR	NR	NR	FR	NR	NR
Total (%) analysed [c]	**1950 (46.9)**	**2012 (48.4)**	**677 (16.3)**	**1243 (29.9)**	**379 (9.1)**	**497 (11.9)**	**1965 (47.9)**	**1993 (47.9)**	**1291 (31.0)**	**1546 (37.2)**

ST - short-term follow-up (< 12 months)
LT - long-term follow-up (≥ 12 months)
FR - full reporting, outcome included in analysis
PR - partial reporting, insufficient information on outcome reported for inclusion in analysis
* no incidence of outcome observed
NR - outcome not reported
HF - heart failure; NYHA - New York Heart Association; LVEF - left ventricular ejection fraction
[a]Composite measure of mortality, reinfarction or rehospitalisation for heart failure.
[b]LVEF measured by any method.
[c]Total number of participants included in meta-analysis of outcome (% of total number of participants from all included studies). NB. trials with 0 events excluded from this number, see *.
[d]Total number analysed given for LVEF measured by magnetic resonance imaging.

Target vessel revascularisation		Arrhythmias		Restenosis		NYHA class		Quality of life (QoL)		Exercise tolerance		LVEF [b]	
ST	**LT**	**ST**	**LT**	**ST**	**LT**	**ST**	**LT**	**ST**	**LT**	**ST**	**LT**	**ST**	**LT**
NR	NR	NR	NR	NR	NR	FR	FR	NR	NR	NR	NR	FR	FR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
PR*	NR	NR	NR	FR	NR	NR	NR	NR	NR	NR	NR	FR	FR
PR*	FR	NR	NR	FR	NR	NR	NR	NR	NR	NR	NR	FR	FR
NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR	NR
NR	PR*	NR	PR*	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR
NR	NR	NR	NR	FR	FR	NR	NR	NR	NR	NR	NR	FR	NR
NR	NR	PR	PR	NR	NR	NR	NR	NR	NR	NR	NR	FR	FR
NR	NR	PR*	NR	NR	NR	PR	NR	PR	NR	NR	NR	FR	NR
NR	PR*	NR	PR*	NR	NR	NR	PR	NR	NR	NR	NR	FR	FR
NR	NR	NR	FR	NR	NR	NR	NR	NR	NR	NR	NR	NR	FR
843 (20.3)	**982 (23.6)**	**551 (13.2)**	**1040 (25.1)**	**641 (15.4)**	**395 (9.5)**	**398 (9.6)**	**342 (8.2)**	**209 (5.0)**	**81 (1.9)**	**267 (6.4)**	**71 (1.7)**	**1476 (35.4)[d]**	**968 (23.3)[d]**

Table 4. Periprocedural adverse events

Study ID	Periprocedural adverse events
Angeli 2012	Not reported
Cao 2009	1 x transient acute heart failure 7 days after cell transplantation
Chen 2004	Not reported
Choudry 2016	2x VF arrest post BM aspiration. 3x post-procedural minor bleeding. 1x post-procedural renal impairment.
Colombo 2011	No adverse events were reported until the end of hospitalisation
Gao 2013	1 x death 3 days after cell transplantation due to suspected acute in-stent thrombosis; 1 x serious complication of acute coronary occlusion during cell injection with subsequent recurrent MI
Ge 2006	No bleeding complications at BM puncture site and no angina aggravation, malignant diseases or substantial arrhythmias after PCI and BM transfer during hospitalisation in either treatment group
Grajek 2010	Not reported
Haddad 2020	Not reported
Hirsch 2011	No complications of cell harvesting. A CK or CK-MB elevation between 1 and 2 times the ULN was detected in 4 patients and between 2 and 3 times the ULN in one patient. 1 x occluded infarct-related artery (patient did not receive cell therapy as randomised). During cell catheterisation: 1 x coronary spasm, 1 x transient brachycardia and 1 x thrombus in the infarct related artery
Huang 2006	Not reported
Huang 2007	Not reported
Huikuri 2008	3 x mild self terminating vasovagal reactions during BM aspiration; no other procedural complications relating to aspiration. Subacute stent thrombosis occurred in 4 patients (1 x cell therapy and 3 x placebo); 1 x cell therapy patient had 'no reflow' phenomenon after stenting of the infarcted artery
Janssens 2006	11 x treatment-related tachycardia (supraventricular arrhythmia: 5 in the cell therapy group and 6 in the control group); 3 patients in the control group experienced non-sustained ventricular tachycardia
Jazi 2012	Not reported
Jin 2008	Not reported
Karpov 2005	No complications of BM aspiration or cell infusion
Kim 2018	There were no serious inflammatory reactions or bleeding complications at the iliac puncture site after BM aspiration. Patients had no or mild angina during balloon inflation for infusion of BM-MSC. There were no serious procedural complications related to intracoronary administration of the BM-MSC, such as ventricular arrhythmias, thrombus formation, or dissection. Periprocedural MI did not occur in all patients.
Kirgizova 2015	Not reported
Lee 2014	No serious inflammatory reactions or bleeding complications from BM aspiration. No (or mild) angina during balloon inflation. No serious procedural complications related to intracoronary administration of MSCs including ventricular arrhythmia, thrombus formation or dissection. Periprocedural MI occurred in 2 patients
Lunde 2006	2 x stent thrombosis in the acute phase in the cell therapy group (no cells administered as randomised); 1 x sustained ventricular tachycardia before cell administration; 1 x ventricular fibrillation at day 6, 24 hours after injection.1 x pulseless ventricular tachycardia in control patient - converted to sinus rhythm by means of a precordial thump on day 2
Mathur 2020	Not reported

Table 4. Continued

Study ID	Periprocedural adverse events
Meluzin 2008	2 patients had fever and 1 patient had brachycardia, all within 20 hours prior to cells (these patients did not receive cell therapy as randomised). 3 x cell therapy-related complications: 1 x intimal dissection during repeat balloon inflations at time of cell implantation, 1 x short-lasting fever on day of scheduled transplantation, 1 x small thrombus in infarct-related artery diagnosed immediately after cell transplantation. 2 x control patients had repeat MI 2 days after the hospital discharge due to in-stent thrombosis
Nogueira 2009	Ck-MB elevation (3 x normal value) in 3 patients in the arterial group and 1 patient in venous group. 1 x tortuous anterior interventricular vein (patient did not receive cell therapy as randomised). No new pericardial effusions
Nair 2015	6x acute or subacute stent trombosis, including one death (vs 4x in the control group) and 1x transient ischaemic attack in the control group
Naseri 2018	There were no reported study related serious adverse events during the initial hospitalization.
Penicka 2007	2 x serious complications (1 x stent thrombosis with reinfarction immediately after BM harvest, patient died 2 weeks later due to sepsis and acute respiratory distress syndrome; 1 x ventricular septal rupture before cell injection, patient died 3 months later from severe heart failure).
Piepoli 2010	All procedures well tolerated. No inflammatory reaction or abscess detected at the site of puncture after BM harvest. The invasive coronary catheterisation was associated with some mild angina during balloon inflations for cell infusions. No procedural complications during cardiac catheterisation related to cell injections (no ventricular arrhythmia, new thrombus formation or embolism after cell infusion or dissections due to balloon inflations)
Plewka 2009	Not reported
Quyyumi 2011	1 high-dose treatment group patient died soon after cell infusion from ventricular fibrillation attributed to recurrent MI from stent thrombosis preceding cell infusion. 1 x high-dose treatment group patient with acute stent thrombosis before cell infusion (patient withdrawn from study). Cell therapy group: 1 x arrhythmia, 1 x chest pain, 3 x musculoskeletal pain, 2 x upper respiratory tract infection, 2 x rash, 3 x dyspnoea, 1 x fever. Control group: 1 x arrhythmia, 3 x musculoskeletal pain, 1 x upper respiratory tract infection, 1 x dyspnoea
Quyyumi 2017	8x serious adverse events from time of BM harvest up to start of cell infusion, although these serious adverse events were not otherwise specified
Roncalli 2010	Cell therapy group: 1 x transient ischaemic attack and 1 x thrombopenia induced by GP2b3a inhibitor (both excluded before BM aspiration). Control group: 1 x steroids given for angioneurotic oedema; 1 x post-MI ventricular septal defect (both withdrawn before day 7)
Ruan 2005	Not reported
San Roman 2015	2x periprocedural MI during BMMNC infusion.
Schachinger 2006	No bleeding complications or haematoma formation at puncture site of BM aspiration. 1 x patient was excluded owing to fever and an increase in the level of C-reactive protein. 1 x patient in placebo group had angiographic evidence of a thrombus in a non-infarct-related artery (placebo medium not infused). 2 x deaths, cause not reported (1 x cell therapy group and 1 x placebo) and 2 x reinfarction (cell therapy group) prior to discharge
Suarez de Lezo 2007	Not reported
Sürder 2013	1 death in cell therapy group prior to transplantation, cause of death not reported
Tendera 2009	1 patient developed arteriovenous fistula of the femoral artery after the procedure and required surgical treatment. No complications arising from BM cell transfer

Table 4. Continued

Study ID	Periprocedural adverse events
Traverse 2010	BM aspiration carried out without complications. No patient experienced a rise in troponin or procedure-related complication following infusion
Traverse 2011	No complications associated with BM aspiration. 2 x patients underwent additional stenting at time of cell infusion (1 x distal stent edge dissection related to primary PCI procedure; 1 x possible dissection related to stop-flow procedure). 1 x postpartum spontaneous coronary dissection with diffuse thrombus throughout stented region of left anterior descending artery; 1 x presence of severe left main coronary stenosis identified before transfusion (this patient did not receive cell therapy as randomised). No patients experienced postprocedural increase in cardiac enzymes
Traverse 2018	No complications associated with BM harvesting or intracoronary infusion. 1 x death in the BM cell therapy group due to subarachnoid haemorrhage prior to cell delivery
Turan 2012	No procedural or cell-induced complications and no side effects in any patient
Wang 2014	Not reported
Wohrle 2010	Not reported
Wollert 2004	No bleeding complications at BM harvest site. No increases in troponin T serum levels in any patients 24 hours after BM transfer
Wollert 2017	5x adverse event during cell infusion in another trial, (one dissection, one stent trombosis, two flow reductions after cell infusion and one major groin hematoma for which transfusion was necessary)
Xiao 2012	Not reported
Yang 2020	Not reported
Yao 2006	1 x temporary hypotension, 2 x brachycardia, 7 x new hyperuricaemia
Yao 2009	1 x brachycardia with subsequent pacemaker implantation, 1 x fever (these patients did not receive cells as randomised)
You 2008	Not reported
Zhang 2021	Not reported
Zhukova 2009	Not reported

MI, acute myocardial infarction; PCI, percutaneous coronary intervention; BM, bone marrow; MSC, mesenchymal stem cells; ULN, upper limit of normal

Table 5. Quality of life and performance measures

Study ID	No. analysed participants		Quality of life (QoL) assessment	Reported data (EP/MC/SR)	Performance assessment	Summary measures of performance	Reported data (EP/MC/SR)	Mean follow-up
	Cells	No cells						
Choudry 2016	25	30	EQ5D	EP, MC	NYHA class		%	12 months
Colombo 2011	5	4	n/r	n/r	Exercise stress test	Peak HR, peak MET, peak double product (SBPxHR), peak predicted HR	EP (median)	12 months
Grajek 2010	31	14	n/r	n/r	Cardiopulmonary exercise treadmill test (modified Bruce protocol)	METs, maximum VO^2, VE/VCO^2 slope, RER, peak SBP, peak HR, VO^2 anaerobic threshold, HR recovery	EP	12 months
Hirsch 2011	65	60	n/r	n/r	NYHA class		EP	60 months
Huikuri 2008	27	27	n/r	n/r	Symptom-limited maximal exercise test	METs, peak HR, T-wave alternans	EP, MC	6 months
Jazi 2012	16	16	n/r	n/r	NYHA class		EP	6 months
Jin 2008	14	12	MLHFQ	EP	NYHA class		EP	12 months
Karpov 2005	16 [a]	28 [a]	MLHFQ	EP	Six minute walk test; functional class (undefined)	Distance (metres)	EP	6 months
Kirgizova 2015	10	16	n/r	n/r	NYHA class	6 minute walking test (metres)	EP	7 years
Lunde 2006	50 [b]	50 [b]	SF-36	EP, MC	Electrically braked bicycle ergometer; NYHA class	Time (min), maximum VO^2, VE/VCO^2 slope etc., peak HR	EP, MC	6 months
Naseri 2018	51	26	n/r	n/r	NYHA class		MC	18 months
Penicka 2007	14	10	SF-36	SR	NYHA class		EP	24 months
Piepoli 2010	17	15	n/r	n/r	Cardiopulmonary exercise treadmill test (modified Bruce protocol)	Exercise duration (min), maximum VO^2, VE/VCO^2 slope	MC	12 months
Roncalli 2010	52	49	MLHFQ	SR	n/r			12 months
Sürder 2013	117	61	n/r	n/r	NYHA class		EP	4 months
Turan 2012	42	20	n/r	n/r	NYHA class		EP	12 months
You 2008	7	16	QoL (no details)		NYHA class		SR	8 weeks
Zhang 2021	18	19	n/r	n/r	NYHA class		EP	12 months

MLHFQ, Minnesota Living with Heart Failure Questionnaire; NYHA, New York Heart Association; SF-36, Short-Form 36 Quality of Life; MET, metabolic equivalent test (mL/kg/min); HR, heart rate (bpm); SBP, systolic blood pressure (mmHg); RER, respiratory exchange ratio; VE, minute ventilation; VO_2, oxygen volume; VCO_2, carbon dioxide volume; EP, endpoint; MC, mean change from baseline; SR, summary results; n/r, not reported.

[a] Karpov 2005: QoL was measured in 37 participants (cells: 18 cells, no cells: 19)

[b] Lunde 2006: QoL was measured in 46 BMMNC and 45 controls; exercise tolerance was measured in 49 BMMNC and 50 controls

Table 6. Surrogate (continuous) outcome: LVEF

Study ID	No. randomised participants		No. analysed participants		Baseline LVEF		Mean follow-up of LVEF
	Cells	No cells	Cells	No cells	Cells	No cells	
Measured by MRI							
Choudry 2016 (REGENERATE-AMI)	55	45	51	41	48.9	47.8	12 months
Hirsch 2011 (HEBE)	69	65	59	52	43.7 (9.0)%	42.4 (8.3)%	24 months
Huang 2006	20	20	20	20	44.5 (7.1)%	43.4 (6.7)%	6 months
Janssens 2006	33	34	30	30	48.5 (7.2)%	46.9 (8.2)%	12 months
Lunde 2006 (ASTAMI)	50	51	44	44	54.8 (13.6)%	53.6 (11.6)%	36 months
Quyyumi 2011 (AMR-1)	16	15	11	10	LD: 47.0 (13)% MD: 47.3 (11)% HD: 49.9 (7)%	53.2(10)%	6 months
Quyyumi 2017 (PreServe-AMI)	100	95	83	78	34.3(7.3)%	34.1(8.4)%	6 months
Roncalli 2010 (BONAMI)	52	49	47	43	37.0 (9.8)%	38.7 (9.2)%	3 months
San Roman 2015 (TECAM)	59	61	48	44	BMMNC: 49(8)% BMMNC+G-CSF: 45(9)%	Regular: 47(8)% G-CSF: 54(9)%	12 months
Schachinger 2006 (REPAIR-AMI)	101	103	26	33	47.8 (6.2)%	47.7 (6.2)%	60 months [(a)]
Sürder 2013 (SWISS-AMI)	133	67	107	60	E: 36.5 (9.9)% L: 36.3 (8.2)%	40.0 (9.9)%	4 months
Tendera 2009(REGENT)	160	40	97	20	S: 33.9 (8.6)% U: 35.6 (6.5)%	38.9 (5.2)%	6 months
Traverse 2010	30	10	30	10	49 (9.5)%	48.6 (8.5)%	6 months
Traverse 2011 (LATE-TIME)	59	29	55	26	48.7 (12)%	45.3 (9.9)%	6 months
Traverse 2018 (TIME)	80	40	65	30	46.2 (9.6)%	46.3 (8.5)%	12 months
Wohrle 2010 (SCAMI)	29	13	28	12	53.5 (9.3)%	55.7 (9.4)%	36 months
Wollert 2004 (BOOST)	33	32	30	30	50 (10)%	51.3 (9.3)%	60 months
Wollert 2017 (BOOST-2)	77	37	71	26	loBMMNC: 44.2(7.8)% hiBMMNC: 44.8(9.1)%	47.8(6.7)%	6 months
Yang 2020	50	50	40	36	34.2(32.7-36.5)%	33.5(31.5-35.0)%	12months
Yao 2009	30	15	27	11	SD: 32.5 (3.6)% DD: 33.7 (4.7)%	32.3 (2.0)%	12 months

Table 6. Continued

Study ID	No. randomised participants		No. analysed participants		Baseline LVEF		Mean follow-up of LVEF
	Cells	No cells	Cells	No cells	Cells	No cells	
Zhukova 2009	8	3	6 [b]	1 [b]	33.4 (3)%	28 (4)%	36 months [b]
Measured by echocardiography							
Angeli 2012	11	11	11	11	n/r	n/r	12 months
Cao 2009	41	45	41	45	41.3 (2.8)%	40.7 (3.1)%	48 months
Colombo 2011	5	5	5	4	44.6 (8.8)%	43.2 (9.1)%	12 months
Gao 2013	21	22	19	20	50.8 (6.5)%	51.4 (7.2)%	24 months
Ge 2006	10	10	10	10	53.8 (9.2)%	58.2 (7.5)%	6 months
Grajek 2010	31	14	27	12	50.3 (9.8)%	50.8 (12)%	12 months
Huang 2007	20	20	20	20	48.5 (5.5)%	48.2 (6.30%	6 months
Huikuri 2008 (FINCELL)	40	40	39	38	56 (10)%	57 (10)%	6 months
Jin 2008	14	12	14	12	54.3 (5.5)%	55.8 (5.9)%	12 months
Karpov 2005	22	22	16	10	49.3 (11.1)%	47.0 (7.5)%	6 months
Kim 2018	14	12	14	12	35.1 (4.5)%	37.4 (1.7)%	12 months
Kirgizova 2015	10	16	10	16	52.5 (13.3)%	47.6 (9.4)%	7 years
Lee 2014 (SEED-MSC)	40	40	30	28	48.1 (8.0)%	51.0 (9.2)%	6 months
Lunde 2006 (ASTAMI)	50	51	50	50	45.7 (9.4)%	46.9 (8.6)%	36 months
Mathur 2020 (BAMI)	185	190	n/r	n/r	39 (5)%	39 (5)%	no follow-up
Nair 2015	125	125	71	117	n/r	n/r	6 months
Naseri 2018	51	26	51	26	CD133: 31.1(5.4) MNC: 32.3(5.9)	32.9(8.4)	n/r
Nogueira 2009 (EMRTCC)	24	6	22	6	AG: 48.3 (10.4)% VG: 48.6 (7.1)%	47.6 (14.3)%	6 months
Penicka 2007	17	10	14	10	39.2 (9.2)%	39.4 (5.6)%	24 months
Piepoli 2010 (CARDIAC)	19	19	17	15	38.4 (6.4)%	38.9 (5.6)%	24 months
Plewka 2009	40	20	38	18	35 (6)%	33 (7)%	24 months
Roncalli 2010 (BONAMI)	52	49	47	43	38.1 (7.9)%	39.8 (7.0)%	12 months [c]
Ruan 2005	9	11	9	11	53.4 (8.9)%	53.5 (5.8)%	6 months

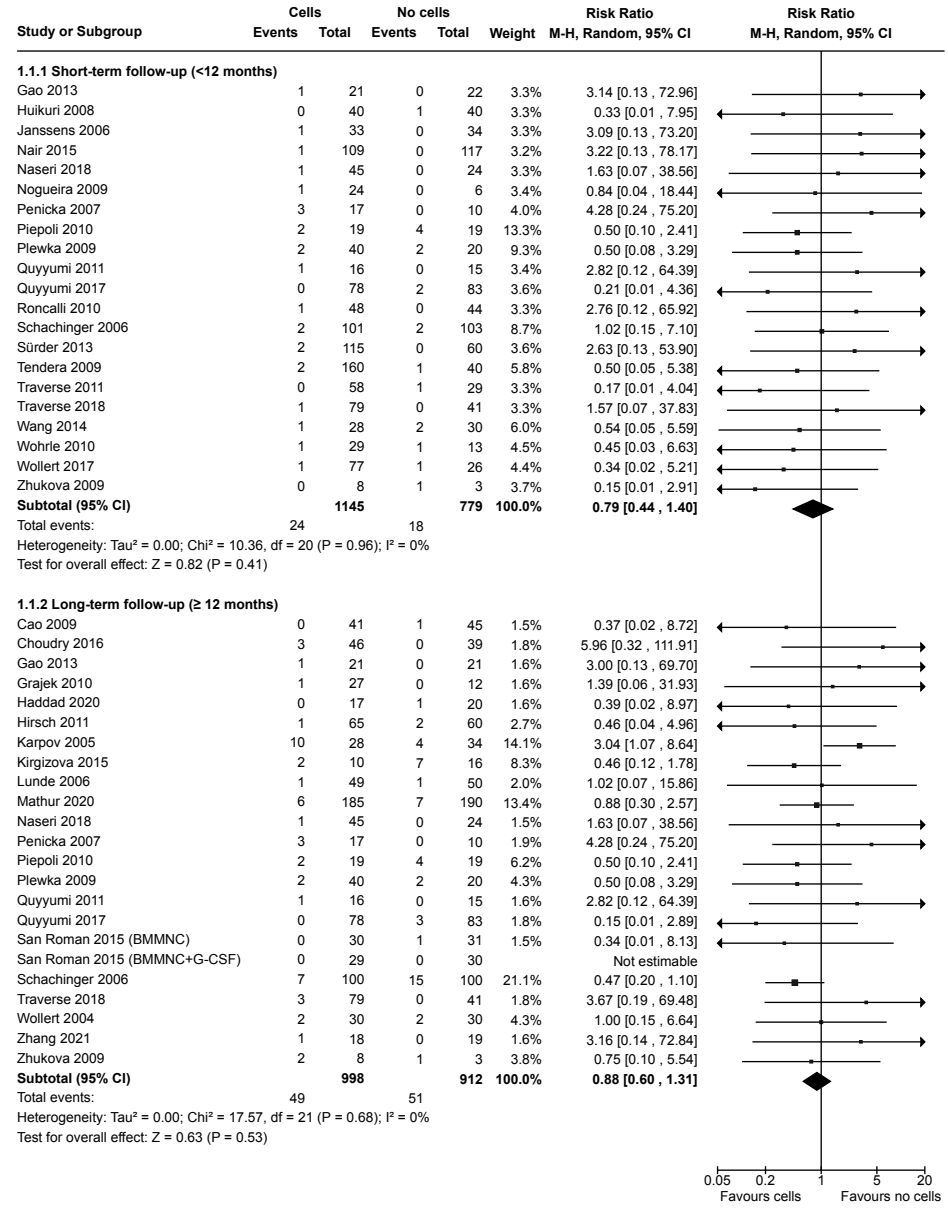
Table 6. Continued

Study ID	No. randomised participants		No. analysed participants		Baseline LVEF		Mean follow-up of LVEF
	Cells	No cells	Cells	No cells	Cells	No cells	
Xiao 2012	17	21	17	21	35.6 (3.1)%	35.7 (3.1)%	3 months
Yang 2020	50	50	40	36	40(40-45)%	41(40-45)%	12 months
You 2008	7	16	7	16	37 (4.6)%	38.6 (5.4)%	8 weeks
Zhang 2021	21	22	21	22	53.7(6.4)%	57.2(10.2)%	12 months
Roncalli 2010 (BONAMI)	52	49	47	43	38.1 (7.9)%	39.8 (7.0)%	12 months [c]
Ruan 2005	9	11	9	11	53.4 (8.9)%	53.5 (5.8)%	6 months
Xiao 2012	17	21	17	21	35.6 (3.1)%	35.7 (3.1)%	3 months
Yang 2020	50	50	40	36	40(40-45)%	41(40-45)%	12 months
You 2008	7	16	7	16	37 (4.6)%	38.6 (5.4)%	8 weeks
Zhang 2021	21	22	21	22	53.7(6.4)%	57.2(10.2)%	12 months
Roncalli 2010 (BONAMI)	52	49	47	43	38.1 (7.9)%	39.8 (7.0)%	12 months [c]
Ruan 2005	9	11	9	11	53.4 (8.9)%	53.5 (5.8)%	6 months
Xiao 2012	17	21	17	21	35.6 (3.1)%	35.7 (3.1)%	3 months
Yang 2020	50	50	40	36	40(40-45)%	41(40-45)%	12 months
You 2008	7	16	7	16	37 (4.6)%	38.6 (5.4)%	8 weeks
Zhang 2021	21	22	21	22	53.7(6.4)%	57.2(10.2)%	12 months
Measured by SPECT							
Angeli 2012	11	11	11	11	n/r	n/r	12 months
Cao 2009	41	45	41	45	41.2 (3.1)%	40.8 (3.3)%	48 months
Kim 2018	14	12	14	12	34.2 (4.7)%	35.4 (3.0)%	12 months
Lee 2014 (SEED-MSC)	40	40	30	28	49.0 (11.7)%	52.3 (9.3)%	6 months
Lunde 2006 (ASTAMI)	50	51	50	50	41.3 (10.4)%	42.6 (11.7)%	6 months
Meluzin 2008	44	22	40	20	LD: 41 (2)% HD: 30 (2)%	40 (2)%	12 months
Naseri 2018	51	26	51	26	CD133: 39.8(10.6) MNC: 37.06(9.0)	40.5(15.1)	18 months
Piepoli 2010 (CARDIAC)	19	19	17	15	36.6 (8.2)%	37.5 (8.9)%	24 months
Plewka 2009	40	20	26	10	41.2 (10.1)%	40.0 (14.2)%	6 months

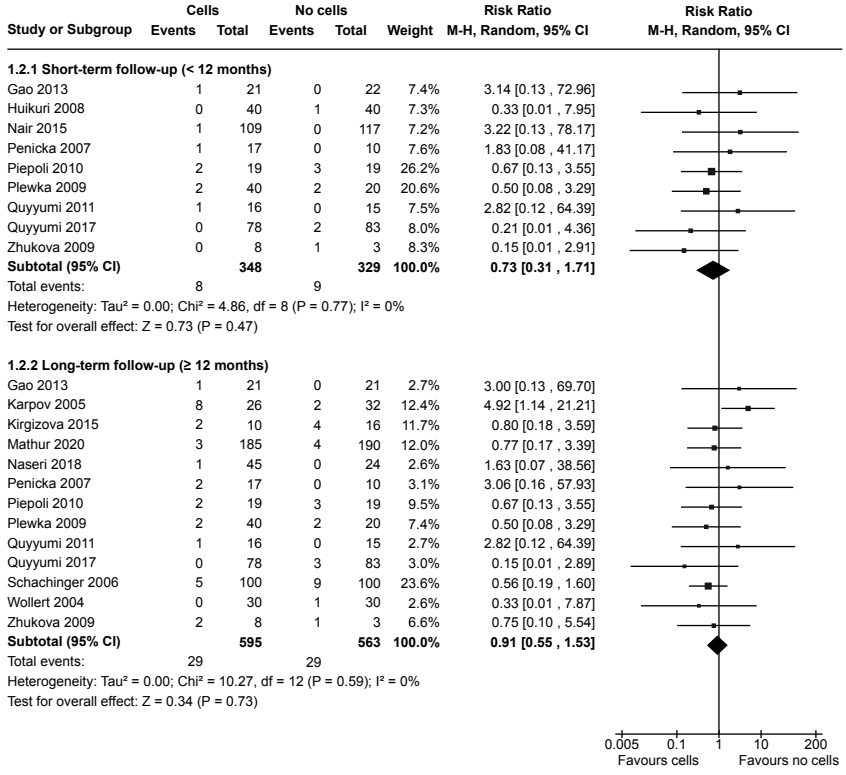
Table 6. Continued

Study ID	No. randomised participants		No. analysed participants		Baseline LVEF		Mean follow-up of LVEF
	Cells	No cells	Cells	No cells	Cells	No cells	
Measured by LV angiography							
Chen 2004	34	35	34	35	49 (9)%	48 (10)%	6 months
Choudry 2016	54	44	49	37	49.2(11.1)%	52.4(10.3)%	6 months
Huang 2006	20	20	20	20	56.7 (9.7)%	57.3 (8.2)%	6 months
Huikuri 2008 (FINCELL)	40	40	36	36	59 (11)%	62 (12)%	6 months
Jazi 2012	n/r	n/r	16	16	33.37 (11.2)%	29.0 (7.5)%	6 months
San Roman 2015	59	61	54	44	BMMNC: 50(12)% BMMNC+G-CSF: 47(13)%	Regular: 49(12)% G-CSF: 54(14%)	12 months
Schachinger 2006 (REPAIR-AMI)	101	103	95	92	48.3 (9.2)%	46.9 (10.4)%	4 months
Suarez de Lezo 2007	10	10	10	10	37 (5)%	39 (6)%	3 months
Turan 2012	42	20	42	20	43 (10)%	45 (10)%	12 months
Wang 2014	30	30	27	28	37.8 (6.3)%	20.2 (2.5)% [(d)]	6 months
Yao 2006	92	92	90	84	n/r	n/r	6 months
Measured by RNV							
Grajek 2010	31	14	27	12	45.4 (10.2)%	42.7 (7.4)%	12 months
Nair 2015	125	125	71	117	n/r	n/r	6 months
Nogueira 2009 (EMRTCC)	24	6	22	6	AG: 41.0 (10.3)% VG: 39.9 (7.4)%	40.1 (12.4)%	6 months
Roncalli 2010 (BONAMI)	52	49	47	43	35.6 (7.0)%	37.0 (6.7)%	3 months
Measured by gated PET							
Colombo 2011	5	5	5	4	36.6 (5.4)%	37.6 (7.0)%	12 months

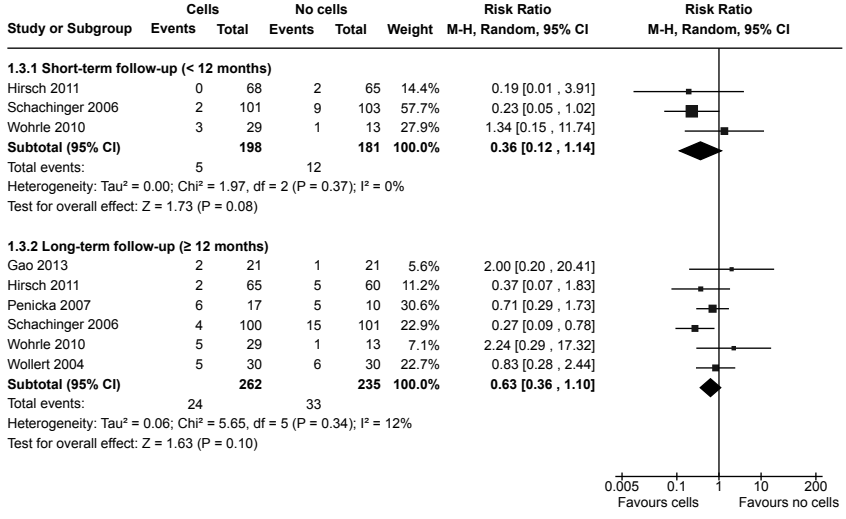
n/r - not reported
LD - low dose, MD - moderate dose, HD - high dose, AG - arterial group, VG - venous group, E - early cells, L - late cells, S - selected cells, U - unselected cells, SD - single dose, DD - double dose [(a)]Schachinger 2006: MRI was performed at five-year follow-up but summary results only were reported; 24-month data are used in meta-analysis.
(b)Zhukova 2009: 24-month data were used in the analysis as only one control was available at 36 months.
[(c)]Roncalli 2010: echocardiography was performed at 12-month follow-up but summary results only were reported; three-month data are used in meta-analysis.
[(d)]Wang 2014: the reported baseline LVEF value in the control group is assumed to be an error since the difference between values at baseline and endpoint (49.1%) is not significant. We have been unable to clarify the correct value with the study authors.
[(e)] median (with 95%CI) reported instead of mean±SD



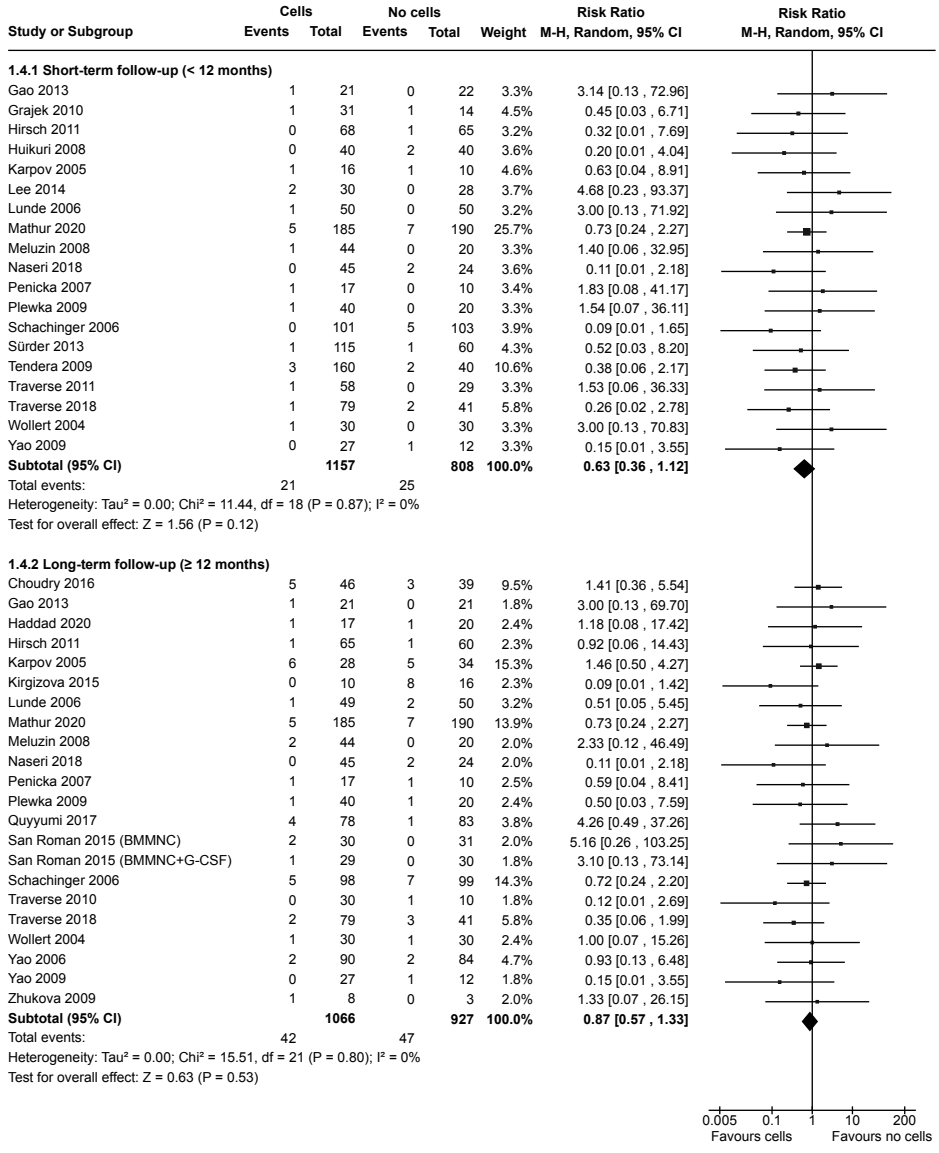
Analysis 1.1 Comparison 1: Cells compared to no cells, Outcome 1: All-cause mortality



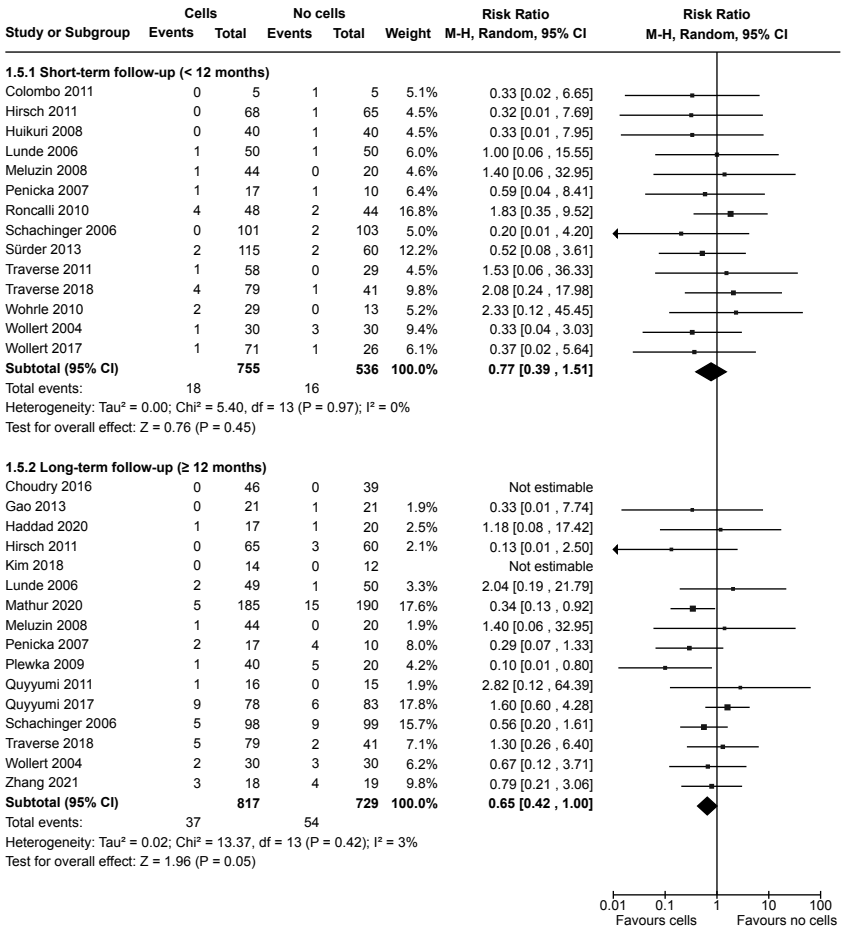
Analysis 1.2 Comparison 1: Cells compared to no cells, Outcome 2: Cardiovascular mortality



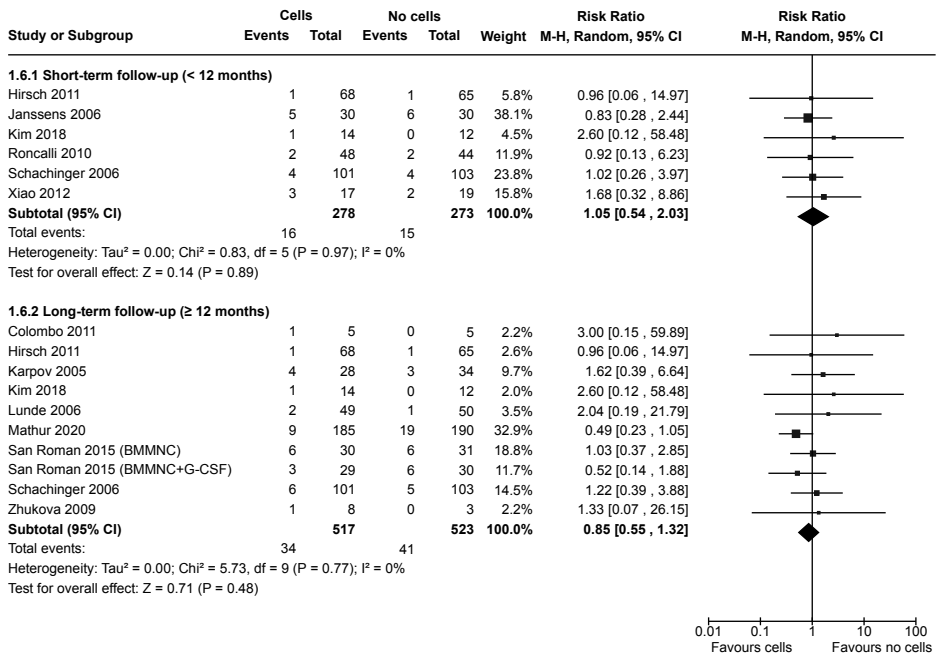
Analysis 1.3 Comparison 1: Cells compared to no cells, Outcome 3: Composite measure of death, reinfarction, re-hospitalisation for heart failure



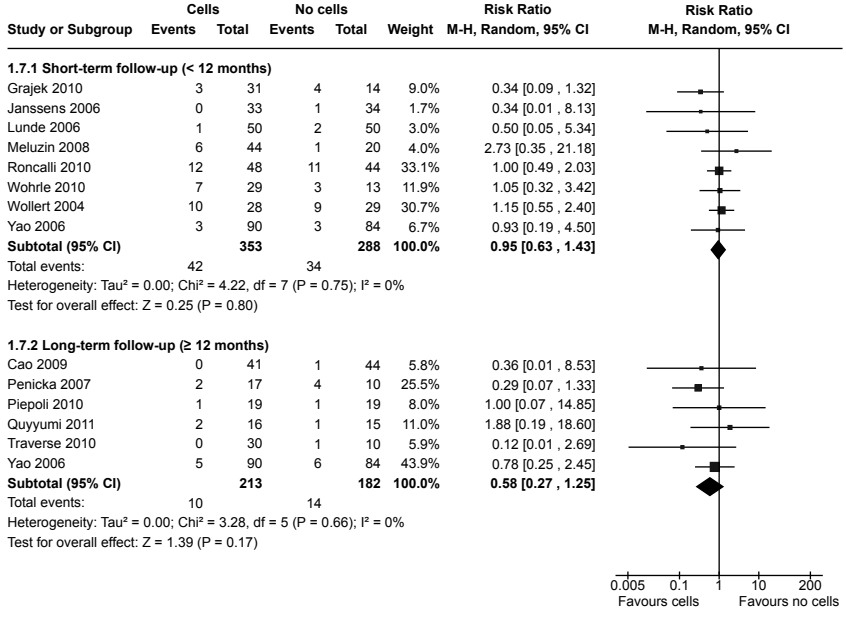
Analysis 1.4 Comparison 1: Cells compared to no cells, Outcome 4: Incidence of reinfarction



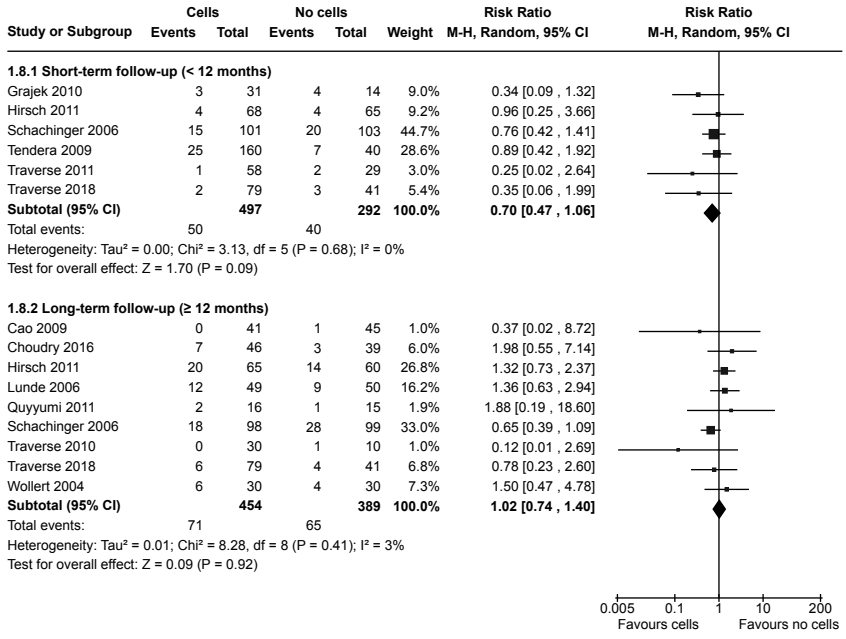
Analysis 1.5 Comparison 1: Cells compared to no cells, Outcome 5: Incidence of re-hospitalisation for heart failure



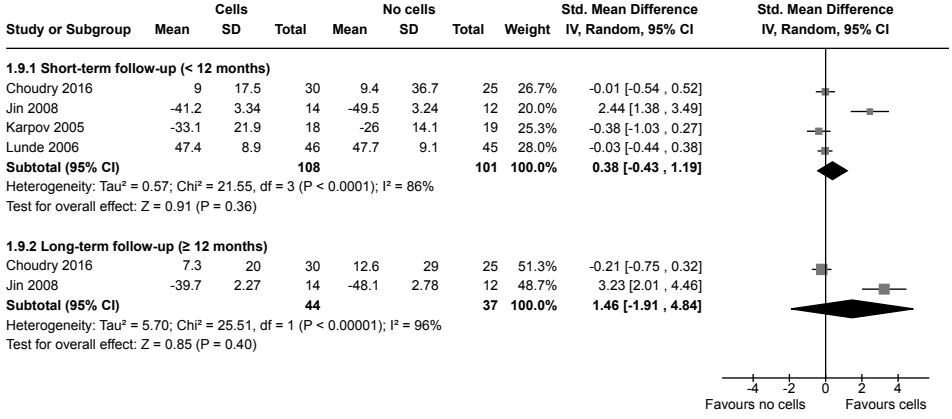
Analysis 1.6 Comparison 1: Cells compared to no cells, Outcome 6: Incidence of arrhythmias



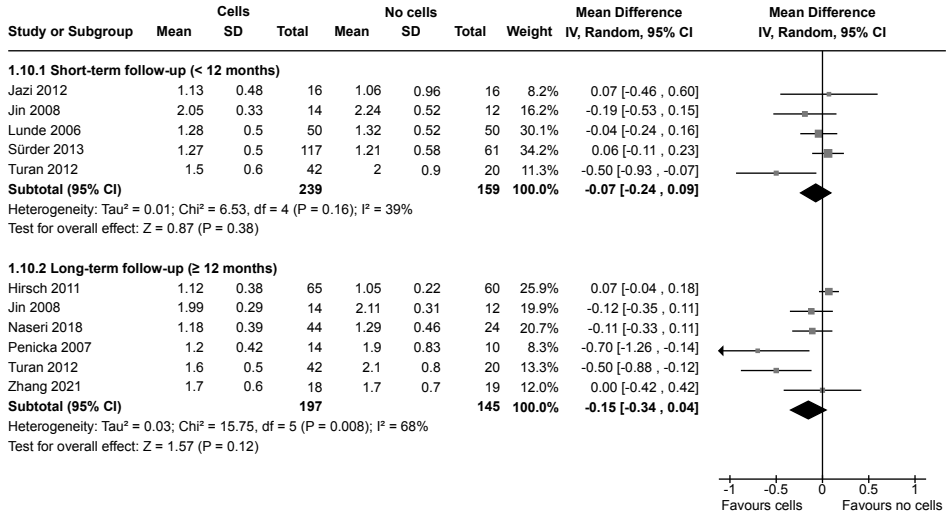
Analysis 1.7 Comparison 1: Cells compared to no cells, Outcome 7: Incidence of restenosis



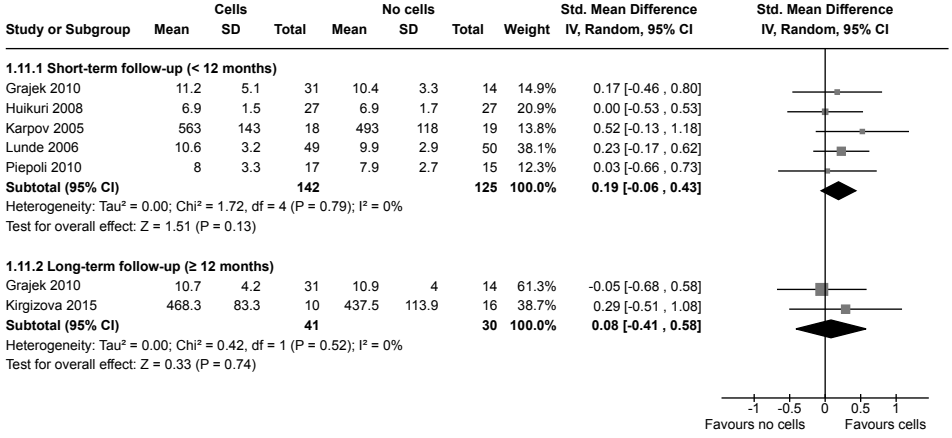
Analysis 1.8 Comparison 1: Cells compared to no cells, Outcome 8: Incidence of target vessel revascularisation



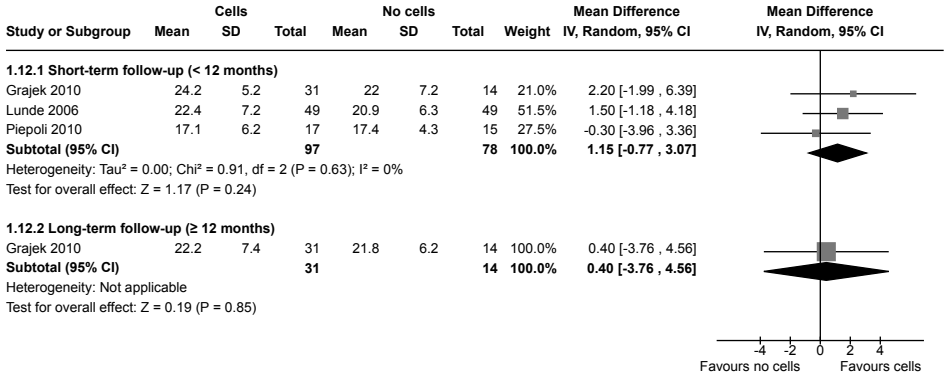
Analysis 1.9 Comparison 1: Cells compared to no cells, Outcome 9: Quality of life measures



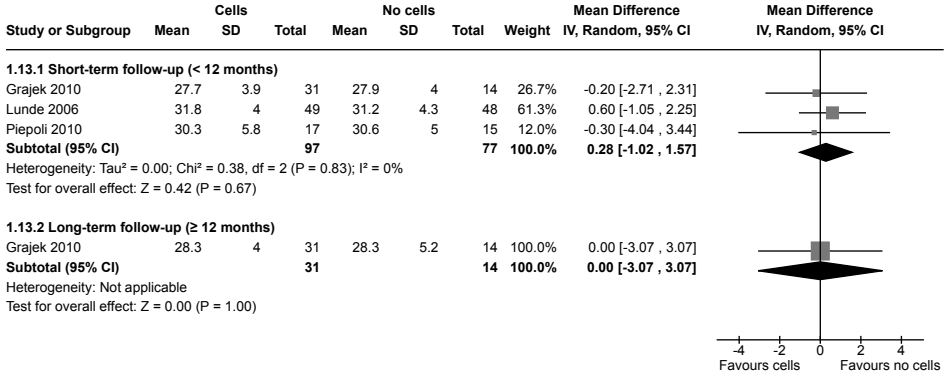
Analysis 1.10 Comparison 1: Cells compared to no cells, Outcome 10: NYHA classification



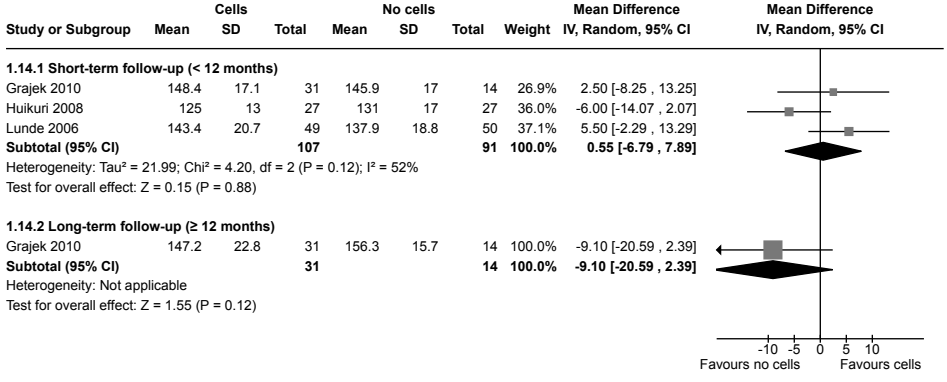
Analysis 1.11 Comparison 1: Cells compared to no cells, Outcome 11: Exercise tolerance



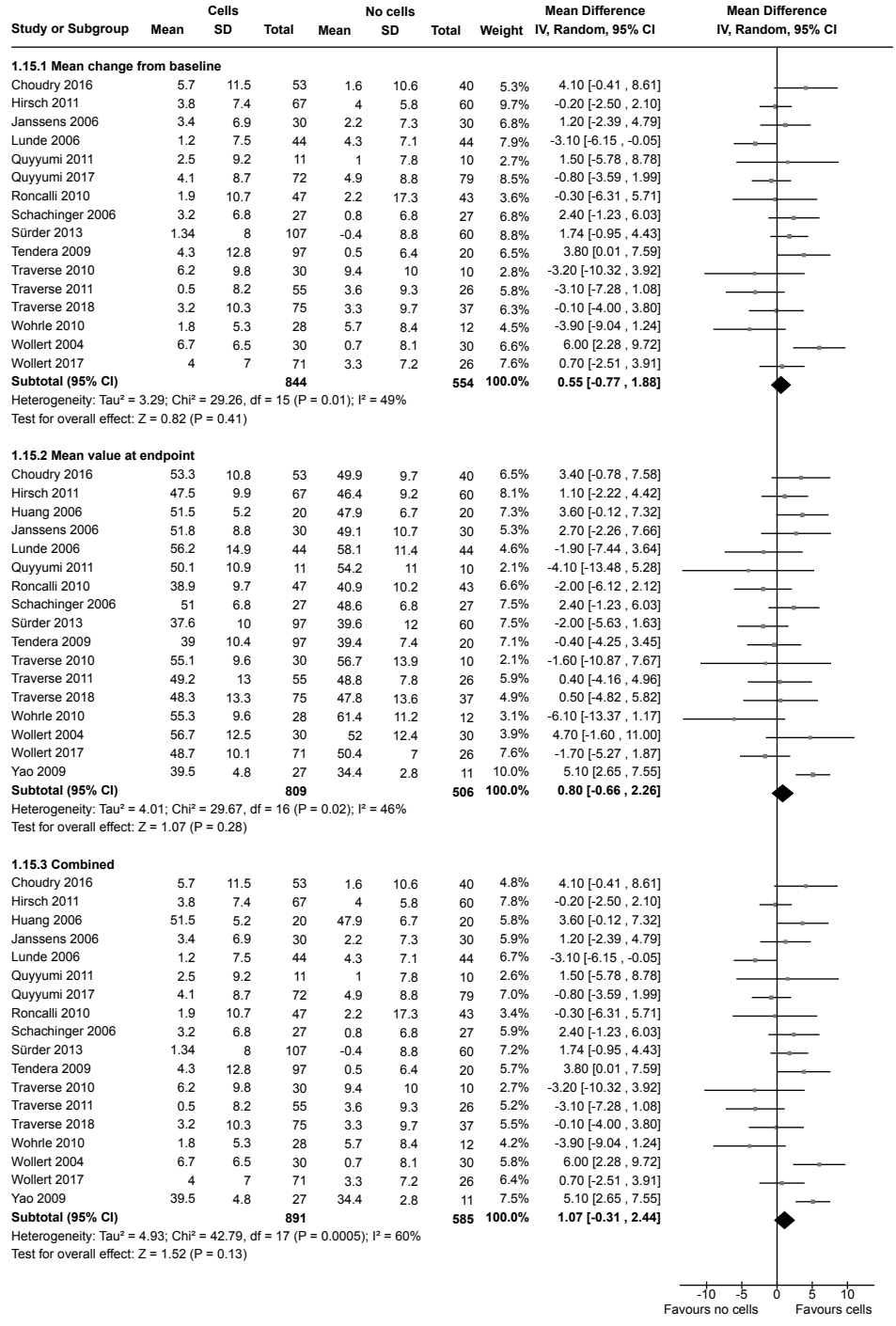
Analysis 1.12 Comparison 1: Cells compared to no cells, Outcome 12: Maximum VO_2 (mL/kg/min)



Analysis 1.13 Comparison 1: Cells compared to no cells, Outcome 13: VE/VCO_2 slope

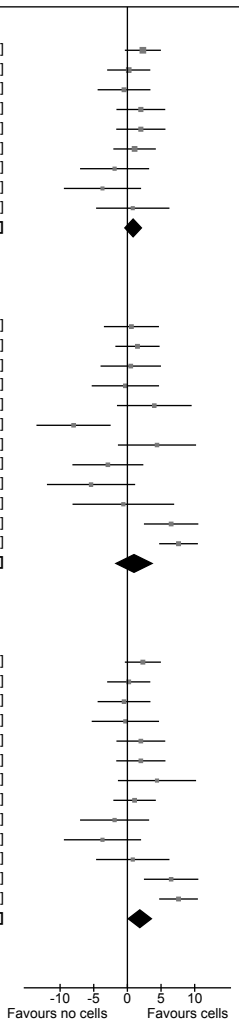


Analysis 1.14 Comparison 1: Cells compared to no cells, Outcome 14: Peak heart rate (bpm)

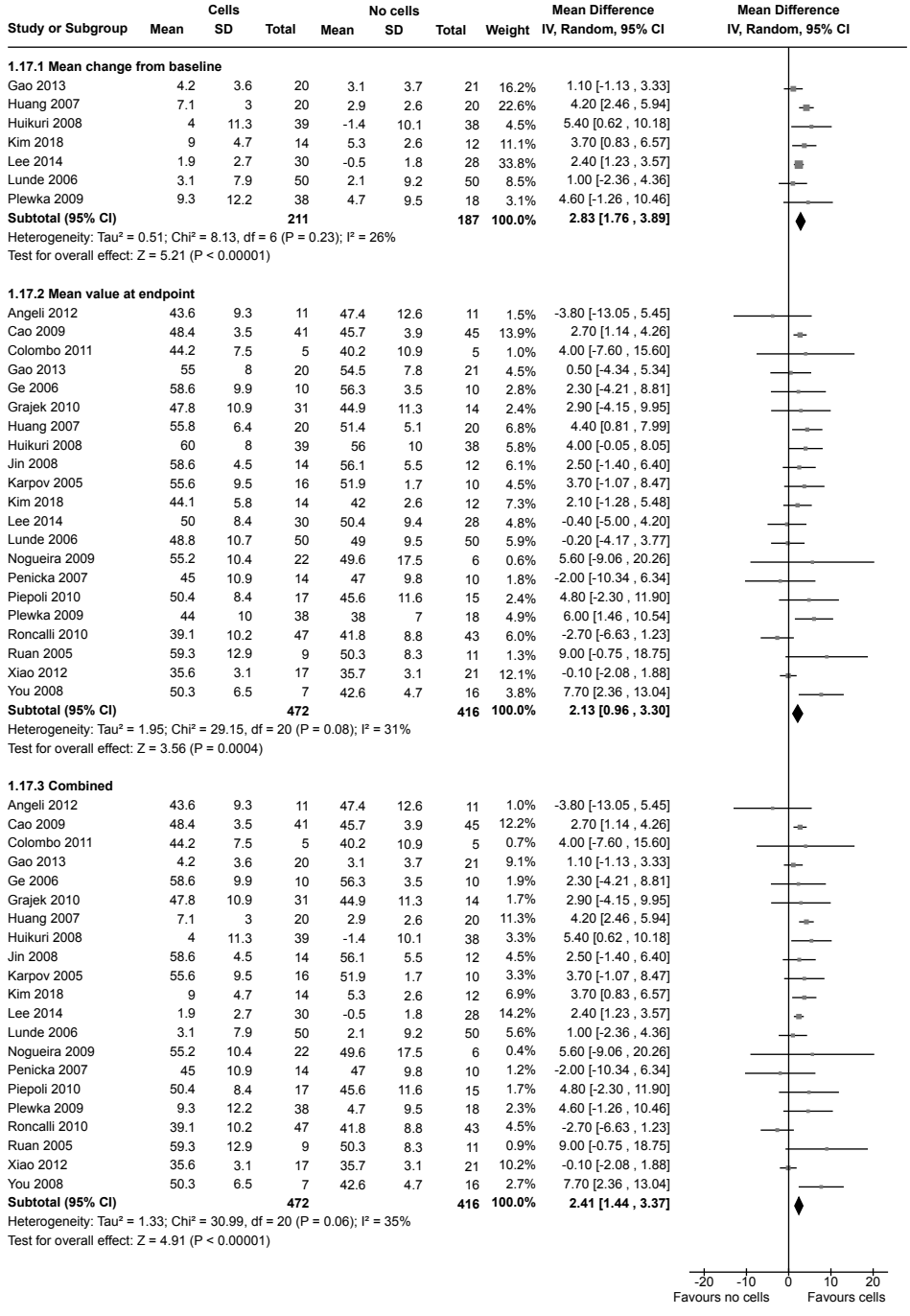


Analysis 1.15 Comparison 1: Cells compared to no cells, Outcome 15: LVEF measured by MRI (<12 months)

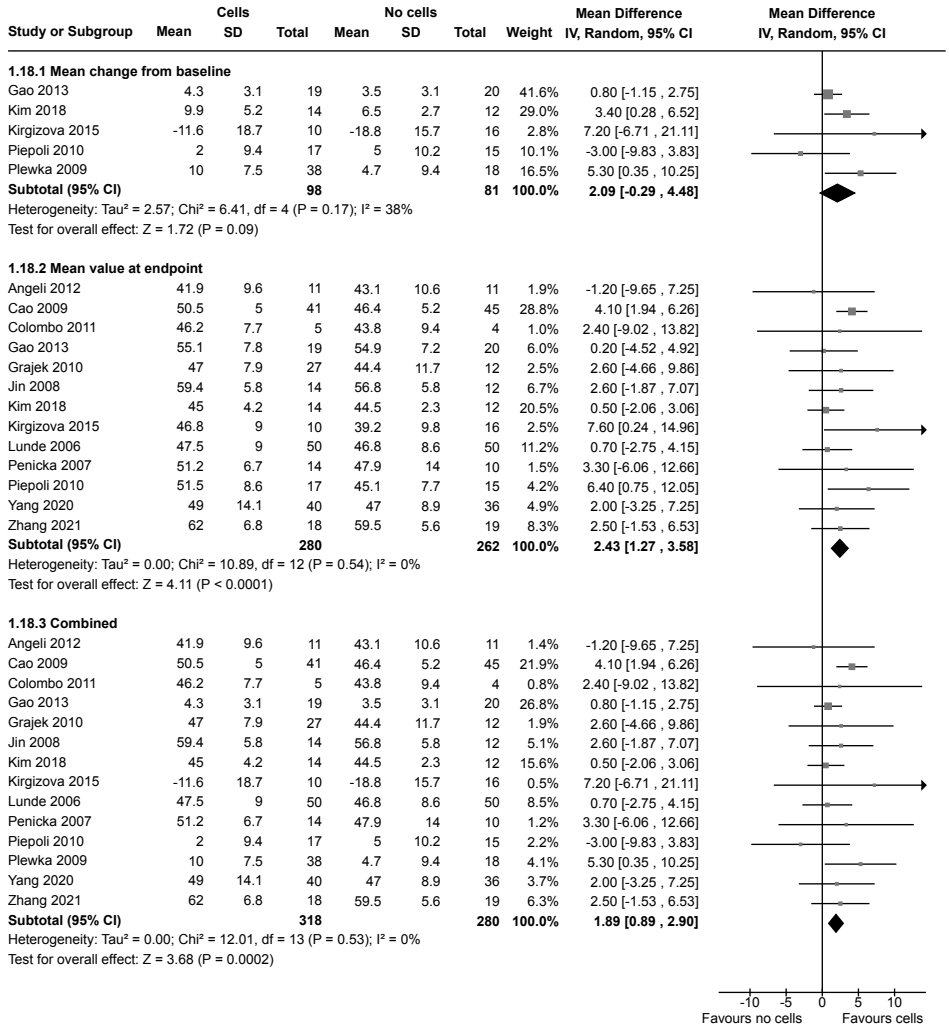
Study or Subgroup	Cells Mean	Cells SD	Cells Total	No Cells Mean	No Cells SD	No Cells Total	Weight	Mean Difference IV, Random, 95% CI
1.16.1 Mean change from baseline								
Choudry 2016	5.1	7.5	51	2.8	5.6	41	21.3%	2.30 [-0.38 , 4.98]
Hirsch 2011	4.2	8.6	59	4	8.6	52	14.9%	0.20 [-3.01 , 3.41]
Janssens 2006	2	7.5	30	2.5	8	30	9.9%	-0.50 [-4.42 , 3.42]
San Roman 2015 (BMMNC)	6	6	26	4	7	24	11.6%	2.00 [-1.63 , 5.63]
San Roman 2015 (BMMNC+G-CSF)	4	7	22	2	5	20	11.4%	2.00 [-1.65 , 5.65]
Sürder 2013	-0.8	10.3	107	-1.87	9.8	60	15.3%	1.07 [-2.09 , 4.23]
Traverse 2018	2.8	9.4	58	4.7	12	27	5.8%	-1.90 [-7.03 , 3.23]
Wohrle 2010	-1.7	5.8	28	2	9.4	12	4.6%	-3.70 [-9.44 , 2.04]
Wollert 2004	-2.5	11.9	30	-3.3	9.5	30	5.1%	0.80 [-4.65 , 6.25]
Subtotal (95% CI)			**411**			**296**	**100.0%**	**0.85 [-0.38 , 2.09]**
Heterogeneity: Tau² = 0.00; Chi² = 6.04, df = 8 (P = 0.64); I² = 0%								
Test for overall effect: Z = 1.35 (P = 0.18)								
1.16.2 Mean value at endpoint								
Choudry 2016	52.6	10.7	51	52	9.3	41	9.2%	0.60 [-3.49 , 4.69]
Hirsch 2011	49.2	8.1	59	47.7	9.4	52	9.9%	1.50 [-1.79 , 4.79]
Janssens 2006	50.4	7.3	30	49.9	10.2	30	8.8%	0.50 [-3.99 , 4.99]
Lunde 2006	54.9	13.2	44	55.2	10.6	44	8.3%	-0.30 [-5.30 , 4.70]
San Roman 2015 (BMMNC)	55	10	26	51	10	24	7.8%	4.00 [-1.55 , 9.55]
San Roman 2015 (BMMNC+G-CSF)	48	8	22	56	10	20	7.8%	-8.00 [-13.51 , -2.49]
Schachinger 2006	48.9	11.6	26	44.5	10.9	33	7.6%	4.40 [-1.41 , 10.21]
Traverse 2018	48.7	11.2	58	51.6	11.7	27	8.1%	-2.90 [-8.17 , 2.37]
Wohrle 2010	54	9.9	28	59.4	9.6	12	6.9%	-5.40 [-11.95 , 1.15]
Wollert 2004	47.5	16.7	30	48.1	12.9	30	6.1%	-0.60 [-8.15 , 6.95]
Yang 2020	46	8.6	40	39.5	9.3	36	9.2%	6.50 [2.46 , 10.54]
Yao 2009	42.9	5.3	27	35.3	3.5	11	10.3%	7.60 [4.72 , 10.48]
Subtotal (95% CI)			**441**			**360**	**100.0%**	**0.98 [-1.70 , 3.67]**
Heterogeneity: Tau² = 16.01; Chi² = 43.54, df = 11 (P < 0.00001); I² = 75%								
Test for overall effect: Z = 0.72 (P = 0.47)								
1.16.3 Combined								
Choudry 2016	5.1	7.5	51	2.8	5.6	41	10.2%	2.30 [-0.38 , 4.98]
Hirsch 2011	4.2	8.6	59	4	8.6	52	9.2%	0.20 [-3.01 , 3.41]
Janssens 2006	2	7.5	30	2.5	8	30	7.9%	-0.50 [-4.42 , 3.42]
Lunde 2006	54.9	13.2	44	55.2	10.6	44	6.3%	-0.30 [-5.30 , 4.70]
San Roman 2015 (BMMNC)	6	6	26	4	7	24	8.4%	2.00 [-1.63 , 5.63]
San Roman 2015 (BMMNC+G-CSF)	4	7	22	2	5	20	8.4%	2.00 [-1.65 , 5.65]
Schachinger 2006	48.9	11.6	26	44.5	10.9	33	5.3%	4.40 [-1.41 , 10.21]
Sürder 2013	-0.8	10.3	107	-1.87	9.8	60	9.3%	1.07 [-2.09 , 4.23]
Traverse 2018	2.8	9.4	58	4.7	12	27	6.1%	-1.90 [-7.03 , 3.23]
Wohrle 2010	-1.7	5.8	28	2	9.4	12	5.4%	-3.70 [-9.44 , 2.04]
Wollert 2004	-2.5	11.9	30	-3.3	9.5	30	5.7%	0.80 [-4.65 , 6.25]
Yang 2020	46	8.6	40	39.5	9.3	36	7.8%	6.50 [2.46 , 10.54]
Yao 2009	42.9	5.3	27	35.3	3.5	11	9.8%	7.60 [4.72 , 10.48]
Subtotal (95% CI)			**548**			**420**	**100.0%**	**1.84 [0.12 , 3.57]**
Heterogeneity: Tau² = 5.72; Chi² = 30.00, df = 12 (P = 0.003); I² = 60%								
Test for overall effect: Z = 2.10 (P = 0.04)								



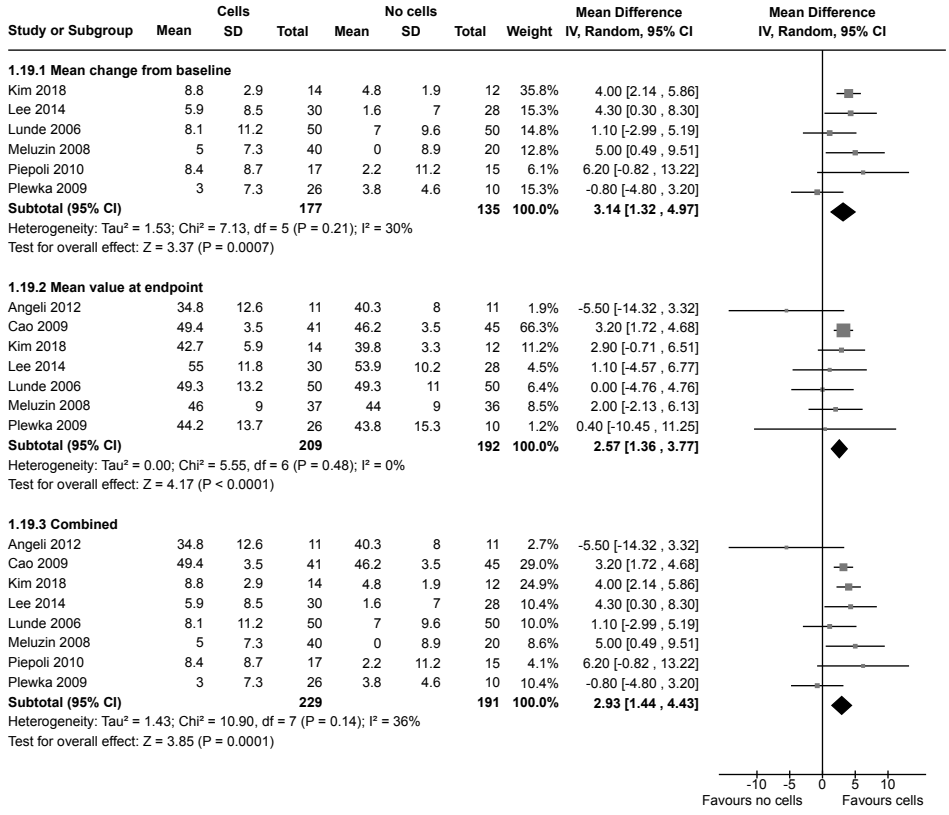
Analysis 1.16 Comparison 1: Cells compared to no cells, Outcome 16: LVEF measured by MRI (≥ 12 months)



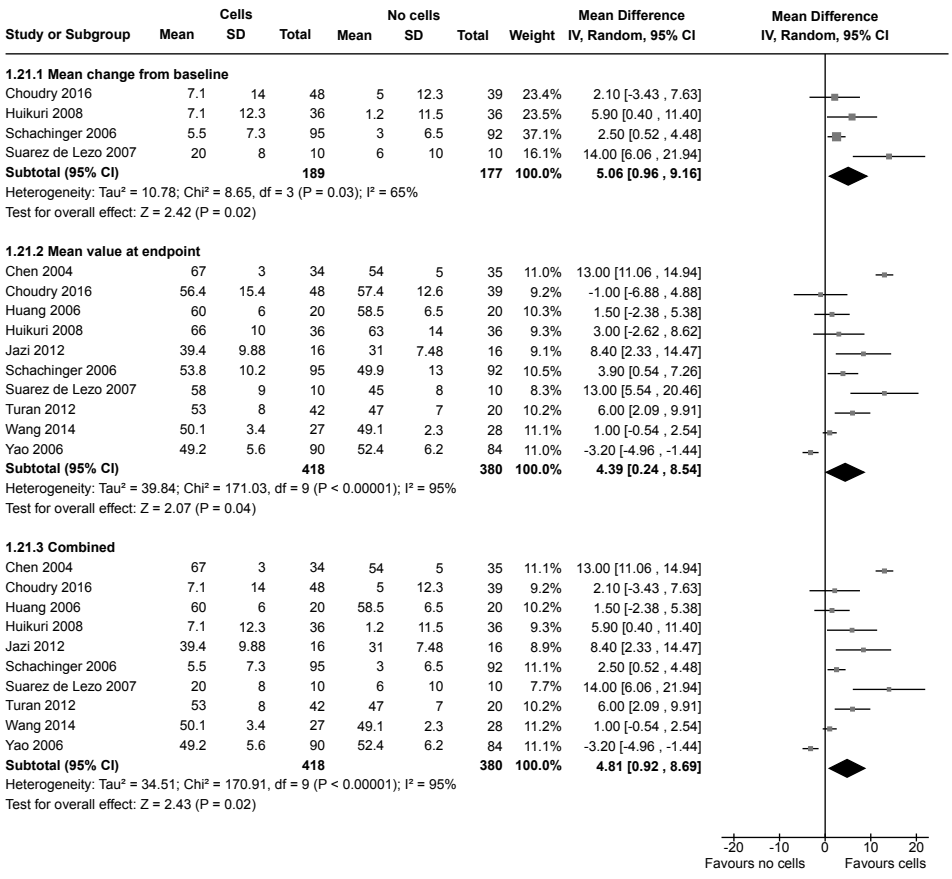
Analysis 1.17 Comparison 1: Cells compared to no cells, Outcome 17: LVEF measured by echocardiography (< 12 months)



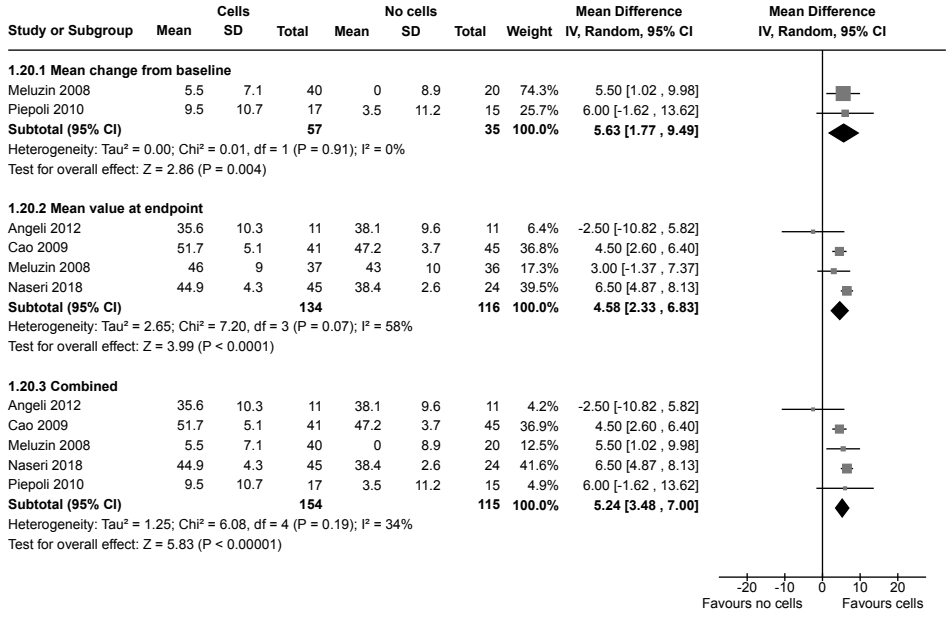
Analysis 1.18 Comparison 1: Cells compared to no cells, Outcome 18: LVEF measured by echocardiography (≥12 months)



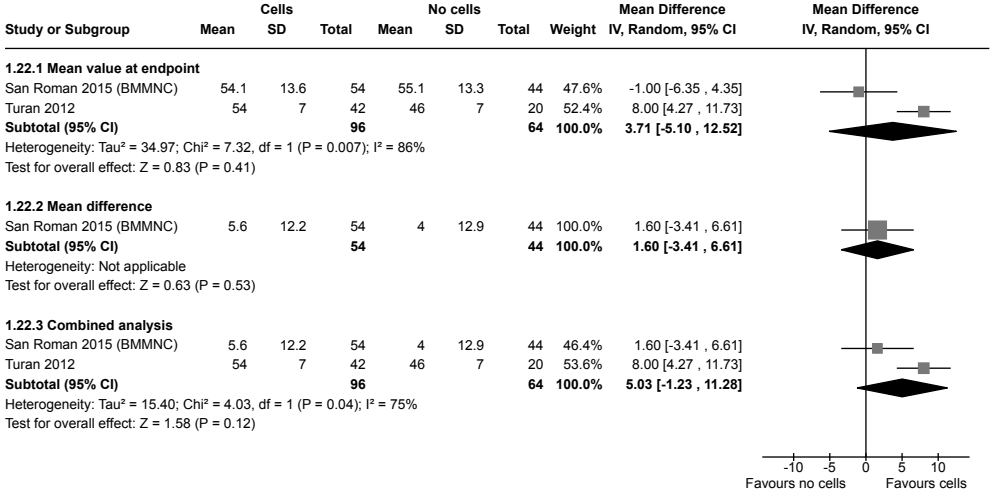
Analysis 1.19 Comparison 1: Cells compared to no cells, Outcome 19: LVEF measured by SPECT (< 12 months)



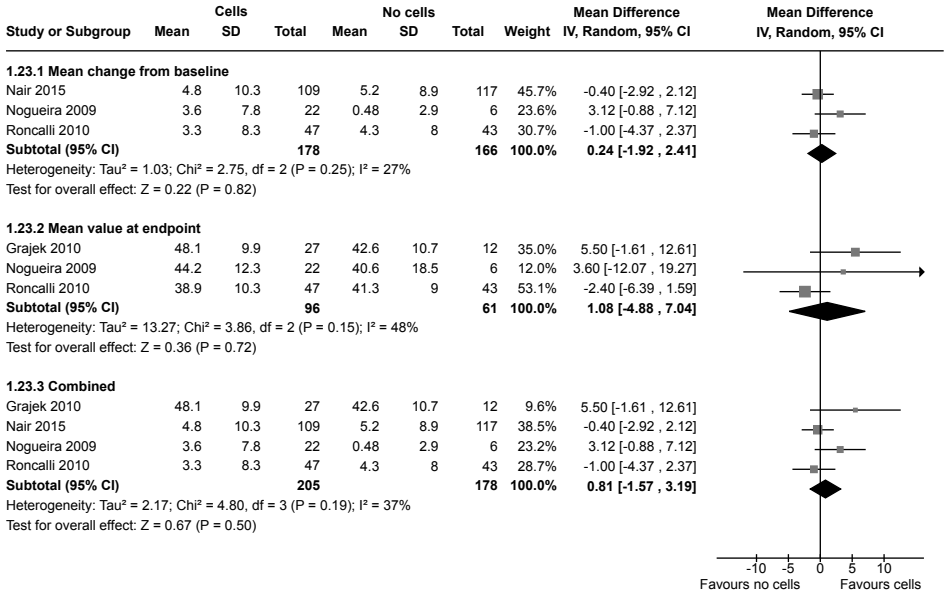
Analysis 1.20 Comparison 1: Cells compared to no cells, Outcome 20: LVEF measured by SPECT (≥ 12 months)



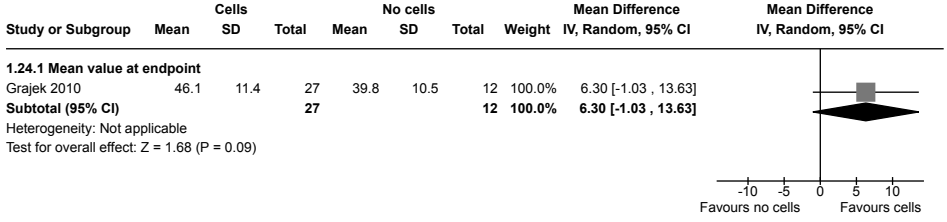
Analysis 1.21 Comparison 1: Cells compared to no cells, Outcome 21: LVEF measured by left ventricular angiography (< 12 months)



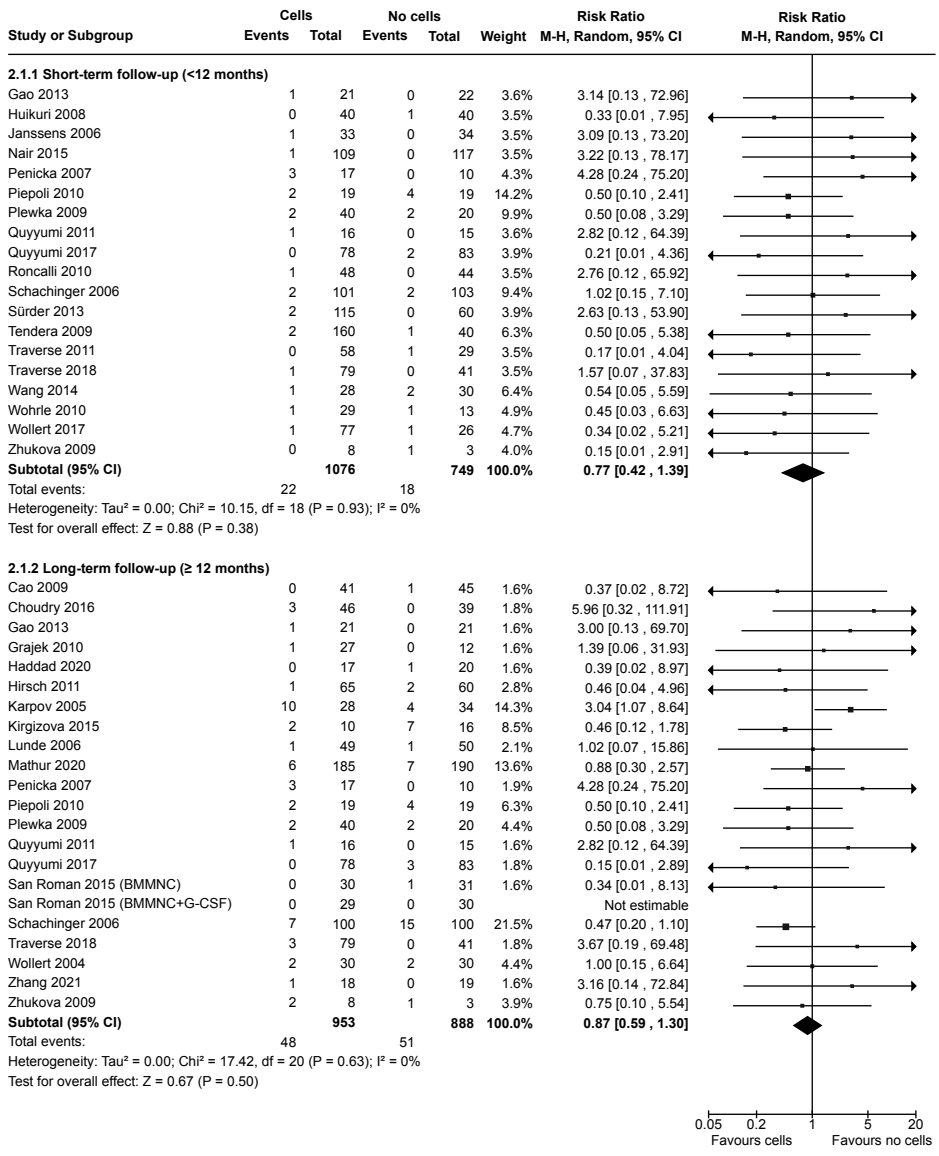
Analysis 1.22 Comparison 1: Cells compared to no cells, Outcome 22: LVEF measured by left ventricular angiography (≥ 12 months)



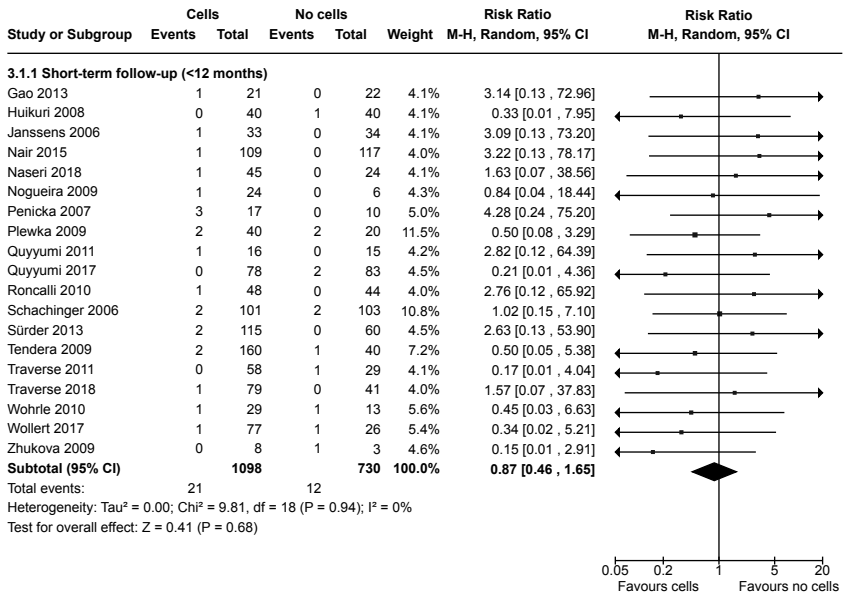
Analysis 1.23 Comparison 1: Cells compared to no cells, Outcome 23: LVEF measured by radionuclide ventriculography (RNV) (<12 months)



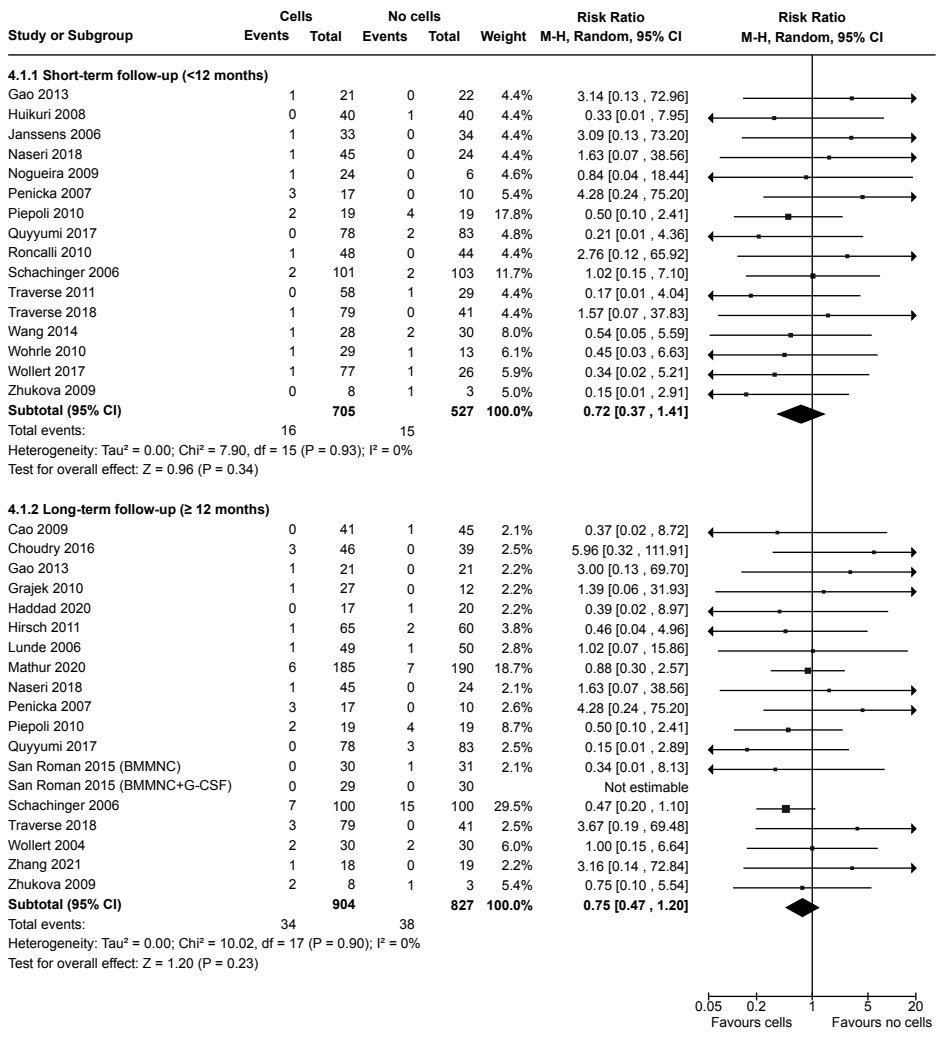
Analysis 1.24 Comparison 1: Cells compared to no cells, Outcome 24: LVEF measured by radionuclide ventriculography (≥ 12 months)



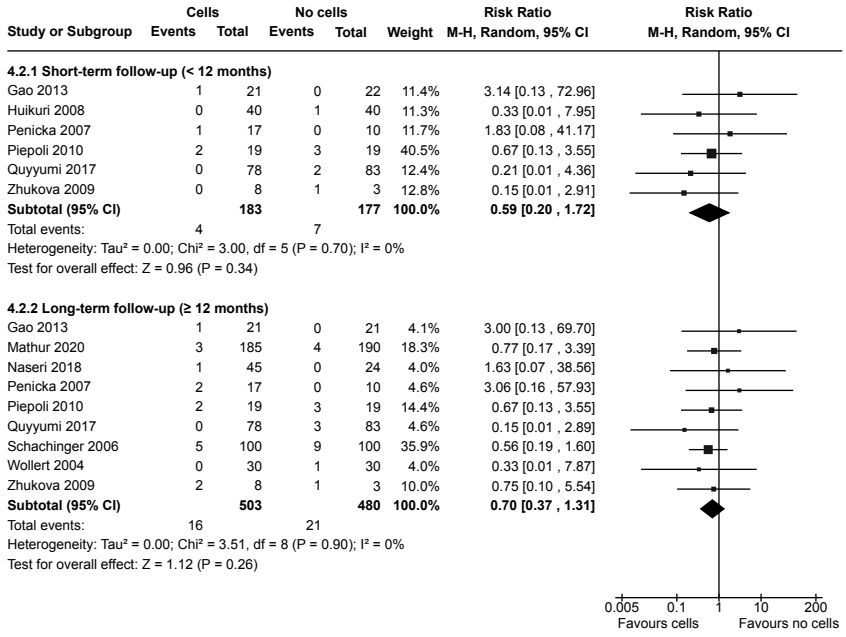
Analysis 2.1 Comparison 2: Sensitivity analysis - route of cell delivery, Outcome 1: All-cause mortality



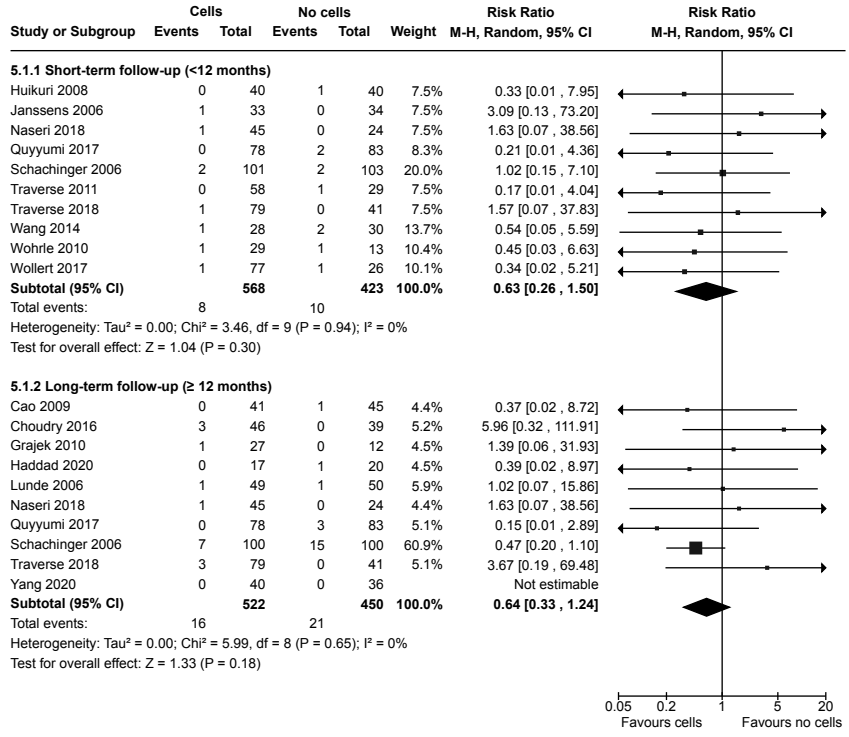
Analysis 3.1 Comparison 3: Sensitivity analysis - selection bias, Outcome 1: All-cause mortality



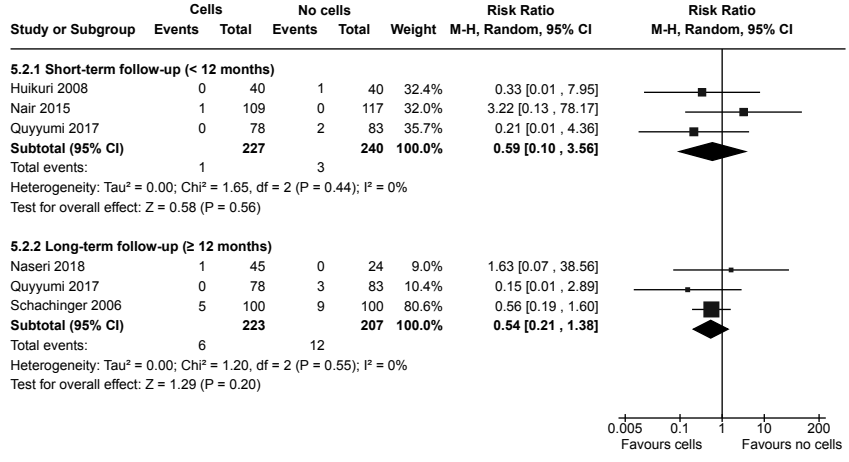
Analysis 4.1 Comparison 4: Sensitivity analysis - attrition bias, Outcome 1: All-cause mortality



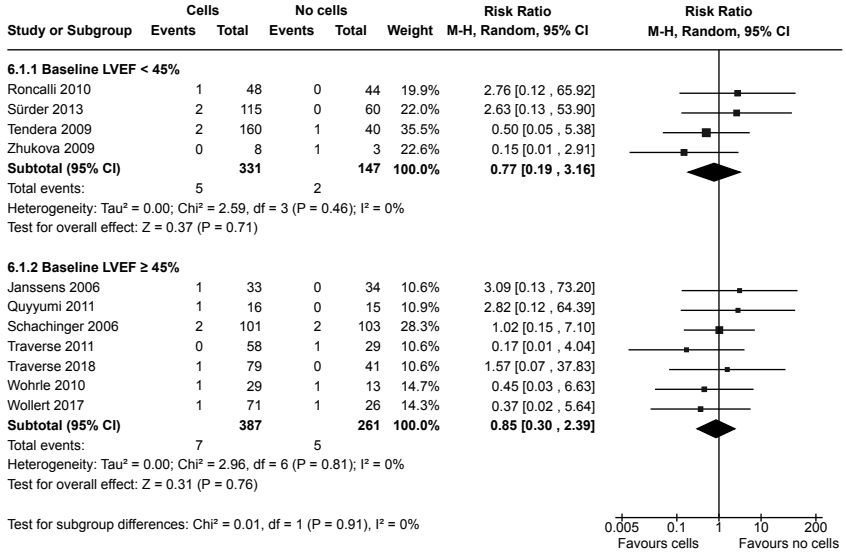
Analysis 4.2 Comparison 4: Sensitivity analysis - attrition bias, Outcome 2: Cardiovascular mortality



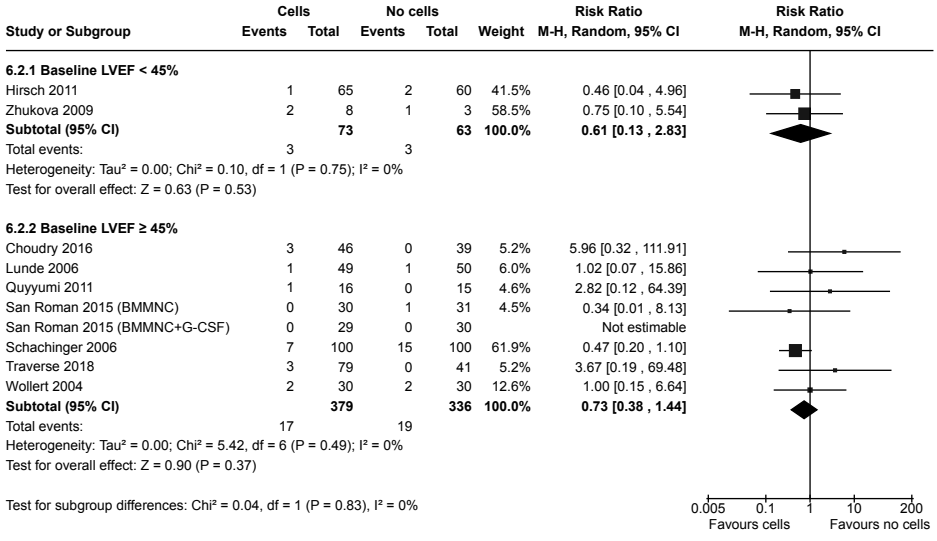
Analysis 5.1 Comparison 5: Sensitivity analysis - performance bias, Outcome 1: All-cause mortality



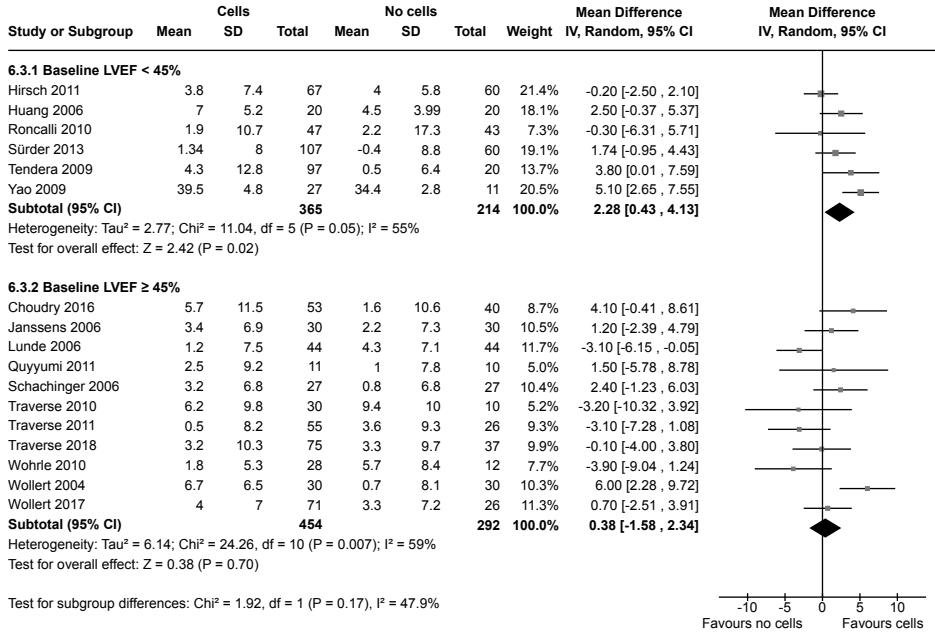
Analysis 5.2 Comparison 5: Sensitivity analysis - performance bias, Outcome 2: Cardiovascular mortality



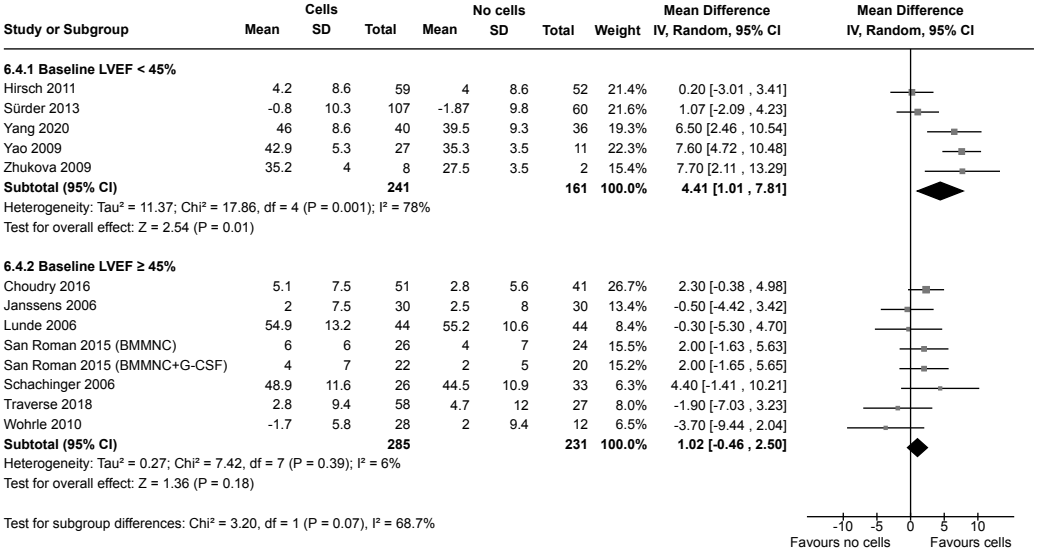
Analysis 6.1 Comparison 6: Subgroup analysis - baseline LVEF measured by MRI, Outcome 1: All-cause mortality (< 12 months)



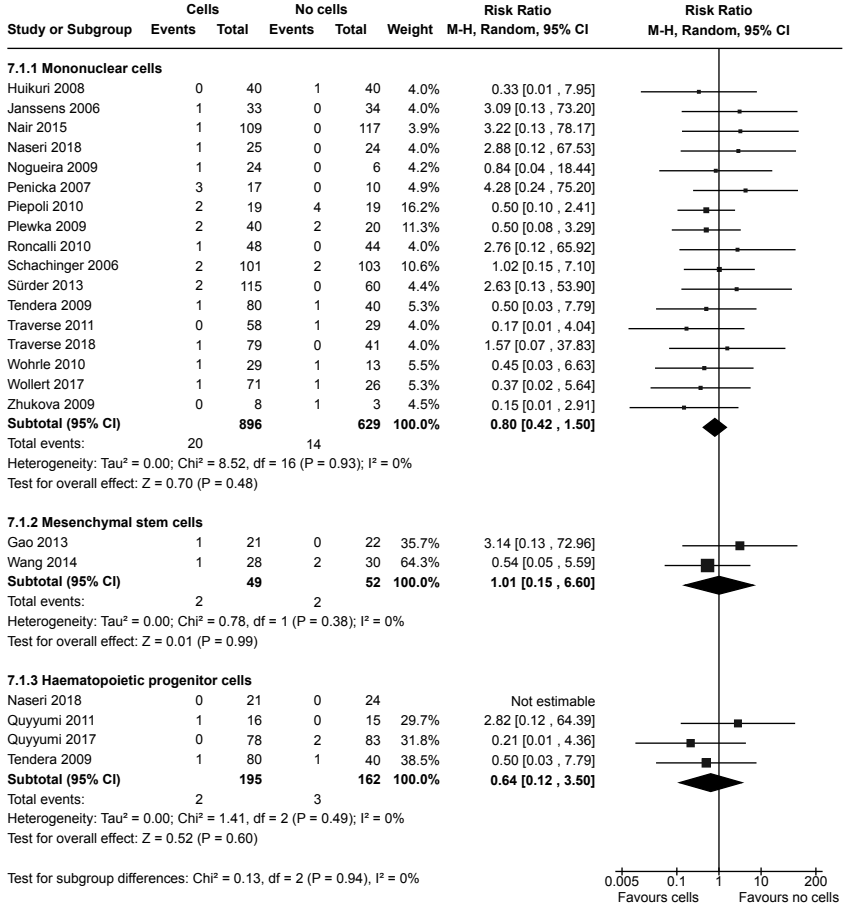
Analysis 6.2 Comparison 6: Subgroup analysis - baseline LVEF measured by MRI, Outcome 2: All-cause mortality (≥ 12 months)



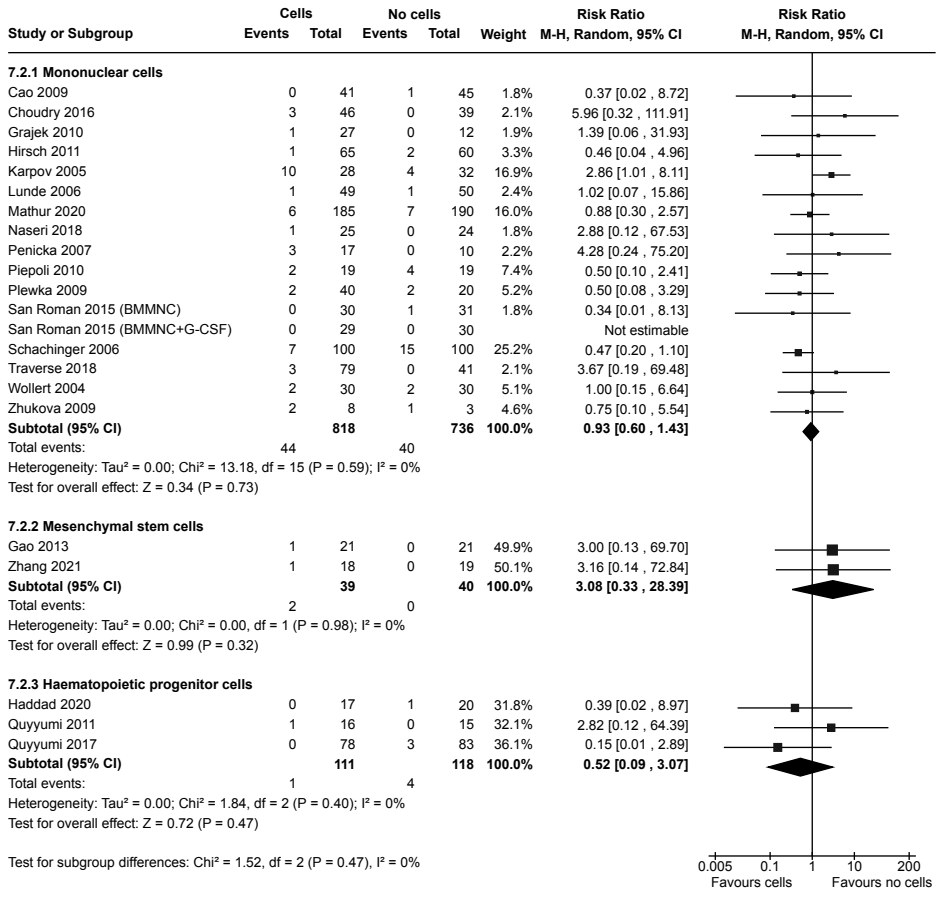
Analysis 6.3 Comparison 6: Subgroup analysis - baseline LVEF measured by MRI, Outcome 3: LVEF measured by MRI (< 12 months)



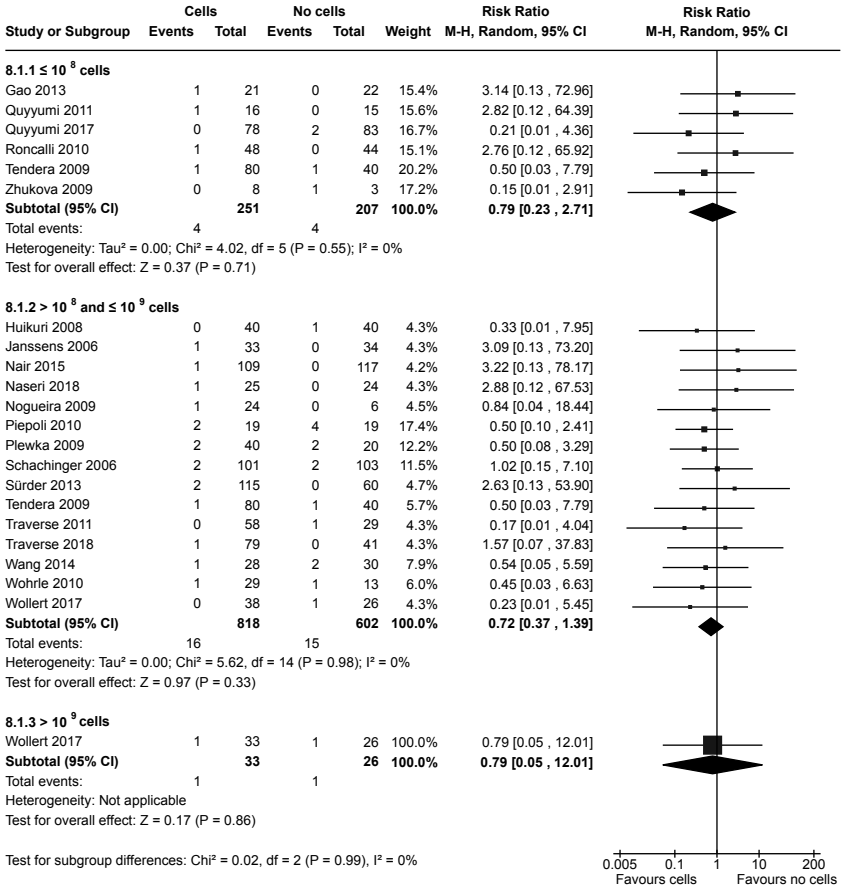
Analysis 6.4 Comparison 6: Subgroup analysis - baseline LVEF measured by MRI, Outcome 4: LVEF measured by MRI (≥ 12 months)



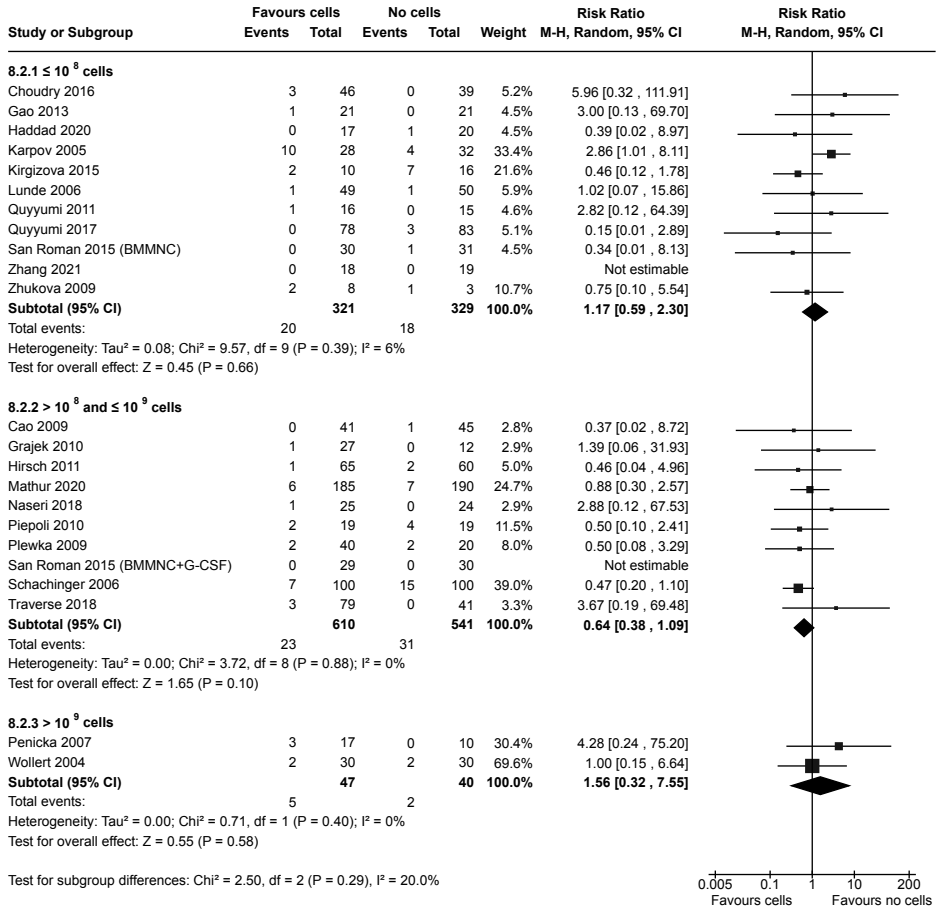
Analysis 7.1 Comparison 7: Subgroup analysis - cell type, Outcome 1: All-cause mortality (< 12 months)



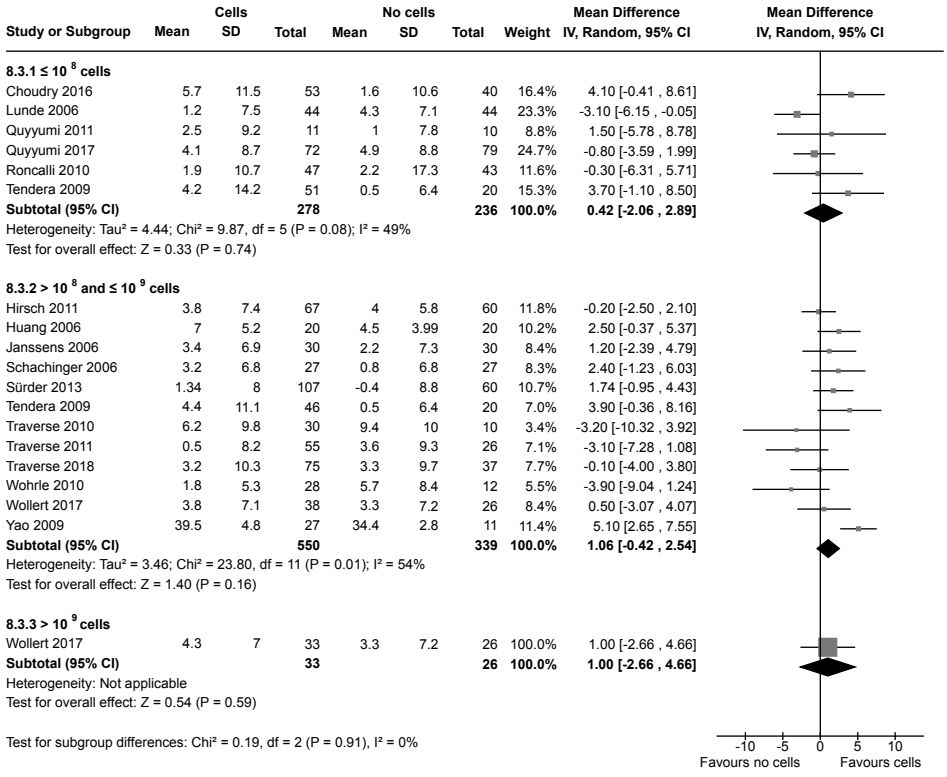
Analysis 7.2 Comparison 7: Subgroup analysis - cell type, Outcome 2: All-cause mortality (≥ 12 months)



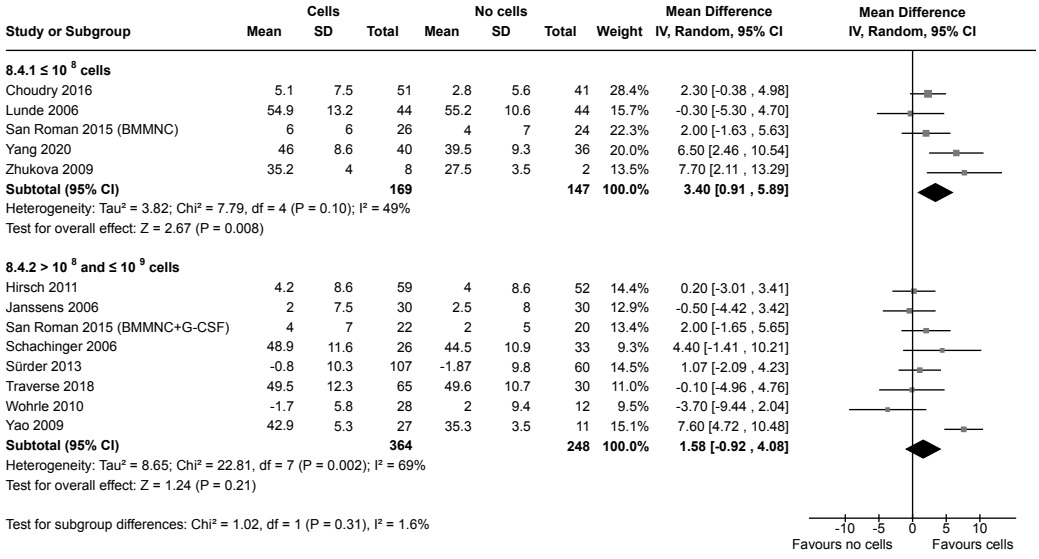
Analysis 8.1 Comparison 8: Subgroup analysis - dose of stem cells, Outcome 1: All-cause mortality (< 12 months)



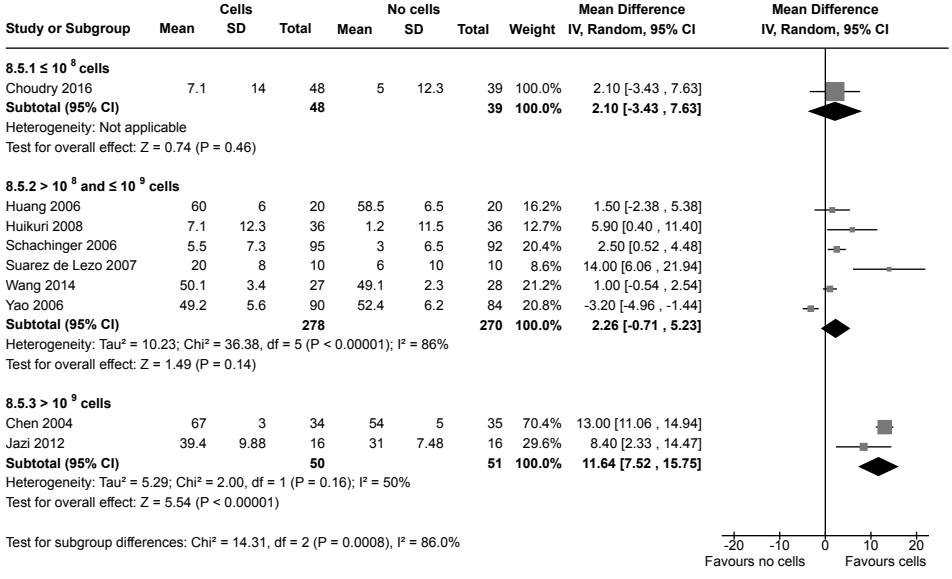
Analysis 8.2 Comparison 8: Subgroup analysis - dose of stem cells, Outcome 2: All-cause mortality (≥ 12 months)



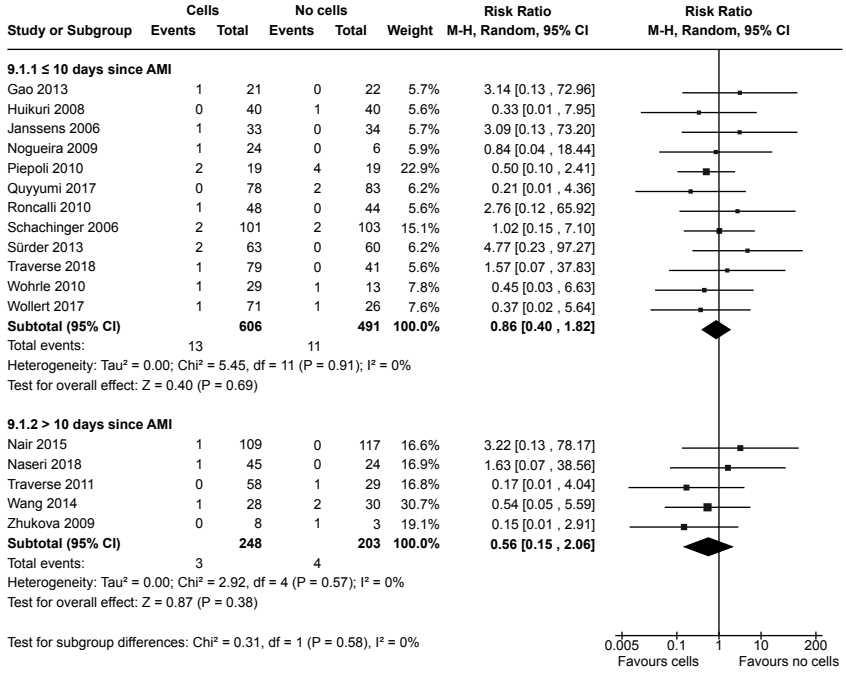
Analysis 8.3 Comparison 8: Subgroup analysis - dose of stem cells, Outcome 3: LVEF measured by MRI (< 12 months)



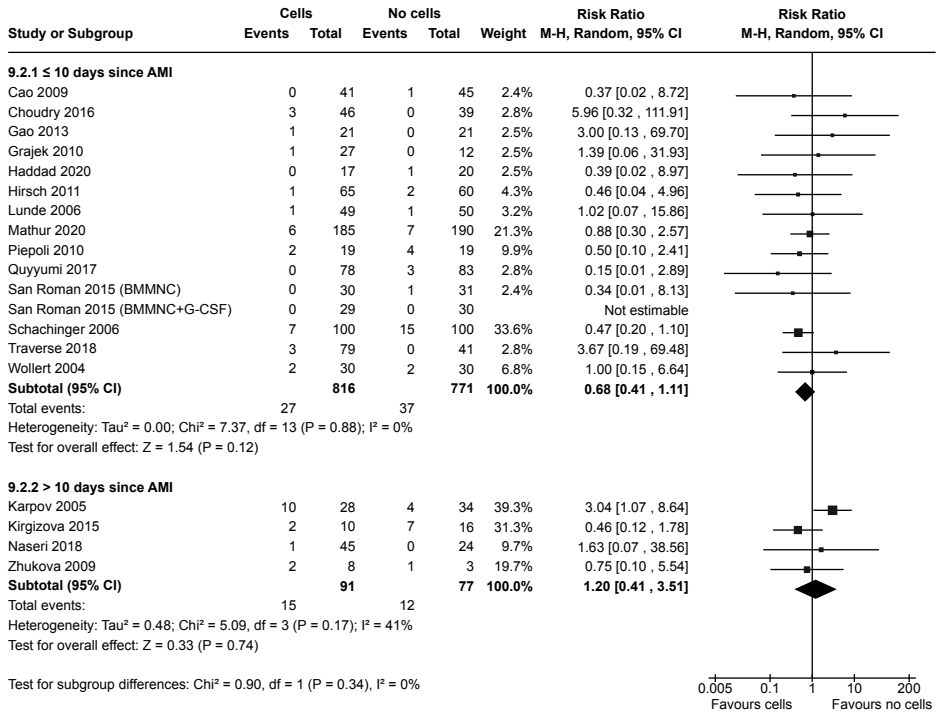
Analysis 8.4 Comparison 8: Subgroup analysis - dose of stem cells, Outcome 4: LVEF measured by MRI (≥ 12 months)



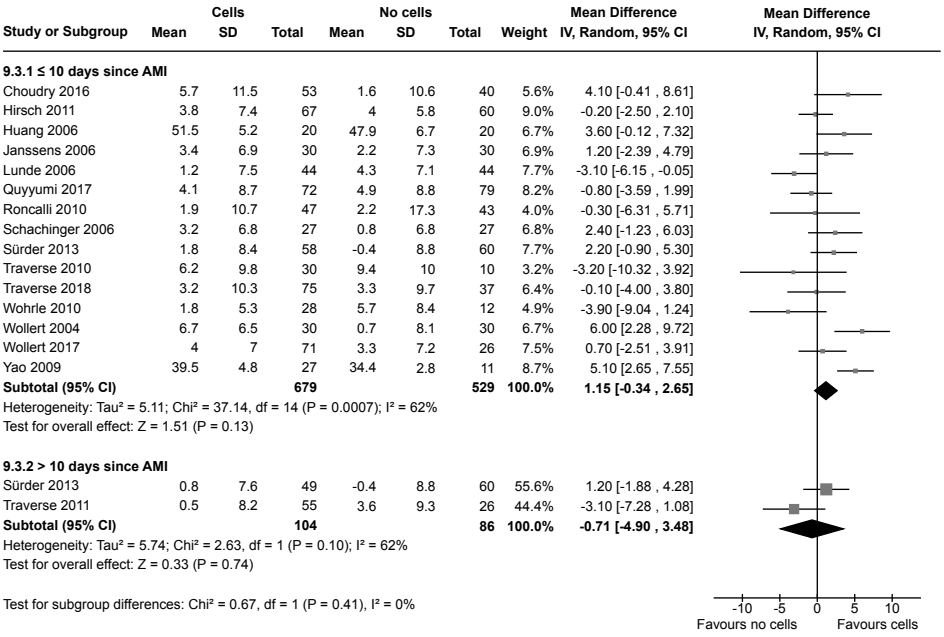
Analysis 8.5 Comparison 8: Subgroup analysis - dose of stem cells, Outcome 5: LVEF measured by left ventricular angiography (< 12 months)



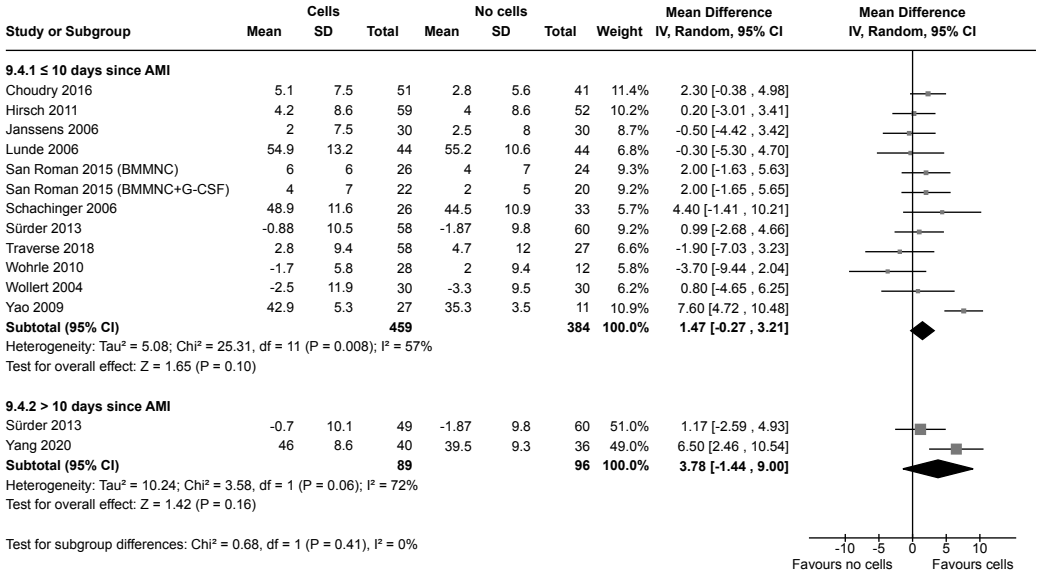
Analysis 9.1 Comparison 9: Subgroup analysis - timing of cell administration, Outcome 1: All-cause mortality (< 12 months)



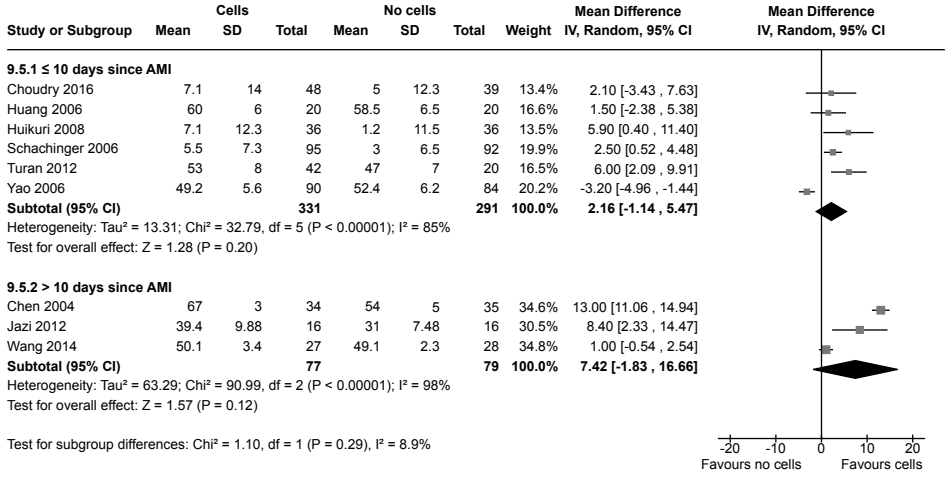
Analysis 9.2 Comparison 9: Subgroup analysis - timing of cell administration, Outcome 2: All-cause mortality (≥ 12 months)



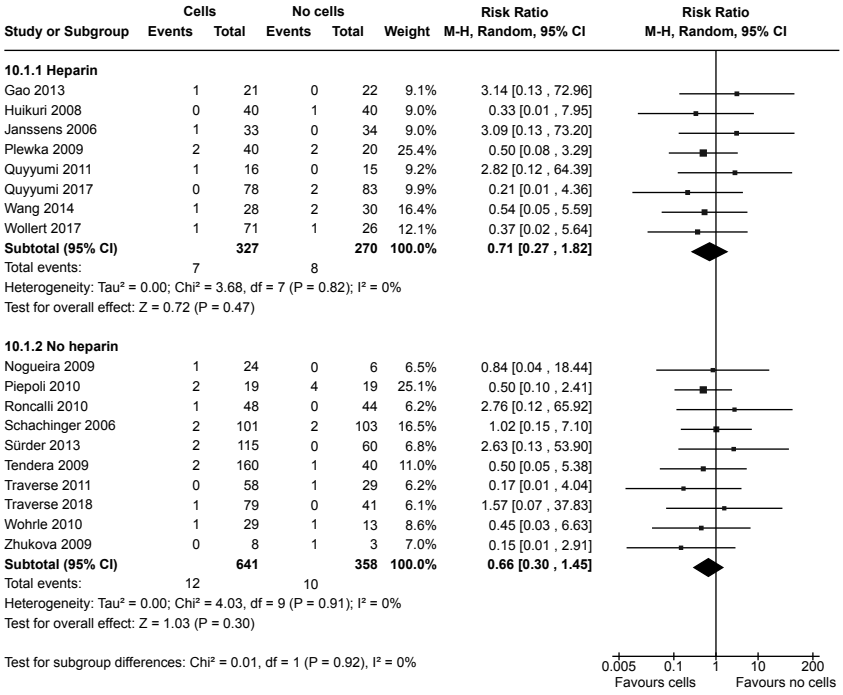
Analysis 9.3 Comparison 9: Subgroup analysis - timing of cell administration, Outcome 3: LVEF measured by MRI (< 12 months)



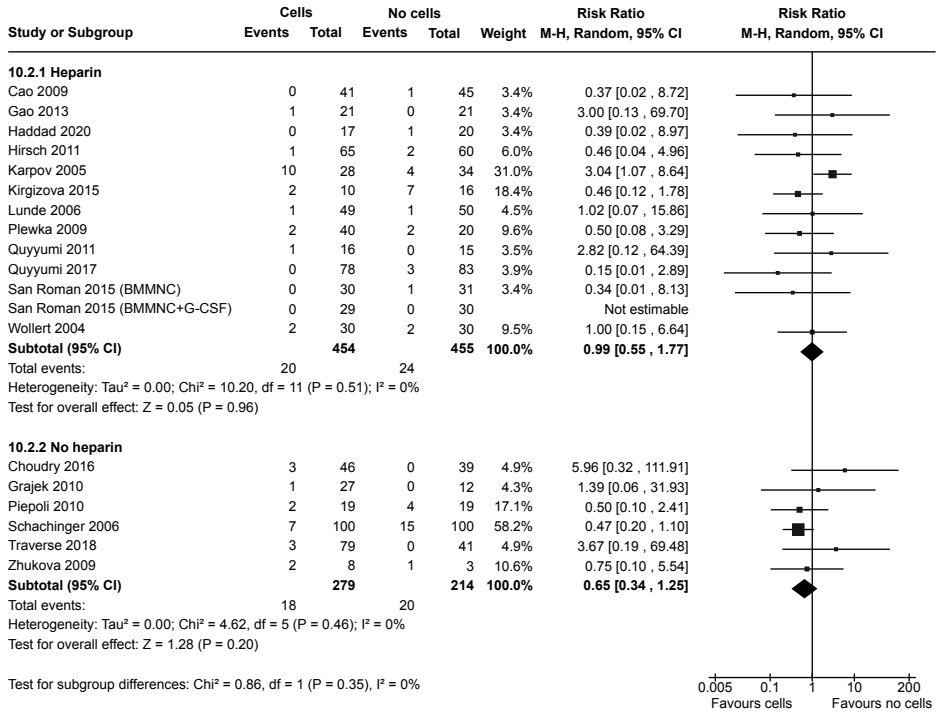
Analysis 9.4 Comparison 9: Subgroup analysis - timing of cell administration, Outcome 4: LVEF measured by MRI (≥ 12 months)



Analysis 9.5 Comparison 9: Subgroup analysis - timing of cell administration, Outcome 5: LVEF measured by left ventricular angiography (< 12 months)

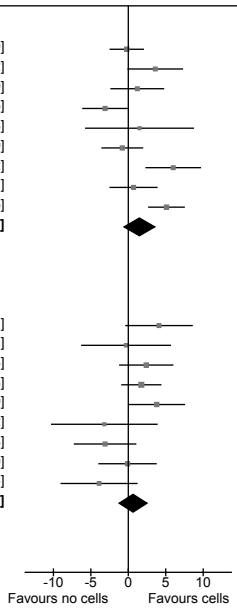


Analysis 10.1 Comparison 10: Subgroup analysis - heparinised cell solution, Outcome 1: All-cause mortality (< 12 months)



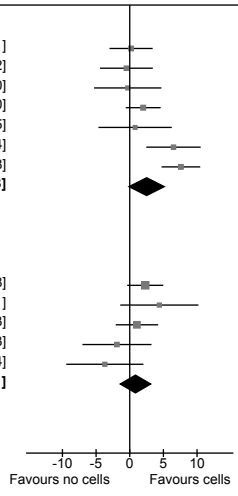
Analysis 10.2 Comparison 10: Subgroup analysis - heparinised cell solution, Outcome 2: All-cause mortality (≥ 12 months)

Study or Subgroup	Cells Mean	Cells SD	Cells Total	No cells Mean	No cells SD	No cells Total	Weight	Mean Difference IV, Random, 95% CI
10.3.1 Heparin								
Hirsch 2011	3.8	7.4	67	4	5.8	60	13.5%	-0.20 [-2.50 , 2.10]
Huang 2006	51.5	5.2	20	47.9	6.7	20	10.6%	3.60 [-0.12 , 7.32]
Janssens 2006	3.4	6.9	30	2.2	7.3	30	10.8%	1.20 [-2.39 , 4.79]
Lunde 2006	1.2	7.5	44	4.3	7.1	44	11.9%	-3.10 [-6.15 , -0.05]
Quyyumi 2011	2.5	9.2	11	1	7.8	10	5.3%	1.50 [-5.78 , 8.78]
Quyyumi 2017	4.1	8.7	72	4.9	8.8	79	12.5%	-0.80 [-3.59 , 1.99]
Wollert 2004	6.7	6.5	30	0.7	8.1	30	10.6%	6.00 [2.28 , 9.72]
Wollert 2017	4	7	71	3.3	7.2	26	11.6%	0.70 [-2.51 , 3.91]
Yao 2009	39.5	4.8	27	34.4	2.8	11	13.2%	5.10 [2.65 , 7.55]
Subtotal (95% CI)			**372**			**310**	**100.0%**	**1.48 [-0.57 , 3.53]**
Heterogeneity: Tau² = 6.73; Chi² = 28.75, df = 8 (P = 0.0004); I² = 72%								
Test for overall effect: Z = 1.42 (P = 0.16)								
10.3.2 No heparin								
Choudry 2016	5.7	11.5	53	1.6	10.6	40	10.5%	4.10 [-0.41 , 8.61]
Roncalli 2010	1.9	10.7	47	2.2	17.3	43	7.1%	-0.30 [-6.31 , 5.71]
Schachinger 2006	3.2	6.8	27	0.8	6.8	27	13.5%	2.40 [-1.23 , 6.03]
Sürder 2013	1.34	8	107	-0.4	8.8	60	17.5%	1.74 [-0.95 , 4.43]
Tendera 2009	4.3	12.8	97	0.5	6.4	20	12.9%	3.80 [0.01 , 7.59]
Traverse 2010	6.2	9.8	30	9.4	10	10	5.5%	-3.20 [-10.32 , 3.92]
Traverse 2011	0.5	8.2	55	3.6	9.3	26	11.6%	-3.10 [-7.28 , 1.08]
Traverse 2018	3.2	10.3	75	3.3	9.7	37	12.5%	-0.10 [-4.00 , 3.80]
Wohrle 2010	1.8	5.3	28	5.7	8.4	12	8.9%	-3.90 [-9.04 , 1.24]
Subtotal (95% CI)			**519**			**275**	**100.0%**	**0.64 [-1.22 , 2.50]**
Heterogeneity: Tau² = 3.24; Chi² = 13.76, df = 8 (P = 0.09); I² = 42%								
Test for overall effect: Z = 0.67 (P = 0.50)								
Test for subgroup differences: Chi² = 0.36, df = 1 (P = 0.55), I² = 0%								

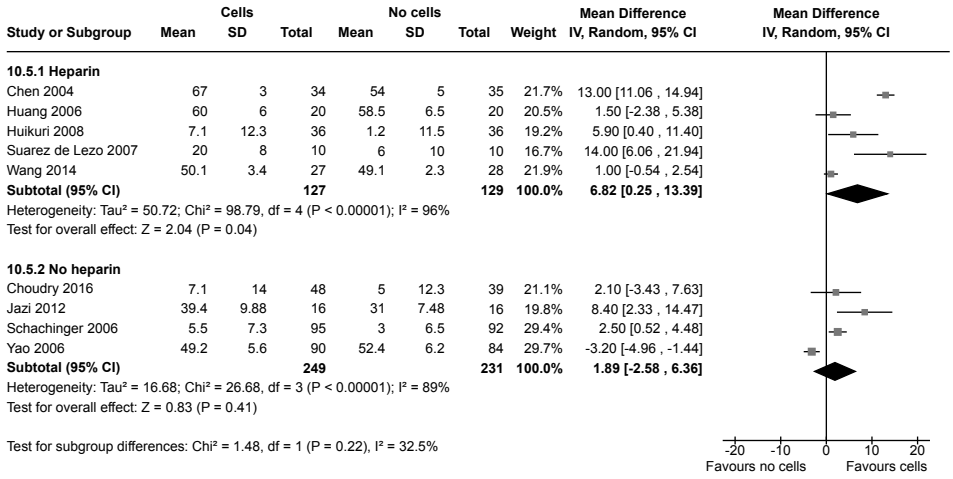


Analysis 10.3 Comparison 10: Subgroup analysis - heparinised cell solution, Outcome 3: LVEF measured by MRI (< 12 months)

Study or Subgroup	Cells Mean	Cells SD	Cells Total	No Cells Mean	No Cells SD	No Cells Total	Weight	Mean Difference IV, Random, 95% CI
10.4.1 Heparin								
Hirsch 2011	4.2	8.6	59	4	8.6	52	15.8%	0.20 [-3.01 , 3.41]
Janssens 2006	2	7.5	30	2.5	8	30	14.1%	-0.50 [-4.42 , 3.42]
Lunde 2006	54.9	13.2	44	55.2	10.6	44	11.8%	-0.30 [-5.30 , 4.70]
San Roman 2015 (BMMNC)	5.1	6.5	48	3.1	6.2	44	17.1%	2.00 [-0.60 , 4.60]
Wollert 2004	-2.5	11.9	30	-3.3	9.5	30	10.9%	0.80 [-4.65 , 6.25]
Yang 2020	46	8.6	40	39.5	9.3	36	13.8%	6.50 [2.46 , 10.54]
Yao 2009	42.9	5.3	27	35.3	3.5	11	16.5%	7.60 [4.72 , 10.48]
Subtotal (95% CI)			**278**			**247**	**100.0%**	**2.51 [-0.11 , 5.13]**
Heterogeneity: Tau² = 8.66; Chi² = 21.51, df = 6 (P = 0.001); I² = 72%								
Test for overall effect: Z = 1.88 (P = 0.06)								
10.4.2 No heparin								
Choudry 2016	5.1	7.5	51	2.8	5.6	41	32.9%	2.30 [-0.38 , 4.98]
Schachinger 2006	48.9	11.6	26	44.5	10.9	33	12.1%	4.40 [-1.41 , 10.21]
Sürder 2013	-0.8	10.3	107	-1.87	9.8	60	27.9%	1.07 [-2.09 , 4.23]
Traverse 2018	2.8	9.4	58	4.7	12	27	14.7%	-1.90 [-7.03 , 3.23]
Wohrle 2010	-1.7	5.8	28	2	9.4	12	12.4%	-3.70 [-9.44 , 2.04]
Subtotal (95% CI)			**270**			**173**	**100.0%**	**0.85 [-1.41 , 3.11]**
Heterogeneity: Tau² = 2.18; Chi² = 6.01, df = 4 (P = 0.20); I² = 33%								
Test for overall effect: Z = 0.74 (P = 0.46)								
Test for subgroup differences: Chi² = 0.88, df = 1 (P = 0.35), I² = 0%								



Analysis 10.4 Comparison 10: Subgroup analysis - heparinised cell solution, Outcome 4: LVEF measured by MRI (≥ 12 months)



Analysis 10.5 Comparison 10: Subgroup analysis - heparinised cell solution, Outcome 5: LVEF measured by left ventricular angiography (< 12 months)

REFERENCES

References to studies included in this review

Angeli 2012 {published data only}
20643248
- Angeli FS, Caramori PR, da Costa Escobar Piccoli J, Danzmann LC, Magedanz E, Bertaso A, et al. Autologus transplantation of mononuclear bone marrow cells after acute myocardial infarction: a PILOT study. International Journal of Cardiology 2012;158(3):449-50. 20643249

Cao 2009 {published data only}
20643250
- Cao F, Sun D, Li C, Narsinh K, Zhao L, Li X, et al. Long-term myocardial functional improvement after autologous bone marrow mononuclear cell transplantation in patients with ST-segment elevation myocardial infarction: 4 years follow-up. European Heart Journal 2009;30(16):1986-94. 20643251
- Cao F, Sun D, Li C, Zhao L and Wang H. Four years follow up of intracoronary delivery autologous bone marrow mononuclear cells in patients with ST-elevation myocardial infarction [American College of Cardiology 58th Annual Scientific Session and i2 Summit: Innovation in Intervention, Orlando, FL United States, 29-31 March 2009]. Journal of American College of Cardiology 2009;53(10):A41. 20643252
- Cao F, Sun DD, Li CX, Narsinh K, Guo W, Li X, et al. Long-term myocardial functional improvement after autologous bone marrow mononuclear cell transplantation in patients with ST-segment elevation myocardial infarction (STEMI): 4-years follow-up. [14th Annual Symposium - Angioplasty Summit 2009 TCT Asia Pacific, Seoul, South Korea, 22-24 April 2009]. American Journal of Cardiology 2009;103(9):2B-3B. 20643253
- NCT00626145. Long-term follow-up of autologous bone marrow mononuclear cells therapy in STEMI. http://clinicaltrials.gov/ct2/show/NCT00626145 (accessed by 31 January 2011). 20643254

Chen 2004 {published data only}
20643255
- Chen SL, Fang WW, Qian J, Ye F, Liu YH, Shan SJ, et al. Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. Chinese Medical Journal 2004;117(10):1443-8. 20643256
- Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. American Journal of Cardiology 2004;94(1):92-5. 20643257

Choudry 2016 {published data only}
20643258
- Anthony Mathur, Doo Sun Sim, Fizzah Choudry, Jessry Veerapen, Martina Colicchia, Tymoteusz Turlejski, Mohsin Hussain, Stephen Hamshere, Didier Locca, Roby Rakhit, Tom Crake, Jens Kastrup, Samir Agrawal, Daniel A Jones, John Martin. Fiveyear follow-up of intracoronary autologous cell therapy in acute myocardial infarction: the REGENERATE-AMI trial. ESC Heart Failure 18-1-2022. 20643259 [DOI: 10.1002/ehf2.13786] [PMID: 35043578]
- Fizzah Choudry, Stephen Hamshere, Natalie Saunders, Jessry Veerapen, Katrine Bavnbek, Charles Knight, Denis Pellerin, Didier Locca, Mark Westwood, Roby Rakhit, Tom Crake, Jens Kastrup, Mahesh Parmar, Samir Agrawal, Daniel Jones, John Martin, Anthony Mathur. A randomized double-blind control study of early intracoronary autologous bone marrow cell infusion in acute myocardial infarction: the REGENERATE-AMI clinical trial. European Heart Journal 14-1-2016:256-63. 20643260 [DOI: 10.1093/eurheartj/ehv493] [PMID: 26405233]
- Hamshere S, Choudhury T, Jones DA, Locca D, Mills P, Rothman M, et al. A randomised double-blind control study of early intracoronary autologous bone marrow cell infusion in acute myocardial infarction (REGENERATE-AMI). BMJ Open 2014;4(2):e004258. 20643261
- Locca D, Burcliell T, Flett A, Yeo C, de Palma R, Knight C, et al. Randomised controlled clinical trial of the use of autologous bone marrow derived progenitor cells to salvage myocardium in patients with acute anterior myocardial infarction. [EuroPCR 2011, Paris, France. 17-20 May 2011]. EuroIntervention 2011;7:M87. 20643262
- NCT00765453. Bone marrow derived adult stem cells for acute anterior myocardial infarction (REGEN-AMI). https://clinicaltrials.gov/ct2/show/NCT00765453 (accessed 11 March 2015). 20643263

Colombo 2011 {published data only}
20643264
- Castellani M, Colombo A, Giordano R, Pusineri E, Canzi C, Longari V, et al. The role of PET with 13N-ammonia and 18F-FDG in the assessment of myocardial perfusion and metabolism in patients with recent AMI and intracoronary stem cell injection. Journal of Nuclear Medicine 2010;51(12):1908-16. 20643265
- Colombo A, Castellani M, Piccaluga E, Pusineri E, Palatresi S, Longari V, et al. Myocardial blood flow and infarct size after CD133+ cell injection in large myocardial infarction with good recanalization and poor reperfusion: results from a randomized controlled trial. Journal of Cardiovascular Medicine 2011;12(4):239-48. 20643266
- Colombo A, Piccaluga E, Castellani M, Canzi C, Palatresi S, Pusineri E. Infarct size and myocardial blood flow after CD133(+) stem cells therapy in large myocardial infarction: a randomized controlled trial. American Journal of Cardiology 2007;100(8A Suppl):2L. 20643267
- NCT00400959. CD133+ autologous cells after myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00400959 (accessed 11 March 2015). 20643268

Gao 2013 {published data only}
20643269
- Gao L. A critical challenge: Dosage-related efficacy and acute complication intracoronary injection of autologous bone marrow mesenchymal stem cells in acute myocardial infarction. [24th Great Wall International Congress of Cardiology, Asia Pacific Heart Congress 2013; International Congress of Cardiovascular Prevention and Rehabilitation 2013, Beijing, China, 10-13 October 2013]. Heart 2013;99:A145-6. 20643270
- Gao LR, Chen Y, Ding QA, Guo F, Li TC, Liu HL, et al. Intracoronary infusion of bone marrow mesenchymal stem cells in acute myocardial infarction - a critical challenge for dosage-related efficacy and safety. [25th Annual Symposium Transcatheter Cardiovascular Therapeutics, TCT 2013, San Francisco, CA United States, 27 October - 1 November 2013]. Journal of the American College of Cardiology 2013;62(18 Suppl 1):B241. 20643271
- Gao LR, Pei XT, Ding QA, Chen Y, Zhang NK, Chen HY, et al. A critical challenge: dosage-related efficacy and acute complication intracoronary injection of autologous bone marrow mesenchymal stem cells in acute myocardial infarction. International Journal of Cardiology 2013;168(4):3191-9. 20643272

Ge 2006 {published data only}
20643273
- Ge J, Li Y, Qian J, Shi J, Wang Q, Niu Y, et al. Efficacy of emergent transcatheter transplantation of stem cells for treatment of acute myocardial infarction (TCT-STAMI). Heart 2006;92(12):1764-7. 20643274

Grajek 2010 {published data only}
20643275
- Grajek S, Popiel M, Breborowicz P, Lesiak M, Araszkiewicz A, Katarzynska A et al. Can intracoronary infusion of autologous bone marrow stem cells (BMSC) prevent remodeling of left ventricle in patients with AMI? Results of the study with 12 month followup. European Heart Journal 2006;27(Suppl 1):348. 20643276
- Grajek S, Popiel M, Gil L, Breborowicz P, Lesiak M, Czepczynski R, et al. Influence of bone marrow stem cells on left ventricle perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: randomized clinical trial: Impact of bone marrow stem cell intracoronary infusion on improvement of microcirculation. European Heart Journal 2010;31(6):691- 702. 20643277
- Grajek S, Popiel M, Sawinski K, Lesiak M, Breborowicz P, Czepczynski R, et al. Improvement of left ventricle function in patients with acute myocardial infarction after infusion of bone marrow stem cells. In: Advances in Heart Disease: Proceedings of the 12th World Congress on Heart Disease. MEDIMOND, 2005:125-9. 20643278
- Popiel M, Grajek S, Czepczynski R, Oleksa R, Breborozicz P, Czyz A, et al. Improvement of microcirculation after bone marrow stem cells injection in patients with AMI of anterior wall. [9th International Conference of Non-Invasive Cardiovascular Imaging, Barcelona, Spain, 10-13 May 2009]. European Heart Journal. Supplement 2009;11:S53-S54. 20643279
- Popiel M, Straburzynska-Migaj E, Breborowicz P, Grajek S, Katarzynska-Szymanska A, Lesiak M, et al. Exercise capacity, arrhythmic risk profile and pulmonary function is not influenced by bone marrow stem cells IC infusion in patients with acute myocardial infarction. [European Society of Cardiology, ESC Congress 2011, Paris, France, 27-31 August 2011]. European Heart Journal 2011;32:819. 20643280

- Straburzynska-Migaj E, Popiel M, Grajek S, Katarzynska-Szymanska A, Lesiak M, Breborowicz P, et al. Exercise capacity, arrhythmic risk profile, and pulmonary function is not influenced by intracoronary injection of bone marrow stem cells in patients with acute myocardial infarction. International Journal of Cardiology 2012;159(2):134-8. 20643281

Haddad 2020 {published data only}
20643282
- Actrn2009. Comparison of intracoronary selected CD 133+ bone marrow stem cells in cardiac recovery after acute myocardial infarct and left ventricular dysfunction: cOMPARE-AMI a randomized controlled double blind clinical study. http://www.who.int/trialsearch/Trial2.aspx?TrialID=ACTRN12609001045202. 20643283
- Fuyu, Qiu and Maehara, Akiko and {El Khoury}, Ramez and Genereux, Philippe and LaSalle, Laura and Mintz, Gary S and Noiseux, Nicolas and Roy, Denis-Claude and Gobeil, Francois and Stevens, Louis-Mathieu and Reeves, Francois and Leclerc, Guy and Rivard, Alain and Mansour, Samer. Impact of intracoronary injection of CD133+ bone marrow stem cells on coronary atherosclerotic progression in patients with STEMI: a COMPARE-AMI IVUS substudy. Coron Artery Dis. 2016 Jan. 20643284 [DOI: 10.1097/MCA.0000000000000302 nm]
- Kevin Haddad, Brian James Potter, Alexis Matteau, Francois Reeves, Guy Leclerc, Alain Rivard, Francois Gobeil, Denis-Claude Roy, Nicolas Noiseux, Samer Mansour. Analysis of the COMPARE-AMI trial: First report of long-term safetyof CD133+ cells. International Journal of Cardiology 15-06-2020;319:32-35. 20643285 [DOI: 10.1016/j.ijcard.2020.06.004]
- Mansour S, Noiseux N, Denis-Claude R, Francois G, Rivard A, Leclerc G. COMPARE-AMI trial: Comparison of intracoronary injection of CD133+ bone marrow stem cells to placebo in patients after acute myocardial infarction: safety and feasibility analysis. Circulation 2009;120(18 Suppl 2):S774. 20643286
- Mansour S, Roy D, Stevens LM, Leclerc G, Gobeil F, Revard A, et al. Comparison of intracoronary injection of CD133+ bone marrow stem cells to placebo in patients after acute myocardial infarction and left ventricular dysfunction: One year safety analysis of the COMPARE-AMI trial. [2010 Canadian Council of Cardiovascular Nurses Annual Scientific Sessions, Montreal, QC Canada. 23-26 October 2010]. Canadian Journal of Cardiology 2010;26:116D. 20643287
- Mansour S, Roy DC, Bouchard V, Nguyen BK, Stevens LM, Gobeil F, et al. COMPARE-AMI trial: comparison of intracoronary injection of CD133+ bone marrow stem cells to placebo in patients after acute myocardial infarction and left ventricular dysfunction: study rationale and design. Journal of Cardiovascular Translational Research 2010;3(2):153-9. 20643288
- Mansour S, Roy DC, Bouchard V, Stevens LM, Gobeil F, Rivard A, et al. One-year safety analysis of the COMPARE-AMI trial: comparison of intracoronary injection of CD133+ bone marrow stem cells to placebo in patients after acute myocardial infarction and left ventricular dysfunction. Bone Marrow Research 2011;2011:Article Number 385124. 20643289
- Mansour S, Roy DC, Lemieux B, Ouellet C, Stevens LM, Noiseux N. Stem cell therapy for the broken heart: mini-organ transplantation. Transplantation Proceedings 2009;41(8):3353-7. 20643290
- Qiu F, Maehara A, El Khoury R, Genereux P, LaSalle L, Mintz GS et al. mpact of intracoronary injection of CD133+ bone marrow stem cells on coronary atherosclerotic progression in patients with STEMI: A COMPARE-AMI IVUS Substudy. [26th Annual Symposium Transcatheter Cardiovascular Therapeutics, TCT 2014, Washington, DC United States. 13-17 September 2014]. Journal of the American College of Cardiology 2014;64(11 Suppl 1):B46. 20643291

Hirsch 2011 {published data only}
20643292
20643293
- Afsharzada F, Nijveldt R, Hirsch A, van Der Vleuten PA, Beek AM, Tijssen JGP, et al. Myocardial perfusion after intracoronary infusion of mononuclear cells of bone marrow or peripheral blood after acute myocardial infarction. [American College of Cardiology's 59th Annual Scientific Session and i2 Summit: Innovation in Intervention, Atlanta, GA United States, 14-16 March 2010]. Journal of American College of Cardiology 2010;55(10 Suppl 1):A110.e1024. 20643294
- Delewi R, van der Laan A, Robbers L, Hirsch A, Nijveldt R, van der Vleuten P, et al. Intracoronary infusion of mononuclear cells compared with standard therapy after acute myocardial infarction: 2 year magnetic resonance imaging results of the randomized controlled HEBE trial. [24th Annual Symposium of the Transcatheter Cardiovascular Therapeutics, TCT 2012, Miami, FL United States, 22-26 October 2012]. Journal of the American College of Cardiology 2012;60:B149. 20643295

- Delewi R, van der Laan AM, Robbers L, Hirsch A, Nijveldt R, Tijssen JGP, et al. Long-term follow-up after intracoronary infusion of mononuclear cells from bone marrow or peripheral blood after acute myocardial infarction: 5 year results of the randomized controlled HEBE trial. [European Society of Cardiology, ESC Congress 2014, Barcelona, Spain. 30 August - 3 September 2014]. European Heart Journal 2014;35:364. 20643296
- Delewi R, van der Laan AM, Robbers LF, Hirsch A, Nijveldt R, van der Vleuten PA, et al. Long term outcome after mononuclear bone marrow or peripheral blood cells infusion after myocardial infarction. Heart 2015;101(5):363-8. 20643297
- Hirsch, A and Nijveldt, R and Van Der Vleuten, P A and Piek, J J and Zijlstra, F and Biemond, B J and Doevendans, P and Van Rossum, A C and Tijssen, J G P. HEBE: multicentre, randomised trial of intracoronary infusion of autologous mononuclear bone blood marrow cells or peripheral mononuclear blood cells after primary PCI. Netherlands Heart Journal 2006. 20643298
- Hirsch A, Nijveldt R, van der Vleuten PA, Biemond BJ, Doevendans PA, van Rossum AC, et al. Intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary percutaneous coronary intervention: rationale and design of the HEBE trial - a prospective, multicenter, randomized trial. American Heart Journal 2006;152:434-41. 20643299
- Hirsch A, Nijveldt R, van der Vleuten PA, Tijssen JGP, van der Giessen WJ, Tio RA, et al. Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial. European Heart Journal 2011;32(14):1736-47. 20643300
- Hirsch A, Nijveldt R, van der Vleuten PA, Tio RA, van der Giessen WJ, Marques KM, et al. Intracoronary infusion of autologous mononuclear bone marrow cells in patients with acute myocardial infarction treated with primary PCI: Pilot study of the multicenter HEBE trial. Catheterization & Cardiovascular Interventions 2008;71(3):273-81. 20643301
- Hirsch A, Nijveldt R, van der Vleuten PA. Multicenter, randomised trial of intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary PCI. Netherlands Heart Journal 2005;13(10):377. 20643302
- Robbers LF, Nijveldt R, Beek AM, Hirsch A, van der Laan AM, Delewi R, et al. Cell therapy in reperfused acute myocardial infarction does not improve the recovery of perfusion in the infarcted myocardium: a cardiac MR imaging study. Radiology 2014;272(1):113-22. 20643303
- Robbers LF, Nijveldt R, Beek AM, Kemme MJ, Delewi R, Hirsch A, et al. Intracoronary infusion of mononuclear cells after PCItreated myocardial infarction and arrhythmogenesis: is it safe? Netherlands Heart Journal 2012;20(3):133-7. 20643304
- Robbers LFH, Delewi R, van Beurden Y, Nijveldt R, Beek AM, Kemme MJB, et al. Intracoronary infusion of mononuclear stem cells after acute revascularized myocardial infarction is not associated with increased prevalence of late ventricular arrhythmias. [European Society of Cardiology, ESC Congress 2011, Paris, France, 27-31 August 2011]. European Heart Journal 2011;32:735. 20643305
- Robbers LFHJ, Nijveldt R, van der Laan AM, Delewi R, Hirsch A, van der Vleuten PA, et al. Intracoronary infusion of bone marrow cells and peripheral mononuclear blood cells has no influence on the recovery of myocardial perfusion after acute revascularized myocardial infarction. [European Society of Cardiology, ESC Congress 2011, Paris, France, 27-31 August 2011]. European Heart Journal 2011;32:155. 20643306
- van der Laan A, Hirsch A, Nijveldt R, van der Vleuten PA, van der Giessen WJ, Doevendans PA, et al. Bone marrow cell therapy after acute myocardial infarction: the HEBE trial in perspective, first results. Netherlands Heart Journal 2008;16(12):436-9. 20643307
- van der Laan AM, Hirsch A, Haeck JD, Nijveldt R, Delewi R, Biemond BJ, et al. Recovery of microcirculation after intracoronary infusion of bone marrow mononuclear cells or peripheral blood mononuclear cells in patients treated by primary percutaneous coronary intervention the Doppler substudy of the Hebe trial. JACC: Cardiovascular interventions 2011;4(8):913-20. 20643308
- van der Laan AM, van der Vleuten PA, Hirsch A, Nijveldt R, van der Giessen WJ, Biemond BJ, et al. Intracoronary infusion of mononuclear cells potentially prevents post-infarct ventricular remodeling in patients with an initial dilated left ventricle: A HEBE substudy. [European Society of Cardiology, ESC Congress 2009, Barcelona, Spain, 29 August - 2 September 2009]. European Heart Journal 2009;30:505. 20643309

Huang 2006 {published data only}
20643310
- Huang RC, Yao K, Zou YZ, Ge L, Qian JY, Yang J, et al. Long term follow-up of emergent intracoronary autologous

bone marrow mononuclear cell transplantation for acute inferior-wall myocardial infarction [Chinese]. Zhonghua Yi Xue Za Zhi [Journal of the Chinese Medical Association] 2006;86(16):1107-11. 20643311

Huang 2007 {published data only}
20643312
- Huang RC, Yao K, Qian JY, Niu YH, Ge L, Chen SG, et al. Evaluation of myocardial viability with 201T1/18F-FDG DISA SPECT technique in patients with acute myocardial infarction after emergent intracoronary autologous bone marrow mononuclear cell transplantation [Chinese]. Zhonghua Xin Xue Guan Bing Za Zhi [Chinese Journal of Cardiovascular Diseases] 2007;35(6):500- 3. 20643313

Huikuri 2008 {published data only}
20643314
- Huikuri HV, Kervinen K, Niemela M, Ylitalo K, Saily M, Koistinen P, et al. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile and restenosis after thrombolytic therapy of acute myocardial infarction. European Heart Journal 2008;29(22):2723-32. 20643315
- Huikuri HV. Efficacy and safety of intracoronary injection of mononuclear BMC after thrombolytic therapy of acute myocardial infarction: IM-BMC Study. Clinical Research in Cardiology 2008;97(1):10. 20643316
- Miettinen J, Ylitalo K, Hedberg P, Saily M, Koistinen P, Airaksinen KEJ, et al. Effects of intracoronary injection of autologous bone marrow derived stem cells on natriuretic peptides and inflammatory markers in patients with acute ST-elevation myocardial infarction. [European Society of Cardiology, ESC Congress 2010, Stockholm, Sweden, 28 August - 1 September 2010]. European Heart Journal 2010;31:307-8. 20643317
- Miettinen JA, Ylitalo K, Hedberg P, Jokelainen J, Kervinen K, Niemela M, et al. Determinants of functional recovery after myocardial infarction of patients treated with bone marrow-derived stem cells after thrombolytic therapy. Heart 2010;96(5):362- 67. 20643318
- Miettinen JA, Ylitalo K, Hedberg P, Kervinen K, Niemela M, Saily M, et al. Effects of intracoronary injection of autologous bone marrow-derived stem cells on natriuretic peptides and inflammatory markers in patients with acute ST-elevation myocardial infarction. Clinical Research in Cardiology 2011;100(4):317-25. 20643319
- NCT00363324. Bone marrow cells in myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00363324 (accessed 11 March 2015). 20643320
- Perkiomaki J, Luokkala J, Makikallio T, Ylitalo K, Huikuri H. Electrocardiographic risk markers in relation to intracoronary bone marrow cell therapy for acute myocardial infarction. [Venice Arrhythmias 2011, Venice, Italy, 9-12 October 2011]. Journal of Cardiovascular Electrophysiology 2011;22:S66. 20643321

Janssens 2006 {published data only}
20643322
- Herbots L, D'hooge J, Eroglu E, Thijs D, Ganame J, Claus P, et al. Improved regional function after autologous bone marrowderived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study. European Heart Journal 2009;30(6):662-70. 20643323
- Janssens S, Bogaert J, Dubois C, van Cllemput P, Theunissen K, Kalantzis M, et al. Long-term safety and efficacy of autologous bone marrow-derived stem cell transfer after acute myocardial infarction. Circulation 2006;114(18 Suppl):II_515. 20643324
- Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 2006;367(9505):113-21. 20643325
- NCT00264316. Bone marrow-derived stem cell transfer in acute myocardial infarctions. https://clinicaltrials.gov/ct2/show/NCT00264316 (accessed 11 March 2015). 20643326
- Ruwende C. Autologous bone marrow derived stem cell transfer in patients with ST-segment elevation myocardial infarction: double blind randomised controlled trial. ACC Cardiosource Review Journal 2006;15(3):28. 20643327

Jazi 2012 {published data only}
20643328
- Jazi SM, Esfahani MH, Fesharaki M, Moulavi F, Gharipour M. Initial clinical outcomes of intracoronary infusion of autologous progenitor cells in patients with acute myocardial infarction. ARYA Atherosclerosis 2012;7(4):162-7. 20643329

Jin 2008 {published data only}
20643330
- Jin B, Yang YG, Shi HM, Luo XP, Li Y, Sun YL, et al. Autologous intracoronary mononuclear bone marrow cell transplantation for acute anterior myocardial infarction: Outcomes after 12-month follow-up. Journal of Clinical Rehabilitative Tissue Engineering Research 2008;12(12):2267-71. 20643331

Karpov 2005 {published data only}
20643332
- Karpov RS, Popov SV, Markov VA, Suslova TE, Ryabov VV, Yu S, et al. Autologous mononuclear bone marrow cells during reparative regeneration after acute myocardial infarction. Bulletin of Experimental Biology and Medicine 2005;1(4):640-3. 20643333
- Ryabov V, Kirgizova M, Suslova T, Markov V, Karpov R. Long-term results of autologous bone marrow mononuclear cell transplantation in STEMI patients. [Heart Failure Congress 2014 and the 1st World Congress on Acute Heart Failure, Athens, Greece, 17-20 May 2014]. European Journal of Heart Failure 2014;16:352. 20643334
- Ryabov VV, Alexandrovna KM. Long-term results after transplantation of autologous bone marrow mononuclear cells to patients with acute myocardial infarction. Koncept 2014;20:4786-90. 20643335
- Ryabov VV, Suslova TE, Krylov AL, Poponina YS, Vesnina ZV, Sazonove Sl, et al. Cardiomyoplasty with autological mononuclear cells of the bone marrow in patients with acute myocardial infarction. Terapevticheskii Arkhiv 2006;78(8):47-52. 20643336
- Vyacheslav V Ryabov , Marina A Kirgizova, Tatiana E Suslova, Sergei I Karas, Valentin A Markov, Rostislav S Karpov. Relationships of growth factors, proinflammatory cytokines, and anti-inflammatory cytokines with long-term clinical results of autologous bone marrow mononuclear cell transplantation in STEMI. PLoS One 30 May 2017. 20643337 [DOI: 10.1371/journal.pone.0176900] [PMID: 28558042]

Kim 2018 {published data only}
20643338
- Su Hyun Kim, Jang Hyun Cho, Yoon Ho Lee, Ji Hye Lee, Soo Sung Kim, Mi Yang Kim, Min Gu Lee, Won Yu Kang, Kyung Sim Lee, Young Keun Ahn, Myung Ho Jeong, Hyun Soo Kim. Improvement in Left Ventricular Function with IntracoronaryMesenchymal Stem Cell Therapy in a Patient with Anterior WallST-Segment Elevation Myocardial Infarction. Cardiovacular Drugs and Therapy June 28, 2018;32:329-338. 20643339 [DOI: 10.1007/s10557-018-6804-z]

Kirgizova 2015 {published data only}
20643340[DOI: 10.1371/journal.pone.0176900]
https://www.clinicaltrials.gov/ct2/show/NCT01748383. 20643341 [CLINICALTRAILS.GOV: NCT01748383]
- M A Kirgizova, T E Suslova, V A Markov, R S Karpov, and V V Ryabov. Long-term clinical results of autologous bone marrow CD 133+ cell transplantation in patients with ST-elevation myocardial infarction. AIP Conference Proceedings 23-11-2015;1688. 20643342 [DOI: 10.1063/1.4936060]

Lee 2014 {published data only}
20643343
- Lee JW, Ahn SG, Kim JY, Yoo BS, Lee SH, Yoon J, et al. A randomized, open-labeled, multicenter trial for safety and efficacy of intracoronary adult human mesenchymal stem cells after acute myocardial infarction. [60th Annual Scientific Session of the American College of Cardiology and i2 Summit: Innovation in Intervention, ACC.11, New Orleans, LA United States, 2-5 April 2011]. Journal of the American College of Cardiology 2011;57(14 Suppl 1):E982. 20643344
- Lee JW, Lee SH, Youn YJ, Ahn MS, Kim JY, Yoo BS, et al. A randomized, open-label, multicenter trial for the safety and efficacy of adult mesenchymal stem cells after acute myocardial infarction. Journal of Korean Medical Science 2014;29(1):23-31. 20643345
- NCT01392105. Safety and efficacy of intracoronary adult human mesenchymal stem cells after acute myocardial infarction (SEEDMSC). https://clinicaltrials.gov/ct2/show/NCT01392105 (accessed 11 March 2015). 20643346

Lunde 2006 {published data only}
20643347

- Beitnes JO, Gjesdal O, Lunde K, Solheim S, Edvardsen T, Arnesen H, et al. Left ventricular systolic and diastolic function improve after acute myocardial infarction treated with acute percutaneous coronary intervention, but are not influenced by intracoronary injection of autologous mononuclear bone marrow cells: a 3-year serial echocardiographic sub-study of the randomized-controlled ASTAMI study. European Journal of Echocardiography 2011;12(2):98-106. 20643348
- Beitnes JO, Hopp E, Lunde K, Solheim S, Arnesen H, Brinchmann JE, et al. Long-term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: the ASTAMI randomised, controlled study. Heart 2009;95(24):1983-9. 20643349
- Beitnes JO, Hopp E, Lunde K, Solheim S, Arnesen H, Forfang K, et al. Long term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: The ASTAMI study. [European Society of Cardiology, ESC Congress 2009, Barcelona, Spain, 29 August - 2 September 2009]. European Heart Journal 2009;30:912. 20643350
- Beitnes JO, Lunde K, Gjesdal O, Solheim S, Edvardsen Y, Arnesen H, et al. Regional and diastolic function improves after acute myocardial infarction treated with acute PCI, but is not influenced by injection of autologous mononuclear bone marrow cells: an ASTAMI sub-study. [European Society of Cardiology, ESC Congress 2010, Stockholm, Sweden, 28 August - 1 September 2010]. European Heart Journal 2010;31:323. 20643351
- Furenes EB, Opstad TB, Solheim S, Lunde K, Arnesen H, Seljeflot I. The influence of autologous bone marrow stem cell transplantation on matric metalloproteinases in patients treated for acute ST-elevation myocardial infarction. Mediators of Inflammation 2014;2014:285901. 20643352
- Grogaard HK, Seljeflot I, Lunde K, Solheim S, Aakhus S, Forfang K, et al. Cell treatment after acute myocardial infarction prevents early decline in circulating IGF-1. Scandinavian Cardiovascular Journal 2010;44(5):267-72. 20643353
- Hopp E, Lunder K, Solheim S, Aakhus S, Arnesen H, Forfang K, et al. Regional myocardial function after intracoronary bone marrow cell injection in reperfused anterior wall infarction - a cardiovascular magnetic resonance tagging study. Journal of Cardiovascular Magnetic Resonance 2011;13:22. 20643354
- Lunde K, Aakhus S. Intracoronary injection of mononuclear bone marrow cells after acute myocardial infarction: lessons from the ASTAMI trial. European Heart Journal Supplements 2018;10:K35-8. 20643355
- Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Forfang K, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. New England Journal of Medicine 2006;355(12):1199-209. 20643356
- Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Forfang K. Autologous stem cell transplantation in acute myocardial infarction: the ASTAMI randomized controlled trial. Intracoronary transplantation of autologous mononuclear bone marrow cells, study design and safety aspects. Scandinavian Cardiovascular Journal 2005;39:150-8. 20643357
- Lunde K, Solheim S, Aakhus S, Arnesen H, Moum T, Abdelnoor M, et al. Exercise capacity and quality of life after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: results from the Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI) randomized controlled trial. American Heart Journal 2007;154(4):710 e1-8. 20643358
- Lunde K, Solheim S, Forfang K, Arnesen H, Brinch L, Bjornerheim R, et al. Anterior myocardial infarction with acute percutaneous coronary intervention and intracoronary injection of autologous mononuclear bone marrow cells: safety. clinical outcome, and serial changes in left ventricular function during 12-months' follow-up. Journal of the American College of Cardiology 2008;51(6):674-6. 20643359
- NCT00199823. Autologous stem cell transplantation in acute myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00199823 (accessed 11 March 2015). 20643360
- Solheim S, Seljeflot I, Lunde K, Bratseth V, Aakhus S, Forfang K, et al. The influence of intracoronary injection of bone marrow cells on prothrombotic markers in patients with acute myocardial infarction. Thrombosis Research 2012;130(5):765-8. 20643361

Mathur 2020 {published data only}
20643362
- Anthony Mathur, Francisco Fernandez-Aviles, Jozef Bartunek, Ann Belmans, Filippo Crea, Sheik Dowlut, Manuel Galinanes, Marie-Claire Good, Juha Hartikainen, Christine Hauskeller, Stefan Janssens, Petr Kala, Jens Kastrup, John Martin, Philippe Menasche, Ricardo Sanz-Ruiz, Seppo Yla-Herttuala, andAndreas Zeiher, On behalf of the BAMI Group. The effect of intracoronary infusion of bonemarrow-derivedmononuclear cells on all-causemortality in acutemyocardial infarction: theBAMI trial. European Heart Journal Aug 30th 2020;41:3702-

3710. 20643363 [DOI: 10.1093/eurheartj/ehaa651]
- Mathur, Anthony and Arnold, Roman and Assmus, Birgit and Bartunek, Jozef and Belmans, Ann and Bonig, Halvard and Crea, Filippo and Dimmeler, Stefanie and Dowlut, Sheik and Fernandez □ Aviles, Francisco and Galianes, Manuel and Garcia □ Dorado, David and Hartikainen, Juha and Hill, Jonathan and Hogardt □ Noll, Annette and Homsy, Christian and Janssens, Stefan and Kala, Petr and Kastrup, Jens and Martin, John. The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: rationale and design of the BAMI trial. European Journal of Heart Failure Sep 2017;11:1545--1550. 20643364 [DOI: 10.1002/ejhf.829] [PMID: 28948706]
- Nct. BAMI. The Effect of Intracoronary Reinfusion of Bone Marrow-derived Mononuclear Cells(BM-MNC) on All Cause Mortality in Acute Myocardial Infarction. https://clinicaltrials.gov/show/NCT01569178. 20643365 [NCT:2012: https://clinicaltrials.gov/show/NCT01569178]

Meluzin 2008 {published data only}
20643366
- Jaroslav Meluzin, Stanislav Janousek, Jiri Mayer, Ladislav Groch, Jan Sitar, Miloslav Tejc, Petr Kala, Roman Panovsky, Milan Kaminek, Martin Klabusay, Zdenek Koristek, Ladislav Dusek. Autologous transplantationof mononuclear bone marrow cells in patientswith chronic myocardial infarction. Cor Vasa 2007. 20643367 [DOI: 10.33678/cor.2007.021]
- Kaminek M, Meluzin J, Panovsky R, Janousek S, Mayer J, Prasek J, et al. Individual differences in the effectiveness of intracoronary bone marrow cell transplantation assessed by gatted sestamibi SPECT/FDG PET imaging. Journal of Nuclear Cardiology 2008;15(3):392-9. 20643368
- Kaminek M, Meluzin J, Panovsky R, Metelkova I, Budikova M, Richter M. Long-term results of intracoronary bone marrow cell transplantation: the potential of gated SPECT/FDG PET imaging to select patients with maximum benefit from cell therapy. Clinical Nuclear Medicine 2010;35(10):780-7. 20643369
- Klabusay M, Navratil M, Koristek Z, Groch L, Meluzin J, Mayer J. Implantation of autologous bone marrow mononuclear cells in patients after acute myocardial infarction via coronary artery. Blood 2005;106(11):Abstract 4220. 20643370
- Meluzin J, Janousek S, Mayer J, Groch L, Hornacek I, Hlinomaz O, et al. Three-, 6- and 12-month results of autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction. International Journal of Cardiology 2008;128(2):185-92. 20643371
- Meluzin J, Mayer J, Groch L, Kanousek S, Hornacek I, Hinomaz O, et al. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: the effect of the dose of transplanted cells on myocardial function. American Heart Journal 2006;152(5):975.e9-15. 20643372
- Panovsky R, Meluzin J, Janousek S, Mayer J, Kaminek M, Groch L, et al. Cell therapy in patients with left ventricular dysfunction due to myocardial infarction. Echocardiography 2008;25(8):888-97. 20643373

Nair 2015 {unpublished data only}
20643374[DOI: 10.4103/0971-5916.164245]
- CTRI/2008/091/000232. Efficacy of stem cell in improvement of left ventricular function in patients with acute myocardial infarction. http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=224 (accessed 11 March 2015). 20643375
- Velu Nair, Hemant Madan, Sunil Sofat, Prosenjit Ganguli, MJ Jacob, Rajat Datta, Prashant Bharadwaj, RS Sarkar, AJ Pandit, Soniya Nityanand, Pravin K Goel, Naveen Garg, Sanjay Gambhir, Paul V George, Sunil Chandy, Vikram Mathews, Oomen K George, KK Talwar, Ajay Bahl, Neelam Marwah, Anish Bhatacharya, Balram Bhargava, Balram Airan, Sujata Mohanty, Chetan D Patel, Alka Sharma, Shinjini Bhatnagar, A Mondal, Jacob Jose & A Srivastava, for MI3 Trial. Efficacy of stem cell in improvement of left ventricular function in acute myocardial infarction - MI3 Trial. Indian Journal of Medical Research August 2015;142:165-74. 20643376 [DOI: 10.4103/0971-5916.164245] [PMID: 26354213]

Naseri 2018 {published data only}
20643377
- Mohammad Hassan Naseri, Hoda Madani, Seyed Hossein Ahmadi Tafti, Maryam Moshkani Farahani, Davood Kazemi Saleh, Hossein Hosseinnejad, Saeid Hosseini, Sepideh Hekmat, Zargham Hossein Ahmadi, Majid Dehghani, Alireza Saadat, Soura Mardpour, Seyedeh Esmat Hosseini, Maryam Esmaeilzadeh, Hakimeh Sadeghian, Gholamreza Bahoush, Ali Bassi, Ahmad Amin, Roghayeh Fazeli, Yaser Sharafi, Leila Arab, Mansour Movahhed, Saeid Davaran, Narges Ramezanzadeh, Azam Kouhkan, Ali Hezavehei, Mehrnaz Namiri, Fahimeh

Kashfi, Ali Akhlaghi, Fattah Sotoodehnejadnematalahi, Ahmad Vosough Dizaji, Hamid Gourabi, Naeema Syedi, Abdolhosein Shahverdi, Hossein Baharvand, Nasser Aghdami. COMPARE CPM-RMI Trial: Intramyocardial Transplantation Of Autologous Bone Marrow-Derived CD133+ Cells And MNCs During CABG In Patients With Recent MI: A Phase II/III, Multicenter, Placebo-Controlled, Randomized, Double-Blind Clinical Trial. Cell Journal 18-5-2018:267-277. 20643378 [DOI: 10.22074/cellj.2018.5197] [PMID: 29845801]

- NCT01167751. https://clinicaltrials.gov/ct2/show/NCT01167751. 20643379

Nogueira 2009 {published data only}
20643380

- Dohmann HF, Silva SA, Sousa AL, Graga AM, Branco RV, Haddad AF, et al. Multicenter double blind trial of autologous bone marrow mononuclear cell transplantation through intracoronary injection post acute myocardium infarction- MiHeart/AMI study. Trials 2008;9:41. 20643381
- Moreira RC, Haddad AF, Silva SA, Souza AL, Tuche FA, Oliveira MA, et al. Intracoronary stem-cell injection after myocardial infarction: microcirculation sub-study. Arquivos Brasileiros de Cardiologia 2011;97(5):420-6. 20643382
- NCT00350766. Cell therapy in myocardial infarction (EMRTCC). https://clinicaltrials.gov/ct2/show/NCT00350766 (accessed 11 March 2015). 20643383
- Nogueira FB, Silva SA, Haddad AF, Peixoto CM, Carvalho RM, Tuche FA, et al. Systolic function of patients with myocardial infarction undergoing autologous bone marrow transplantation. Arquivos Brasileiros de Cardiologia 2009;93(4):374-9. 20643384
- Silva SA, Sousa Al, Haddad AF, Azevedo JC, Soares VE, Peixoto CM, et al. Autologous bone marrow mononuclear cell transplantation after acute myocardial infarction: comparison of two delivery techniques. Cell Transplantation 2009;18(3):343- 52. 20643385

Penicka 2007 {published data only}
20643386

- Horak J, Penicka M, Kobylka P, Ascheremann M, Linhart A, Skalicka H et al. Effectivity and safety of intracoronary autologous bone marrow stem cell transplantation in large anterior myocardial infarction - a randomized study. Journal of the American College of Cardiology 2007;49(9 (Suppl A)):201A. 20643387
- Penicka M, Horak J, Kobylka P, Pytlik R, Kozak T, Belohlavek O, et al. Intracoronary injection of autologous bone marrow-derived mononuclear cells in patients with large anterior acute myocardial infarction. A prematurely terminated randomised study. Journal of the American College of Cardiology 2007;49(24):2373-4. 20643388
- Penicka M. Intracoronary stem cells in large myocardial infarction. www.ClinicalTrials.gov. 2006 (accessed by 31 January 2011). 20643389 [NCT00389545]
- Skalicka H, Horak J, Kobylka P, Palecek T, Linhart A, Aschermann M. Intracoronary injection of autologous bone marrow-derived mononuclear cells in patients with large anterior acute myocardial infarction and left ventricular dysfunction: a 24-month follow up study. Bratislavske Lekarske Listy 2012;113(4):220-7. 20643390

Piepoli 2010 {published data only}
20643391

- Malagoli A, Piepoli M, Armentano C, Vallisa D, Arbasi M, Rossi A, et al. Acute myocardial infarction: long term echocardiographic remodeling after bone marrow cell transplantation. [ESC Congress 2010, Stockholm, Sweden. 28 August - 1 September 2010]. European Heart Journal 2010;31:735. 20643392
- Malagoli A, Piepoli MF, Armentano C, Vallisa D, Arbasi MC, Rossi A, et al. Bone marrow cell transplantation in patients after acute myocardial infarction and left ventricular dysfunction: long term effects on heart function and remodeling. [Heart Failure 2010 Congress, Berlin, Germany. 29 May - 1 June 2010]. In: European Journal of Heart Failure. Vol. 9. 2010:S97-8. 20643393
- NCT00437710. Safety and efficacy of bone marrow cell transplantation in humans myocardial infarction (CARDIAC). https://clinicaltrials.gov/ct2/show/NCT00437710 (accessed 11 March 2015). 20643394
- Piepoli MF, Vallisa D, Arbasi C, Cavanna L, Cerri L, Mori M, Passerini F, et al. 2 year follow-up results of the CARDIAC (CARDIomyoplasty by Autologous intraCoronary bone marrow in acute myocardial infarction) randomised controlled trial. International Journal of Cardiology 2013;168(5):e132. 20643395
- Piepoli MF, Vallisa D, Arbasi M, Cavanna L, Cerri L, Mori M, et al. Bone marrow cell transplantation improves cardiac, autonomic and functional indexes in acute anterior myocardial infarction patients (Cardiac study). European Journal of Heart Failure 2010;12(2):172-80. 20643396

- Vallisa D, Piepoli M, Cavanna L, Arbasi C, Moroni C, Lazzaro A, et al. Autologous bone marrow stem cell support in acute anterior myocardial infarction. [49th Annual Meeting of the American Society of Hematology, Atlanta, Georgia. 8-11 December 2007]. Blood 2007;110(11):1078A-9A. 20643397

Plewka 2009 {published data only}
20643398
- Lipiec P, Kreminska-Paluka M, Plewka M, Kusmierek J, Plachcinska A, Szuminski R, et al. Impact of intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction on left ventricular perfusion and function: a 6-month follow-up gated 99mTc-MiBi single-photon emission computed tomography study. European Journal of Nuclear Medicine and Molecular Imaging 2009;36(4):587-93. 20643399
- Plewka M, Krzeminska-Pakula M, Lipiec P, Peruga JZ, Jezewski T, Kidawa M, et al. Clinical 2-years outcome after intracoronary injection of mononuclear bone marrow stem cells in patients with acute myocardial infarction. [European Society of Cardiology, ESC Congress 2010, Stockholm, Sweden. 28 August - 1 September 2010]. European Heart Journal 2010;31:1045-6. 20643400
- Plewka M, Krzeminska-Pakula M, Lipiec P, Peruga JZ, KJezewski T, Kidawa M, et al. Effect of intracoronary injection of mononuclear bone marrow stem cels on left ventricular function in patients with acute myocardial infarction. American Journal of Cardiology 2009;104(10):1336-42. 20643401
- Plewka M, Krzeminska-Pakula M, Peruga JZ, Lipiec P, Kurpesa M, Wierzbowska-Drabik K, et al. The effects of intracoronary delivery of mononuclear bone marrow cells in patients with myocardial infarction: a two year follow-up results. Kardiologia Polska 2011;69(12):1234-40. 20643402

Quyyumi 2011 {published data only}
20643403
- Bhatti S, Hakeem A, Taylor M, Chung E, Quyyumi AA, Oshinski J et al. MRI strain analysis as a novel modality for the assessment of myocardial function following stem cell therapy - results from Amorcyte trial. [SCMR/ Euro CMR Joint Scientific Sessions, Nice, France. 2-6 February 2011]. Journal of Cardiovascular Magnetic Resonance 2011;13:2011. 20643404
- NCT00313339. Intra-coronary infusion of bone marrow derived autologous CD34+ selected cells in patients with acute myocardial infarction (AMR-1). http://clinicaltrials.gov/ct2/show/NCT00313339 (accessed 11 March 2015). 20643405 [NCT00313339]
- Quyyumi AA, Murrow JR, Esteves F, Galt J, Oshinski J, Lerakis S, et al. CD34+ infusion after ST elevation myocardial infarction is associated with improved perfusion. Journal of American College of Cardiology 2009;53(10):A327. 20643406
- Quyyumi AA, Waller EK, Murrow J, Esteves F, Galt J, Oshinski J, et al. CD34+ cell infusion after ST-elevation myocardial infarction is associated with improved perfusion and is dose dependent. American Heart Journal 2011;161(1):98-105. 20643407

Quyyumi 2017 {published data only}
20643408
- Arshed A Quyyumi, Alejandro Vasquez, Dean J Kereiakes, Marc Klapholz, Gary L Schaer, Ahmed Abdel-Latif, Stephen Frohwein, Timothy D Henry, Richard A Schatz, Nabil Dib, Catalin Toma, Charles J Davidson, Gregory W Barsness, David M Shavelle, Martin Cohen, Joseph Poole, Thomas Moss, Pamela Hyde, Anna Maria Kanakaraj, Vitaly Druker, Amy Chung, Candice Junge, Robert A Preti, Robin L Smith, David J Mazzo, Andrew Pecora, Douglas W Losordo. A Randomized, Double-Blind, Placebo-Controlled Clinical Trialof Intracoronary Administration of Autologous CD34+ Cells inPatients With Left Ventricular Dysfunction Post STEMI. Circulation Research 7 November 2016;120:324-331. 20643409 [DOI: 10.1161/CIRCRESAHA.115.308165] [NCT: https://clinicaltrials.gov/show/NCT01495364] [PMID: 27821724]
- NCT01495364. NBS10 (also known as AMR-001) versus placebo post ST segment elevation myocardial infarction (PreSERVEAMI). https://clinicaltrials.gov/ct2/show/NCT01495364 (accessed 11 March 2015).. 20643410 [CLINICALTRIALS: NCT01495364]

Roncalli 2010 {published data only}
20643411
- Guillaume Lamirault, Elodie de Bock, Veronique Sebille, Beatrice Delasalle, Jerome Roncalli, Sophie Susen, Christophe Piot7, Jean-Noel Trochu, Emmanuel Teiger8, Yannick Neuder9, Thierry Le Tourneau, Alain Manrique Jean-Benoıt Hardouin, Patricia Lemarchand. Sustained quality of life improvement after

intracoronaryinjection of autologous bone marrow cells in the setting of acutemyocardial infarction: results from the BONAMI trial. Quality of Life Research July 20th 2016;26:121-125. 20643412 [DOI: 10.1007/s11136-016-1366-7]

- Lamirault G, de Bock E, Roncalli JH, van Belle E, Piot C, le Corvoisier P, et al. Sustained quality of life improvement after intracoronary injection of autologous bone marrow cells in the setting of acute myocardial infarction. [European Society of Cardiology, ESC Congress 2013, Amsterdam, Netherlands. 31 August - 4 September 2013]. European Heart Journal 2013;34:277-8. 20643413
- Le Tourneau T, Sportouch C, Foucher C, Delasalle B, Rosso J, Neuder Y, et al. Left ventricular morphological and mechanical remodeling after acute myocardial infarction in the BONAMI trial: An isotopic and echocardiographic study. [16th Annual Meeting of the European Association of Echocardiography, EUROECHO 2012, Athens, Greece. 5-8 December 2012]. European Heart Journal Cardiovascular Imaging 2012;13:i78-9. 20643414
- Manrique, Alain and Lemarchand, Patricia and Delasalle, Beatrice and Lairez, Olivier and Sportouch-Duckan, Catherine and Lamirault, Guillaume and Corvoisier, Philippe and Neuder, Yannick and Richardson, Marjorie and Lebon, Alain and Roncalli, Jerome and Piot, Christophe and Trochu, Jean-Noel and Teiger, Emmanuel and Hossein-Foucher, Claude and Tourneau, Thierry. Predictors of ventricular remodelling in patients with reperfused acute myocardial infarction and left ventricular dysfunction candidates for bone marrow cell therapy: insights from the BONAMI trial. European Journal of Nuclear Medicine & Molecular Imaging Dec 2015. 20643415 [DOI: 10.1007/s00259-015-3279-z] [PMID: 26666236]
- Mouquet F, Roncalli J, Piot C, Trochu JN, Le Corvoisier P, Neuder V, et al. Autologous bone marrow mononucleated cell infusion for acute myocardial infarction in patients with severe left ventricular dysfunction: Results of the BONAMI Trials (a multicenter randomized controlled trial). Circulation 2008;118(Suppl 18):S764. 20643416
- NCT00200707. BONAMI (BOne Marrow in Acute Myocardial Infarction). http://clinicaltrials.gov/ct2/show/NCT00200707 (accessed 11 March 2015). 20643417 [NCT00200707]
- Roncalli J, Mouquet F, Piot C, Trochu JN, Le Corvoisier P, Neuder Y, et al. Intracoronary autologous mononucleated bone marrow cell infusion for acute myocardial infarction: results of the randomized multicenter BONAMI trial. European Heart Journal 2011;32(14):1748-57. 20643418

Ruan 2005 {published data only}
20643419

- Ruan W, Pan CZ, Huang GQ, Li YL, Ge JB, Shu XH, et al. Assessment of left ventricular segmental function after autologous bone marrow stem cells transplantation in patients with acute myocardial infarction by tissue tracking and strain imaging. Chinese Medical Journal 2005;118(14):1175-81. 20643420

San Roman 2015 (BMMNC) {published data only}
20643421

- NCT00984178. Trial of hematopoietic stem cells in acute myocardial infarction. http://clinicaltrials.gov/ct2/show/NCT00984178 (accessed 11 March 2015).. 20643422
- Sanchez Fernandez PL, San Roman JA, Villa A, Gimeno F, Arnold R, Sanz-Ruiz R, et al. A multicenter, prospective, randomized, open-labeled, trial comparing different bone-marrow-derived stem cell approaches in patients with reperfused ST-elevation myocardial infarction. [European Society of Cardiology, ESC Congress 2012, Munich, Germany. 25-29 August 2012].. European Heart Journal 2012;33:516.. 20643423
- San Roman, J Alberto, Sánchez, Pedro L, Villa, Adolfo, Sanz-Ruiz, Ricardo, Fernandez-Santos, María Eugenia, Gimeno, Federico, Ramos, Benigno, Arnold, Roman, Serrador, Ana, Gutiérrez, Hipólito, Martin-Herrero, Francisco, Rollán, María Jesús, Fernández-Vázquez, Felipe, López-Messa, Juan, Ancillo, Pablo, Pérez-Ojeda, German, Fernández-Avilés, Francisco. Comparison of Different Bone Marrow-Derived Stem Cell Approaches in Reperfused STEMI. A Multicenter, Prospective, Randomized, Open-Labeled TECAM Trial. JACC June 2015;22:2372-2382. 20643424 [DOI: 10.1016/j.jacc.2015.03.563] [PMID: 26046730]

San Roman 2015 (BMMNC+G-CSF) {published data only}
20643425[CTG: NCT00984178]

- NCT00984178. Trial of hematopoietic stem cells in acute myocardial infarction. http://clinicaltrials.gov/ct2/show/NCT00984178 (accessed 11 March 2015). 20643426
- San Roman, J Alberto, Sánchez, Pedro L, Villa, Adolfo, Sanz-Ruiz, Ricardo, Fernandez-Santos, María Eugenia, Gimeno, Federico, Ramos, Benigno, Arnold, Roman, Serrador, Ana, Gutiérrez, Hipólito, Martin-Herrero,

Francisco, Rollán, María Jesús, Fernández-Vázquez, Felipe, López-Messa, Juan, Ancillo, Pablo, Pérez-Ojeda, German, Fernández-Avilés, Francisco. Comparison of Different Bone Marrow-Derived Stem Cell Approaches in Reperfused STEMI. A Multicenter, Prospective, Randomized, Open-Labeled TECAM Trial. JACC June 2015;22:2372-2382. 20643427 [DOI: 10.1016/j.jacc.2015.03.563] [PMID: 26046730]

Schachinger 2006 {published data only}
20643428

- Assmus B, Leistner DM, Schachinger V, Erbs S, Elsasser A, Haberbosch W, et al. Long-term clinical outcome after intracoronary application of bone marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival. European Heart Journal 2014;35(19):1275-83. 20643429
- Assmus B, Rolf A, Erbs A, Elsasser A, Haberbosch W, Hambrecht R, et al. Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circulation: Heart Failure 2010;3(1):89- 96. 20643430
- Assmus B, Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, et al. Cell functionality of administered autologous bone marrow-derived mononuclear cells predict 5-year clinical outcome in patients with acute myocardial infarction - Results of the REPAIR-AMI trial. [American Heart Association 2012 Scientific Sessions and Resuscitation Science Symposium, Los Angeles, CA United States. 3-6 November 2012]. Circulation 2012;126(21 (Suppl 1)). 20643431
- Assmus B, Tonn T, Seeger FH, Yoo C-H, Leistner D, Klotsche J, et al. Red blood cell contamination of the final cell product impairs the efficacy of autologous bone marrow mononuclear cell therapy. Journal of American College of Cardiology 2010;55(13):1385-94. 20643432
- Dill T, Schachinger V, Rolf A, Mollmann S, Thiele H, Tillmanns H, et al. Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk of adverse remodelling after acute ST-segment elevation myocardial infarction: Results of the reinfusion of enriched progenitor cells and infarct remodelling in acute myocardial infarction study (REPAIR-AMI) cardiac magnetic resonance imaging study. American Heart Journal 2009;157(3):541-7. 20643433
- Erbs S, Linke A, Assmus B, Hoffmann C, Schachinger V, Schuler G, et al. Restoration of coronary microvascular function in the infarct-related artery by bone marrow-derived progenitor cells after acute MI: Results from the double-blind, placebo-controlled REPAIR-AMI trial. European Heart Journal 2006;27(Suppl 1):151. Meeting Abstract 923. 20643434
- Erbs S, Linke A, Assmus B, Hoffmann C, Schachinger V, Tonn T, et al. Normalization of coronary microvascular function in the infarct artery by bone marrow-derived progenitor cells after acute myocardial infarction: results from the double-blind, placebocontrolled REPAIR-AMI trial. Circulation 2006;114(18 Suppl):856-7. 20643435
- Erbs S, Linke A, Schachinger V, Assmus B, Thiele H, Diederich KW, et al. Restoration of microvascular function in the infarctrelated artery by intracoronary transplantation of bone marrow progenitor cells in patients with acute myocardial infarction: the Doppler Substudy of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial. Circulation 2007;116(4):366-74. 20643436
- Konorza TFM. Repair AMI late: a prospective multicenter randomised placebo-controlled trial of autologous bone marrow-derived progenitor cells in patients with acute myocardial infarction. Herz 2007;32(2):169. 20643437
- Leistner D, Assmus B, Erbs S, Hambrecht R, Thiele H, Elsaesser A, et al. Intracoronary infusion of bone marrow-derived mononuclear cells in acute myocardial infarction: 5 year clinical outcome and MRI data of the randomized, double-blind, placebo-controlled REPAIR-AMI trial. [American Heart Association's Scientific Sessions 2011, Orlando, FL United States. 12- 16 November 2011]. Circulation 2011;124(21 (Suppl 1)). 20643438
- Mill, J S and Rao, S V. REPAIR-AMI: stem cells for acute myocardial infarction. Future cardiology:137-140. 20643439 [DOI: 10.2217/14796678.3.2.137]
- NCT00279175. REPAIR-AMI: intracoronary progenitor cells in acute myocardial infarction (AMI). https://clinicaltrials.gov/ct2/show/NCT00279175 (accessed 11 March 2015). 20643440
- Rolf A, Assmus B, Schachinger V, Rixe J, Mollmann S, Mollmann H, et al. Maladaptive hypertrophy after acute myocardial infarction positive effect of bone marrow-derived stem cell therapy on regional remodeling measured by cardiac MRI. Clinical Research in Cardiology 2011;100(11):983-92. 20643441
- Rolf A, Dill T, Moellmann S, Conradi G, Schaechinger V, Zeiher A, et al. One year follow-up of left ventricular function and remodelling after intracoronary infusion of bone marrow-derived progenitor cells after acute

STEMI: MRI substudy of the double-blind, randomized, placebo-controlled multicenter REPAIR-AMI trial. Journal of the American College of Cardiology 2007;49(9 Suppl B):22B. 20643442

- Rolf A, Mollmann S, Rixe J, Conradi G, Schachinger V, Dimmeler S, et al. Positive effect of bone marrow progenitor cell transfer on regional post infarction ventricular remodelling by magnetic resonance imaging. Insights from the REPAIR AMI trial [Circulation]. 2007 116;16 Suppl S(772):Abstract 3419. 20643443
- Schachinger V, Assmus B, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, et al. Intracoronary infusion of bone marrow-derived mononuclear cells abrogates adverse left ventricular remodelling post-acute myocardial infarction: insights from the reinfusion of enriched progenitor cells and infarct remodelling in acute myocardial infarction (REPAIR-AMI) trial. European Journal of Heart Failure 2009;11(10):973-9. 20643444
- Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, et al. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. European Heart Journal 2006;27(23):2775-83. 20643445
- Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. New England Journal of Medicine 2006;355(12):1210-21. 20643446
- Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, et al. Predictors of contractile recovery of left ventricular function after intracoronary infusion of bone marrow-derived progenitor cells in patients with acute myocardial infarction: lessons from the REPAIR-AMI trial. Circulation 2006;114(18 Suppl):788. 20643447
- Schachinger V, Tonn T, Dimmeler S, Zeiher AM. Bone-marrow-derived progenitor cell therapy in need of proof of concept: design of the REPAIR-AMI trial. Nature Clinical Practice Cardiovascular Medicine 2006;3(Suppl 1):S23-8. 20643448

Suarez de Lezo 2007 {published data only}
20643449

- Suarez de Lezo J, Herrera C, Pan M, Romero M, Pavlovic D, Segura J. Regenerative therapy in patients with a revascularised acute anterior myocardial infarction and depressed ventricular function. Revista Espanola De Cardiologia 2007;60(4):357-65. 20643450

Sürder 2013 {published data only}
20643451

- M Gastl, D Sürder, R Corti, D M M Faruque Osmany, A Gotschy, J von Spizcak, J Sokolska, D Metzen, H Alkadhi, F Ruschitzka, S Kozerke, R Manka. Effect of intracoronary bone marrow-derived mononuclear cell injection early and late after myocardial infarction on CMR-derived myocardial strain. International Journal of Cardiology 15-1-2020;310:108-115. 20643452 [DOI: 10.1016/j.ijcard.2020.01.025] [PMID: 31982162]
- NCT00355186. SWiss Multicenter Intracoronary Stem Cells Study in Acute Myocardial Infarction (SWISS-AMI). https://clinicaltrials.gov/ct2/show/NCT00355186 (accessed 11 March 2015). 20643453
- Sürder D, Manka R, Cicero V, Moccetti T, Rufibach K, Soncin S, et al. Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. Circulation 2013;127(19):1968-79. 20643454
- Sürder D, Manka R, Moccetti T, Rufibach K, Astori G, Cicero VL, et al. Results of the Swiss multicentre intracoronary stem cells study in acute myocardial infarction (Swiss AMI) trial. [American Heart Association 2012 Scientific Sessions and Rescitation Science Symposium, Los Angeles, CA United States, 3-6 November 2012]. Circulation 2012;126(23):2783. 20643455
- Sürder D, Manka R, Turchetto L, Moccetti T, Erne P, Zuber M, et al. Intracoronary injection of bone marrow derived mononuclear cells, early or late after acute myocardial infarction: long-term effects on global left ventricular function - twelve months MRI and long-term clinical results of the SWISS-AMI trial. [26th Annual Symposium Transcatheter Cardiovascular Therapeutics, TCT2014, Washington, DC United States. 13-17 September 2014]. Journal of the American College of Cardiology 2014;64(11 Suppl 1):B45. 20643456
- Sürder D, Moccetti T, Astori G, Soldati G, Schwitter J, Erne P, et al. From SWISS-AMI to CARDIASTIM - translation of a clinical progenitor-cell based protocol into GMP. [European Society of Cardiology, ESC Congress 2009, Barcelona, Spain, 29 August - 2 September 2009]. European Heart Journal 2009;30:503. 20643457
- Sürder D, Schwitter J, Moccetti T, Astori G, Rufibach K, Plein S, et al. Cell-based therapy for myocardial repair in patients with acute myocardial infarction: Rationale and study design of the SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infraction (SWISS-AMI). American Heart Journal 2010;160(1):58-64. 20643458

Tendera 2009 {published data only}
20643459
- Bavry AA. Myocardial regeneration by intracoronary infusion of selected population of stem cells in acute myocardial infarction (REGENT). ACC Cardiosource Review Journal 2008;17(10):30. 20643460
- Chojnowska L, Kepka C, Demkow M, Chmielak Z, Kukula K, Dabrowski M, et al. Intracoronary administration of mononuclear bone marrow cells in acute STEMI: long-term follow-up. European Heart Journal 2007;28(Suppl 1):443. 20643461
- Kazmierski M, Wojakowski W, Michalewska-Wludarczyk A, Ciosek J, Rychlik W, et al. Improvement of flow-mediated dilatation in patients with acute myocardial infarction treated with intracoronary infusion of CD34+CXCR4+ cells. [European Society of Cardiology, ESC Congress 2009, Barcelona, Spain. 29 August - 2 September 2009]. European Heart Journal 2009;30:502. 20643462
- NCT00316381. Stem cell therapy to improve myocardial function in patients with acute myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00316381 (accessed 11 March 2015). 20643463
- Tendera M, Wojakowski W, Ruzylllo W, Chojnowska L, Kepka C, Tracz W, et al. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration Infarction (REGENT) Trial. European Heart Journal 2009;30(11):1313-21. 20643464
- Tendera M, Wojakowski W and REGENT Investigators. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction. 5-year follow-up of randomized, multicenter myocardial regeneration by intracoronary infusion of selected population of stem cells in acute myocardial infarction (REGENT) trial. Circulation 2013;128(22 Suppl):Meeting Abstract: 17138. 20643465
- Tendera M. Myocardial regeneration by intracoronary infusion of selected population of stem cells in acute myocardial infarction (REGENT) randomized multicenter trial. Clinical Research in Cardiology 2008;97(12):853. 20643466
- Wojakowski W, Kazmierski M, Kucia M, Zuba-Surma E, Buszman P, Ochala A, et al. Number of circulating progenitor cells is a predictor of left ventricular ejection fraction improvement in patients with acute myocardial infarction treated with intracoronary infusion of bone marrow-derived CD34+ CXCR4+ cells. [15th Annual Interventional Vascular Therapeutics Angioplasty Summit - Transcatheter Cardiovascular Therapeutics Asia Pacific Symposium, TCTAP 2010, Seoul, South Korea. 28-30 April 2010]. American Journal of Cardiology 2010;105(9 Suppl 1):34B. 20643467
- Wojakowski W, Kucia M, Ochala Am Biszman P, Krol M, Ryderka R, Maslankiewicz K, et al. Mobilization of CXCR4+ stem cells in acute myocardial infarction is correlated with left ventricular ejection fraction and myocardial perfusion assessed by MRI in 1 year follow-up (REGENT trial). American Journal of Cardiology 2006;98(8A Suppl):46M. 20643468
- Wojakowski W, Kucia M, Wyderka R, Maslankiewicz K, Zebzda A, Ochala A, et al. Mobilization of CXCR4+ stem cells in acute myocardial infarction is correlated with left ventricular ejection fraction and myocardial perfusion assessed by MRI in 1 year follow-up (REGENT trial). Circulation 2006;114(18 Suppl):669. 20643469
- Wojakowski W, Michalowska A, Majka M, Kucia M, Maslankievvicz K, Wyderka R, et al. The mobilization of tissue-committed (CD34(+),CD117(+),CXCR4(+), C-Met(+)) stem cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in acute myocardial infarction: REGENT study. Circulation 2004;110(17 Suppl):238-9. Meeting Abstract 1143. 20643470
- Wojakowski W. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: Extended 6-year follow-up of randomized multicenter REGENT trial. [19th Cardiovascular Summit: Transcatheter Cardivascular Therapiutics Asia Pacific, TCTAP 2014, Coex, Seoul, South Korea. 22-25 April 2014]. Journal of the American College of Cardiology 2014;63(12 Suppl 2):S44. 20643471

Traverse 2010 {published data only}
20643472
- NCT00268307. Bone marrow stem cell infusion following a heart attack. http://clinicaltrials.gov/ct2/show/NCT00268307 (accessed 11 March 2015). 20643473 [NCT00268307]
- Traverse JH, McKenna DH, Harvey K, Jorgenso BC, Olson RE, Brostom N, et al. Results of a phase 1, randomized, double-blind, placebo-controlled trial of bone marrow mononuclear stem cell administration in patients following ST-elevation myocardial infarction. American Heart Journal 2010;160(3):428-34. 20643474

Traverse 2011 {published data only}
20643475
- Bhatnagar, Aruni and Bolli, Roberto and Johnstone, Brian H and Traverse, Jay H and Henry, Timothy D and Pepine, Carl J and Willerson, James T and Perin, Emerson C and Ellis, Stephen G and Zhao, David X M and Yang, Phillip C and Cooke, John P and Schutt, Robert C and Trachtenberg, Barry H and Orozco, Aaron and Resende, Micheline and Ebert, Ray F and Sayre, Shelly L and Simari, Robert D Moyé, Lem and Cogle, Christopher R and Taylor, Doris A, on behalf of the CCTRN. Bone marrow cell characteristics associated with patient profile and cardiac performance outcomes in the LateTIME-Cardiovascular Cell Therapy Research Network (CCTRN) trial. American Heart Journal Sept 2016;179:142-50. 20643476 [DOI: 10.1016/j.ahj.2016.06.018] [PMID: 27595689]
- Bhatnagar A, Johnston BH, Traverse JH, Henry TD, Pepine CJ, Willerson JT et al. Patient and cell characteristics associated with clinical outcomes in the CCTRN LateTime trial. [American Heart Association's Scientific Sessions and Resuscitation Science Symposium, Chicago, IL United States. 15-18 November 2014]. Circulation 2014;130. 20643477
- Henry TD, Traverse JH, Pepine CJ, Willerson JT, Ellis S, Zhao DX, et al. Results from lateTIME: a randomized, placebo controlled trial of intracoronary stem cell delivery two to three weeks following acute myocardial infarction from the cardiovascular cell therapy research network. [25th Annual Symposium Transcatheter Cardiovascular Therapeutics, TCT 2013, San Francisco, CA United States, 27 October - 1 November 2013]. Journal of the American College of Cardiology 2013;62(18 Suppl 1):B249. 20643478
- NCT00684060. Use of adult autologous stem cells in treating people 2 to 3 weeks after having a heart attack (The Late TIME Study). https://clinicaltrials.gov/ct2/show/NCT00684060 (accessed 11 March 2015). 20643479
- Traverse JH, Henry TD, Ellis SG, Pepine CJ, Willerson JT, Zhao DX, et al. Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. JAMA 2011;306(19):2110-9. 20643480
- Traverse JH, Henry TD, Ellis SG, Pepine CJ, Willerson JT, Zhao DX, et al. Results from LateTIME: a randomized, placebo controlled trial of intracoronary stem cell delivery two to three weeks following acute myocardial infarction. [American Heart Association's Scientific Sessions 2011, Orlando, FL United States, 12-16 November 2011]. Circulation 2011;124(21):2372. 20643481
- Traverse JH, Henry TD, Vaughan DE, Ellis SG, Pepine CJ, Willerson JT, et al. LateTIME: a phase-II, randomized, double-blinded, placebo-controlled, pilot trial evaluating the safety and effect of administration of bone marrow mononuclear cells 2 to 3 weeks after acute myocardial infarction. Texas Heart Institute Journal 2010;37(4):412-20. 20643482
- Wang, X Y and Derakhshandeh, R and Rodriguez, H J and Han, D D and Kostyushev, D S and Henry, T D and Traverse, J H and Moye, L and Simari, R D and Taylor, D A and Springer, M L. Impaired Therapeutic Efficacy of Bone Marrow Cells from PostMyocardial Infarction Patients in the TIME and LateTIME Clinical Trials. PLoS One 2020:337. 20643483 [DOI: 10.1371/journal.pone.0237401] [PMID: 32841277]

Traverse 2018 {published data only}
20643484
- Jay H Traverse, Timothy D Henry, Carl J Pepine, James T Willerson, Atul Chugh, Phillip C Yang, David X M Zhao, Stephen G Ellis, John R Forder, Emerson C Perin, Marc S Penn, Antonis K Hatzopoulos, Jeffrey C Chambers, Kenneth W Baran, Ganesh Raveendran, Adrian P Gee, Doris A Taylor, Lem Moyé, Ray F Ebert, and Robert D Simari. The TIME Trial - Effect of Timing of Stem Cell Delivery Following ST-Elevation Myocardial Infarction on the Recovery of Global and Regional Left Ventricular Function: Final 2-Year Analysis. Circulation Research 5-12-2017;122:479-488. 20643485 [DOI: 10.1161/CIRCRESAHA.117.311466] [PMID: 29208679]
- NCT00684021. Use of adult autologous stem cells in treating people who have had a heart attack (The TIME Study). https://clinicaltrials.gov/ct2/show/NCT00684021 (accessed 11 March 2015). 20643486
- Schutt RC, Trachtenberg BH, Cooke JP, Traverse JH, Henry TD, Pepine CJ, et al. Bone marrow characteristics associated with changes in infarct size after STEMI: a biorepository evaluation from the CCTRN TIME trial. Circulation Research 2015;116(1):99-107. 20643487
- Traverse JH, Henry TD, Pepine CJ, Willerson JT, Ellis SG. One-year follow-up of intracoronary stem cell delivery on left ventricular function following ST-elevation myocardial infarction. JAMA 2014;311(3):301-2. 20643488
- Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, et al. Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA - Journal of the American Medical Association 2012;308(22):2380-9. 20643489

- Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, et al. Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial [Erratum]. JAMA - Journal of the American Medical Association 2013;309(4):343. 20643490
- Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, et al. The effect of timing of stem cell delivery following acute myocardial infarction: the NHLBI and CCTRM TIME trial. Circulation 2012;126(23):2783-4. 20643491
- Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, et al. The NHLBI and cardiovascular cell therapy research network (CCTRN) TIME trial: two-year results. [American Heart Association's Scientific Sessions and Resuscitation Science Symposium, Chicago, IL United States. 15-18 November 2014]. Circulation 2014;130. 20643492
- Traverse JH, Henry TD, Pepine CJ, Willerson JT, Zhao DX, Ellis SG, et al. The NHLBI TIME trial: One-year results. Circulation 2013;128(24):2714. American Heart Association's Scientific Sessions 2013, Dallax, TX United States, 16-20 November 2013. 20643493
- Traverse JH, Henry TD, Vaughan DE, Ellis SG, Pepine CJ, Willerson JT, et al. Rationale and design for TIME: a phase II, randomized, double-blind, placebo-controlled pilot trial evaluating the safety and effect of timing of administration of bone marrow mononuclear cells after acute myocardial infarction. American Heart Journal 2009;158(3):356-63. 20643494
- Traverse LH, Moye L, Henry TD. Identification of clinical factors in cell therapy trials that determine recovery versus deterioration of LV function following STEMI. [American Heart Association's Scientific Sessions and Resuscitation Science Symposium, Chicago, IL United States. 15-18 November 2014]. Circulation 2014;130. 20643495

Turan 2012 {published data only}
20643496
- Turan RG, Bozdag TI, Turan CH, Ortak J, Akin I, Kische S, et al. Enhanced mobilization of the bone marrow-derived circulating progenitor cells by intracoronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction. Journal of Cellular and Molecular Medicine 2012;16(4):852-64. 20643497
- Turan RG, Ilkay BT, Akin I, Kische S, Schneider H, Turan CH, et al. Enhanced cardiac chemoreflex sensitivity after intra coronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction. [27th National Congress of Cardiology, Istanbul, Turkey, 27-30 October 2011] [Akut miyokart enfarktuslu hastalarda taze isole edilmis intrakoroner kemik iliti kaynakli kok hucre transplantasyonu sonrasi artmis kardiyak kemorefleks duyarliliti]. Tűrk Kardiyoloji Derneği Arşivi 2011;39:83. 20643498
- Turan RG, Turan IB, Turan CH, Akin I, Kische S, Paranskaya L, et al. Long term improvement of cardiac function by intracoronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction. [28th National Congress of Cardiology, Antalya, Turkey. 11-14 October 2012] [Akut miyokard infarktusu hastalarinda intra koroner taze izole edilmis kemik iligi kok hucre tranplantasyonu sonrasi kardiyak fonksiyonlarinda meydana gelen uzun sureli duzelme]. Turk Kardiyoloji Dernegi Arsivi 2012;40:62. 20643499

Wang 2014 {published data only}
20643500
- Wang X, Xi WC, Wang F. The beneficial effects of intracoronary autologous bone marrow stem cell transfer as an adjunct to percutaneous coronary intervention in patients with acute myocardial infarction. Biotechnology Letters 2014;36(11):2163-8. 20643501

Wohrle 2010 {published data only}
20643502
- Mailander V, Wohrle J, Schauwecker P, Merkle N, Nusser T, Bommer M, et al. Intracoronary stem cell therapy in patients with acute myocardial infarction - a randomized, double-blind, placebo-controlled trial (SCAMI). Transfusion Medicine and Hemotherapy 2009;36(Suppl 1):19-20. 20643503
- NCT00669227. Intracoronary stem cell therapy in patients with acute myocardial infarction (SCAMI). https://clinicaltrials.gov/ct2/show/NCT00669227 (accessed 11 March 2015). 20643504
- Woehrle J, von Scheidt F, Markovic S, Schauwecker P, Schwarz K, Wiesneth M, et al. Intracoronary stem cell therapy in patients with acute myocardial infarction - 36 months results of a randomized, double-blind, placebo controlled trials with serial MRI follow-ups. [61st Annual Scientific Session of the American College of Cardiology and i2 Summit: Innovation in Intervention, ACC.12, Chicago, IL United States. 24-27 March

2012]. Journal of the American College of Cardiology 2012;59(13 Suppl 1):E346. 20643505
- Wohrle J, Merkle N, Mailander V, Nusser T, Schauwecker P, Von Scheidt F, et al. Intracoronary stem cell therapy after myocardial infarction - twelve months follow-up of a randomized, rigorous double-blind, placebo controlled trial. In: Journal of American College of Cardiology. Vol. 55 (10 Suppl 1). 2010:A100.e938. 20643506
- Wohrle J, Merkle N, Mailander V, Nusser T, Schauwecker P, von Scheidt F, et al. Results of the intracoronary stem cell therapy after acute myocardial infarction. American Journal of Cardiology 2010;105(6):804-12. 20643507
- Wohrle J, von Scheidt F, Schauwecker P, Wiesneth M, Markovic S, Schrezenmeier H, et al. Impact of cell number and microvascular obstruction in patients with bone-marrow derived cell therapy: final results from the randomized, double-blind, placebo controlled intracoronary Stem Cell therapy in patients with Acute Myocardial Infarction (SCAMI) trial. Clinical Research in Cardiology 2013;102(10):765-70. 20643508

Wollert 2004 {published data only}
20643509
- Gerd P, Schaefer A, Wollert KC, Lippolt P, Fuchs M, Kaplan M, et al. Can bone marrow transfer in patients after myocardial infarction prevent the development of diastolic dysfunction? Results from the BOOST trial. Circulation 2004;110(17 Suppl):239-40. Abstract 1148. 20643510
- Hertenstein B, Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, et al. Bone marrow cell transfer for treatment of acute myocardial infarction: 18 months follow-up data from the randomised-controlled BOOST trial. Bone Marrow Transplantation 2006;27(Suppl 1):S62. 20643511
- Hertenstein B, Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Breidenbach C, et al. Randomised controlled clinical trial of intracoronary autologous bone marrow cell transfer post-myocardial infarction. Bone Marrow Transplantation 2004;33(Suppl 1):S2. 20643512
- Meyer GP, Wollert KC, Lotz J, Pirr J, Rager U, Loppolt P, et al. Intracoronary bone marrow cell transfer after myocardial infarction: 5-year follow-up from the randomized-controlled BOOST trial. European Heart Journal 2009;30:2978-84. 20643513
- Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance STelevation infarct regeneration) trial. Circulation 2006;113:1287-94. 20643514
- NCT00224536. Bone marrow transfer to enhance ST-elevation infarct regeneration. https://www.clinicaltrials.gov/ct2/show/NCT00224536 (accessed 11 March 2015). 20643515
- Schaefer A, Meyer GP, Fuchs M, Klein G, Kaplan M, Wollert KC, et al. Impact of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: results from the BOOST trial. European Heart Journal 2006;27:929-35. 20643516
- Schaefer A, Zwadlo C, Fuchs M, Meyer GP, Lippolt P, Wollert KC, et al. Long-term effects of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: 5-year results from the randomized-controlled BOOST trial-an echocardiographic study. European Journal of Echocardiography 2010;11:165-71. 20643517
- Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004;364(9429):141-8. 20643518

Wollert 2017 {published data only}
20643519[DOI: 10.1093/eurheartj/ehx188][DOI: 28431003][EUCTR: http://www.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2005- 000774-46-DE][ISRCTN: ISRCTN17457407]
- Andreas Seitz, Kai C Wollert, Gerd P Meyer, Jochen Müller-Ehmsen, Carsten Tschope, Andreas E May, Klaus Empen, Emmanuel Chorianopoulos, Benedikta Ritter, Jens Pirr, Lubomir Arseniev, Hans-Gert Heuft, Arnold Ganser, Eed Abu-Zaid, Hugo A Katus, Stephan B Felix, Meinrad P Gawaz, Heinz-Peter Schultheiss, Dennis Ladage, Johann Bauersachs, Heiko Mahrholdt, Simon Greulich. Adenosine stress perfusion cardiac magnetic resonance imagingin patients undergoing intracoronary bone marrow cell transferafter ST-elevation myocardial infarction: the BOOST-2 perfusionsubstudy. Clinical Research in Cardiology 10-08-2019;109:539-548. 20643520 [DOI: 10.1007/s00392-019-01537-4]
- Euctr2005b. Bone marrow cell transplantation for improved recovery after heart attack. http://www.who.int/trialsearch/Trial2.aspx? TrialID=EUCTR2005-000774-46-DE. 20643521
- ISRCTN17457407. BOne marrOw transfer to enhance ST-elevation infarct regeneration-2 (BOOST-2). http://www.controlledtrials.com/ISRCTN17457407 (accessed on 11 March 2015). 20643522

- Kai C Wollert, Gerd P Meyer, Jochen Müller-Ehmsen, Carsten Tschöpe, Vernon Bonarjee, Alf Inge Larsen, Andreas E May, Klaus Empen, Emmanuel Chorianopoulos, Ulrich Tebbe, Johannes Waltenberger, Heiko Mahrholdt, Benedikta Ritter, Jens Pirr, Dieter Fischer, Mortimer Korf-Klingebiel, Lubomir Arseniev, Hans-Gert Heuft Jan E Brinchmann , Diethelm Messinger, Bernd Hertenstein, Arnold Ganser, Hugo A Katus, Stephan B Felix, Meinrad P Gawaz, Kenneth Dickstein, Heinz-Peter Schultheiss, Dennis Ladage, Simon Greulich, Johann Bauersachs. Intracoronary autologous bone marrow cell transfer after myocardial infarction: the BOOST-2 randomised placebo-controlled clinical trial. European Heart Journal 19-4-2017;38:2936-2943. 20643523 [DOI: 10.1093/eurheartj/ehx188]
- Seitz, A and Wollert, K C and Meyer, G P and Muller-Ehmsen, J and Tschope, C and May, A E and Empen, K and Chorianopoulos, E and Ritter, B and Pirr, J and Arseniev, L and Heuft, H G and Ganser, A and Abu-Zaid, E and Katus, H A and Felix, S B and Gawaz, M P and Schultheiss, H P and Ladage, D and Bauersachs, J and Mahrholdt, H and Greulich, S. Adenosine stress perfusion cardiac magnetic resonance imaging in patients undergoing intracoronary bone marrow cell transfer after STelevation myocardial infarction: the BOOST-2 perfusion substudy. Clinical research in cardiology 2019. 20643524

Xiao 2012 {published data only}
20643525
- Xiao WT, Gao CY, Dai GY, Li MW, Wang XP, Liu HZ, et al. Autologous bone marrow mesenchymal stem cells for myocardial renewal and repair. Chinese Journal of Tissue Engineering Research 2012;16(27):5081-6. 20643526

Yang 2020 {published and unpublished data}
20643527[CTG: NCT00979758][DOI: 10.1136/openhrt-2019-001139]
- NCT00979758. Strengthening transplantation effects of bone marrow mononuclear cells with atorvastatin in myocardial infarction. http://clinicaltrials.gov/ct2/show/NCT00979758 (accessed 11 March 2015). 20643528
- Yue-Jin Yang , Hai-Yan Qian, Lei Song, Yong-Jian Geng, Run-lin Gao, Na Li, Hong Wang, Xia-Qiu Tian, Ji Huang, Pei-Sen Huang, Jun Xu, Rui Shen, Min-Jie Lu, Shi-Hua Zhao, Wei-Chun Wu, Yuan Wu, Jun Zhang, Jie Qian, Jun-Yan Xu, Yu-Yan Xiong. Strengthening effects of bone marrow mononuclear cells with intensive atorvastatin in acute myocardial infarction. Open Heart 26-3-2020. 20643529 [DOI: 10.1136/ openhrt-2019-001139] [PMID: 32393654]

Yao 2006 {published data only}
20643530
- Yao K, Huang RC, Ge L, Qian JY, Li YL, Xu SK, et al. Observation on the safety: clinical trial on intracoronary autologous bone marrow mononuclear cells transplantation for acute myocardial infarction. Chinese Journal of Cardiovascular Diseases 2006;34(7):577-81. 20643531

Yao 2009 {published data only}
20643532
- Yao K, Huang R, Sun A, Qian J, Liu X, Ge L, et al. Repeated autologous bone marrow mononuclear cell therapy in patients with large myocardial infarction. European Journal Heart Failure 2009;11(7):691-8. 20643533

You 2008 {published data only}
20643534
- You QJ, Shen ZY, Xiao MD, Jiang XC. Influence of autologous bone marrow stem cell transplantation on short-term heart function in cardiac failure patients. Journal of Clinical Rehabilitative Tissue Engineering Research 2008;12(8):1467-71. 20643535

Zhang 2021 {published data only}
20643536
- Runfeng Zhang, Jiang Yu, Ningkun Zhang, Wensong Li, Jisheng Wang, Guocai Cai, Yu Chen, Yong Yang and Zhenhong Liu. Bone marrow mesenchymal stem cells transfer in patients with ST-segment elevation myocardial infarction: single- blind, multicenter, randomized controlled trial [Bone marrow mesenchymal stem cells transfer in patients with ST-segment elevation myocardial infarction: single- blind, multicenter, randomized controlled trial]. Stem Cell Research & Therapy 07-01-2021. 20643537 [DOI: doi.org/10.1186/s13287-020-02096-6]

Zhukova 2009 {published data only}
20643538
• Zhukova NS, Staroverov II, Stukalova OV, Samoilenko LE, Romanov YA, Sinitsin VE, et al. An experience of the use of stem cells in the treatment of patients with myocardial infarction and low ejection fraction. Kardiologia 2009;7(8):19-24. 20643539

References to studies excluded from this review

Anastasiadis 2020 {published data only}
20643540
• Kyriakos Anastasiadis, Polychronis Antonitsis, Stephen Westaby, Ajan Reginald, Sabena Sultan, Argirios Doumas, George Efthimiadis &Martin John Evans. Editorial Expression of Concern: Implantation of a Novel Allogeneic Mesenchymal Precursor Cell Type in Patients with Ischemic Cardiomyopathy Undergoing Coronary Artery Bypass Grafting: an Open Label Phase IIa Trial.. J. Cardiovasc. Transl. Res.;14:587–588. 20643541

Ang 2008 {published data only}
20643542
• Ang KL. Intramuscular or intracoronary administration of autologous BMC fails to improve contractility of scarred myocardium: IC/IM-BMC study. Clinical Research in Cardiology 2008;97(1):10. 20643543

Anonymous 2013 {published data only}
20643544
• Anonymous. Erratum: Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: The POSEIDON randomized trial ((2012). Journal of the American Medical Association;310:750. 20643545 [PMID: 23117550]

Arnesen 2007 {published data only}
20643546
• Arnesen H, Lunde K, Aakhus S, Forfang K. Cell therapy in myocardial infarction. Lancet 2007;369(9580):2142-3. 20643547

Atsma 2008 {unpublished data only}
20643548
• Atsma DE. Autologous bone marrow-derived in vitro expanded mesenchymal stem cell transplantation in patients with an acute myocardial infarction treated by successful primary percutaneous transluminal coronary angioplasty - a safety and feasibility study. http://www.trialregister.nl (accessed by 31 January 2011). 20643549

Bayes-Genis 2021 {published data only}
20643550
• Bayes□Genis, A. The CONCERT-HF trial: a sweet and sour symphony.. Eur. J. Heart Fail. Apr 2021;23,:675–676. 20643551 [PMID: 10.1002/ejhf.2188] [PMID: 33840139]

Beeres 2007 {published data only}
20643552
• Beeres, Slma. Cardiac regeneration in 2006: barcelona, 4 September 2006. Netherlands heart journal 2006;(2):16-17. 20643553 * Beeres SL, Bax JJ, Dibbets-Schneider P, Stokkel MP, Fibbe WE, van der Wall EE, et al. Intramyocardial injection of autologous bone marrow mononuclear cells in patients with chronic myocardial infarction and severe left ventricular dysfunction. American Journal of Cardiology 2007;100(7):1094-8. 20643554

Benedek 2014 {published data only}
20643555
• Benedek I, Bucur O, Benedek T. Intracoronary infusion of mononuclear bone marrow-derived stem cells is

associated with a lower plaque burden after four years. Journal of Atherosclerosis and Thrombosis 2014;21(3):217-29. 20643556 Benedek IS, Chitu M, Kovacs I, Benedek IJR, Suciu ZS, Benedek T. Intracoronary infusion of stem cells reduces local artherosclerosis progression on long term follow-up - an Angio CT multislice 64 study. European Heart Journal 2013;34:842- 53. 20643557

Bolli 2021 {published data only}
20643558
- Roberto Bolli, Raul D Mitrani, Joshua M Hare, Carl J Pepine, Emerson C Perin, James T Willerson, Jay H Traverse, Timothy D Henry, Phillip C Yang, Michael P Murphy, Keith L March, Ivonne H Schulman, Sohail Ikram, David P Lee, Connor O'Brien, Joao A Lima, Mohammad R Ostovaneh, Bharath Ambale-Venkatesh, Gregory Lewis, Aisha Khan, Ketty Bacallao, Krystalenia Valasaki, Bangon Longsomboon, Adrian P Gee, Sara Richman, Doris A Taylor, Dejian Lai, Shelly L Sayre, Judy Bettencourt, Rachel W Vojvodic, Michelle L Cohen, Lara Simpson, David Aguilar, Catalin Loghin, Lem Moyé, Ray F Ebert, Barry R Davis, Robert D Simari, Cardiovascular Cell Therapy Research Network (CCTRN). A Phase II study of autologous mesenchymal stromal cells and c-kit positive cardiac cells, alone or in combination, in patients with ischaemic heart failure: the CCTRN CONCERT-HF trial.. Eur. J. Heart Fail. Apr 2021;23,:661–674. 20643559 [PMID: 10.1002/ejhf.2178] [PMID: 33811444]

Byun 2009 {published data only}
20643560
- Byun, K H and Kim, S W. Is stem cell-based therapy going on or out for cardiac disease?. Korean circulation journal 2009;39:87- -92. 20643561

Caramia 2009 {published data only}
20643562
- Caramia, G. New perspectives in medicine: stem cells. Pediatria medica e chirurgica [Medical and surgical pediatrics] 2009;31:104- 116. 20643563

Chen 2004a {published data only}
20643564
- Chen YX, Ou RM, Zhang XY, Xu X, Zhao HY, Guan HH, et al. Effect of self-marrow stem cell transplantation in situ on the size of infarct area and cardiac functions after acute myocardium infarction. Chinese Journal of Clinical Rehabilitation 2004;8(3):568- 9. 20643565

Chen 2014 {published data only}
20643566
- Chen XM, Cui DY, Zhang M. Clinical observation of stem cell transplantation in patients with acute myocardial infarction complicated with heart failure. Journal of Dalian Medical University 2014;36(2):157-9. 20643567

Chernyavskiy 2017 {published data only}
20643568
- Chernyavskiy, A and Fomichev, A and Minin, S and Nikitin, N. Bone Marrow Mononuclear Cell Implantation in Myocardial Laser Channels in the Ischemic Heart Disease Surgery. Long-Term Results. Proceedings of the Xxv Conference on High-Energy Processes in Condensed Matter 2017;1893. 20643569 [ISBN: 978-0-7354-1578-2]

ChiCtr2018 {published data only}
20643570
- A clinical study for enhanced external counterpulsation combined with MSCs transplantation in the treatment of acute myocardial infarction. http://www.who.int/trialsearch/Trial2.aspx?TrialID=ChiCTR1800018788 2018. 20643571 [http://www.who.int/trialsearch/Trial2.aspx?TrialID=ChiCTR1800018788]

Chin 2021 {published data only}
20643572
- Sze Piaw Chi, Oteh Maskon, Chiang Soo Tan, John E Anderson, Chee Yin Wong, Hamat Hamdi Che Hassan, Chee Ken Choor, S Abdul Wahid Fadilah, Soon Keng Cheong. Synergistic effects of intracoronary infusion of autologous bone marrow-derived mesenchymal stem cells and revascularization procedure on improvement

of cardiac function in patients with severe ischemic cardiomyopathy.. Stem cell Investig. Jan 2021;8. 20643573 [DOI: 10.21037/sci-2020-026] [PMID: 33575315]

Ctri2009a {published data only}
20643574
- A clinical study to assess the safety and efficacy of stem cell in patients with Myocardial Infarction. http://www.who.int/trialsearch/Trial2.aspx?TrialID=CTRI/2009/091/000176. 20643575 [http://www.who.int/trialsearch/Trial2.aspx?TrialID=CTRI/2009/091/000176]

Dash 2020 {published data only}
20643576
- Dash, N and Bohl, V and Toth, C. Studying Cardiovascular Disease Using Human Stem Cell-derived 3D Cardiomyocytes. Faseb Journal;34. 20643577 [ISBN: 0892-6638] [https://digitalcommons.providence.edu/bio_students/7]

Dib 2008 {published data only}
20643578
- Dib, N and Tomaselli, G. CAuSMIC Trial (Catheter-Based Delivery of Autologous Skeletal Myoblasts [ASM] for Ischemic Cardiomyopathy). Clinical cardiology 2008;(2):92. 20643579

Dolan 2019 {published data only}
20643580
- Dolan, Eimear B and Hofmann, Bjorn and de Vaal, M Hamman and Bellavia, Gabriella and Straino, Stefania and Kovarova, Lenka and Pravda, Martin and Velebny, Vladimir and Daro, Dorothee and Braun, Nathalie and Monahan, David S and Levey, Ruth E and O'Neill, Hugh and Hinderer, Svenja and Greensmith, Robert and Monaghan, Michael G and Schenke-Layland, Katja and Dockery, Peter and Murphy, Bruce P and Kelly, Helena M and Wildhirt, Stephen and Duffy, Garry P. A bioresorbable biomaterial carrier and passive stabilization device to improve heart function post-myocardial infarction. Materials Science and Engineering C May 2019. 20643581 [DOI: 10.1016/j.msec.2019.109751]

Drabik 2021 {published data only}
20643582
- L Drabik, A Mazurek, L Czyz, M Skubera, E Kwiecien, M Sikorska, A Kulaga, A Mikunda, W Szot, M Kostkiewicz, M BrzyszczykMarzec, M Urbanczyk, W Plazak, P Podolec, P Musialek. Relationship between left ventricular global longitudinal strain, infarct size and left ventricular function in patients with acute myocardial infarction in a stem cell therapy study. In: European heart Journal. 2021. 20643583 [https://esc365.escardio.org/presentation/237745]

Edlinger 2016 {published data only}
20643584
- Edlinger, C and Wernly, B and Leisch, M and Kammler, J and Kypta, A and Eder, S and Wuppinger, T and Moesenlechner, T and Ausserwinkler, M and Jung, C and Hoppe, U C and Lichtenauer, M. Analysis of ambient influences affecting Interleukin-6 as a paracrine mediator in clinical trials of stem cell therapy for myocardial infarction. ;37:515-516. 20643585 [DOI: 10.7754/Clin.Lab.2015.151002]

Engelmann 2006 {published data only}
20643586
- Engelmann MG, Theiss HD, Hennig-Theiss C, Huber A, Wintersperger BJ, Werle-Ruedinger AE, et al. Autologous bone marrow stem cell mobilization induced by granulocyte colony stimulating factor after subacute ST-segment elevation myocardial infarction undergoing late revascularization. Journal of the American College of Cardiology 2006;48(8):1712-21. 20643587

Epstein 2019 {published data only}
20643588
- Jonathan A Epstein. A Time to Press Reset and Regenerate Cardiac Stem Cell Biology. JAMA Cardiology Feb 2019. 20643589 [DOI: 10.1001/jamacardio.2018.4435] [PMID: 30480702]

Euctr 2006b {published data only}
20643590
- Effect of intracoronary injection of autologous stemm cells on left ventricular ejection fraction and volumes one year after an acute myocardial infarction. [Efecto de la inyeccion intracoronaria de celulas madre obtenidas de sangre periferica sobre la fraccion de eyeccion y los volumenes del ventriculo izquierdo al ano de haber padecido un infarto agudo de miocardio]. http://www.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2006-001772-20-ES 2006. 20643591 [http://www.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2006-001772-20-ES]

EUCTR 2010-020497-41-GB {unpublished data only}
20643592
- EUCTR 2010-020497-41-GB. A prospective, double blind, randomized, placebo-controlled clinical trial of intracoronary infusion of immunoselected, bone marrow-derived Stro3 mesenchymal precursor cells (MPC) in the treatment of patients with STelevation myocardial infarction - the AMICI trial. https://www.clinicaltrialsregister.eu/ctr-search/trial/2010-020497-41/GB (accessed 11 March 2015). 20643593

Fedak 2001 {published data only}
20643594
- Fedak, P W M and Verma, S and Weisel, R D and Mickle, D A G and Li, R K. Angiogenesis: protein, gene, of cell therapy? Heart surgery forum;4:301--304. 20643595

Fernandez 2004 {published data only}
20643596
- Fernandez RJ, Saslavsky J, Andrin O, Vrsalovic F, Geffner L, Camozzi L, et al. First-reported data from Argentina of the implantation and cellular therapy in myocardial infarction (Tescelcor) study. American Journal of Cardiology 2004;94(6A Suppl):187E-8E. 20643597

Fernandez Ruiz 2016 {published data only}
20643598
- Fernandez-Ruiz, Irene. Stem cells: Cell therapy improves outcomes in heart failure. Nature Reviews Cardiology 2016:311. 20643599 [DOI: 10.1038/nrcardio.2016.58]

Florenzano 2007 {published data only}
20643600
- Florenzano, U F. Stem-cell therapy [Therapio Celular]. Revista medica de Chile 2007:1221--1222. 20643601 [DOI: 10.4067/S0034- 98872007000900020]

Francis 2006 {published data only}
20643602
- Francis, D. The 2005 Scientific Sessions of the American Heart Association (AHA). Heart failure monitor:34--36. 20643603

Galinanes 2008 {published data only}
20643604
- Galinanes, M and Tomaselli, G. Intramuscular or intracoronary administration of autologous bone marrow cells fails to improve contractility of scar 20643605 [https://einstein.pure.elsevier.com/en/publications/intramuscular-or-intracoronary-administration-of-autologous-bone-] [https://www.researchgate.net/publication/278252716_Intramuscular_or_intracoronary_administration_of_autologous_bone_marrow_cells_fa

Gao 2015 {published data only}
20643606
- Gao, Lian R and Yu, Chen and Zhang, Ning K and Yang, Xi L and Liu, Hui L and Wang, Zhi G and Yan, Xiao Y and Yu, Wang and Zhu, Zhi M and Li, Tian C and Wang, Li H and Chen, Hai Y and Chen, Yun D and Huang, Chao L and Peng, Qu and Chen, Yao and Bin, Wang and Chen, Guang H and Wang, Zhong M and Xu, Zhao Y.

Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: double-blind, randomized controlled trial. BMC Medicine:1-15. 20643607 [DOI: 10.1186/s12916-015-0399-z.]

Goto 2017 {published data only}
20643608
- Goto, T and Miyagawa, S and Tamai, K and Kawamura, T and Matsuura, R and Harada, A and Sawa, Y. High Mobility Group Box 1 Induced Endogenous Bone Marrow Mesenchymal Stem Cells Have Potential to Improve Cardiac Function by Inhibition of Ventricular Remodeling After Myocardial Infarction. Circulation;140:A9905. 20643609 [https://www.ahajournals.org/doi/10.1161/circ.140.suppl_1.9905

Gyongyosi 2009 {published data only}
20643610
- Gieseking E, Syeda B, Sochor H, Charwat S, Bergler-Klein J, Maurer G, et al. Long-term follow-up of patients treated with combined delivery of intracoronary and intramyocardial bone-marrow mononuclear cells. [Osterreichische Kardiologische Gesellschaft Jahrestagung 2012, Salzburg, Austria. 30 May - 2 June 2012]. Journal fur Kardiologie 2012;19(5-6):162. 20643611
- Gyongyosi M, Charwat S, Lang IM, Dettke M, Glogar D, Maurer G. 5 years clinical follow-up of patients treated with combined delivery of intracoronary and intramyocardial bone-marrow mononuclear cells. [Osterreichische Kardiologische Gesellschaft Jahrestagung 2011, Salzburg, Austria. 25-28 May 2011]. Journal fur Kardiologie 2011;18(5-6):185. 20643612
- Gyongyosi M, Lang I, Dettke M, Beran G, Graf S, Sochor H, et al. Combined delivery approach of bone marrow mononuclear stem cells early and late after myocardial infarction: the MYSTAR prospective, randomized study. Nature Clinical Practice Cardiovascular Medicine 2009;6(1):70-81. 20643613
- Gyongyosi M, Lang I, Dettke M, Charwat S, Nyolczas N, Sochor H, et al. Results from the multicenter, prospective, randomized single-blind trial on intracoronary or combined (percutaneous intramyocardial and intracoronary) administration of non-selected autologous bone marrow cells to patients after acute myocardial infarction. American Journal of Cardiology 2007;100(8A Suppl):23L. 20643614
- Nyolczas N, Gyongyosi M, Beran G, Dettke M, Graf S, Sochor H, et al. Design and rationale for the myocardial stem cell administration after acute myocardial infarction (MYSTAR) study: a multicenter, prospective, randomised, single-blind trial comparing early and late intracoronary or combined (percutaneous intramyocardial and intracoronary) administration of nonselected autologous bone marrow cells to patients after acute myocardial infarction. American Heart Journal 2007;153(2):212.e1-7. 20643615

Haller 2021 {published data only}
20643616
- Paul M Haller, Mariann Gyöngyösi, Lourdes Chacon-Alberty, Camila Hochman-Mendez, Luiz C Sampaio, Doris A Taylor. SexBased Differences in Autologous Cell Therapy Trials in Patients With Acute Myocardial Infarction: Subanalysis of the ACCRUE Database.. Front. Cardiovasc. Med. May 2021. 20643617 [DOI: 10.3389/fcvm.2021.664277] [PMID: 34124198]

Hare 2007 {published data only}
20643618
- Hare J. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (Provacel) following acute myocardial infarction (AMI). Clinical Cardiology 2007;30(7):364. 20643619
- Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo controlled, doseescalation study of intravenous adult human mesenchymal stem cells (PROCHYMAL) after acute myocardial infarction. Journal of the American College of Cardiology 2009;54(24):2277-86. 20643620
- Richartz BB. A randomized, double blind placebo controlled, dose escalation study of intravenous human adult mesenchymal stem cells (PROVACEL (trademark)) following acute myocardial infarction. Herz 2007;32(4):338. 20643621

Heeger 2012 {published data only}
20643622
- Heeger CH, Jaquet K, Thiele H, Zulkarnaen Y, Cuneo A, Haller D, et al. Percutaneous, transendocardial injection

of bone marrowderived mononuclear cells in heart failure patients following acute ST-elevation myocardial infarction: ALSTER-Stem Cell trial. Eurointervention 2012;8(6):732-42. 20643623

Hendrikx 2006 {published data only}
20643624
- Hendrikx M, Hensen K, Clijsters C, Jongen H, Koninckx R, Bijnens E, et al. Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation. Results from a randomised controlled clinical trial. Circulation 2006;114(Suppl I):I101-7. 20643625

Henon 2020 {published data only}
20643626
- Philippe Hénon. Key Success Factors for Regenerative Medicine in Acquired Heart Diseases.. Stem cell Rev. reports June 2020:441–458. 20643627 [DOI: 10.1007/s12015-020-09961-0] [PMID: 32297205]

Herdrich 2010 {published data only}
20643628
- Herdrich, B J & Gorman, R C. Progenitor Cells for the Treatment of Acute Myocardial Infarction.. Adv. Skin Wound Care Jan 2010:519–525. 20643629 [PMID: 22025903]

Holinski 2011 {published data only}
20643630
- Holinski S, Schmeck B, Claus B, Radtke H, Elgeti T, Holzhausen M, et al. Encouraging experience with intracardiac transplantation of unselected autologous bone marrow cells concomitant with coronary artery bypass surgery after myocardial infarction. Annals of Thoracic and Cardiovascular Surgery 2011;17(4):383-9. 20643631

Hu 2015 {published data only}
20643632
20643633
- Clinical Study of Hypoxia-Stressed Bone Marrow Mononuclear Cell Transplantation to Treat Heart Diseases. https://clinicaltrials.gov/show/NCT01234181. 20643634
- Hu, Xinyang and Huang, Xin and Yang, Qian and Wang, Lihua and Sun, Jianzhong and Zhan, Hongwei and Lin, Jianjing and Pu, Zhaoxia and Jiang, Jun and Sun, Yong and Xiang, Meixiang and Liu, Xianbao and Xie, Xiaojie and Yu, Xia and Chen, Zexin. GW26-e0740 Safety and Efficacy of IntraCoronary Hypoxia-precondItioned Bone Marrow MonoNuclear Cell Administration for Acute Myocardial Infarction Patients: The CHINA-AMI Randomized Controlled Trial. In: Journal of the American College of Cardiology (JACC). C79-80. 20643635
- Hu, X Y and Huang, X and Yang, Q and Wang, L H and Sun, J Z and Zhan, H W and Lin, J J and Pu, Z X and Jiang, J and Sun, Y and Xiang, M X and Liu, X B and Xie, X J and Yu, X and Chen, Z X. Safety and Efficacy of IntraCoronary HypoxiaprecondItioned Bone Marrow MonoNuclear Cell Administration for Acute Myocardial Infarction Patients: The CHINA-AMI Randomized Controlled Trial. In: Journal of the American College of Cardiology. C79-80. 20643636
- Hu X, Huang X, Yang Q, Wang L, Sun J, Zhan H, et al. Safety and efficacy of intracoronary hypoxia-preconditioned bone marrow mononuclear cell administration for acute myocardial infarction patients: the CHINA-AMI randomized controlled trial. International Journal of Cardiology 2015;184i:446-51. 20643637
- Hu XY, Huang X, Yang Q, Wang L, Sun J, Zhan H, et al. Safety and efficacy of intracoronary hypoxia-preconditioned bone marrow mononuclear cells administration for acute myocardial infarction patients. [American Heart Association's Scientific Sessions and Resuscitation Science Symposium, Chicago, IL United States. 15-18 November 2014]. Circulation 2014;130. 20643638

ISRCTN14054375 {published data only}
20643639
- https://www.isrctn.com/ISRCTN14054375? q=14054375&filters=&sort=&offset=1&totalResults=1&page=1&pageSize=10&searchType=basic-search. 20643640 [DOI: 10.1186/ISRCTN14054375]

ISRCTN75217135 {published data only}
20643641
- http://www.who.int/trialsearch/Trial2.aspx?TrialID=ISRCTN75217135. 20643642

Jeong 2018 {published data only}
20643643
• Hyunsuk Jeong, Hyeon Woo Yim, Hun-Jun Park, Youngseung Cho, Hanter Hong, Na Jin Kim, Il-Hoan Oh. Mesenchymal Stem Cell Therapy for Ischemic Heart Disease: Systematic Review and Meta-analysis.. International Journal of Stem Cells May 2018:1- 12. 20643644 [DOI: 10.15283/ijsc17061] [PMID: 29482311]

Jiang 2011 {published data only}
20643645
• Jiang M, Pu J, He B. The short to mid-term effect of bone marrow derived cell transfer on diastolic function after acute myocardial infarction. International Journal of Cardiology 2011;153(1):87-8. 20643646

JPRN-jRCT2053190103 {published data only}
20643647
• http://www.who.int/trialsearch/Trial2.aspx?TrialID=JPRN-jRCT2053190103. 20643648

Kahn 2006 {published data only}
20643649
• Kahn J. Stem cells show mixed results in MI patients. Journal of Interventional Cardiology 2006;19(4):297-301. 20643650

Kahn 2016 {published data only}
20643651
• Kahn, Joel K. New Life for a Damaged Heart. Bottom Line Health 2016:8--9. 20643652 [ISBN: 1092-0129] [http://search.ebscohost.com/login.aspx? direct=true{\&}AuthType=ip,shib{\&}db=jlh{\&}AN=113268445{\&}site=ehostlive{\&}scope=site{\&}custid=s8454451]

Kang 2004 {published data only}
20643653
• Kang HJ, Kim HS, Park YB. Stem cell therapy for myocardial infarction. Canadian Medical Association Journal 2004;171(5):442-3. 20643654

Kang 2004b {published data only}
20643655
• Hyun-Jae Kang, Hyo-Soo Kim, Shu-Ying Zhang, Kyung-Woo Park, Hyun-Jai Cho, Bon-Kwon Koo, Yong-Jin Kim, Dong Soo Lee, Dae-Won Sohn, Kyou-Sup Han, Byung-Hee Oh, Myoung-Mook Lee, Young-Bae Park. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: The MAGIC cell randomised clinical.. Lancet 2004:751–756. 20643656 [DOI: 10.1016/S0140-6736(04)15689-4] [PMID: 15016484]

Kang 2006 {published data only}
20643657
• Kang HJ, Kim MK, Kim MG, Choi DJ, Yoon JH, Park YB, et al. A multicenter, prospective, randomized, controlled trial evaluating the safety and efficacy of intracoronary cell infusion mobilized with granulocyte colony-stimulating factor and darbepoetin after acute myocardial infarction: study design and rationale of the 'MAGIC cell-5-combination cytokine trial'. Trials 2011;12(1):33. 20643658
• Kang HJ, Kim MK, Lee HY, Park KW, Lee W, Cho YS, et al. Five-year results of intracoronary infusion of the mobilized peripheral blood stem cells by granulocyte colony-stimulating factor in patients with myocardial infarction. European Heart Journal 2012;33(24):3062-9. 20643659
• Kang HJ, Kim YS, Koo BK, Park KW, Lee HY, Sohn DW, et al. Effects of stem cell therapy with G-CSF on coronary artery after drug-eluting stent implantation in patients with acute myocardial infarction. Heart 2008;94(5):604-9. 20643660
• Kang HJ, Lee HY, Na SH, Chang SA, Park KW, Kim HK, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. Circulation 2006;114(1 Suppl):I145-51. 20643661

- Kim HS. Myocardial regeneration and angiogenesis in myocardial infarction with G-CSF and intracoronary stem cell infusion-3- DES. www.ClinicalTrials.gov 2006;(accessed by 31 January 2011). 20643662 [NCT00291629]
- Lee HY, Kang HJ, Na SH, Chang SA, Hahn JY, Kim YS, et al. Differential effect of intracoronary infusion of the mobilized peripheral blood stem cell by G-CSF on angionnyogenesis in patients AMI versus OMI: interim result of 'MAGIC Cell-DES' trial. Circulation 2005;112(17 Suppl):U644. Meeting Abstract 2758. 20643663

Kang 2007 {published data only}
20643664
- Kang HJ, Kim HS, Koo BK, Kim YJ, Lee DE, Sohn DW, et al. Intracoronary infusion of the mobilized peripheral blood stem cell by G-CSF is better than mobilization alone by G-CSF for improvement of cardiac function and remodeling: 2-year follow-up results of the Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion (MAGIC Cell) 1 trial. American Heart Journal 2007;153(2):237.e1-8. 20643665
- Kang HJ, Kim HS, Na SH, Zhang SY, Kang WJ, Youn TJ, et al. Six months follow-up results of "granulocyte stimulating factor" based stem cell therapy in patients with myocardial infarction. Korean Circulation Journal 2006;36:99-107. 20643666
- Kang HJ, Kim HS, Zhang SY, Park KW, Choo HJ, Koo BK, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 2004;363:751-6. 20643667

Kang 2008 {published data only}
20643668
- Kang HJ, Kim HS. Safety and efficacy of intracoronary infusion of mibilized peripheral blood stem cell in patients with myocardial infarction: Magic Cell-1 and Magic Cell-3-DES trials. European Heart Journal Supplements 2008;10(K):K39-K43. 20643669

Kang 2011 {published data only}
20643670
- Kang HJ, Kim MK, Kim MG, Choi DJ, Yoon JH, Park YB, et al. A multicenter, prospective, randomized, controlled trial evaluating the safety and efficacy of intracoronary cell infusion mobilised with granulocyte colony-stimulating factor and darbepoetin after acute myocardial infarction: study design and rationale of the 'MAGIC cell-5-combination cytokine trial'. Trials 2011;12:33. 20643671
- NCT00501917. MAGIC Cell-5-Combicytokine Trial. https://clinicaltrials.gov/ct2/results?term=NCT00501917&Search=Search (accessed 11 March 2015). 20643672

Khoei 2020 {published data only}
20643673
- Saeideh Gholamzadeh Khoei, Fateme Karimi Dermani, Sara Malih, Nashmin Fayazi, Mohsen Sheykhhasan. The Use of Mesenchymal Stem Cells and their Derived Extracellular Vesicles in Cardiovascular Disease Treatment. Curr. Stem Cell Res. Ther. 2020:623–638. 20643674 [DOI: 10.2174/1574888X15666200501235201] [PMID: 32357818]

Kloner 2016 {published data only}
20643675
- Kloner, Robert A and Dai, Wangde and Hale, Sharon L and Shi, Jianru. Approaches to Improving Cardiac Structure and Function During and After an Acute Myocardial Infarction: Acute and Chronic Phases. Journal of Cardiovascular Pharmacology Therapeutics 2016:363--367. 20643676 [DOI: 10.1177/1074248415616187] [PMID: 26612091]

Komok 2019 {published data only}
20643677
- Komok, V V and Bunenkov, N S and Beliy, S A and Pizin, V M and Kondratev, V M and Dulaev, A V and Lukashenko, V I and Kobak, A E and Maksimova, T S and Sergienko, I P and Parusova, E V and Smirnova, L A and Babenko, E V and Afanasev, B V and Nemkov, A S and Khubulava, G G. Assessment of safety of additional transplantation of autologous bone marrow mononuclear cells in the combined treatment of coronary heart

disease. Results from a randomized, blind, placebo-controlled trial (TAMIS). Vestnik Transplantologii i Iskusstvennykh Organov July 2019. 20643678 [DOI: 10.15825/1995-1191-2019-2-112- 120]

Kwiecien 2020 {published data only}
20643679
- E E Kwiecien, L Drabik, A Mazurek, M Sikorska, L Czyz, M Skubera, M Urbanczyk, W Szot, M Kostkiewicz, RP Banys, W Plazak, M Olszowska, M Majka, P Podolec, P Musialek, Kwiecien, L Drabik, A Mazurek, M Sikorska, L Czyz, M Skubera, M Urbanczyk, W Szot, M Kostkiewicz, RP Banys, W Plazak, M Olszowska, M Majka, P Podolec, P Musialek. Evolution of left ventricular function after Wharton's jelly mesenchymal stem cells transcoronaryadministration: 5-year follow up in a pilot cohort of CIRCULATE-AMI Randomized Trial. In: European Heart Journal. 2020:1719. 20643680

Landmesser 2009 {published data only}
20643681
- Landmesser, U. Bone marrow cell therapy after myocardial infarction. What should we select? European heart journal June 2009. 20643682 [DOI: 10.1093/eurheartj/ehp181] [PMID: 19429633]

Li 2006 {published data only}
20643683
- Li Z, Zhang M, Jin Y, Zhang W, Liu L, Cui L, et al. The clinical randomized study of autologous peripheral blood progenitor cell transplantation by intracoronary infusion in patients with acute myocardial infarction. Circulation 2006;114(Suppl 18):II514-5. 20643684
- Li ZQ, Zhang M, Jing YZ, Zhang WW, Liu Y, Cui JL, et al. The clinical study of autologous peripheral blood stem cell transplantation by intracoronary infusion in patients with acute myocardial infarction (AMI). International Journal of Cardiology 2007;115(1):52-6. 20643685
- Li ZQ, Zhang M, Jin YZ, Zhang WW, Liu Y, Yuan L, et al. Safety and efficacy of intracoronary transplantation of G-CSF mobilised autologous peripheral blood stem cells in patients with acute myocardial infarction. Chinese Journal of Cardiovascular Diseases 2006;34(2):99-102. 20643686
- Zhang M, Li ZQ, Jun YZ, Zhang WW, Liu Y, Cui LJ et al. Autologous peripheral blood stem cell transplantation via coronary artery for acute myocardial infarction: a follow-up of short-term curative effect. Journal of Clinical Rehabilitative Tissue Engineering Research 2007;11(20):3944-7. 20643687

Li 2008 {published data only}
20643688
- Li XJ, Shang XM, Xu D, Liu CQ. Effect of bone marrow mesenchymal stem cell transplantation on vascular endothelial function in acute myocardial infarction patients. Journal of Clinical Rehabilitative Tissue Engineering Research 2008;12(38):7443-6. 20643689

Li 2016 {published data only}
20643690
- Rong, Li and Xiao-Ming, Li and Jun-Rong, Chen and Li, Rong and Li, Xiao-Ming and Chen, Jun-Rong. Clinical efficacy and safety of autologous stem cell transplantation for patients with ST-segment elevation myocardial infarction. Therapeutics & Clinical Risk Management Aug 2016. 20643691 [DOI: 10.2147/TCRM.S107199] [PMID: 27536122]

Li 2021 {published data only}
20643692
- Junlang Li, Shiqi Hu, Dashuai Zhu, Ke Huang, Xuan Mei, Blanca López de Juan Abad, Ke Cheng. All Roads Lead to Rome (the Heart): Cell Retention and Outcomes From Various Delivery Routes of Cell Therapy Products to the Heart. JAHA Apr 2021. 20643693 [DOI: 10.1161/JAHA.120.020402] [PMID: 33821664]

Liu 2020 {published data only}
20643694
- Ziwei Liu 1, Reyaj Mikrani, Hafiz Muhammad Zubair, Abdoh Taleb, Muhammad Naveed, Mirza Muhammad Faran Asraf Baig, Qin Zhang, Cuican Li, Murad Habib, Xingxing Cui, Kiganda Raymond Sembatya, Han Lei, Xiaohui Zhou. Systemic and local delivery of mesenchymal stem cells for heart renovation: Challenges and innovations.. Eur. J. Pharmacol June 2020. 20643695 [DOI: 10.1016/j.ejphar.2020.173049] [PMID: 32142771]

Lu 2012 {published data only}
20643696
• Lu M, Zhao S, Liu Q, Jiang S, Song P, Qian H, et al. Transplantation with autologous mesenchymal stem cells after acute myocardial infarction evaluated by magnetic resonance imaging: an experimental study. Journal of Thoracic Imaging 2012;27(2):125-35. 20643697

Madeddu 2021 {published data only}
20643698
• Paolo Madeddu. Cell therapy for the treatment of heart disease: Renovation work on the broken heart is still in progress.. Free Radic. Biol. Med. Feb 2021:206–222. 20643699 [DOI: 10.1016/j.freeradbiomed.2020.12.444] [PMID: 33421587]

Makkar 2012 {published data only}
20643700
• Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase I trial. Lancet 2012;379(9819):895- 904. 20643701
• Malliaras K, Cheng K, Smith R, Mendizabal A, Gerstenblith G, Marban L, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction: determinants of regenerative efficacy in the final 1-year results of the CADUCEUS trial. [American Heart Association 2012 Scientific Sessions and Resuscitation Science Symposium, Los Angeles, CA United States. 3-6 November 2012]. Circulation 2012;126(21 Suppl 1). 20643702
• Malliaras K, Makkar RR, Smith RR, Cheng K, Wu E, Bonow RO, et al. Intracoronary cardiosphere-derived cells after myocardial infarction: Evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trials (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction). Journal of the American College of Cardiology 2014;63(2):110-22. 20643703
• NCT00893360. A phase I randomized, dose escalation study of the safety and efficacy of intracoronary delivery of cardiospherederived stem cells in patients with ischemic left ventricular dysfunction and a recent myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00893360 (accessed 11 March 2015). 20643704

Marenzi 2007 {published data only}
20643705
• Marenzi G, Bartorelli AL. Improved clinical outcome after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. European Heart Journal 2007;28(17):2172-3. 20643706

Menasche 2002 {published data only}
20643707
• Menasche, P. Myoblast transplantation for the treatment of heart failure. Seminars in Thoracic and Cardiovascular Surgery 2002:565--568. 20643708 [DOI: 10.1053/stcs.2002.32319]

Menasche 2008 {published data only}
20643709
• Menasche, P and Alffieri, O and Janssens, S. Myoblast autologous grafting in ischemic cardiomyopathy (MAGIC). ACC cardiosource review journal. 20643710 [https://www.acc.org/latest-in-cardiology/clinical-trials/2013/05/28/14/07/magic]

Messori 2013 {published data only}
20643711
• Messori A, Fadd V, Maratea D, Trippoli S. Intracoronary infusion of bone-marrow derived mononuclear cells in acute myocardial infarction: are outcomes influenced by the number of infused cells? Heart, Lung and Circulation 2013;22(9):786-7. 20643712

Micheu 2015 {published data only}
20643713
• Micheu MM, Oprescu N, Calmac L, Pitic D, Dorobantu M. Early effect of autologous bone marrow stem cell

therapy on left ventricular systolic fun pilot study. [21st World Congress on the International Society for Heart Research, San Diego, CA United States. 30 June - 4 July 2013]. Jour Miruna Micheu, Nicoleta Oprescu, Lucian Calmac, Daniela Pitic, Maria Dorobantu. AUTOLOGOUS STEM CELL THERAPY FOR ACUTE MYOC FEASIBILITY AT 1 MONTH FOLLOW-UP - BUCHARESTEXPERIENCE. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţi 2014;24:19 * Miruna Mihaela Micheu, Nicoleta Oprescu, Alina Ioana Scarlatescu, Lucian Calmac, Daniela Pitic, Maria Dorobanţu. TRANSTEM pilot study - des patientswith acute myocardial infarction and impaired left ventricularfunction. Journal of Translational Medicine and Research 2015;20:215-2 Micheu/publication/288496899_TRANSTEM_pilot_study_- _Design_safety_and_results_6_months_after_autologous_bone_marrow_stem_cell_transplant_in_patients_with_acute_myocardial_infarctio pilot-study-Design-safety-and-results-6-months-after-autologous-bone-marrow-stem-cell-transplant-in-patients-with-acute-myocardial-infarcti

Micheu 2017 {published data only}
20643717
- Micheu, M M & Dorobantu, M. Fifteen years of bone marrow mononuclear cell therapy in acute myocardial infarction.. World J. Stem Cells Apr 2017:68–76. 20643718 [DOI: 10.4252/wjsc.v9.i4.68] [PMID: 28491241]

Mills 2007 {published data only}
20643719
- Mill JS, Rao SV. REPAIR-AMI: stem cells for acute myocardial infarction. Future Cardiology 2007;3(2):137-40. 20643720

Musialek 2006 {published data only}
20643721
- Musiatek P, Tracz W, Skotnicki AB, Zmudka K, Pienlazek P, Walter Z, et al. Transcoronary stem cell delivery using physiological endothelium-targeting perfusion technique: the rationale and a pilot study involving a comparison with conventional over thewire balloon coronary occlusions in patients with recent myocardial infarction. Kardiologia Polska 2006;64:489-98. 20643722

Musialek 2010 {published data only}
20643723
- Musialek P, Tekieli L, Kostkiewicz M, Majka M, Szot W, Walter Z, et al. Randomized transcoronary delivery of CD34(+) cells with perfusion versus stop-flow method in patients with recent myocardial infarction: early cardiac retention of 99(m)Tc-labeled cells activity. Journal of Nuclear Cardiology 2011;18(1):104-16. 20643724

Nasseri 2013 {published data only}
20643725
- Nasseri MH, Aghdami N, Ahmadi H, Moshkani FM, Farahani M, Madani H, et al. Phase iii randomized clinical trial comparing the effects of autologous bone marrow derived MNC and CD133 cells transplantation in AMI patients during CABG. [19th Annual Meeting of the International Society for Cellular Therapy, ISCT 2013, Auckland, New Zealand. 22-25 April 2013]. Cytotherapy 2013;15(4 Suppl 1):S12. 20643726

NCT00081913 {published data only}
20643727
- https://clinicaltrials.gov/show/NCT00081913. 20643728

NCT00114452 {published data only}
20643729
- https://clinicaltrials.gov/show/NCT00114452. 20643730

NCT00548613 {unpublished data only}
20643731[CTG: NCT00548613]
- NCT00548613. Combination stem cell (MESENDO) therapy for utilization and rescue of infarcted myocardium. https://clinicaltrials.gov/ct2/show/NCT00548613 (accessed 11 March 2015). 20643732

NCT00874354 {unpublished data only}
20643733[CTG: NCT00874354]
- NCT00874354. REVITALIZE: randomized evaluation of intracoronary transplantation of bone marrow stem

cells in myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00874354 (accessed 11 March 2015). 20643734

NCT00877903 {unpublished data only}
20643735[CTG: NCT00877903]
- NCT00877903. A phase II, multi-center, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of Prochymal® (ex vivo cultured adult human mesenchymal stem cells) intravenous infusion following acute myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT00877903 (accessed 11 March 2015). 20643736

NCT00950274 {published data only}
20643737
- https://clinicaltrials.gov/ct2/show/NCT00950274. 20643738

NCT01768702 {published data only}
20643739
- https://clinicaltrials.gov/show/NCT01768702. 20643740

nct03798353 {published data only}
20643741
- Https://clinicaltrials.gov/show/nct03798353. 20643742

nct04011059 {published data only}
20643743
- Https://clinicaltrials.gov/show/nct04011059. 20643744

nct04050163 {published data only}
20643745
- Https://clinicaltrials.gov/show/nct04050163. 20643746

nct04052191 {published data only}
20643747
- Https://clinicaltrials.gov/show/nct04052191. 20643748

NCT04340609 {published data only}
20643749
- https://clinicaltrials.gov/show/NCT04340609. 20643750

NCT05043610 {published data only}
20643751
- https://clinicaltrials.gov/show/NCT05043610. 20643752

Nie 2007 {published data only}
20643753
- Nie Y, Guo YH, Guo LJ. Intracoronary transfer autologous bone marrow stem cells can improve cardiac function in patients with left ventricular dysfunction after myocardial infarction [Chinese]. Beijing da Xue Xue Bao. Yi Xue Ban: Journal of Peking University. Health Sciences 2007;39(6):634-7. 20643754

Obradovic 2009 {published data only}
20643755
- Obradovic S, Balint B, Romanovic R, Trifunovic Z, Rusovic S, Baskot B, et al. Influence of intracoronary injections of bone-marrowderived mononuclear cells on large myocardial infarction outcome: quantum of initial necrosis is the key. Vojnosaniteski Pregled 2009;66(12):998-1004. 20643756

Osterziel 2007 {published data only}
20643757
- Osterziel KJ. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor

cells in acute myocardial infarction: final 1 year results of the REPAIR-AMI trial. European Heart Journal 2007;28(5):638. 20643758

Ott 2013 {published data only}
20643759
- Ott I, Schulz S, Cassese S, Byrne R, Kastrati A. Stem cell mobilization by granulocyte-colony stimulating factor in patients with acute myocardial infarction: five-year results of the REVIVAL-2 trial. Journal of the American College of Cardiology 2013;62(18 Suppl 1):B248. 20643760 Ott I, Schulz S, Fusaro M, Cassese S, Byrne R, Joner M, et al. Stem cell mobilization by granulocyte-colony stimulating factor in patients with acute myocardial infarction: five-year results of the REVIVAL-2 trial. European Heart Journal 2013;34(Suppl 1):74. 20643761

Peregud-Pogorzelska {published data only}
20643762
- Małgorzata Peregud-Pogorzelska, Krzysztof Przybycień, Bartłomiej Baumert, Maciej Kotowski, Ewa Pius-Sadowska, Krzysztof Safranow, Jarosław Peregud-Pogorzelski, Zdzisława Kornacewicz-Jach, Edyta Paczkowska, Bogusław Machaliński. The Effect of Intracoronary Infusion of Autologous Bone Marrow-Derived Lineage-Negative Stem/Progenitor Cells on Remodeling of PostInfarcted Heart in Patient with Acute Myocardial Infarction. International Journal of Medical Science Apr 2020. 20643763 [DOI: 10.7150/ijms.42561] [PMID: 32410827]

Peruga 2009 {published data only}
20643764
- Peruga J, Plewka M, Kasprzak J, Jezewski T, Wierzbicka A, Robak T, et al. Intracoronary administration of stem cells in patients with acute myocardial infarction - angiographic follow-up. Kardiologia Polska 2009;67(5):477-84. 20643765

Potapov 2007 {published data only}
20643766
- Potapov, I V and Kirillov, I A. Enhancement of angiogenesis as the basis of reparative morphogenesis in ischemic myocardial lesion. Vestnik rossiiskoi akademii meditsinskikh nauk 2007. 20643767 [PMID: 18030711]

Qayyum 2017 {published data only}
20643768
- Abbas Ali Qayyum, Anders Bruun Mathiasen, Naja Dam Mygind, Jørgen Tobias Kühl, Erik Jørgensen, Steffen Helqvist, Jens Jørgen Elberg, Klaus Fuglsang Kofoed, Niels Groove Vejlstrup, Anne Fischer-Nielsen, Mandana Haack-Sørensen, Annette Ekblond, Jens Kastrup. Adipose-Derived Stromal Cells for Treatment of Patients with Chronic Ischemic Heart Disease (MyStromalCell Trial): A Randomized Placebo-Controlled Study.. Stem Cells Int. 2017. 20643769 [DOI: 10.1155/2017/5237063.] [PMID: 29333165]

Ramireddy 2017 {published data only}
20643770
- Ramireddy, Archana and Brodt, Chad R and Mendizabal, Adam M and DiFede, Darcy L and Healy, Chris and Goyal, Vishal and Alansari, Yahya and Coffey, James O and Viles-Gonzalez, Juan F and Heldman, Alan W and Goldberger, Jeffrey J and Myerburg, Robert J and Hare, Joshua M and Mitrani, Raul D. Effects of Transendocardial Stem Cell Injection on Ventricular Proarrhythmia in Patients with Ischemic Cardiomyopathy: Results from the POSEIDON and TAC-HFT Trials. Stem cells translational medicine May 2017. 20643771 [DOI: 10.1002/sctm.16-0328] [PMID: 28252842]

Raval 2018 {published data only}
20643772
- Raval, Amish N. Clinical Trial Design for Investigational Cardio-Regenerative Therapy. Advances in experimental medicine and biology 2018. 20643773 [DOI: 10.1007/978-3-319-97421-7_11] [PMID: 30238373]

Razeghian-Jahromi 2021 {published data only}
20643774
- Iman Razeghian-Jahromi, Anthony G Matta, Ronan Canitrot, Mohammad Javad Zibaeenezhad, Mahboobeh

Razmkhah, Anahid Safari, Vanessa Nader, Jerome Roncalli. Surfing the clinical trials of mesenchymal stem cell therapy in ischemic cardiomyopathy. Stem Cell Research & Therapy Jun 2021. 20643775 [PMID: 10.1186/s13287-021-02443-1] [PMID: 34162424]

Reinsch 2018 {published data only}
20643776
- Reinsch, M and Weinberger, F. [Stem cell-based cardiac regeneration after myocardial infarction]. Stammzellbasierte kardiale Regeneration nach Infarkt.:109-114. 20643777 [DOI: 10.1007/s00059-017-4662-2] [PMID: 29243046]

Ripa 2009 {published data only}
20643778
- Ripa, R S and Kastrup, J. Stem cells: REGENT trial-the end of cell therapy for MI? Nature reviews. Cardiology:567-8. 20643779 [DOI: 10.1038/nrcardio.2009.137] [PMID: 19696777]

Roberts 2004 {published data only}
20643780
- Roberts, R. Will stem cells face the same premature demise as gene therapy? American heart hospital journal 2004. 20643781 [DOI: 10.1111/j.1541-9215.2004.03534.x] [PMID: 15604854]

Schachinger 2004 {published data only}
20643782
- Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. New England Journal of Medicine 2006;355(12):1222-32. 20643783
- Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106:3009-17. 20643784
- Britten MB, Abolmaali ND, Assmus B, Lehmann R, Honold J, Scmitt J, et al. Infarct remodelling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI). Mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation 2003;108:2212-8. 20643785
- Leistner DM, Fischer-Rasokat U, Honold J, Seeger FH, Schachinger V, Lehmann R, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. Clinical Research in Cardiology 2011;100(10):925-34. 20643786
- Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction. Final one-year results of the TOPCARE-AMI trial. Journal of the American College of Cardiology 2004;44:1690-9. 20643787
- Schachinger V, Assmus B, Honold J, Lehmann R, Hofmann WK, Martin H, et al. Normalization of coronary blood flow in the infarct related artery after intracoronary progenitor cell therapy: intracoronary Doppler substudy of the TOPCARE-AMI trial. Clinical Research in Cardiology 2006;95:13-22. 20643788

Schueller 2007 {published data only}
20643789
- Schueller PO, Meyer C, Brehm M, Wernet P, Schannwell CM, Strauer BE. Intracoronary autologous bone marrow cell transplantation beneficially modulates heart rate variability. International Journal of Cardiology 2007;119(3):398-9. 20643790

Shahid 2016 {published data only}
20643791
- Shahid, Muhammad Siddique and Lasheen, Wael and Haider, Khawaja Husnain. The modest outcome of clinical trials with bone marrow cells for myocardial repair: is the autologous source of cells the prime culprit? Journal of Thoracic Disease Oct 2016:E1371-1374. 20643792 [DOI: 10.21037/jtd.2016.10.37] [PMID: 27867632]

Shrimahachota 2011 {published data only}
20643793
- Srimahachota S, Boonyaratavej S, Rerkpattanapipat P, Wangsupachart S, Tumkosit M, Bunworasate U, et al.

Intra-coronary bone marrow mononuclear cell transplantation in patients with ST-elevation myocardial infarction: a randomized controlled study. Journal of the Medical Association of Thailand 2011;94(6):657-63. 20643794

Smiseth 2014 {published data only}
20643795
- Smiseth, O A and Steg, P G and Sipido, K and Battler, A and Wijins, W. News from the European Society of Cardiology Congress in Vienna, August 30 to September 3, 2003. In: Journal of the American College of Cardiology. Feb 2004. 20643796 [DOI: 10.1016/j.jacc.2003.11.025] [PMID: 14975484]

Soetisna 2020 {published data only}
20643797
- Soetisna, Tri Wisesa and Sukmawan, Renan and Setianto, Budhi and Mansyur, Muchtaruddin and Murni, Tri Wahyu and Listiyaningsih, Erlin and Santoso, Anwar. Combined transepicardial and transseptal implantation of autologous CD 133+ bone marrow cells during bypass grafting improves cardiac function in patients with low ejection fraction. Journal of cardiac surgery Apr 2020:740-746. 20643798 [DOI: 10.1111/jocs.14454] [PMID: 32048356]

Soetisna 2021 {published data only}
20643799
- Soetisna, T W. CD133+ Stem Cell Therapy Effects on Myocardial Regeneration Through Increased Vascular Endothelial Growth Factor Correlate with Cardiac Magnetic Resonance Imaging Results in Coronary Artery Bypass Graft Surgery Patients with Low Ejection Fraction. Heart Surgery Forum Jul 2021. 20643800 [DOI: 10.1532/hsf.3763] [PMID: 34473043]

Taljaard 2010 {published data only}
20643801
- NCT00936819. Enhanced Angiogenic Cell Therapy in Acute Myocardial Infarction: a multicentre, phase IIb randomised placebocontrolled trial. https://clinicaltrials.gov/ct2/show/NCT00936819 (accessed 11 March 2015). 20643802
- Taljaard M, Ward MR, Kutryk MJB, Courtman DW, Camack N, Goodman SG, et al. Rationale and design of Enhanced Angiogenic Cell Therapy in Acute Myocardial Infarction (ENACT-AMI): the first randomized placebo-controlled trial of enhanced progenitor cell therapy for acute myocardial infarction. American Heart Journal 2010;159(3):354-60. 20643803

Tatsumi 2006 {published data only}
20643804
- Tatsumi, T and Matsubara, H. Therapeutic angiogenesis for peripheral arterial disease and ischemic heart disease by autologous bone marrow cells implantation. Nippon rinsho. Japanese journal of clinical medicine Nov 2006:2126-34. 20643805 [PMID: 17087307]

Tendera 2009b {published data only}
20643806
- Tendera, M and Wojakowski, W. Cell therapy - Success does not come easy. European heart journal Feb 2009. 20643807 [DOI: 10.1093/eurheartj/ehp066]

Terrovitis 2011 {published data only}
20643808
- Pantsios C, Ntalianis A, Kanakakis J, Papadimitriou C, Kapelios C, Repasos E, et al. Functional and structural benefits after therapy with autologous GCSF-mobilized peripheral blood progenitor cells in subacute myocardial infarction: a prospective, randomized, controlled study. [Heart Failure Congress 2014 and the 1st World Congress on Acute Heart Failure, Athens, Greece. 17-20 May 2014]. European Journal of Heart Failure 2014;16:349. 20643809
- Terrovitis J, Ntalianis A, Kanakakis J, Papadimitriou C, Pantsios C, Eleftherakis E, et al. The effect of intracoronary infusion of mobilized peripheral blood stem cells on left ventricular function after myocardial infarction. [American Heart Association's Scientific Sessions 2011, Orlando, FL United States. 12-16 November 2011]. Circulation 2011;124(21 Suppl 1). 20643810

Trzos 2009 {published data only}
20643811
- Trzos E, Krzeminska-Pakula M, Rechcinski T, Bugala M, Kasprzak JD, Plewka M, et al. The influence of intracoronary autologous mononuclear bone marrow cell transplantation. [European Society of Cardiology, ESC Congress 2009, Barcelona, Spain. 29 August - 2 September 2009]. European Heart Journal 2009;30:496. 20643812
- Trzos E, Krzeminska-Pakula M, Rechcinski T, Plewka M, Kasprzak J, Peruga JZ, et al. The effects of intracoronary autologous mononuclear bone marrow cell transplantation on cardiac arrhythmia and heart rate variability. Kadiologia Polska 2009;67(7):713-21. 20643813

Tyler 2018 {published data only}
20643814
- Tyler, J M and Kereiakes, D J and Henry, T D. No Risk, No Reward; Are Post-STEMI Cell Therapy Clinical Trials Approaching Futility? Circulation research Aug 2018. 20643815 [DOI: 10.1161/CIRCRESAHA.118.313593]

Ulus 2020 {published data only}
20643816
- A Tulga Ulus, Ceren Mungan, Murat Kurtoglu, Ferda Topal Celikkan, Mesut Akyol, Merve Sucu, Mustafa Toru, Serdar Savas Gul , Ozgur Cinar, Alp Can. Intramyocardial Transplantation of Umbilical Cord Mesenchymal Stromal Cells in Chronic Ischemic Cardiomyopathy: A Controlled, Randomized Clinical Trial (HUC-HEART Trial). International Journal of Stem cells Nov 2020. 20643817 [DOI: 32840230] [PMID: 32840230]

Vanderheyden 2007 {published data only}
20643818
- Vanderheyden M, Vercauteren S, Mansour S, Delrue L, Vandekerckhove B, Heyndrickx GR, et al. Time-dependent effects on coronary remodelling and epicardial conductance after intracoronary injection of enriched hematopoietic bone marrow stem cells in patients with previous myocardial infarction. Cell Transplantation 2007;16(9):919-25. 20643819

Vassali 2007 {published data only}
20643820
- Vassalli, G and Vanderheyden, M and Renders, F and Eeckhout, E and Bartunek, J. Bone marrow stem cell therapy for cardiac repair: challenges and perspectives. Minerva cardioangiologica Oct 2007. 20643821 [PMID: 17912169]

Vrtovec 2019 {published data only}
20643822
- Vrtovec, Bojan and Bolli, Roberto. Potential Strategies for Clinical Translation of Repeated Cell Therapy. Circulation research Mar 2019:690-692. 20643823 [PMID: 10.1161/CIRCRESAHA.118.314653] [PMID: 30817255]

Wang 2006 {published data only}
20643824
- Wang WM, Sun NL, Liu J, Zhang P, Liu KY, Wang Q, et al. Effects of intracoronary autologous bone marrow mononuclear cells transplantation in patients with anterior myocardial infarction. Chinese Journal of Cardiology 2006;34(2):103-6. 20643825

Warbington 2013 {published data only}
20643826
- Warbington B, Weinstein D, Mallinson D, Olijnyk D, Paterson S, Ridha S, et al. Characterization on bone marrow derived CD34+ cells with different mobility potentials by micro RNA fingerprinting. [55th Annual Meeting of the American Society of Hematology, ASH2013, New Orleans, LA United States. 7-10 December 2013]. Blood 2013;122(21). 20643827

Welt 2006 {published data only}
20643828
- Welt, F G P and Losordo, D W. Cell therapy for acute myocardial infarction: curb your enthusiasm? Circulation Mar 2006:1272- -1274. 20643829 [DOI: 10.1161/CIRCULATIONAHA.105.613034] [PMID: 16534025]

Wollert 2009 {published data only}
20643830
• Wollert, K C and Drexter, H. Does cell therapy for myocardial infarction and heart failure work? Dialogues in cardiovascular medicine Jan 2009:37--43. 20643831 [https://www.researchgate.net/publication/293763569_Does_cell_therapy_for_myocardial_infarction_and_heart_failure_work]

Wollert 2015 {published data only}
20643832
• Wollert, Kai C. Bone marrow mononuclear cell therapy for acute myocardial infarction: we know what we want, but we just don't know how yet. Heart Mar 2015:337-338. 20643833 [PMID: 10.1136/heartjnl-2014-306787] [PMID: 25601817]

Wu 2017 {published data only}
20643834
• Wu, Zhiye and Chen, Guoqin and Zhang, Jianwu and Hua, Yongquan and Li, Jinliang and Liu, Bei and Huang, Anqing and Li, Hekai and Chen, Minsheng and Ou, Caiwen. Treatment of Myocardial Infarction with Gene-modified Mesenchymal Stem Cells in a Small Molecular Hydrogel. Scientific reports Nov 2017. 20643835 [DOI: 10.1038/s41598-017-15870-z]

Yanamandala 2017 {published data only}
20643836
• Yanamandala, Mounica and Kitsis, Richard N and Bolli, Roberto and Zhu, Wuqiang and Jun, Ho-wook and Zhang, Jianyi and Garry, Daniel J and Kamp, Timothy J and Hare, Joshua M and Yoon, Young-sup and Bursac, Nenad and Prabhu, Sumanth D and Iidorn, Gerald W and {Dorn 2nd}, Gerald W. Overcoming the Roadblocks to Cardiac Cell Therapy Using Tissue Engineering. JACC Aug 2017:766-775. 20643837 [DOI: 10.1016/j.jacc.2017.06.012] [PMID: 28774384]

Yang 2010 {published data only}
20643838
• Jang Z, Zhang F, Ma W, Chen B, Zhou F, Xu Z, et al. A novel approach to transplanting bone marrow stem cells to repair human myocardial infarction: delivery via a noninfarct-related artery. Cardiovascular Therapeutics 2010;28(6):380-5. 20643839

Yoon 2010 {published data only}
20643840
• Yoon, C H and Koyanagi, M and Iekushi, K and Seeger, F and Urbich, C and Zeiher, A M and Dimmeler, S. Mechanism of improved cardiac function after bone marrow mononuclear cell therapy: role of cardiovascular lineage commitment. Circulation:2001- 2011. 20643841 [DOI: 10.1161/CIRCULATIONAHA.109.909291] [PMID: 20421519]

Yu 2005 {published data only}
20643842
• Yu LF, Lu B, Yu LY, Li TF, Chen YJ, Liu WH, et al. Effect of intracoronary transplantation of bone marrow mononuclear cells on cardiac function and regional myocardial movement of patients with acute myocardial infarction. Chinese Journal of Clinical Rehabilitation 2005;9(26):71-4. 20643843

Yu 2014 {published data only}
20643844
• Yu L, Zhang M. Intracoronary transplantation of autologous peripheral blood stem cells in old patients with acute myocardial infarction: five-year postoperative evaluation of cardiac function. Chinese Journal of Tissue Engineering Research 2014;18(1):125-30. 20643845

Zhang 2021b {published data only}
20643846
• Jianyi Zhang, Roberto Bolli, Daniel J Garry, Eduardo Marbán, Philippe Menasché, Wolfram-Hubertus Zimmermann, Timothy J Kamp, Joseph C Wu, and Victor J Dzau. Basic and Translational Research in Cardiac

Repair and Regeneration: JACC Stateof-the-Art Review. JACC Nov 2021. 20643847 [DOI: 10.1016/j.jacc.2021.09.019] [PMID: 34794691]

References to studies awaiting assessment

Alves 2011 {published data only}
20643848
- Alves SA, Pereira SB, Frajtag R, Moreira RC, Souza ALS, Haddad A, et al. Long-term follow-up of stem cell therapy after acute myocardial infarction. [15th Annual Scientific Meeting, Heart Failure Society of America, Boston, MA United States. 18-21 September 2011]. Journal of Cardiac Failure 2011;17(8 Suppl 1):S87. 20643849

Chang 2008 {published data only}
20643850
- Chang SA, Kim HK, Lee HY, Choi SY, Koo BK, Kim YJ, et al. Restoration of left ventricular synchronous contraction after acute myocardial infarction by stem cell therapy: new insights into the therapeutic implication of stem cell therapy for acute myocardial infarction. Heart 2008;94(8):995-1001. 20643851
- Chang SA, Kim HK, Lee HY, Kang HJ, Kim YJ, Zo JH. Restoration of synchronicity of the left ventricular myocardial contraction with stem cell therapy: New insights into the therapeutic implication of stem cell therapy in myocardial infarction. [American Heart Association Scientific Sessions 2006, Chicago, IL United States, 12-15 November 2006]. Circulation 2006;114(18 Suppl):Abstract 2718. 20643852

Fernandez-Pereira 2006 {published data only}
20643853
- Fernandez-Pereira C, Vigo CF, Bataglia S, Perez de la Hoz R, Vetulli H, Koziner B, et al. Autologous bone marrow stem cell transplant after myocardial infarction. In-hospital and long-term follow-up results of the randomized Argentina trial (STAR AMI). European Heart Journal 2006;27(Suppl 1):279. 20643854

Huang 2007b {published data only}
20643855
- Huang R, Yao K, Ge JB, Zhou YZ, Qian YJY, Hge LEI, et al. Timing and therapeutic response of intracoronary bone marrow mononuclear cells administration in acute ST-elevation myocardial infarction patients. European Heart Journal 2007;28(Suppl 1):229. 20643856

Huang 2008 {published data only}
20643857
- Huang RC, Yao K, Qian J, Sun A, Ge L, Li YL, et al. Transcatheter transplantation of stem cells for treatment of acute myocardial infarction (TCT-STAMI-2). European Heart Journal 2008;28(Suppl 1):366-7. 20643858

Lee 2005 {published data only}
20643859
- Lee YL, Ge JB, Qian JY, Shi JH, Wang QB, Niu YH, et al. Clinical trial on emergent intracoronary autologous bone marrow mononuclear cells transfer in patients with acute anterior myocardial infarction. American Journal of Cardiology 2005;96(7A Suppl):25H. 20643860

Lu 2012b {published data only}
20643861
- Lu M, Song L, Jiang S, Yang Y, Zhang Y, Yin G, et al. Effects of autologous bone marrow mononuclear cells transplantation via coronary artery in patients with acute myocardial infarction assessed by MRI. [23rd Great Wall International Congress of Cardiology, Asia Pacific Heart Congress 2012, Beijing, China. 11-14 October 2012]. Heart 2012;98:E172. 20643862

Park 2011 {published data only}
20643863
- Park SD, Shin SH, Woo SI, Park KS, Kim DH, Kwan J. Effect of heart rate variability of autologous bone marrow

mesenchymal stem cells in patients with ST segment elevation myocardial infarction. [EHRA Europace 2011, Madrid, Spain. 26-29 June 2011]. Europace 2011;13(Suppl 3). 20643864

Perez-Oteyza 2006 {published data only}
20643865
- Catalan-Sanz M, Vaticon C, Asin E, Munoz M, Perez de Oteyza J, Cuevas P, et al. Randomised trial of intracoronary autologous bone-marrow cell transfer after myocardial infarction. Preliminary results by cardiac magnetic resonance imaging. European Heart Journal 2005;26(Suppl 1):627. 20643866
- Perez-Oteyza J, Ramos P, Catalan SM, Perez-Abad M, Blanchard MJ, Heras C, et al. Intracoronary autologous bone-marrow stem cell transfer after myocardial infarction. In: 11th Congress of the European Haematology Association, Amsterdam, The Netherlands. Amsterdam, The Netherlands: European Haematology Association, 2006. 20643867

Silva 2014 {published data only}
20643868
- Silva T, Fiarresga A, Abreu J, Reffeira A, Branco L, Timoteo A, et al. Effect of intracoronary infusion of autologous bone marrow derived progenitor cells on the global longitudinal strain in patients with ST-elevation myocardial infarction. [European Society of Cardiology, ESC Congress 2014, Barcelona, Spain. 30 August-3 September 2014]. European Heart Journal 2014;35:306. 20643869

References to ongoing studies

EUCTR 2006-001772-20-ES {unpublished data only}
20643870
- EUCTR 2006-001772-20-ES. Effect of intracoronary injection of autologous stem cells on left ventricular ejection fraction and volumes one year after an acute myocardial infarction. https://www.clinicaltrialsregister.eu/ctr-search/search? query=eudract_number:2006-001772-20 (accessed 11 March 2015). 20643871

EUCTR 2006-005628-17-ES {unpublished data only}
20643872
- EUCTR 2006-005628-17-ES. Open study with blind regulator on the effectiveness of autologous bone marrow mononuclear cells in patients with left ventricular dysfunction after myocardial infarction. https://www.clinicaltrialsregister.eu/ctr-search/search? query=eudract_number:2006-005628-17 (accessed 11 March 2015). 20643873

ISRCTN65630838 {unpublished data only}
20643874[ISRCTN: ISRCTN65630838]
- 20643875 ISRCTN65630838. Selected bone marrow cell transplant following myocardial infarction in patients undergoing coronary surgery: a prospective double blind controlled trial. http://www.isrctn.com/ISRCTN65630838 (accessed 11 March 2015). 20643876

NCT00529932 {unpublished data only}
20643877[CTG: NCT00529932]
- EUCTR 2007-001448-33-NL. A multi-centre, double-blind, randomised, placebo-controlled trial using CD133 enriched bone marrow cells following primary angioplasty for acute myocardial infarction with central core laboratory analysis. - SELECT-AMI. https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2007-001448-33 (accessed 11 March 2015). 20643878 NCT00529932. A trial using CD133 enriched bone marrow cells following primary angioplasty for acute myocardial infarction. http://clinicaltrials.gov/ct2/show/NCT00529932 (accessed on 11 March 2015). 20643879

NCT00711542 {unpublished data only}
20643880[CTG: NCT00711542]
- NCT00711542. Effects of intracoronary progenitor cell therapy on coronary flow reverse after acute MI. http://clinicaltrials.gov/ct2/show/NCT00711542 (accessed 11 March 2015). 20643881

NCT00936819 {unpublished data only}
20643882[CTG: NCT00936819]
• NCT00936819. The Enhanced Angiogenic Cell Therapy - Acute Myocardial Infarction Trial (ENACT-AMI). https://clinicaltrials.gov/ct2/show/NCT00936819 (accessed 11 March 2015). 20643883

NCT01187654 {unpublished data only}
20643884[CTG: NCT01187654]
• NCT01187654. Bone marrow derived AC 133+ and mono-nuclear cells (MNC) implantation in myocardial infarction (MI) patient. https://clinicaltrials.gov/ct2/show/NCT01187654 (accessed 11 March 2015). 20643885

NCT01394432 {unpublished data only}
20643886[CTG: NCT01394432]
• NCT01394432. "ESTIMATION Study" for endocardial mesenchymal stem cells implantation in patients after acute myocardial infarction. https://clinicaltrials.gov/ct2/show/NCT01394432 (accessed 11 March 2015). 20643887

NCT01536106 {unpublished data only}
20643888[CTG: NCT01536106]
• NCT01536106. Rapid delivery of autologous bone marrow derived stem cells in acute myocardial infarction patients (AMIRST). https://clinicaltrials.gov/ct2/show/NCT01536106 (accessed 11 March 2015). 20643889

NCT01625949 {unpublished data only}
20643890[CTG: NCT01625949]
• NCT01625949. Stem cell therapy in patients with myocardial infarction and persistent total occlusion of infarct related artery (COAT). https://clinicaltrials.gov/ct2/show/NCT01625949 (accessed 11 March 2015). 20643891

NCT01652209 {unpublished data only}
20643892[CTG: NCT01652209]
• CT01652209. RELIEF (A Randomized, Open labEled, muLticenter Trial for Safety and Efficacy of Intracoronary Adult Human Mesenchymal stEm Cells Acute Myocardial inFarction). https://clinicaltrials.gov/ct2/show/NCT01652209 (accessed 11 March 2015). 20643893

NCT02323620 {unpublished data only}
20643894[CTG: NCT02323620]
• NCT02323620. Impact of intracoronary injection of autologous BMMC for LV contractility and remodeling in patients with STEMI (RACE-STEMI). https://www.clinicaltrials.gov/ct2/show/NCT02323620 (accessed March 2015). 20643895

Pena-Duque 2011 {published data only}
20643896
• NCT00725738. Intracoronary autologous stem cell transplantation in ST-elevation myocardial infarction: TRACIA STUDY. http://clinicaltrials.gov/ct2/show/NCT00725738 (accessed 11 March 2015). 20643897
• Pena-Duque MA, Martinez-Rios MA, Calderon GE, Mejia AM, Gomez E, Martinez-Sanchez C, et al. Design and implementation of the TRACIA: intracoronary autologous transplant of bone marrow-derived stem cells for acute ST elevation myocardial infarction. Archivos de Cardiologia de Mexico 2011;81(3):183-7. 20643898

Xu 2019 {published data only}
20643899
• Jun-Yan Xu, Hai-Yan Qian, Pei-Sen Huang, Jun Xu, Yu-Yan Xiong, Wen-Yang Jiang, Yi Xu, Wen-Xiu Leng, Xiang-Dong Li, GuiHao Chen, Rui-Jie Tang, Cun-Rong Huang, Meng-Jin Hu, Chen Jin, Yuan Wu, Jun Zhang, Jie Qian, Bo Xu, Shi-Hua Zhao, Min-Jie Lu, Rui Shen, Wei Fang, Wei-Chun Wu, Xi Chen, Yang Wang, Wei Li, Xiang-Feng Lu, Xi-Feng Jiang, Chun-Cheng Ma, Jian-Wen Li, Yong-Jian Geng, Shu-Bin Qiao, Run-Lin Gao, Yue-Jin Yang. Transplantation efficacy of autologous bone marrow mesenchymal stem cells combined with atorvastatin for acute myocardial infarction (TEAM-AMI): rationale and design of a randomized, double-blind, placebo-controlled, multi-center, Phase II TEAM-AMI trial. Regenerative Medicine Dec 2019;14:1077-1087. 20643900 [DOI: 10.2217/rme-2019-0024] [PMID: 31829095]

• Jun-Yan Xu, Hai-Yan Qian, Pei-Sen Huang, Jun Xu, Yu-Yan Xiong, Wen-Yang Jiang, Yi Xu, Wen-Xiu Leng, Xiang-Dong Li, GuiHao Chen, Rui-Jie Tang, Cun-Rong Huang, Meng-Jin Hu, Chen Jin, Yuan Wu, Jun Zhang, Jie Qian, Bo Xu, Shi-Hua Zhao, Min-Jie Lu, Rui Shen, Wei Fang, Wei-Chun Wu, Xi Chen, Yang Wang, Wei Li, Xiang-Feng Lu, Xi-Feng Jiang, Chun-Cheng Ma, Jian-Wen Li, Yong-Jian Geng, Shu-Bin Qiao, Run-Lin Gao, Yue-Jin Yang. Transplantation efficacy of autologous bone marrow mesenchymal stem cells combined with atorvastatin for acute myocardial infarction (TEAM-AMI): rationale and design of a randomized, double-blind, placebo-controlled, multi-center, Phase II TEAM-AMI trial. Regen Med. 20643901 [PMID: 31829095]

Additional references

Afzal 2015 Afzal MR, Samanta A, Shah ZI, Jeevanantham V, Abdel-Latif A, Zuba-Surma EK, et al. Adult bone marrow cell therapy for ischemic heart disease: evidence and insights from randomized controlled trials. Circulation Research 2015 Jul 9 [Epub ahead of print].

Al-Mohammad 2010 Al-Mohammad A, Mant J, Laramee P, Swain S, Chronic Heart Failure Guideline Development Group. Diagnosis and management of adults with chronic heart failure: summary of updated NICE guidance. BMJ 2010;341:c4130.

Assmus 2002 Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106(24):3009-17.

Atoui 2012 Atoui R, Chiu R. Mesenchymal stromal cells as universal donor cells. Expert Opinion on Biological Therapy 2012;12(10):1293- 1297. [DOI: DOI: 10.1517/14712598.2012.711307]

Bartunek 2006 Bartunek J, Dimmeler S, Drexler H, Frenandez-Aviles F, Gallinares M, Janssens S, et al. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. European Heart Journal 2006;27(11):1338-40.

Bartunek 2010 Bartunek J, Vanderheyden M, Hill J, Terzic A. Cells as biologics for cardiac repair in ischaemic heart failure. Heart 2010;96:792- 800.

Behfar 2014 Behfar A, Crespo-Diaz R, Terzic A, Gersh BJ. Cell therapy for cardiac repair--lessons from clinical trials. Nature Reviews Cardiology 2014;11(4):232-46.

Beitnes 2009 Beitnes JO, Hopp E, Lunde K, Solheim S, Arnesen H, Brinchmann JE, et al. Long-term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: the ASTAMI randomised, controlled study. Heart 2009;95(24):1983-9.

BHF 2014 Townsend N, Williams J, Bhatnagar P, Wickramasinghe K, Rayner M. Cardiovascular disease statistics, 2014 (https://www.bhf.org.uk/~/media/files/publications/research/bhf_cvd-statistics-2014_web_2.pdf). London: British Heart Foundation, 2014 (accessed 8 May 2014).

Bolli 2020 Bolli R. Cell therapy for acute myocardial infarction: Requiescat in Pace. Eur Heart J 2020;41:3711-3714.

Borm 2009 Borm GF, Donders AR. Updating meta-analyses leads to larger type I errors than publication bias. Journal of Clinical Epidemiology 2009;62(8):825-30.

Cheng 2014 Cheng K, Ibrahim A, Hensley MT, Shen D, Sun B, Middleton R, et al. Relative roles of CD90 and c-kit to the regenerative efficacy of cardiosphere-derived cells in humans and in a mouse model of myocardial infarction. Journal of the American Heart Association 2014;3(5):e001260.

de Jong 2014 de Jong R, Houtgraaf JH, Samiei S, Boersma E, Duckers HJ. Intracoronary stem cell infusion after acute myocardial infarction: a meta-analysis and update on clinical trials. Circulation. Cardiovascular Interventions 2014;7(2):156-67.

Delewi 2014 Delewi R, Hirsch A, Tijssen JG, Schächinger V, Wojakowski W, Roncalli J, et al. Impact of intracoronary bone marrow cell therapy on left ventricular function in the setting of ST-segment elevation myocardial infarction: a collaborative meta-analysis. European Heart Journal 2014;35(15):989-98.

Egger 1997 Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.

Engblom 2009 Engblom H, Hedström E, Heiberg E, Wagner GS, Pahlm O, Arheden H. Rapid initial reduction of hyperenhanced myocardium after reperfused first myocardial infarction suggests recovery of the peri-infarction zone: one-year follow-up by MRI. Circulation. Cardiovascular Imaging 2009;2(1):47-55.

ESC/ACC 2000 The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined - a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition if myocardial infarction. European Heart Journal 2000;21(18):1502-13.

Falk 1995 Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995;92(3):657-71.

Fernandez-Aviles 2004 Fernandez-Aviles F, San Roman JA, Garcia-Frade J, Fernandez ME, Penarrubia MJ, de la Fuente L, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circulation Research 2004;95(7):742-8.

Fisher 2012 Clifford DM, Fisher SA, Brunskill SJ, Doree C, Mathur A, Clarke MJ, et al. Long-term effects of autologous bone marrow stem cell treatment in acute myocardial infarction: factors that may influence outcomes. PLoS One 2012;7(5):e37373.

Fisher 2014 Fisher SA, Brunskill SJ, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. Cochrane Database of Systematic Reviews 2014, Issue 4. Art. No: CD007888. [DOI: 10.1002/14651858.CD007888.pub2]

Fisher 2015 Fisher SA, Doree C, Mathur A, Martin-Rendon E. Meta-analysis of cell therapy trials for patients with heart failure. Circulation Research 2015;116(8):1361-77.

Fisher 2015b Fisher SA, Zhang H, Doree C, Mathur A, Martin-Rendon E. Stem cell treatment for acute myocardial infarction.. Cochrane Database Syst Rev. 2015;2015(9):CD006536. [DOI: 10.1002/14651858]

GRADEpro GDT 2014 [Computer program] GRADEpro GDT [www.gradepro.org]. McMaster University, 2014 (accessed 19 May 2015).

Gyöngyösi 2015 Gyöngyösi M, Wojakowski W, Lemarchand P, Lunde K, Tendera M, Bartunek J, et al. Meta-Analysis of Cell-based CaRdiac stUdiEs (ACCRUE) in patients with acute myocardial infarction based on individual patient data. Circulation Research 2015;116(8):1346-60.

Hartwell 2005 Hartwell D, Colquitt J, Loveman E, Clegg AJ, Brodin H, Waugh N, et al. Clinical effectiveness and cost-effectiveness of immediate angioplasty for acute myocardial infarction: systematic review and economic evaluation. Health Technology Assessment 2005;9(17):1-99.

Henry 2018 Henry TD, Losordo DW, Traverse JH, Schatz RA, Jolicoeur EM, Schaer GL, Clare R, Chiswell K, White CJ, Fortuin FD, Kereiakes DJ, Zeiher AM, Sherman W, Hunt AS, Povsic TJ. Autologous CD34+ cell therapy improves exercise capacity, angina frequency and reduces mortality in no-option refractory angina: a patient-level pooled analysis of randomized double-blinded trials. Eur Heart J 2018;39:2208-2216.

Higgins 2003 Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327(7414):557-60.

Higgins 2011 Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Hu 2007 Hu M, Cappeleri J, Lan KK. Applying the law of the iterated logarithm to control type I error in cumulative meta-analysis of binary outcomes. Clinical Trials 2007;4(4):329-40.

Jeevanantham 2012 Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. Circulation 2012;126(5):551.

Jones 2019 Jones DA, Weeraman D, Colicchia M, Hussain MA, Veerapen D, Andiapen M, Rathod KS, Baumbach A, Mathur A. The Impact of Cell Therapy on Cardiovascular Outcomes in Patients With Refractory Angina. Circ Res 2019;124:1786-1795.

Lan 2003 Lan KK, Hu M, Cappelieri J. Applying the law of the iterated logarithm to cumulative meta-analysis of a continuous endpoint. Statistica Sinica 2003;13:1135-45.

Leri 2009 Leri A, Kajstura J, Anversa P, Frishman WH. Myocardial regeneration and stem cell repair. Current Problem in Cardiology 2009;33:91-153.

Mathur 2020 Mathur A, Fernández-Avilés F, Bartunek J, Belmans A, Crea F, Dowlut S, Galiñanes M, Good MC, Hartikainen J, Hauskeller C, Janssens S, Kala P, Kastrup J, Martin J, Menasché P, Sanz-Ruiz R, Ylä-Herttuala S, Zeiher A, BAMI Group. The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: the BAMI trial. Eur Heart J 2020;41(38):3702-3710. [DOI: 10.1093/eurheartj/ehaa651.]

Meyer 2006 Meyer GP, Wollert KC, Drexler H. Stem cell therapy: a new perspective in the treatment of patients with acute myocardial infarction. European Journal of Medical Research 2006;11(10):439-46.

Nair 2015 Nair V, Madan H, Sofat S, Ganguli P, Jacob MJ, Datta R, Bharadwaj P, Sarkar RS, Pandit AJ, Nityanand S, Goel PK, Garg N, Gambhir S, George PV, Chandy S, Mathews V, George OK, Talwar KK, Bahl A, Marwah N, Bhatacharya A, Bhargava B, Airan B, Mohanty S, Patel CD, Sharma A, Bhatnagar S, Mondal A, Jose J, Srivastava A, MI3 Trial. Efficacy of stem cell in improvement of left ventricular function in acute myocardial infarction--MI3 Trial. Indian J Med Res 2015;142(2):165-74. [DOI: 10.4103/0971-5916.164245.]

Nowbar 2014 Nowbar AN, Mielewczik M, Karavassilis M, Dehbi HM, Shun-Shin MJ, Jones S, et al. Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. BMJ 2014;348:g2688.

Poglajen 2014 Poglajen G, Frljak S, Zemljič G, Cerar A, Okrajšek R, Šebeštjen M, Vrtovec B Stem Cell Therapy for Chronic and Advanced Heart Failure. Impact of intracoronary bone marrow cell therapy on left ventricular function in the setting of ST-segment elevation myocardial infarction: a collaborative meta-analysis. Eur Heart J 2014;35:989-98.

R Core Team 2013 [Computer program] R: A language and environment for statistical computing (http://www.R-project.org/) [(2015). R: A language and environment for statistical computing.]. R Development Core Team. Vienna, Austria: R Foundation for Statistical Computing, 2015. [ISBN 3- 900051-07-0.]

Reed 2017 Reed G, Rossi J, Cannon C. Acute myocardial infarction. The Lancet 2017;389:197-210.

Review Manager 2014 [Computer program] Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

San Roman 2015 San Roman, J Alberto, Sánchez, Pedro L, Villa, Adolfo, Sanz-Ruiz, Ricardo, Fernandez-Santos, María Eugenia, Gimeno, Federico, Ramos, Benigno, Arnold, Roman, Serrador, Ana, Gutiérrez, Hipólito, Martin-Herrero, Francisco, Rollán, María Jesús, Fernández-Vázquez, Felipe, López-Messa, Juan, Ancillo, Pablo, Pérez-Ojeda, German, Fernández-Avilés, Francisco. Comparison of Different Bone Marrow-Derived Stem Cell Approaches in Reperfused STEMI. A Multicenter, Prospective, Randomized, Open-Labeled TECAM Trial.. JACC June 2015;22:2372-2382. [DOI: 10.1016/j.jacc.2015.03.563] [PMID: 26046730]

Savarese 2017 Savarese G, Lund LH. Global public health burden of heart failure. Card Fail Rev 2017;3:7-11.

Schachinger 2009 Schachinger V, Assmus B, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, et al. Intracoronary infusion of bone marrow-derived mononuclear cells abrogates adverse left ventricular remodelling post-acute myocardial infarction: insights from the reinfusion of enriched progenitor cells and infarct remodelling in acute myocardial infarction (REPAIR-AMI) trial. European Journal of Heart Failure 2009;11(10):973-9.

Schwarzer 2016 Schwarzer. Meta: General Package for Meta-Analysis. R package version 4.4-1. Available from https://CRAN.Rproject.org/package=meta..

Stamm 2003 Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361(9351):45-6.

Strauer 2002 Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106(15):1913-8.

Townsend 2016 Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J 2016;37(42):3232-3245. [DOI: 10.1093/eurheartj/ehw334.]

TSA 2011 [Computer program] Trial Sequential Analysis (TSA) program (www.ctu.dk/tsa). Denmark: Copenhagen Trial Unit, 2011.

Tse 2003 Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361:47-9.

Velagaleti 2008 Velagaleti RS, Pencina MJ, Murabito JM, Wang TJ, Parikh NI, D'Agostino RB, et al. Long-term trends in the incidence of heart failure after myocardial infarction. Circulation 2008;118(20):2057- 62.

Wen 2012 Wen Y, Chen B, Wang C, Ma X, Gao Q. Bone marrow-derived mononuclear cell therapy for patients with ischemic heart disease and ischemic heart failure. Expert Opinion on Biological Therapy 2012;12(12):1563-73.

Wettersley 2008 Wettersley J, Thorlund K, Brok J, Gluud C. Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. Journal of Clinical Epidemiology 2008;61:64-75.

Wickham 2016 H Wickham. ggplot2: Elegant Graphics for Data Analysis.. Springer-Verlag New York, 2016..

Wu 2009 Wu T, Li Y, Bian Z, Liu G, Moher D. Randomized trials published in some Chinese journals: how many are randomized? Trials 2009;20:46.

Zwetsloot 2016 Peter-Paul Zwetsloot, Hendrik Gremmels, Birgit Assmus, Stefan Koudstaal, Joost P G Sluijter, Andreas M Zeiher, Steven A J Chamuleau. Responder Definition in Clinical Stem Cell Trials in Cardiology: Will the Real Responder Please Stand Up? Circulation Research Aug 2016. [DOI: 10.1161/CIRCRESAHA.116.308733] [PMID: 27492842]

References to other published versions of this review

Clifford 2012 Clifford DM, Fisher SA, Brunskill SJ, Doree C, Mathur A, Watt S, et al. Stem cell treatment for acute myocardial infarction. Cochrane Database of Systematic Reviews 2012, Issue 2. Art. No: CD006536. [DOI: 10.1002/14651858.CD006536.pub3]

Martin-Rendon 2007 Martin-Rendon E, Brunskill S, Doree C, Hyde C, Watt S, Mathur A. Stem cell treatment for acute myocardial infarction. Cochrane Database of Systematic Reviews 2007, Issue 2. Art. No: CD006536. [DOI: 10.1002/14651858.CD006536]

Martin-Rendon 2008a Martin-Rendon E, Brunskill SJ, Doree C, Hyde CJ, Mathur A, Stanworth SJ, et al. Stem cell treatment for acute myocardial infarction. Cochrane Database of Systematic Reviews 2008, Issue 4. Art. No: CD006536. [DOI: 10.1002/14651858.CD006536.pub2]

Martin-Rendon 2008b Martin-Rendon E, Brunskill SJ, Hyde CJ, Mathur A, Stanworth S, Watt SM. Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. European Heart Journal 2008;29(15):1807-18.

APPENDICES

Appendix 1. Search strategies 2007

CENTRAL (The Cochrane Library)

#1 STEM CELL TRANSPLANTATION single term (MeSH)
#2 PERIPHERAL BLOOD STEM CELL TRANSPLANTATION single term (MeSH)
#3 HEMATOPOIETIC STEM CELL TRANSPLANTATION single term (MeSH)
#4 HEMATOPOIETIC STEM CELL MOBILIZATION single term (MeSH)
#5 STEM CELLS single term (MeSH)
#6 HEMATOPOIETIC STEM CELLS explode all trees (MeSH)
#7 BONE MARROW CELLS single term (MeSH)
#8 haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR haemopoietic OR haemopoietic OR marrow NEAR
cell* OR stem cell* OR progenitor cell* OR precursor cell*
#9 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8
#10 MYOCARDIAL ISCHEMIA explode all trees (MeSH)
#11 myocardial NEAR infarct* OR myocardium NEAR infarct* OR subendocardial NEAR infarct* OR transmural NEAR infarct* OR
cardiac NEAR infarct* OR cardial NEAR infarct* OR heart NEAR infarct* OR acute NEAR infarct*
#12 ischemi* NEAR myocard* OR ischemi* NEAR heart OR ischaemi* NEAR myocard* OR ischaemi* NEAR heart
#13 acute NEAR coronary OR occlusion* NEAR coronary OR disease* NEAR coronary
#14 unstable NEAR angina OR heart NEXT attack* OR AMI
#15 heart NEAR repair* OR heart NEAR reparation OR heart NEAR improve* OR heart NEAR regenerate* OR cardiac NEAR repair*
OR cardiac NEAR reparation OR cardiac NEAR improve* OR cardiac NEAR regenerat* OR myocard* NEAR repair* OR myocard*
NEAR reparation OR myocard* NEAR improve* OR myocard* NEAR regenerat*
#16 myoblast* NEAR transplantation OR myoblast* NEAR graft* OR myoblast* NEAR implant*
#17 #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16
#18 #9 AND #17
#19 cellular NEXT cardiomyoplasty or cardiomyocyte* NEAR transplantation* OR intramyocardial NEAR transplantation* OR
transendocardial NEAR stem NEXT cell* OR intracoronary NEXT progenitor NEXT cell*
#20 #18 OR #19

MEDLINE (Dialog DataStar)

1. STEM-CELL-TRANSPLANTATION.DE.
2. PERIPHERAL-BLOOD-STEM-CELL-TRANSPLANTATION.DE.
3. HEMATOPOIETIC-STEM-CELL-TRANSPLANTATION.DE.
4. HEMATOPOIETIC-STEM-CELL-MOBILIZATION.DE.
5. STEM-CELLS.DE.
6. HEMATOPOIETIC-STEM-CELLS#.DE.
7. BONE-MARROW-CELLS.DE.
8. (haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR hemopoietic OR haemopoietic OR marrow NEAR
cell$1 OR stem cell$1 OR progenitor cell$1 OR precursor cell$1).TI,AB.
9. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8
10. MYOCARDIAL-ISCHEMIA#.DE.
11. (myocardial OR myocardium OR subendocardial OR transmural OR cardiac OR cardial OR heart OR acute) NEAR infarct$3
12. (ischemi$1 OR ischaemi$1) NEAR (myocardium OR myocardial OR heart)
13. (acute OR occlusion$1 OR disease$1) NEAR coronary
14. unstable NEAR angina OR heart NEXT attack$1 OR AMI
15. (heart or cardiac OR myocardium OR myocardial) NEAR (repair$3 OR reparation OR improve$1 OR regenerat$3)
16. (myoblast$1 NEAR (transplantation OR graft$3 OR implant$3)
17. 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16

18. 9 AND 17
19. cellular ADJ cardiomyoplasty or cardiomyocyte$1 NEAR transplantation OR intramyocardial NEAR transplantation OR
transendocardial NEAR stem ADJ cell$1 OR intracoronary ADJ progenitor ADJ cell$1
20. 18 OR 19

EMBASE (Dialog DataStar)

1. STEM-CELL-TRANSPLANTATION#.DE.
2. STEM-CELL-MOBILIZATION.DE.
3. STEM-CELL.DE.
4. HEMATOPOIETIC-STEM-CELL.DE.
5. BONE-MARROW-CELL.DE.
6. (haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR hemopoietic OR haemopoietic OR marrow NEAR
cell$1 OR stem cell$1 OR progenitor cell$1 OR precursor cell$1).TI,AB.
7. 1 OR 2 OR 3 OR 4 OR 5 OR 6
8. HEART-INFARCTION#.DE.
9. (myocardial OR myocardium OR subendocardial OR transmural OR cardiac OR cardial OR heart OR acute) NEAR infarct$3
10. (ischemi$1 OR ischaemi$1) NEAR (myocardium OR myocardial OR heart)
11. (acute OR occlusion$1 OR disease$1) NEAR coronary
12. unstable NEAR angina OR heart NEXT attack$1 OR AMI
13. (heart or cardiac OR myocardium OR myocardial) NEAR (repair$3 OR reparation OR improve$1 OR regenerat$3)
14. (myoblast$1 NEAR (transplantation OR graft$3 OR implant$3)
15. 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14
16. 7 AND 15
17. cellular ADJ cardiomyoplasty or cardiomyocyte$1 NEAR transplantation OR intramyocardial NEAR transplantation OR
transendocardial NEAR stem ADJ cell$1 OR intracoronary ADJ progenitor ADJ cell$1
18. 16 OR 17

CINAHL (Dialog DataStar)

1. HEMATOPOIETIC-STEM-CELL-TRANSPLANTATION.DE.
2. STEM-CELLS#.DE.
3. (haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR hemopoietic OR haemopoietic OR marrow NEAR
cell$1 OR stem cell$1 OR progenitor cell$1 OR precursor cell$1).TI,AB.
4. 1 OR 2 OR 3
5. MYOCARDIAL-ISCHEMIA#.DE.
6. (myocardial OR myocardium OR subendocardial OR transmural OR cardiac OR cardial OR heart OR acute) NEAR infarct$3
7. (ischemi$1 OR ischaemi$1) NEAR (myocardium OR myocardial OR heart)
8. (acute OR occlusion$1 OR disease$1) NEAR coronary
9. unstable NEAR angina OR heart NEXT attack$1 OR AMI
10. (heart or cardiac OR myocardium OR myocardial) NEAR (repair$3 OR reparation OR improve$1 OR regenerat$3)
11. (myoblast$1 NEAR (transplantation OR graft$3 OR implant$3)
12. 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11
13. 4 AND 12
14. cellular ADJ cardiomyoplasty or cardiomyocyte$1 NEAR transplantation OR intramyocardial NEAR transplantation OR
transendocardial NEAR stem ADJ cell$1 OR intracoronary ADJ progenitor ADJ cell$1
15. 13 OR 14

LILACS and INDMED

((marrow cell$ OR stem cell$ OR progenitor cell$ OR precursor cell$) AND (infarct$ OR coronar$ OR myocard$

OR heart attack$ OR heart failure OR cardiac$ OR cardiomyo$ OR intramyocardial$ OR ischemia))

KOREAMED

((marrow cell$ OR stem cell$ OR progenitor cell$ OR precursor cell$) AND (infarct$ OR coronar$ OR myocard$ OR heart attack$ OR heart failure OR cardiac$ OR cardiomyo$ OR intramyocardial$ OR ischemia))

mRCT

((“marrow cell%” OR “stem cell%” OR “progenitor cell%” or “precursor cell%”) AND (infarct% OR coronar% OR myocard% OR “heart attack%” OR “heart failure” OR cardiac% OR cardiomyo% OR intramyocardial% OR ischemia))

APPENDIX 2. SEARCH STRATEGIES 2011

CENTRAL (The Cochrane Library)

#1 STEM CELL TRANSPLANTATION single term (MeSH)
#2 PERIPHERAL BLOOD STEM CELL TRANSPLANTATION single term (MeSH)
#3 HEMATOPOIETIC STEM CELL TRANSPLANTATION single term (MeSH)
#4 HEMATOPOIETIC STEM CELL MOBILIZATION single term (MeSH)
#5 STEM CELLS single term (MeSH)
#6 HEMATOPOIETIC STEM CELLS explode all trees (MeSH)
#7 BONE MARROW CELLS single term (MeSH)
#8 haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR haemopoietic OR haemopoietic OR (marrow NEAR/3
cell*) OR stem cell* OR progenitor cell* OR precursor cell* or cell* therap* or ((mesenchymal or stromal) AND marrow)
#9 (cell* NEAR/3 transplantation) OR (cell* NEAR/3 graft*) OR (cell* NEAR/3 implant*)
#10 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9
#11 MYOCARDIAL ISCHEMIA explode all trees (MeSH)
#12 myocardial NEAR/3 infarct* OR myocardium NEAR/3 infarct* OR subendocardial NEAR/3 infarct* OR transmural NEAR/3 infarct*
OR cardiac NEAR/3 infarct* OR cardial NEAR/3 infarct* OR heart NEAR/3 infarct* OR acute NEAR/3 infarct*
#13 ischemi* NEAR/3 myocard* OR ischemi* NEAR/3 heart OR ischaemi* NEAR/3 myocard* OR ischaemi* NEAR/3 heart
#14 acute NEAR/3 coronary OR occlusion* NEAR/3 coronary OR disease* NEAR/3 coronary
#15 unstable NEAR/3 angina OR heart NEXT attack* OR AMI
#16 heart NEAR/3 repair* OR heart NEAR/3 reparation OR heart NEAR/3 improve* OR heart NEAR/3 regenerate* OR cardiac
NEAR/3 repair* OR cardiac NEAR/3 reparation OR cardiac NEAR/3 improve* OR cardiac NEAR/3 regenerat* OR myocard* NEAR/3
repair* OR myocard* NEAR/3 reparation OR myocard* NEAR/3 improve* OR myocard* NEAR/3 regenerat*
#17 #11 OR #12 OR #13 OR #14 OR #15 OR #16
#18 #10 AND #17
#19 (cellular NEXT cardiomyoplasty) or (cardiomyocyte* NEAR/3 transplantation*) OR (intramyocardial NEAR/3 transplantation*) OR
(transendocardial NEAR/3 stem NEXT cell*)
#20 (intracoronary NEAR/4 cell*) or (intracoronary NEAR/3 bone NEXT marrow) or (intracoronary NEAR/3 BMC*) or (intracoronary
NEAR/3 infus*)
#21 #18 OR #19 OR #20

MEDLINE (Ovid)

1. exp STEM CELL TRANSPLANTATION/
2. exp STEM CELLS/

3. BONE MARROW TRANSPLANTATION/
4. BONE MARROW CELLS/
5. CELL TRANSPLANTATION/
6. (haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR hemopoietic OR haemopoietic OR (marrow adj3 cell*)
OR stem cell* OR progenitor cell* OR precursor cell* OR cell* therap* OR ((mesenchymal OR stromal) AND marrow).ti,ab.
7. (cell* adj3 (transplant* or graft* or implant*)).ti,ab
8. cell transplantation.jn. or cell stem cell.jn. or stem cell reviews.jn. or bone marrow transplantation.jn.
9. or/1-8
10. exp MYOCARDIAL ISCHEMIA/
11. ((myocardial OR myocardium OR subendocardial OR transmural OR cardiac OR cardial OR heart OR acute) adj3 infarct*).ti,ab.
12. ((ischemi* OR ischaemi*) adj3 (myocardium OR myocardial OR heart)).ti,ab.
13. ((acute OR occlusion* OR disease*) adj3 coronary).ti,ab.
14. ((unstable adj3 angina) OR heart attack* OR AMI).ti,ab.
15. ((heart or cardiac OR myocardium OR myocardial) adj3 (repair* OR reparation OR improve* OR regenerat*)).ti,ab.
16. or/10-15
17. 9 AND 16
18. (cellular cardiomyoplasty or (cardiomyocyte* adj3 transplant*) OR (intramyocardial* adj3 transplant*) OR (transendocardial* adj3
stem cell*)).ti,ab.
19. (intracoronary adj4 (cell* or BMC* or infus*)).ti,ab.
20. or/17-19
21. RANDOMIZED CONTROLLED TRIAL.pt.
22. CONTROLLED CLINICAL TRIAL.pt.
23. exp CLINICAL TRIAL/
24. MULTICENTER STUDY.pt.
25. CLINICAL TRIALS AS TOPIC/
26. CLINICAL TRIALS PHASE III AS TOPIC/
27. CLINICAL TRIALS PHASE IV AS TOPIC/
28. exp CONTROLLED CLINICAL TRIALS AS TOPIC/
29. RANDOM ALLOCATION/
30. DOUBLE BLIND METHOD/
31. SINGLE BLIND METHOD/
32. CROSSOVER STUDIES/
33. PLACEBOS/
34. or/21-3335. (controlled adj3 (trial* or stud*)).ti,ab.
36. (blind* or mask*).ti,ab.
37. (placebo* or random* or factorial*).ti,ab.
38. (crossover or (cross adj over)).ti,ab.
39. aleatori*.ti,ab.
40. (treatment adj arm*).ti,ab.
41. ((phase adj iii) or (phase adj three) or (phase adj '3')).ti,ab.
42. (latin adj square).ti,ab.
43. or/35-42
44. 34 or 43
45. ANIMALS/
46. HUMANS/
47. 45 and 46
48. 45 not 47
49. 44 not 48
50. 20 and 49

EMBASE (Ovid)

1. exp CELL THERAPY/
2. exp STEM CELL/
3. BONE MARROW CELL/
4. (haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR hemopoietic OR haemopoietic

OR (marrow adj3 cell*)
OR stem cell* OR progenitor cell* OR precursor cell* OR cell* therap*).ti,ab.
5. ((mesenchymal OR stromal) AND marrow).ti,ab.
6. (cell* adj3 (transplant* or graft* or implant*)).ti,ab.
7. or/1-6
8. exp HEART INFARCTION/
9. ((myocardial OR myocardium OR subendocardial OR transmural OR cardiac OR cardial OR heart OR acute) adj3 infarct*).ti,ab.
10. ((ischemi* OR ischaemi*) adj3 (myocardium OR myocardial OR heart)).ti,ab.
11. ((acute OR occlusion* OR disease*) adj3 coronary).ti,ab.
12. ((unstable adj3 angina) OR heart attack* OR AMI).ti,ab.
13. ((heart or cardiac OR myocardium OR myocardial) adj3 (repair* OR reparation OR improve* OR regenerat*)).ti,ab.
14. or/8-13
15. 7 AND 14
16. (cellular cardiomyoplasty OR (cardiomyocyte* adj3 transplant*) OR (intramyocardial* adj3 transplant*) OR (transendocardial* adj3
stem cell*)).ti,ab.
17. (intracoronary adj4 (cell* OR BMC* OR infus*)).ti,ab.
18. or/15-17
19. random*.ti,ab.
20. factorial*.ti,ab.
21. (crossover* OR cross over* OR cross-over*).ti,ab.
22. placebo*.ti,ab.
23. (double* adj blind*).ti,ab.
24. (singl* adj blind*).ti,ab.
25. assign*.ti,ab.
26. allocat*.ti,ab.
27. volunteer*.ti,ab.28. CROSSOVER PROCEDURE/
29. DOUBLE BLIND PROCEDURE/
30. RANDOMIZED CONTROLLED TRIAL/
31. SINGLE BLIND PROCEDURE/
32. or/19-31
33. exp ANIMAL/
34. NONHUMAN/
35. exp ANIMAL EXPERIMENT/
36. or/33-35
37. exp HUMAN/
38. 36 NOT 37
39. 32 NOT 38
40. 18 AND 39

CINAHL (NHS Evidence)

1. exp CELL TRANSPLANTATION/
2. exp STEM CELLS/
3. exp BONE MARROW TRANSPLANTATION/
4. (haematopoietic OR hematopoietic OR haematopoetic OR hematopoetic OR hemopoietic OR haemopoietic OR (marrow adj3 cell*)
OR “stem cell*” OR “progenitor cell*” OR “precursor cell*” or “cell* therap*”).ti,ab
5. ((mesenchymal OR stromal) AND marrow).ti,ab
6. ((cell* adj3 transplant*) or (cell* adj3 graft*) or (cell* adj3 implant*)).ti,ab
7. 1 OR 2 OR 3 OR 4 OR 5 OR 6
8. exp MYOCARDIAL ISCHEMIA/
9. ((myocardial adj3 infarct*) OR (myocardium adj3 infarct*) OR (subendocardial adj3 infarct*) OR (transmural adj3 infarct*) OR
(cardiac adj3 infarct*) OR (cardial adj3 infarct*) OR (heart adj3 infarct*) OR (acute adj3 infarct*).ti,ab
10. ((ischemi* adj3 myocardium) OR (ischemi* adj3 myocardial) OR (ischemi* adj3 heart)).ti,ab
11. ((ischaemi* adj3 myocardium) OR (ischaemi* adj3 myocardial) OR (ischaemi* adj3 heart)).ti,ab
12. ((acute adj3 coronary) OR (occlusion* adj3 coronary) OR (disease* adj3 coronary)).ti,ab
13. ((unstable adj3 angina) OR “heart attack*” OR AMI).ti,ab

14. ((heart adj3 repair*) or (cardiac adj3 repair*) OR (myocardium adj3 repair*) OR (myocardial* adj3 repair*)).ti,ab
15. ((heart adj3 reparation) or (cardiac adj3 reparation) OR (myocardium adj3 reparation) OR (myocardial* adj3 reparation)).ti,ab
16. ((heart adj3 improv*) or (cardiac adj3 improv*) OR (myocardium adj3 improv*) OR (myocardial* adj3 improv*)).ti,ab
17. ((heart adj3 regenerat*) or (cardiac adj3 regenerat*) OR (myocardium adj3 regenerat*) OR (myocardial* adj3 regenerat*)).ti,ab
18. 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
19. 7 AND 18
20. ("cellular cardiomyoplasty" or (cardiomyocyte* adj3 transplant*) OR (intramyocardial* adj3 transplant*) OR (transendocardial* adj3
stem cell*)).ti,ab21. ((intracoronary adj4 cell*) or (intracoronary adj3 BMC*) or (intracoronary adj3 infus*)).ti,ab
22. 19 or 20 or 21
23. "CLINICAL TRIAL".pt
24. ((controlled adj trial*) OR (clinical adj trial*)).ti,ab
25. ((singl* adj blind*) OR (doubl* adj blind*) OR (trebl* adj blind*) OR (singl* adj mask*) OR (doubl* adj mask*) OR (tripl* adj
mask*)).ti,ab
randomi*.ti,ab
26. RANDOM ASSIGNMENT/
27. ("phase III" OR "phase 3" OR "phase three").ti,ab
28. (random* adj1 allocat*).ti,ab
29. (random* adj1 assign*).ti,ab
30. PLACEBOS/
31. 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30
32. 19 AND 31

PubMed (for e-publications only)

(infarct[ti] OR infarction or coronary[ti] OR myocardial[ti] OR heart attack[ti] OR heart failure[ti] OR cardiac[ti] OR cardiomyopathy[ti]
OR intramyocardial[ti] OR ischemi*[ti] OR ischaemi*[ti]) AND (marrow cell[ti] OR marrow cells[ti] OR stem cell[ti] OR stem cells[ti] OR
progenitor cell[ti] OR progenitor cells[ti] OR precursor cell[ti] OR precursor cells[ti] OR cell therapy[ti] OR cellular therapy[ti] OR cellbased therapy[ti] OR intracoronary cells[ti] or mononuclear cells[ti] OR mesenchymal cells[ti]) AND (publisher[sb] NOT
pubstatusnihms)

LILACS and INDMED

(marrow cell$ OR stem cell$ OR progenitor cell$ OR precursor cell$ OR cell$ therap$ or mesenchymal cell$) AND (infarct$ OR coronar$ OR intracoronary OR myocard$ OR heart attack$ OR heart failure OR cardiac$ OR cardiomyo$ OR intramyocardial$ OR ischemi$)

KoreaMed, PakMediNet and the UKBTS/SRI Transfusion Evidence Library

(marrow cell* OR stem cell* OR progenitor cell* OR precursor cell* OR cell* therap* or mesenchymal cell*) AND (infarct* OR coronar* OR intracoronary OR myocard* OR heart attack* OR heart failure OR cardiac* OR cardiomyo* OR intramyocardial* OR ischemi*)

ClinicalTrials.gov

(myocardial infarction OR cardiomyopathy OR intramyocardial OR intracoronary OR myocardial ischemia) AND ("marrow cells" OR
"stem cells" OR "cell therapy" OR "cellular therapy" OR "cell-based therapy" OR "intracoronary cells" or "mononuclear cells")
ISRCTN Register

(stem cell OR stem cells OR marrow cell OR marrow cells OR progenitor cell or progenitor cells or precursor cell or precursor cells) AND (myocardial infarction OR infarct OR heart attack OR cardiomyopathy OR intramyocardial OR intracoronary OR ischemia OR ischaemia)

WHO International Clinical Trials Registry Platform (ICTRP)

(infarct AND cell* OR infarction AND cell* OR coronary AND cell* OR myocardial AND cell* OR heart attack AND cell* OR heart failure AND cell* OR cardiac AND cell* OR cardiomyopathy AND cell* OR intramyocardial AND cell* OR ischemia AND cell* OR ischemic AND cell* OR ischaemia AND cell* OR ischaemic AND cell*)

APPENDIX 3. SEARCH STRATEGIES 2015

CENTRAL (The Cochrane Library)

#1 MeSH descriptor: [Stem Cell Transplantation] explode all trees
#2 MeSH descriptor: [Bone Marrow Cells] explode all trees
#3 MeSH descriptor: [Stem Cells] explode all trees
#4 MeSH descriptor: [Cell Transplantation] this term only
#5 MeSH descriptor: [Bone Marrow Transplantation] this term only
#6 MeSH descriptor: [Stromal Cells] explode all trees
#7 ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or
precursor or bone marrow or mononuclear or “adipose tissue” or mesenchymal or stromal or autologous or allogeneic or allogenic or
ALDH* or C-KIT*) next/2 cell*)
#8 “cell transplantation”:so or “stem cell”:so or “bone marrow transplantation”:so
#9 (autologous next/3 transplant*) or “cell* therap*”
#10 ((cell* or myoblast*) near/3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*))
#11 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10
#12 MeSH descriptor: [Heart Diseases] explode all trees
#13 ((ischemi* or ischaemi* or nonischemi* or nonischaemi*) near/2 (myocardium or myocardial or cardiomyopath* or heart or
coronary or cardiac or cardial or subendocardial))
#14 ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) near/2 (failure* or
decompensation or insufficien*))
#15 (IHD or CIHD or DCM or IDCM)
#16 ((myocardial near/3 dysfunction*) or stenocardia or angina*)
#17 ((end stage or endstage or dilated or idiopathic or congestive) near/2 cardiomyopath*)
#18 (arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) near/2 coronary
#19 ((heart or cardiac or cardial or myocardium or myocardial) near/3 (repair* or reparation or improv* or regenerat*))
#20 (heart disease* or coronary disease* or cardiovascular disease*)
#21 ((end stage or endstage or dilated or idiopathic or congestive) near/2 cardiomyopath*)
#22 ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) near/3 (infarct* or
postinfarct* or hypoxi* or anoxi*))
#23 heart attack* or coronary attack* or acute coronary syndrome* or AMI
#24 #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23
#25 #11 and #24
#26 cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) near/6 transplant*) or ((intramyocardial* or intracoronary or
transendocardial* or transcoronary) near/6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal
or progenitor cell* or precursor cell*))
#27 #25 or #26

MEDLINE (OvidSP)

1. exp STEM CELL TRANSPLANTATION/
2. BONE MARROW TRANSPLANTATION/
3. CELL TRANSPLANTATION/
4. exp STEM CELLS/
5. BONE MARROW CELLS/
6. exp STROMAL CELLS/
7. ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or
precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or
ALDH* or C-KIT*) adj2 cell*).ti,ab.
8. (cell transplantation or stem cell* or bone marrow transplantation).jn.
9. ((autologous adj3 transplant*) or cell* therap*).tw.
10. ((cell* or myoblast*) adj3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*)).ti,ab.
11. or/1-10
12. exp HEART DISEASES/
13. ((ischemi* or ischaemi* or nonischemi* or nonischaemi*) adj2 (myocardium or myocardial or cardiomyopath* or heart or coronary
or cardiac or cardial or subendocardial)).ti,ab.
14. ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) adj2 (failure* or
decompensation or insufficien*)).ti,ab.
15. (IHD or CIHD or DCM or IDCM).ti,ab.
16. ((myocardial adj3 dysfunction*) or stenocardia or angina*).ti,ab.
17. ((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) adj2 coronary).ti,ab.
18. (heart disease* or coronary disease* or cardiovascular disease*).ti,ab.
9. ((end stage or endstage or dilated or idiopathic or congestive) adj2 cardiomyopath*).ti,ab.
20. ((heart or cardiac or cardial or myocardium or myocardial) adj3 (repair* or reparation or improv* or regenerat*)).ti,ab.
21. ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) adj3 (infarct* or
postinfarct* or hypoxi* or anoxi*)).ti,ab.
22. (heart attack* or coronary attack* or acute coronary syndrome* or AMI).ti,ab.
23. or/12-22
24. 11 and 2325. (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) adj6 transplant*) or ((intramyocardial* or intracoronary or
transendocardial* or transcoronary) adj6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or
progenitor cell* or precursor cell*))).mp.
26. 24 or 25
27. Meta-Analysis.pt.
28. ((meta analy* or metaanaly*) and (trials or studies)).ab.
29. (meta analy* or metaanaly* or evidence-based).ti.
30. ((systematic* or evidence-based) adj2 (review* or overview*)).tw.
31. (cochrane or embase or cinahl or cinhal or lilacs or citation index or psyclit or psychlit or psycinfo or psychinfo or “web of science”
or scopus).ab.
32. Cochrane Database of systematic reviews.jn.
33. ((literature or systematic* or comprehensive* or electronic*) adj2 search*).ab.
34. (additional adj (papers or articles or sources)).ab.
35. (bibliograph* or handsearch* or hand search* or manual* search* or searched or reference list*).ab.
36. (relevant adj (journals or articles)).ab.
37. or/27-36
38. Review.pt.
39. RANDOMIZED CONTROLLED TRIALS AS TOPIC/
40. selection criteria.ab. or critical appraisal.ti.
41. (data adj (extraction or analys$)).ab.
42. RANDOMIZED CONTROLLED TRIALS/
43. or/39-42

44. 38 and 43
45. 37 or 4446. randomized controlled trial.pt.
47. controlled clinical trial.pt.
48. randomi*.tw.
49. (placebo or randomly or groups).ab.
50. clinical trials as topic.sh.
51. trial.ti.
52. or/46-51
53. 45 or 52
54. (ANIMALS/ or exp ANIMAL EXPERIMENTATION/ or exp MODELS, ANIMAL/) not HUMANS/
55. (Comment or Editorial).pt.
56. 54 or 55
57. 53 not 56
58. 26 and 57

EMBASE (OvidSP)

1. exp STEM CELL TRANSPLANTATION/
2. exp BONE MARROW TRANSPLANTATION/
3. exp STEM CELL/
4. BONE MARROW CELL/
5. exp STROMA CELLS/
6. ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or
precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or
ALDH* or C-KIT*) adj2 cell*).ti,ab.
7. (cell transplantation or stem cell* or bone marrow transplantation).jn.
8. ((autologous adj3 transplant*) or cell* therap*).tw.
9. ((cell* or myoblast*) adj3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*)).ti,ab.
10. or/1-9
11. exp ISCHEMIC HEART DISEASE/
12. exp HEART FAILURE/
13. exp MYOCARDIAL DISEASE/
14. ((ischemi* or ischaemi* or nonischemi* or nonischaemi*) adj2 (myocardium or myocardial or cardiomyopath* or heart or coronary
or cardiac or cardial or subendocardial)).ti,ab.
15. ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) adj2 (failure* or
decompensation or insufficien*)).ti,ab.
16. (IHD or CIHD or DCM or IDCM).ti,ab.
17. ((myocardial adj3 dysfunction*) or stenocardia or angina*).ti,ab.
18. ((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) adj2 coronary).ti,ab.
19. (heart disease* or coronary disease* or cardiovascular disease*).ti,ab.
20. ((end stage or endstage or dilated or idiopathic or congestive) adj2 cardiomyopath*).ti,ab.
21. ((heart or cardiac or cardial or myocardium or myocardial) adj3 (repair* or reparation or improv* or regenerat*)).ti,ab.
22. ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) adj3 (infarct* or
postinfarct* or hypoxi* or anoxi*)).ti,ab.
23. (heart attack* or coronary attack* or acute coronary syndrome* or AMI).ti,ab.
24. 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25. 10 and 2426. (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) adj6 transplant*) or ((intramyocardial* or intracoronary or
transendocardial* or transcoronary) adj6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or
progenitor cell* or precursor cell*))).mp.
27. 25 or 26
28. Meta Analysis/

29. Systematic Review/
30. (meta analy* or metaanalys*).tw.
31. (systematic* adj2 (review* or overview* or search*)).tw.
32. (literature adj2 (review* or overview* or search*)).tw.
33. (cochrane or embase or cinahl or cinhal or lilacs or BIDS or science citation index or psyclit or psychlit or psycinfo or psychinfo or
cancerlit).ti,ab.
34. (electronic* adj (sources or resources or databases)).ab.
35. (reference lists or bibliograph* or handsearch* or hand search* or (manual* adj1 search*)).ab.
36. (additional adj (papers or articles or sources)).ab.
37. (relevant adj (journals or articles)).ab.
38. (search term* or published articles or search strateg*).ab.
39. Review.pt. and (data extraction or selection criteria).ab.
40. or/28-39
41. Controlled Clinical Trial/
42. Phase 3 Clinical Trial/
43. Phase 4 Clinical Trial/
44. Randomized Controlled Trial/
45. Randomization/
46. Single Blind Procedure/
47. Double Blind Procedure/
48. Crossover Procedure/
49. Placebo/50. (randomized or randomised or RCT).tw.
51. (random* adj5 (allocat* or assign* or divid* or receiv*)).tw.
52. (single blind* or double blind* or treble blind* or triple blind*).tw.
53. (phase III or phase three or “phase 3”).tw.
54. (crossover* or cross over* or cross-over* or placebo*).tw.
55. Prospective Study/
56. or/41-55
57. Case Study/
58. case report*.tw.
59. (note or editorial).pt.
60. or/57-59
61. 56 not 60
62. 40 or 61
63. limit 62 to embase
64. 27 and 63

CINAHL (EBSCOHost)

S1 (MH “Cell Transplantation+”)
S2 (MH “Stem Cells+”)
S3 TI ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or ALDH* or C-KIT*) N2 cell*) OR AB ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or ALDH* or C-KIT*) N2 cell)
S4 TX ((autologous N3 transplant*) or cell* therap*)
S5 TI ((cell* or myoblast*) N3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*)) OR AB ((cell* or myoblast*) N3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*))
S6 S1 OR S2 OR S3 OR S4 OR S5
S7 (MH “Heart Diseases+”)
S8 TI ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) N3 (infarct* or postinfarct* or hypoxi* or anoxi*)) OR AB ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) N3 (infarct* or postinfarct* or hypoxi* or anoxi*))
S9 TI ((“heart disease*” or “coronary disease*” or IHD or CIHD or DCM or IDCM)) AND AB ((“heart disease*” or “coronary disease*” or IHD or CIHD or DCM or IDCM))
S10 TI (((myocardial N3 dysfunction) OR angina OR stenocardia)) OR AB (((myocardial N3 dysfunction) OR

angina OR stenocardia))
S11 TI (((ischemi* or ischaemi* or nonischemi* or nonischaemi*) N5 (myocardium or myocardial or heart or coronary or cardiac or cardial or subendocardial or cardiomyopath*))) OR AB (((ischemi* or ischaemi* or nonischemi* or nonischaemi*) N5 (myocardium or myocardial or heart or coronary or cardiac or cardial or subendocardial or cardiomyopath*)))
S12 TI (((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) N2 coronary)) OR AB (((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) N2 coronary))
S13 TI (((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) N2 (failure* or decompensation or insufficien*))) OR AB (((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) N2 (failure* or decompensation or insufficien*)))
S14 TI ((end stage or endstage or dilated or idiopathic or congestive) N2 cardiomyopath*) OR AB ((end stage or endstage or dilated or idiopathic or congestive) N2 cardiomyopath*)
S15 TI ((heart or cardiac or cardial or myocardium or myocardial) N3 (repair* or reparation or improv* or regenerat*)) OR AB ((heart or cardiac or cardial or myocardium or myocardial) N3 (repair* or reparation or improv* or regenerat*))
S16 TI (heart attack* or coronary attack* or acute coronary syndrome* or AMI) OR AB (heart attack* or coronary attack* or acute coronary syndrome* or AMI)
S17 S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16
S18 S6 AND S17

PubMed (for epublications)

#1 (stem[TI] OR haematopoietic[TI] OR hematopoietic[TI] OR haematopoetic[TI] OR hematopoetic[TI] OR hemopoietic[TI] OR haemopoietic[TI] OR progenitor[TI] OR precursor[TI] OR bone marrow[TI] OR mononuclear[TI] OR "adipose tissue"[TI] OR mesenchymal[TI] OR stromal[TI] OR autologous[TI] OR allogeneic[TI] OR allogenic[TI] OR ALDH*[TI] OR C-KIT*[TI]) AND cell*[TI]
#2 cell transplantation[TA] OR stem cell*[TA] OR bone marrow transplant*[TA]
#3 "autologous transplant*"[TI] OR "cell therapy"[TI] OR "cell therapies"[TI] OR "cellular therapy"[TI]
#4 (cell[TI] OR cells[TI] OR cellular[TI] OR myoblast*[TI]) AND (transplant[TI] OR transplantation[TI] OR transplants[TI] OR transplanting[TI] OR transplanted[TI] OR autotransplant*[TI] or allotransplant*[TI] or graft*[TI] or implant[TI] OR implants[TI] OR implantation[TI] OR implanted[TI])
#5 #1 OR #2 OR #3 OR #4
#6 (ischemi*[TI] OR ischaemi*[TI] OR nonischemi*[TI] OR nonischaemi*) AND (myocardium[TI] OR myocardial[TI] OR cardiomyopath* [TI] OR heart[TI] OR coronary[TI] OR cardiac[TI] OR cardial[TI] OR subendocardial[TI])
#7 (myocardial[TI] OR myocardium[TI] OR subendocardial[TI] OR transmural[TI] OR cardiac[TI] OR cardial[TI] OR coronary[TI] OR heart) AND (failure*[TI] OR decompensation[TI] OR insufficien*[TI])
#8 "myocardial dysfunction*"[TI] OR stenocardia[TI] OR angina*[TI] OR IHD[TI] OR CIHD[TI] OR DCM[TI] OR IDCM[TI] OR "heart disease"[TI] OR "coronary disease"[TI] OR "coronary artery disease"[TI] OR "cardiovascular disease"[TI]
#9 ("arterial occlusion*"[TI] OR "arterial disease*"[TI] OR arterioscleros*[TI] OR atheroscleros*[TI]) AND coronary[TI]
#10 ("end stage"[TI] OR endstage[TI] OR dilated[TI] OR idiopathic[TI] OR congestive[TI]) AND cardiomyopath*[TI]
#11 (heart[TI] OR cardiac[TI] OR cardial[TI] OR myocardium[TI] OR myocardial[TI]) AND (repair*[TI] OR reparation[TI] OR improv*[TI] OR regenerat*[TI])
#12 (myocardial[TI] OR myocardium [TI] OR subendocardial [TI] OR transmural [TI] OR cardiac [TI] OR cardial [TI] OR coronary [TI] OR heart [TI] OR acute[TI]) AND (infarct* [TI] OR postinfarct* [TI] OR hypoxi* [TI] OR anoxi*)
#13 heart attack* [TI] OR coronary attack* [TI] OR acute coronary syndrome* [TI] OR AMI[TI]
#14 #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13
#15 #5 AND #14#16 (cellular cardiomyoplast* OR ((cardiomyocyte* OR cardiac cell*) AND transplant*) OR ((intramyocardial* OR intracoronary OR transendocardial* OR transcoronary) AND (transplant* OR stem OR bone marrow OR marrow cell* OR BMC* OR stromal OR mesenchymal OR progenitor cell* OR precursor cell*)))
#17 #15 OR #16
#18 (random* OR blind* OR control group* OR placebo OR controlled trial OR controlled study OR trials OR systematic review OR meta-analysis OR metaanalysis OR literature search OR medline OR cochrane OR embase) AND ((publisher[sb] OR inprocess[sb]) NOT pubstatusnihms)
#19 #17 AND #18

LILACS

(tw:((infarct OR infarction OR coronary OR myocardial OR heart OR cardiac OR cardiomyopathy OR myocardial

OR subendocardial OR intramyocardial OR intracoronary OR ischemia OR ischemic OR nonischemic))) AND (tw:((bone marrow OR marrow cell OR marrow cells OR stem cell OR stem cells OR progenitor cells OR precursor cells OR cell therapy OR cellular therapy OR cell-based therapy OR mononuclear cells OR mesenchymal cells OR stromal cells))) AND (instance:"regional") AND (db:("LILACS") AND type_of_study:("clinical_trials"))

KoreaMed

Search lines were run separately, but presented this way for brevity: (stem [ALL] OR marrow [ALL] OR mesenchymal[ALL] OR stromal[ALL]) AND (myocardial [ALL] OR heart[ALL] OR cardiac[ALL] OR coronary[ALL] OR cardiomyopathy[ALL]) AND "Randomized Controlled Trial" [PT]

IndMed

(bone marrow OR marrow cell OR marrow cells OR stem cell OR stem cells OR progenitor cell OR precursor cell OR cell therapy OR cellular therapy OR mesenchymal cells OR stromal cells) AND (infarct OR infarction OR coronary OR intracoronary OR myocardial OR heart OR cardiac OR congestive OR cardiomyopathy OR intramyocardial OR intramyocardial OR intracoronary OR ischemia OR ischemic OR ischaemia OR ischaemic OR nonischemic OR nonischaemic) AND (randomised OR randomly OR randomized OR blind OR blinded OR trial OR study OR control group)

PakMediNet

Combinations of the following free text terms were used: stem cell, stem cells, bone marrow, marrow cells, progenitor cells, precursor cells, mesenchymal cells, stromal cells AND myocardial infarction, heart attack, cardiac ischemia, coronary ischemia, myocardial ischemia, cardiomyopathy, heart failure, cardiac failure, angina, coronary artery disease

Web of Science

Title: "cardiac failure" OR "heart attack" OR "heart failure" OR "coronary disease" OR "cardiovascular disease" OR "coronary artery" OR "coronary arterial" OR "myocardial infarction" OR cardiomyopathy OR "heart disease" OR "heart diseases" OR "cardiac insufficiency" OR AMI OR IHD OR CIHD OR DCM OR IDCM OR "myocardial dysfunction" OR stenocardia OR angina
AND
Title: "stem cell" OR "stem cells" OR "bone marrow" OR "marrow cells" OR "cellular therapy" OR "mesenchymal cells" OR "stromal cells" OR "cell transplant" OR "precursor cells" OR "progenitor cells" OR (c-kit* NEAR/5 cells) OR HSCT OR SCT OR MSC OR MSCs OR BMT OR BMC OR BMAC OR BMCs OR HST OR HSTs
AND
Topic: randomised OR randomly OR randomized OR blind OR blinded OR trial OR study OR "control group" OR group

ClinicalTrials.gov

Search Terms: randomized OR randomised OR random OR randomly Study Type: Intervention Studies Condition: cardiac OR heart attack OR heart failure OR coronary OR myocardial OR cardiomyopathy OR heart disease OR angina Intervention: stem cells OR bone marrow cells OR cellular therapy OR mesenchymal cells OR stromal cells OR cell transplant OR precursor cells OR progenitor cells OR HSCT OR SCT OR MSC OR MSCs OR BMT OR BMC OR BMAC OR BMCs OR HST OR HSTs

ISRCTN Register

(("marrow cell" OR "marrow cells" OR "stem cell" OR "stem cells" OR "progenitor cells" OR "precursor cells" OR "mesenchymal cells"
OR "stromal cells") AND ("myocardial infarction" OR "heart attack" OR cardiomyopathy OR intramyocardial OR intracoronary))
OR
(("marrow cell" OR "marrow cells" OR "stem cell" OR "stem cells" OR "progenitor cells" OR "precursor cells" OR

"mesenchymal cells"
OR "stromal cells") AND ("cardiac ischemia" OR "coronary ischemia" OR "myocardial ischemia" OR "heart failure" OR "cardiac failure"
OR congestive OR "coronary artery disease"))
OR
(("cell therapy" OR "cellular therapy") AND ("myocardial infarction" OR "heart attack" OR cardiomyopathy OR intramyocardial OR
intracoronary OR "cardiac ischemia" OR "coronary ischemia" OR "myocardial ischemia" OR "heart failure" OR "cardiac failure" OR
congestive OR "coronary artery disease" OR angina))

WHO ICTRP Portal

Intervention: stem cells OR bone marrow cells OR cellular therapy OR mesenchymal cells OR stromal cells OR cell transplant OR precursor cells OR progenitor cells OR HSCT OR SCT OR MSC OR MSCs OR BMT OR BMC OR BMAC OR BMCs OR HST OR HSTs
Condition: cardiac OR heart OR coronary OR myocardial OR angina Recruitment
Status: ALL

APPENDIX 4. SEARCH STRATEGIES 2022

CENTRAL

(([mh "Stem Cell Transplantation"] OR [mh "Bone Marrow Cells"] OR [mh "Stem Cells"] OR [mh ^"Cell Transplantation"] OR [mh ^"Bone Marrow Transplantation"] OR [mh "Stromal Cells"] OR ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or precursor or bone marrow or mononuclear or "adipose tissue" or mesenchymal or stromal or autologous or allogeneic or allogenic or ALDH* or C-KIT*) NEAR/2 cell*) OR ("cell transplantation" or "stem cell" or "bone marrow transplantation"):SO OR (autologous NEAR/3 transplant*) or "cell* therap*" OR ((cell* or myoblast*) NEAR/3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*))) AND ([mh "Heart Diseases"] OR ((ischemi* or ischaemi* or nonischemi* or nonischaemi*) NEAR/2 (myocardium or myocardial or cardiomyopath* or heart or coronary or cardiac or cardial or subendocardial)) OR ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) NEAR/2 (failure* or decompensation or insufficien*)) OR (IHD or CIHD or DCM or IDCM) OR ((myocardial NEAR/3 dysfunction*) or stenocardia or angina*) OR ((end stage or endstage or dilated or idiopathic or congestive) NEAR/2 cardiomyopath*) OR ((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) NEAR/2 coronary) OR ((heart or cardiac or cardial or myocardium or myocardial) NEAR/3 (repair* or reparation or improv* or regenerat*)) OR (heart disease* or coronary disease* or cardiovascular disease*) OR ((end stage or endstage or dilated or idiopathic or congestive) NEAR/2 cardiomyopath*) OR ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) NEAR/3 (infarct* or postinfarct* or hypoxi* or anoxi*)) OR heart attack* or coronary attack* or acute coronary syndrome* or AMI)) OR (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) NEAR/6 transplant*) or ((intramyocardial* or intracoronary or transendocardial* or transcoronary) NEAR/6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or progenitor cell* or precursor cell*))) with Cochrane Library publication date Between Dec 2020 and Dec 2022, in Trials

MEDLINE Ovid

1 exp Stem Cell Transplantation/
2 Bone Marrow Transplantation/
3 Cell Transplantation/
4 exp Stem Cells/
5 Bone Marrow Cells/
6 exp Stromal Cells/
7 ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or
precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or

ALDH* or C-KIT*) adj2 cell*).ti,ab.
8 (cell transplantation or stem cell* or bone marrow transplantation).jn.
9 ((autologous adj3 transplant*) or cell* therap*).tw.
10 ((cell* or myoblast*) adj3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*)).ti,ab.
11 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
12 exp Heart Diseases/
13 ((ischemi* or ischaemi* or nonischemi* or nonischaemi*) adj2 (myocardium or myocardial or cardiomyopath* or heart or coronary or
cardiac or cardial or subendocardial)).ti,ab.
14 ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) adj2 (failure* or
decompensation or insufficien*)).ti,ab.
15 (IHD or CIHD or DCM or IDCM).ti,ab.
16 ((myocardial adj3 dysfunction*) or stenocardia or angina*).ti,ab.
17 ((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) adj2 coronary).ti,ab.
18 (heart disease* or coronary disease* or cardiovascular disease*).ti,ab.
19 ((end stage or endstage or dilated or idiopathic or congestive) adj2 cardiomyopath*).ti,ab.
20 ((heart or cardiac or cardial or myocardium or myocardial) adj3 (repair* or reparation or improv* or regenerat*)).ti,ab.
21 ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) adj3 (infarct* or
postinfarct* or hypoxi* or anoxi*)).ti,ab.
22 (heart attack* or coronary attack* or acute coronary syndrome* or AMI).ti,ab.
23 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
24 11 and 23
25 (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) adj6 transplant*) or ((intramyocardial* or intracoronary or
transendocardial* or transcoronary) adj6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or
progenitor cell* or precursor cell*))).mp.
26 24 or 25
27 Meta-Analysis.pt.
28 ((meta analy* or metaanaly*) and (trials or studies)).ab.
29 (meta analy* or metaanaly* or evidence-based).ti.
30 ((systematic* or evidence-based) adj2 (review* or overview*)).tw.
31 (cochrane or embase or cinahl or cinhal or lilacs or citation index or psyclit or psychlit or psycinfo or psychinfo or “web of science”
or scopus).ab.
32 Cochrane Database of systematic reviews.jn.
33 ((literature or systematic* or comprehensive* or electronic*) adj2 search*).ab.
34 (additional adj (papers or articles or sources)).ab.
35 (bibliograph* or handsearch* or hand search* or manual* search* or searched or reference list*).ab.
36 (relevant adj (journals or articles)).ab.
37 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36
38 Review.pt.
39 Randomized Controlled Trials as Topic/
40 selection criteria.ab. or critical appraisal.ti.
41 (data adj (extraction or analys$)).ab.
42 Randomized Controlled Trial/
43 39 or 40 or 41 or 42
44 38 and 43
45 37 or 4446 randomized controlled trial.pt.
47 controlled clinical trial.pt.
48 randomi*.tw.
49 (placebo or randomly or groups).ab.
50 clinical trials as topic.sh.
51 trial.ti.
52 46 or 47 or 48 or 49 or 50 or 51
53 45 or 52
54 (Animals/ or exp Animal Experimentation/ or exp Models, Animal/) not Humans/
55 (Comment or Editorial).pt.

56 54 or 55
57 53 not 56
58 26 and 57
59 limit 58 to ed=20201209-20221231

Embase Ovid

1. exp Stem Cell Transplantation/
2. exp Bone Marrow Transplantation/
3. exp Stem Cell/
4. Bone Marrow Cell/
5. exp Stroma Cells/
6. ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or
precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or
ALDH* or C-KIT*) adj2 cell*).ti,ab.
7. (cell transplantation or stem cell* or bone marrow transplantation).jn.
8. ((autologous adj3 transplant*) or cell* therap*).tw.
9. ((cell* or myoblast*) adj3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*)).ti,ab.
10. or/1-9
11. exp Ischemic Heart Disease/
12. exp Heart Failure/
13. exp Myocardial Disease/
14. ((ischemi* or ischaemi* or nonischemi* or nonischaemi*) adj2 (myocardium or myocardial or cardiomyopath* or heart or coronary
or cardiac or cardial or subendocardial)).ti,ab.
15. ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) adj2 (failure* or
decompensation or insufficien*)).ti,ab.
16. (IHD or CIHD or DCM or IDCM).ti,ab.
17. ((myocardial adj3 dysfunction*) or stenocardia or angina*).ti,ab.
18. ((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) adj2 coronary).ti,ab.
19. (heart disease* or coronary disease* or cardiovascular disease*).ti,ab.
20. ((end stage or endstage or dilated or idiopathic or congestive) adj2 cardiomyopath*).ti,ab.
21. ((heart or cardiac or cardial or myocardium or myocardial) adj3 (repair* or reparation or improv* or regenerat*)).ti,ab.
22. ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) adj3 (infarct* or
postinfarct* or hypoxi* or anoxi*)).ti,ab.
23. (heart attack* or coronary attack* or acute coronary syndrome* or AMI).ti,ab.
24. 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25. 10 and 24
26. (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) adj6 transplant*) or ((intramyocardial* or intracoronary or
transendocardial* or transcoronary) adj6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or
progenitor cell* or precursor cell*))).mp.
27. 25 or 26
28. Meta Analysis/
29. Systematic Review/
30. (meta analy* or metaanalys*).tw.
31. (systematic* adj2 (review* or overview* or search*)).tw.
32. (literature adj2 (review* or overview* or search*)).tw.
33. (cochrane or embase or cinahl or cinhal or lilacs or BIDS or science citation index or psyclit or psychlit or psycinfo or psychinfo or
cancerlit).ti,ab.
34. (electronic* adj (sources or resources or databases)).ab.
35. (reference lists or bibliograph* or handsearch* or hand search* or (manual* adj1 search*)).ab.
36. (additional adj (papers or articles or sources)).ab.

37. (relevant adj (journals or articles)).ab.
38. (search term* or published articles or search strateg*).ab.
39. Review.pt. and (data extraction or selection criteria).ab.
40. or/28-3941. Controlled Clinical Trial/
42. Phase 3 Clinical Trial/
43. Phase 4 Clinical Trial/
44. Randomized Controlled Trial/
45. Randomization/
46. Single Blind Procedure/
47. Double Blind Procedure/
48. Crossover Procedure/
49. Placebo/
50. (randomized or randomised or RCT).tw.
51. (random* adj5 (allocat* or assign* or divid* or receiv*)).tw.
52. (single blind* or double blind* or treble blind* or triple blind*).tw.
53. (phase III or phase three or "phase 3").tw.
54. (crossover* or cross over* or cross-over* or placebo*).tw.
55. Prospective Study/
56. or/41-55
57. Case Study/
58. case report*.tw.
59. (note or editorial).pt.
60. or/57-59
61. 56 not 60
62. 40 or 61
63. limit 62 to embase
64. 27 and 63
65. limit 64 to em=202049-202204

CINAHL

S1 (MH "Cell Transplantation+")
S2 (MH "Stem Cells+")
S3 TI ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or ALDH* or C-KIT*) N2 cell*) OR AB ((stem or haematopoietic or hematopoietic or haematopoetic or hematopoetic or hemopoietic or haemopoietic or progenitor or precursor or bone marrow or mononuclear or adipose tissue or mesenchymal or stromal or autologous or allogeneic or allogenic or ALDH* or C-KIT*) N2 cell)
S4 TX ((autologous N3 transplant*) or cell* therap*)
S5 TI ((cell* or myoblast*) N3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*)) OR AB ((cell* or myoblast*) N3 (autologous or transplant* or autotransplant* or allotransplant* or graft* or implant*))
S6 S1 OR S2 OR S3 OR S4 OR S5
S7 (MH "Heart Diseases+")
S8 TI ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) N3 (infarct* or postinfarct* or hypoxi* or anoxi*)) OR AB ((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart or acute) N3 (infarct* or postinfarct* or hypoxi* or anoxi*))
S9 TI (("heart disease*" or "coronary disease*" or IHD or CIHD or DCM or IDCM)) AND AB (("heart disease*" or "coronary disease*" or IHD or CIHD or DCM or IDCM))
S10 TI (((myocardial N3 dysfunction) OR angina OR stenocardia)) OR AB (((myocardial N3 dysfunction) OR angina OR stenocardia))
S11 TI (((ischemi* or ischaemi* or nonischemi* or nonischaemi*) N5 (myocardium or myocardial or heart or coronary or cardiac or cardial or subendocardial or cardiomyopath*))) OR AB (((ischemi* or ischaemi* or nonischemi* or nonischaemi*) N5 (myocardium or myocardial or heart or coronary or cardiac or cardial or subendocardial or cardiomyopath*)))
S12 TI (((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) N2 coronary)) OR AB (((arter* occlusion* or arter* disease* or arterioscleros* or atheroscleros*) N2 coronary))
S13 TI (((myocardial or myocardium or subendocardial or transmural or cardiac or cardial or coronary or heart) N2 (failure* or decompensation or insufficien*))) OR AB (((myocardial or myocardium or subendocardial or

transmural or cardiac or cardial or coronary or heart) N2 (failure* or decompensation or insufficien*)))
S14 TI ((end stage or endstage or dilated or idiopathic or congestive) N2 cardiomyopath*) OR AB ((end stage or endstage or dilated or idiopathic or congestive) N2 cardiomyopath*)
S15 TI ((heart or cardiac or cardial or myocardium or myocardial) N3 (repair* or reparation or improv* or regenerat*)) OR AB ((heart or cardiac or cardial or myocardium or myocardial) N3 (repair* or reparation or improv* or regenerat*))
S16 TI (heart attack* or coronary attack* or acute coronary syndrome* or AMI) OR AB (heart attack* or coronary attack* or acute coronary syndrome* or AMI)
S17 S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16
S18 S6 AND S17
S19 TI (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) N6 transplant*) or ((intramyocardial* or intracoronary or transendocardial* or transcoronary) N6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or progenitor cell* or precursor cell*))) OR AB (cellular cardiomyoplast* or ((cardiomyocyte* or cardiac cell*) N6 transplant*) or ((intramyocardial* or intracoronary or transendocardial* or transcoronary) N6 (transplant* or stem or bone marrow or marrow cell* or BMC* or stromal or mesenchymal or progenitor cell* or precursor cell*))
S20 S18 OR S19
S21 (MH CLINICAL TRIALS+)
S22 PT Clinical Trial
S23 TI ((controlled trial*) or (clinical trial*)) OR AB ((controlled trial*) or (clinical trial*))
S24 TI ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*)) OR AB ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*))
S25 TI randomi* OR AB randomi*
S26 MH RANDOM ASSIGNMENT
S27 TI ((phase three) or (phase III) or (phase three)) or AB ((phase three) or (phase III) or (phase three))
S28 (TI (random* N2 (assign* or allocat*))) OR (AB (random* N2 (assign* or allocat*)))
S29 MH PLACEBOS
S30 TI placebo* OR AB placebo*
S31 MH QUANTITATIVE STUDIES
S32 S21 or S22 or S23 or S24 or S25 or S26 or S27 or S28 or S29 or S30 or S31
S33 S20 and S32 Published Date: 20201231-20220202

LILACS

Words: (infarct OR infarction OR coronary OR myocardial OR heart OR cardiac OR cardiomyopathy OR myocardial OR subendocardial OR intramyocardial OR intracoronary OR ischemia OR ischemic OR nonischemic) AND Words: (bone marrow OR marrow cell OR marrow cells OR stem cell OR stem cells OR progenitor cells OR precursor cells OR cell therapy OR cellular therapy OR cell-based therapy OR mononuclear cells OR mesenchymal cells OR stromal cells) AND Country, year publication: (2020 or 2021 or 2022)

CPCI-S

4 #3 AND #2 AND #1 Timespan=2020-2022
3 TS=(randomised OR randomly OR randomized OR blind OR blinded OR trial OR study OR “control group” OR group)
2 TI=(“stem cell” OR “stem cells” OR “bone marrow” OR “marrow cells” OR “cellular therapy” OR “mesenchymal cells” OR “stromal cells” OR “cell transplant” OR “precursor cells” OR “progenitor cells” OR (c-kit* NEAR/5 cells) OR HSCT OR SCT OR MSC OR MSCs OR BMT OR BMC OR BMAC OR BMCs OR HST OR HSTs)
1 TI=(“cardiac failure” OR “heart attack” OR “heart failure” OR “coronary disease” OR “cardiovascular disease” OR “coronary artery” OR “coronary arterial” OR “myocardial infarction” OR cardiomyopathy OR “heart disease” OR “heart diseases” OR “cardiac insufficiency” OR AMI OR IHD OR CIHD OR DCM OR IDCM OR “myocardial dysfunction” OR stenocardia OR angina)

CHAPTER 9

Retrograde coronary venous infusion as A delivery strategy in regenerative cardiac therapy: an overview of preclinical and clinical data

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ABSTRACT

An important aspect of cell therapy in the field of cardiac disease is safe and effective delivery of cells. Commonly used delivery strategies such as intramyocardial injection and intracoronary infusion both present with advantages and disadvantages. Therefore, alternative delivery routes are explored, such as retrograde coronary venous infusion (RCVI). Our aim is to evaluate safety and efficiency of RCVI by providing a complete overview of preclinical and clinical studies applying RCVI in a broad range of disease types and experimental models. Available data on technical and safety aspects of RCVI are incomplete and insufficient. Improvement of cardiac function is seen after cell delivery via RCVI. However, cell retention in the heart after RCVI appears inferior compared to intracoronary infusion and intramyocardial injection. Adequately powered confirmatory studies on retention rates and safety are needed to proceed with RCVI in the future.

INTRODUCTION

Cell therapy has proven to be safe and feasible for treatment of cardiac disease. Yet, the clinical relevance of cell therapy is uncertain. Recent meta-analyses show a marginal (2–5%) increase of cardiac function measured by left ventricular ejection fraction(LVEF).[1,2] Taking into account the dynamic nature and the high perfusion characteristics of the cardiac tissue[3], an important aspect of cell therapy is the location and mode of delivery. Two commonly used administration techniques are intramyocardial (IM) injection and intracoronary (IC) infusion[1,2]. IM injection has the benefit of targeted delivery of cells in a target region, e.g., the border zone of the infarct[4], but this procedure is time-consuming, suffers from rapid wash-out of cells via venous drainage after injection[3], and needs specific systems in the catheterization laboratory. IC infusion is quick and easy to perform but the coronary system is often diseased in the target population, leading to inaccessibility of coronary arteries. Manipulation inside the coronary artery can potentially induce embolisms leading to decreased coronary blood flow[5–7]. Therefore, alternative delivery routes are explored. The coronary venous system is easily accessible and typically free of atherosclerotic disease. Retrograde coronary venous infusion (RCVI) is considered to be a good alternative to IM and IC administration. RCVI is performed by placing a balloon-catheter in the coronary sinus (CS) or into one of the coronary veins. In order to maximize the therapeutic potential, the balloon is kept inflated temporarily to prevent the loss of infused cells due to antegrade venous flow and to allow the cells to disseminate in the heart. For optimal effect, this occlusion is often prolonged for a certain period after cell infusion. Our aim is to provide a complete overview of preclinical and clinical studies applying RCVI as a cell delivery strategy and focus on safety aspects and efficiency measures.

METHODS

Search strategy and eligibility

The full search strategy is available as Online Resource 1. In brief, we have performed a search using the PubMed and Embase databases on May 15, 2017. Trials were eligible for inclusion if they met the following criteria: (1) original (preclinical or clinical) study, (2) full text available in English, (3) covering cell therapy, (4) investigating safety or efficacy of retrograde CS/venous administration. An additional cross-reference screening was performed of included articles. The flowchart of the search is presented in Fig. 1.

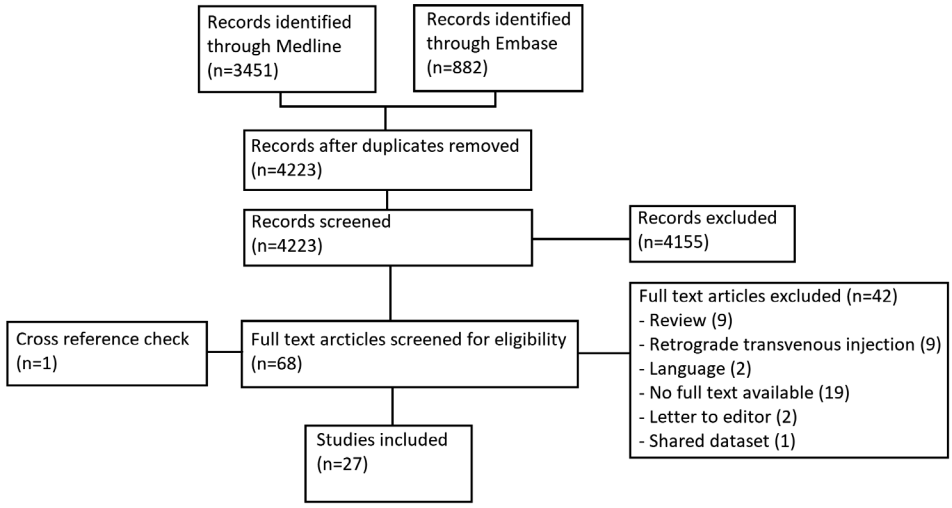

Figure 1. Flowchart of the systematic search articles

RESULTS

Search results

The entire search yielded a total of 4333 (3451 Medline and 882 Embase) hits, of which 110 reports were removed after duplicate screening. Another 4155 reports were excluded after title/abstract screening because they did not fulfill the inclusion criteria. The remaining 68 articles were screened on the availability of full text, leading to another 42 exclusions. One article was excluded due to a shared dataset[8]. The cross-reference screening led to one additional inclusion that did not come up in the original search due to the absence of one part of the search string in the title and abstract[9]. The total number of articles included in this review is 27 (Fig. 1). All articles were published between 2003 and 2016.

Preclinical and clinical experience

Retrograde coronary venous infusion has been performed in a number of different studies. In total, 21 preclinical studies are included in this review; 8 rat studies[10–17], 3 dog studies[18–20] and 10 pig studies[9,21–29]. Patients were treated in 6 studies[30–35].

Preclinical experience

Treatment was given in acute (acute myocardial infarction (AMI))[9,13–15,19,20,22–25,29] and chronic setting (chronic myocardial infarction (CMI))[10–12,17,21,26–29] and in chronic heart failure (CHF)[18]. One study treated healthy subjects (n = 1)[16]. Cell products administered included skeletal myoblasts (n = 6)[10,12,15,16,21,26], bone marrow mononuclear cells (n = 2)[11,29],

peripheral blood mononuclear cells (n = 2)[22,24], adipose-derived stem cells (n = 3)[18,23,24], mesenchymal stem cells (n = 6)[13,14,19,20,25,27], embryonic endothelial progenitor cell (n = 1)[9], autologous unfractionated bone marrow (n = 1)[28], and cardiac explant-derived c-Kit+ cells (n = 1)[17]. One study administered both adipose-derived stem cells and peripheral blood mononuclear cells[24].

Clinical experience

In the clinical setting, treatment was given in AMI[31], CHF[30,32], and chronic refractory angina (CRA)[33–35]. Infused cell products included bone marrow mononuclear cells (n = 3)[30,31,33], umbilical cord subepithelial cells (n = 1)[32], and autologous unfractionated bone marrow (n = 2)[34,35].

Table 1 shows study characteristics on disease model, recipients, and used cell type and number. In summary, there is broad experience with RCVI across species, disease models, and used cells.

Practical aspects of RCVI'

There is a high degree of heterogeneity in the way that RCVI is performed. Important differences between models are (1) the infusion duration, (2) the volume of infused cell suspension, (3) the time that the CS or coronary vein is occluded to prevent cells from draining directly into the right atrium, (4) the number of cells infused, and (5) the location of infusion (Tables 1 and 2).

Preclinical experience

Cells are predominantly infused via the coronary veins in preclinical trials. The infused cell number ranged from approximately 1 × 10^6 to 3 × 10^9. Infusion duration, infused cell volume, and the time that the CS or coronary vein was occluded differed both within and between animal species (Tables 1 and 2).

Clinical experience

In clinical trials, cells were mainly infused via the CS. The amount of cells infused was generally higher, ranging from approximately 1 × 10^8 to 4 × 10^9 cells. Notable differences between preclinical and clinical trials are that infused cell volumes were many times greater in clinical trials compared to preclinical trials and that the CS or coronary vein was occluded longer in clinical trials (Tables 1 and 2).

We found a striking reporting difference regarding practical aspects of RCVI, with roughly 20% of studies not adequately describing procedural characteristics. This hampers the possibility to repeat certain experiments if desired.

Safety issues

Here, safety is described as occurrence of arrhythmias related to RCVI, elevation of heart enzymes, cardiac tamponade, presence of pericardial fluid, microvascular obstruction (MVO), damage to the CS, and mortality. It should be noted that some studies did not report safety aspects due to the purpose and setup of these studies.

Table 1. Practical aspects of RCVI regarding disease type, location of infusion, and infused cell type and number.

	Study	Species	Number of subjects	Model	Administration	Cell type	Number of cells
Small animals	Di Lascio[1]	Rat	66	CMI	RCV	SMB	2 × 10^6 /100 g
	Fukushima[2]	Rat	35	CMI	RCV	BMMNC	10^7
	Fukushima[3]	Rat	85	CMI	RCV	SMB	5 × 10^6
	Huang[4]	Rat	90	AMI	RCV	MSC	10^6
	Huang[5]	Rat	38	AMI	RCV	MSC	10^6
	Suzuki[6]	Rat	62	AMI	RCV	SMB	10^6
	Suzuki[7]	Rat	20	NP	RCV	SMB	10^6
	Zakharova[8]	Rat	32	CMI	RCV	CEDC	10^6
Large animals	Pogue[9]	Dog	15	CHF	RCV	ASC	10^7
	Sun[10]	Dog	28	AMI	RCV	MSC	10^7
	Wang[11]	Dog	18	AMI	RCV	MSC	10^8
	Formigli[12]	Pig	15	CMI	RCV	SMB	8 × 10^7
	Hagikura[13]	Pig	15	AMI	RCV	PBMNC	5 × 10^6
	Hong[14]	Pig	7	AMI	RCV	ASC	10^7
	Hou[15]	Pig	5	AMI	RCV	PBMNC/ASC	10^7
	Kupatt[16]	Pig	ns	AMI	RCV	EEPC	5 × 10^6
	Lu[17]	Pig	36	AMI	RCV	MSC	10^8
	Prifti[18]	Pig	15	CMI	RCV	SMB	Ns
	Sato[19]	Pig	13	CMI	RCV	MSC	10^7
	Vicario[20]	Pig	16	CMI	RCS	AUBM	Ns
	Yokoyama[21]	Pig	21	AMI & CMI	RCV	BMMNC	3.2 ± 1.2 × 10^9
Clinical trials	Patel[22]	Human	46	CHF	RCS	BMMNC	3.7 × 10^9
	Silva[23]	Human	9	AMI	RCV	BMMNC	10^8
	Tuma[24]	Human	14	CRA	RCS	BMMNC	8.2 × 10^8
	Tuma[25]	Human	18	CHF	RCS	UCSEC	1×, 2×, 4 × 10^8
	Vicario[26]	Human	14	CRA	RCS	AUBM	0,04 or 0,08 × 10^8/kg
	Vicario[27]	Human	15	CRA	RCS	AUBM	>0,04 × 10^8/kg

CMI chronic myocardial infarction (administration of cells > 1 week post MI), AMI acute myocardial infarction (administration of cells up to 7 days post MI), CHF chronic heart failure, NP no pathology, CRA chronic refractory angina, MI myocardial infarction, SMB skeletal myoblasts, BMMNC bone marrow mononuclear cells, PBMNC peripheral blood mononuclear cells, ASC adipose-derived stem cells, MSC mesenchymal stem cells, EEPC embryonic endothelial progenitor cells, UCSEC umbilical cord subepithelial cells, AUBM autologous unfractionated bone marrow, CEDC cardiac explant-derived c-Kit+ cells, RCV retrograde coronary venous infusion, RCS retrograde coronary sinus infusion, ns not specified

Table 2. Heterogeneity regarding practical aspects of RCI both within and between species.

Study type	Infusion duration (min)	Infused volume (ml)	Occlusion time (min)
Rat studies (*n* = 8)	1.0 [0.5–1.0] (*n* = 3)	1.0 [0.5–1.0] (*n* = 8)	5.0 [1.0–5.0] (*n* = 8)
Dog studies (*n* = 3)	No data (*n* = 0)	10.0 [10.0–20.0] (*n* = 3)	Insufficient data (*n* = 2)
Pig studies (*n* = 10)	10.0 [0.25–40.0] (*n* = 9)	15.0 [10.0–25.0] (*n* = 10)	10.0 [5.0–20.0] (*n* = 7)
Human studies (*n* = 6)	5.0 [4.0–6.0] (*n* = 6)	60.0 [40.25–120.0] (*n* = 6)	15.0 [11.0–17.0] (*n* = 5)
Overall (*n* = 27)	5.0 [0.88–11.25 (*n* = 18)	10 ml [1.0–40.0] (*n* = 27)	10.0 [5.0–12.75] (*n* = 22)

Data are presented as median with interquartile ranges calculated using IBM SPSS statistics 21. min minute(s), ml milliliter(s), n number of studies that statistics are based on

Safety aspects other than mortality

Preclinical experience

Thirteen preclinical studies reported safety aspects of RCVI. One study only described that RCVI is safe without providing data on safety[29]. Seven studies only reported absence of arrhythmias without providing in-depth data[10,14–16,22,26,28]. Five articles provided more in-depth data on safety aspects of RCVI[11,12,18,20,23]. These five studies will be discussed in more detail below.

In two studies, IM injection was associated with an increased chance of both spontaneous ventricular tachycardias and ventricular premature contractions after cell administration compared to RCVI, suggesting that RCVI is safer in these experimental models[11,12]. Another study closely monitored dogs for occurrence of arrhythmias and reported transient atrial fibrillation during CS catheterization in 6 out of 15 dogs and a pre-existent ventricular arrhythmia in one dog[18]. In another dog study, no occurrence of arrhythmias or cardiac tamponade associated with RCVI was seen[20]. RCVI did not lead to MVO after cell administration in one pig study[23].

Clinical experience

All six clinical studies reported safety aspects of RCVI. Two studies only reported absence of arrhythmias without providing in-depth data[34,35]. The other four studies provided more in-depth information on safety. In one clinical trial, absence of arrhythmias associated with RCVI was reported, but a rise in cardiac enzymes was seen in some patients after RCVI[30]. Rise in cardiac enzymes after RCVI was also reported in some patients in another clinical trial[31]. In a population of patients with heart failure, a transient increase in Troponin-I levels was seen in all patients that resolved within 24 h after catheterization. No arrhythmias were seen in this patient population and there was no evidence of damage to the CS after infusion[32]. No occurrence of arrhythmias, no rise in cardiac enzymes, and no pericardial effusion after retrograde delivery of cells was seen in patients with chronic refractory angina[33].

Mortality

Preclinical experience

Mortality rates were reported in 16 articles, with no RCVI-related deaths occurring in 11 of these 16 studies. The available mortality data are difficult to interpret because it is likely that other factors besides RCVI, such as surgical procedure, have had influence on mortality rates. Loss of subjects that could possibly be attributed to RCVI was seen in 5 studies, described below.

A loss of 11/66 rats (16.7%) after RCVI was seen in one study. This loss could be attributed to the fact that a thoracotomy was performed to access the coronary vein and might not be related to the RCVI procedure itself. Since all animals received cells through RCVI, there is no control group for mortality[10]. A comparison was made between mortality rates after IM injection and RCVI in two rat studies. Mortality rates were comparable between IM injection and RCVI with the first study showing mortality rates of 2/34 rats (5.9%) after IM injection and 2/35 rats (5.7%) after RCVI[11]. Similar results were seen in the second study with a mortality of 4/48 rats (8.3%) in the IM injection group compared to 4/49 rats (8.2%) in the RCVI group[12]. Surgical stress and bleeding were suggested to be the cause of mortality. A common complication with RCVI in small animals is sustained bleeding from the catheter insertion site because the catheter has to be inserted into the fragile left cardiac vein via the left superior vena cava or CS. A comparison was made between conventional RCVI and a modified method of RCVI to see if bleeding could be limited in small animals. Conventional RCVI was described as delivery of cells by direct insertion of a catheter in the left cardiac vein via the CS. Modified RCVI was described as cardiac vein catheterization via the left internal jugular vein. A mortality of 3/7 rats (42.9%) was seen in the group that received cells via conventional RCVI versus 0/20 rats (0%) in the group with modified RCVI[14]. One small animal study reported a loss of 18/62 rats (29%) within 24 h after RCVI, which the authors linked to development of acute heart failure rather than the RCVI[15].

Clinical experience

In all six clinical trials, mortality rates were reported but mortality related to RCVI did not occur.

In conclusion, there seems to be no relation between the way RCVI is performed and the occurrence of adverse events, arrhythmias, and mortality. Especially large animal studies and clinical trials do not report mortality or arrhythmias related to RCVI. Although RCVI is reported to be safe in the majority of studies presented here, safety data on RCVI are underreported with the majority of studies providing no or insufficient safety data to conclude that RCVI is a safe method for cell delivery in the heart. Safety and mortality data are provided in Table 3.

Table 3. Safety and mortality data.

	Study	Species	Reported safety aspects	Mortality related to retrograde infusion procedure
Small animals	Di Lascio[1]	Rat	No arrhythmias, described as safe	16,7% (11/66) probably related to thoracotomy)
	Fukushima[2]	Rat	More VPC and VT in IM group vs RCVI group, described as safe	RCVI: 5.7% (2/35) vs IM: 5.9% (2/34)
	Fukushima[3]	Rat	More VPC and VT in IM group vs RCVI group, described as safe	RCVI: 8.2% (4/49) vs IM: 8.3% (4/48)
	Huang[4]	Rat	ns	ns
	Huang[5]	Rat	No arrhythmias	conventional technique: 42.9% (3/7) modified technique: 0
	Suzuki[6]	Rat	No arrhythmias, described as safe	29% (18/62) within 24 h, probably due to acute heart failure
	Suzuki[7]	Rat	No arrhythmias	0%
	Zakharova[8]	Rat	ns	0%
Large animals	Pogue[9]	Dog	Transient AF during procedure in 6/15 dogs, described as safe	0%
	Sun[10]	Dog	ns	0%
	Wang[11]	Dog	No arrhythmias, no cardiac tamponade, described as safe	0%
	Formigli[12]	Pig	ns	0%
	Hagikura[13]	Pig	No arrhythmias, described as safe	0%
	Hong[14]	Pig	No MVO, described as safe	0%
	Hou[15]	Pig	ns	0%
	Kupatt[16]	Pig	ns	ns
	Lu[17]	Pig	ns	ns
	Prifti[18]	Pig	No arrhythmias, described as safe	0%
	Sato[19]	Pig	ns	0%
	Vicario[20]	Pig	No arrhythmias	ns
	Yokoyama[21]	Pig	Described as safe	ns
Clinical trials	Patel[22]	Human	Rise in cardiac enzymes in some patients, no arrhythmias associated with RCVI, described as safe	0%
	Silva[23]	Human	Rise in cardiac enzymes in some patients	0%
	Tuma[24]	Human	No rise in cardiac enzymes, no arrhythmias, no pericardial effusion, described as safe	0%
	Tuma[25]	Human	No arrhythmias, rise in cardiac enzymes in all patients, no evidence of CS leak or damage, described as safe	0%
	Vicario[26]	Human	No arrhythmias, described as safe	0%
	Vicario[27]	Human	No arrhythmias, described as safe	0%

VPC ventricular premature contraction, VT ventricular tachycardia, IM intramyocardial injection, RCVI retrograde coronary venous infusion, ns not specified, AF atrial fibrillation, MVO microvascular obstruction, CS coronary sinus

Efficiency measures

Retention rate

Preclinical experience

The therapeutic benefit of cell therapy is in part based on the retention of cells in the heart. In total, eight preclinical studies provide data on the percentage of administered cells that retain in the heart after RCVI (Table 4). Different methods are used to determine cardiac retention of cells. One method is the use of real-time polymerase chain reaction for the Y-chromosome-specific Sry gene to detect the amount of transplanted male cells in female subjects. Other methods include administration of β-galactosidase-expressing cells, or to label cells radioactively with 111Indium or Tc99m-hexamethylpropylenamineoxime for quantitative analysis using scintigraphy. The retention rates show a high degree of heterogeneity that can partially be explained by differences in animal model, disease model, cell type, infusion time point, follow-up time point, and quantification technique. Most studies report a retention ≤ 10% and two studies report a remarkably higher retention of respectively 31.4 ± 4.8 and 29.8 ± 6.9%[15,16]. The latter studies applied an indirect measurement of retention by using β-galactosidase-expressing cells, and comparing the level of β-galactosidase activity to the standard curve. One study used a method to optimize retention (magnetic targeting) that resulted in an increase of retention from approximately 2% after routine RCVI to 8.5% with magnetic targeting[13]. It should be noted that the three large animal experiments[9,23,24] consist of very small sample sizes. RCVI appeared to be either inferior to[23,24] or equal to[11,12] IM injection or IC infusion regarding cell retention. Retention rates in Table 4 are presented as the percentage of total administered cells that is retained in the heart. In one study[23], retention of cells in the heart was reported as a percentage of cells retained in five major thoracoabdominal organs. We converted the data to a percentage of total administered cells that are retained in the heart in order to achieve comparability between studies. If retention of cells was measured at multiple time points, we reported retention at the first time point, because retention decreased in time in the majority of these studies. A decrease was not seen in three studies[15,16,23]. This can be explained by the fact that two of these studies used expression of β-galactosidase as a measure of cardiac cell retention[15,16]. Increased expression of β-galactosidase over time was attributed to proliferation of administered cells. The third article[23] presented the retention of cells in the heart as a percentage of the total retention in five major organs. A possible explanation for the increase in retention at a later time point could be that the decrease in the number of cells in the heart was relatively less than the decrease in the number of cells in the five major organs, making this decrease in the heart look like an increase[23].

Functional outcomes

The goal of cardiac reparative therapy is improvement of cardiac function or decrease

Table 4. Retention of cells in the heart. In case retention was not measured as % of total administered dose (e.g., as a % of uptake in major organs), we calculated the retention % of total administered dose. This was the case in one study[23] Sry polymerase chain reaction for the Y-chromosome-specific Sry gene, β-galactosidase presence of β-galactosidase-expressing cells, radiolabel retention measured by scintigraphy after radiolabeled cell infusion, RCVI retrograde coronary sinus/venous infusion, IC intracoronary infusion, IM intramyocardial injection, IV intravenous, ns not specified, na not applicable, # number of subjects

	Study	Species	#	Retention method	Retention time point	Application method				
						RCVI retention	IC retention	IM retention	Peripheral IV retention	Sign comparison
Small animals	Fukushima[2]	Rat	35	Sry	3 days	1.84 ± 0.27%	–	1.45 ± 0.27%	–	ns
	Fukushima[3]	Rat	85	Sry	3 days	10 ± 5%	–	14 ± 5%	–	ns
	Huang[4]	Rat	90	Sry	24 h	2%/8.5%[a]	–	–	–	$P < 0.001$[d]
	Huang[5]	Rat	20	β-galactosidase	10 min	31.4 ± 4.8%	–	–	–	na
	Suzuki[7]	Rat	20	β-galactosidase	10 min	31.4 ± 4.8%	–	–	–	na
	Suzuki[6]	Rat	62	β-galactosidase	10 min	29.8 ± 6.9%	–	–	–	na
Large animals	Hong[14]	Pig	7	Radiolabel	1 h	±8%[c]	±25%[c]	–	–	$P = 0.037$
	Hou[15]	Pig	5	Radiolabel	1 h	3.2 ± 1%	2.6 ± 0.3%	11.3 ± 3%	–	Not sign[b]
	Kupatt[16]	Pig	6	Radiolabel	1 h	2.7%	–	–	0.5%	ns
Clinical trials	Silva[23]	Human	9	Radiolabel	4 h	4.62%	16.14%	–	–	$P = 0.01$

[a]2% in case of normal delivery, 8.5% in case of magnetic targeting
[b]Comparison between RCVI infusion and IM retention
[c]Corrected for total injected dose
[d]Normal delivery versus magnetic targeting

Clinical experience

Retention of cells in the heart was determined in one clinical trial, showing inferiority of RCVI versus IC infusion[31]. Cells labeled with Tc99m-hexamethylpropylenamineoxime were used to assess retention in the heart. Just like the three pig studies, sample size was small and retention rates were comparable[9,23,24].

of disease characteristics such as angina complaints in order to improve quality of life and decrease mortality. Here, we focused on the effect of cell administration on (1) LVEF (AMI, CMI, CHF), (2) improvement on the Canadian Cardiovascular Society scale (CSS) (CRA), and (3) myocardial perfusion (CRA).

Preclinical experience

Most of the preclinical studies that reported changes in LVEF (12/15) showed a significant increase in LVEF versus baseline and/or controls. Three studies only showed improvement of LVEF when cells were combined with growth factors[22] or no effects on LVEF at all[18,19].

Clinical experience

Three out of four clinical studies reported significant improvement of LVEF. The study that did not show improvement of LVEF after RCVI compared IC infusion with RCVI and reported that patients receiving cells through IC infusion did show improvement in LVEF[31]. The difference in cell retention between IC infusion and RCVI in these patients might be the explanation for this difference in functional outcome. Two other studies show comparable retention rates between IM injection and RCVI and both groups show comparable functional gains[11,12]. In case of CRA, changes in CCS scale and improvement in myocardial perfusion were reported[33–35].

In the majority of cases, cells administered with RCVI are able to effectuate improvement of cardiac function in a range of different experimental models. An overview of functional outcomes is presented in Online Resource 2.

DISCUSSION

Cell delivery strategies should meet two important demands. First and foremost, the technique should be safe. Second, it should be effective in delivering cells to the heart. In this paper, we provided an overview of RCVI.

There is a high degree of heterogeneity regarding technical aspects of RCVI both between and within species. Furthermore, roughly 20% of studies do not adequately describe procedural characteristics, which hampers the possibility to repeat these experiments technically.

The main finding is that relevant data regarding technique and safety are poorly reported. For instance, 30% of included studies do not report on safety aspects of RCVI at all, while 33% only report absence of arrhythmias without mentioning other safety parameters. Only a limited number of studies provide more in-depth safety information regarding RCVI. The six clinical trials included in this overview report cardiac enzyme rise as the only safety issue associated with RCVI and show no arrhythmias associated

with RCVI, no development of pericardial fluid, and no sustained damage to the CS after RCVI. It is understandable that the first priority of research focused on cell therapy lies with validating the effectiveness of cell therapy in itself. From this perspective, it is logical that some studies do not report on safety of delivery because this was not the purpose of the study. Nevertheless, due to the poor reporting of safety aspects, we cannot make an accurate assessment of the safety profile of RCVI.

However, retrograde accessing of the coronary venous system has been performed for a long time in the field of cardiac surgery in a great number of patients. With retrograde cardioplegia (RC), the myocardium is retrogradely perfused during cardiac surgery to induce cardiac arrest and protect the myocardium. With RC, a balloon-catheter is used to occlude the opening of the CS, in a way comparable to RCVI. RC is reported to be safe, with injury to the CS occurring in 0.06 to 0.6% of patients[36,37], resulting in formation of hematoma on the atrioventricular groove, perforation of the CS wall, pericardial effusion, or laceration of the right ventricle or CS[37–40]. These data would suggest that the technical part of RCVI, namely the insertion of a balloon-tipped catheter in the CS followed by infusion of fluid, should be safe.

Cells delivered through RCVI are able to improve cardiac function and alleviate angina symptoms. However, in terms of cell retention, the data suggest that RCVI is a limitedly effective delivery strategy for cell therapy. In fact, IC infusion and IM injection show either higher or equal retention rates. It is likely that inferior retention rates decrease the efficacy of RCVI.

Due to the limited number of studies included in this review, we cannot conclude that RCVI is favorable in certain disease types or that certain cell types performed better than others in the included studies.

In conclusion, the available data on technical and safety aspects of RCVI are insufficient and incomplete. Furthermore, retention data show inferior results compared to IC infusion and IM injection. We conclude that at present, there are not enough arguments to proceed with this technique in the clinical arena. Well-designed confirmatory studies on retention rates and safety data are required to proceed with RCVI in the future.

REFERENCES

1. Fisher SA, Zhang H, Doree C, Mathur A, Martin-Rendon E. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev*. 2015;(9). doi:10.1002/14651858.CD006536.pub4
2. Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. *Cochrane Database Syst Rev 2016*. 2016;(12). doi:10.1136/heartjnl-2017-311684
3. van den Akker F, Feyen DAM, van den Hoogen P, et al. Intramyocardial stem cell injection: go(ne) with the flow. *Eur Hear*. 2017;38(3):184-186. doi:10.1093/eurheartj/ehw056
4. van Slochteren FJ, van Es R, Gyongyosi M, et al. Three dimensional fusion of electromechanical mapping and magnetic resonance imaging for real-time navigation of intramyocardial cell injections in a porcine model of chronic myocardial infarction. *Int J Cardiovasc Imaging*. 2016. doi:10.1007/s10554-016-0852-x
5. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet*. 2004;363(9411):P783-784. doi:10.1016/S0140-6736(04)15695-X
6. Suzuki K, Brand NJ, Smolenski RT, Jayakumar J, Murtuza B, Yacoub MH. Development of a novel method for cell transplantation through the coronary artery. *Circulation*. 2000;102(10 suppl 3):III359-64. doi:10.1161/01.cir.102.suppl_3.iii-359
7. Freyman T, Polin G, Osman H, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J*. 2006;27:1114-1122. doi:10.1093/eurheartj/ehi818
8. Moreira R de C, Haddad AF, Silva SA, et al. Intracoronary stem-cell injection after myocardial infarction: microcirculation sub-study. *Arq Bras Cardiol*. 2011;97(5):420-426. doi:10.1590/s0066-782x2011005000095
9. Kupatt C, Hinkel R, Lamparter M, et al. Retroinfusion of Embryonic Endothelial Progenitor Cells Attenuates Ischemia-Reperfusion Injury in Pigs. *Circulation*. 2005;112(9_supplement):I-117-I-122. doi:10.1161/CIRCULATIONAHA.104.524801
10. Lascio G Di, Harmelin G, Targetti M, et al. Cellular retrograde cardiomyoplasty and relaxin therapy for postischemic myocardial repair in a rat model. *Tex Hear Inst J*. 2012;39(4):488-499.
11. Fukushima S, Varela-carver A, Coppen SR, et al. Direct Intramyocardial But Not Intracoronary Injection of Bone Marrow Cells Induces Ventricular Arrhythmias in a Rat Chronic Ischemic Heart Failure Model. *Circulation*. 2007;115:2254-2261. doi:10.1161/CIRCULATIONAHA.106.662577
12. Fukushima S, Coppen SR, Lee J, et al. Choice of Cell-Delivery Route for Skeletal Myoblast Transplantation for Treating Post-Infarction Chronic Heart Failure in Rat. *PLoS One*. 2008;3(8):e3071. doi:10.1371/journal.pone.0003071
13. Huang Z, Shen Y, Sun A, et al. Magnetic targeting enhances retrograde cell retention in a rat model of myocardial infarction. *Stem Cell Res Ther*. 2013;4:149. doi:doi.org/10.1186/scrt360.
14. Huang Z, Shen Y, Zhu H, et al. A New Coronary Retroinfusion Technique in the Rat Infarct Model: Transjugular Cardiac Vein Catheterization. *Exp Anim*. 2013;62(3):197-203. doi:10.1538/expanim.62.197
15. Suzuki K, Murtuza B, Fukushima S, et al. Targeted Cell Delivery Into Infarcted Rat Hearts by Retrograde Intracoronary Infusion: Distribution, Dynamics, and Influence on Cardiac Function. *Circulation*. 2004;110(suppl:II-225-II-230. doi:10.1161/01.CIR.0000138191.11580.e3
16. Suzuki K, Murtuza B, Smolenski RT, Yacoub MH. Selective cell dissemination into the heart by retrograde intracoronary infusion in the rat. *Transplantation*. 2004;77(5):757-759. doi:10.1097/01.tp.0000114482.21064.8f
17. Zakharova L, Nural-guvener H, Feehery L, Popovic S, Nimlos J, Gaballa MA. Retrograde coronary vein infusion of cardiac explant – derived c-Kit 1 cells improves function in ischemic heart failure. *J Hear Lung Transplant*. 2014;33:644-653. doi:10.1016/j.healun.2014.03.006
18. Pogue B, Estrada AH, Sosa-Samper I, et al. Stem-cell therapy for dilated cardiomyopathy: a pilot study evaluating retrograde coronary venous delivery. *J Small Anim Pract*. 2013;54:361-366. doi:10.1111/jsap.12098
19. Sun Q-W, Zhen L, Wang Q, et al. Assessment of Retrograde Coronary Venous Infusion of Mesenchymal Stem Cells Combined with Basic Fibroblast Growth Factor in Canine Myocardial Infarction Using Strain Values Derived from Speckle-Tracking Echocardiography. *Ultrasound Med Biol*. 2016;42(1):272-281. doi:10.1016/j.ultrasmedbio.2015.09.010
20. Wang X, Zhen L, Miao H, et al. Concomitant Retrograde Coronary Venous Infusion of Basic Fibroblast Growth Factor Enhances Engraftment and Differentiation of Bone Marrow Mesenchymal Stem Cells for Cardiac Repair after Myocardial Infarction. *Theranostics*. 2015;5(9):995-1006. doi:10.7150/thno.11607
21. Formigli L, Perna A, Meacci E, et al. Paracrine effects of transplanted myoblasts and relaxin on post-infarction heart remodelling. *J Cell Mol Med*. 2007;11(5):1087-1100. doi:10.1111/j.1582-4934.2007.00111.x
22. Hagikura K, Fukuda N, Yokoyama S, et al. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. *Int J Cardiol*. 2010;142:56-64.

doi:10.1016/j.ijcard.2008.12.108
23. Hong SJ, Hou D, Brinton TJ, et al. Intracoronary and Retrograde Coronary Venous Myocardial Delivery of Adipose-Derived Stem Cells in Swine Infarction Lead to Transient Myocardial Trapping with Predominant Pulmonary Redistribution. *Catheter Cardiovasc Interv*. 2014;83:E17-E25. doi:10.1002/ccd.24659
24. Hou D, Youssef EA, Brinton TJ, et al. Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary. *Circulation*. 2005;112(suppl I):I150-I156. doi:10.1161/CIRCULATIONAHA.104.526749
25. Lu F, Zhao X, Wu J, et al. MSCs transfected with hepatocyte growth factor or vascular endothelial growth factor improve cardiac function in the infarcted porcine heart by increasing angiogenesis and reducing fibrosis. *Int J Cardiol*. 2013;167:2524-2532. doi:10.1016/j.ijcard.2012.06.052
26. Prifti E, Di G, Harmelin G, et al. Cellular cardiomyoplasty into infracted swine's hearts by retrograde infusion through the venous coronary sinus: An experimental study. *Cardiovasc Revascularization Med*. 2016;17(4):262-271. doi:10.1016/j.carrev.2016.02.008
27. Sato T, Iso Y, Uyama T, et al. Coronary vein infusion of multipotent stromal cells from bone marrow preserves cardiac function in swine ischemic cardiomyopathy via enhanced neovascularization. *Lab Investig*. 2011;91(4):553-564. doi:10.1038/labinvest.2010.202
28. Vicario J, Piva J, Pierini A, et al. Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. 2003;3(2002):91-94. doi:10.1016/S1522-1865(03)00002-7
29. Yokoyama S, Fukuda N, Li Y, et al. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. *J Mol Cell Cardiol*. 2006;40(1):24-34. doi:10.1016/j.yjmcc.2005.06.008
30. Patel AN, Mittal S, Turan Go, et al. REVIVE Trial: Retrograde Delivery of Autologous Bone Marrow in Patients With Heart Failure. *Stem Cells Transl Med*. 2015;4(doi.org/10.5966/sctm.2015-0070):1-7.
31. Silva SA, Peixoto M, Soares AJS, et al. Autologous Bone-Marrow Mononuclear Cell Transplantation After Acute Myocardial Infarction: Comparison of Two Delivery Techniques. *Cell Transplant*. 2009;18:343-352. doi:10.3727/096368909788534951
32. Tuma J, Carrasco A, Castillo J, et al. RESCUE-HF Trial: Retrograde Delivery of Allogeneic Umbilical Cord Lining Subepithelial Cells in Ptients with Heart Failure. *Cell Transplant*. 2015;25(9):1713-1721. doi:10.3727/096368915X690314
33. Tuma J, Fernández-viña R, Carrasco A, et al. Safety and feasibility of percutaneous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina. *J Transl Med*. 2011;9(183). doi:10.1186/1479-5876-9-183
34. Vicario J, Campos C, Piva J, et al. Transcoronary sinus administration of autologous bone marrow in patients with chronic refractory stable angina Phase 1. *Cardiovasc Radiat Med*. 2004;5:71-76. doi:10.1016/j.carrad.2004.06.004
35. Vicario J, Campo C, Piva J, et al. One-year follow-up of transcoronary sinus administration of autologous bone marrow in patients with chronic refractory angina. *Cardiovasc Revascularization Med*. 2005;6(3):99-107. doi:10.1016/j.carrev.2005.08.002
36. Menasché P, Subayi JB, Piwnica A. Retrograde coronary sinus cardioplegia for aortic valve operations: A clinical report on 500 patients. *Ann Thorac Surg*. 1990;49(4):556-564. doi:10.1016/0003-4975(90)90301-L
37. Kaul TK, Fields BL, Jones CR. Iaterogenic injuries during retrograde delivery of cardioplegia. *Cardiovasc Surg*. 2000;8(5):400-403. doi:10.1177/096721090000800513
38. Kurusz M, Girouard MK, Brown PS. Coronary sinus rupture with retrograde cardioplegia. *Perfusion*. 2002;17(1):77-80. doi:10.1191/0267659102pf534cr
39. Krishnan S, Papworth DP, Farivar RS, Ueda K. Identification of Coronary Sinus Injury by Transesophageal Echocardiography During Placement of a Retrograde Cardioplegia Catheter for Minimally Invasive Cardiac Surgery. *Anesth Analg*. 2013;116(3). https://journals.lww.com/anesthesia-analgesia/Fulltext/2013/03000/Identification_of_Coronary_Sinus_Injury_by.10.aspx.
40. Aigner C, Wolner E, Mohl W. Management of central coronary sinus ruptures using the pericardial patch repair technique. *Ann Thorac Surg*. 2006;81(4):1275-1278. doi:10.1016/j.athoracsur.2005.11.037

SUPPLEMENTAL

Supplemental table 1. Functional outcomes after RCVI

	Study	Change in LVEF (%) myocardial perfusion or CCS	Follow up timepoint	Versus	P- value
Small animals	Di Lascio[1]	*LVEF: Echocardiopgraphy* 12 ± 11.2 % (cells) 16 ± 8.9 % (cells + RLX)	1 month	 Baseline Baseline	 P=0.0016 P<0.0001
	Fukushima[2]	*LVEF: Echocardiopgraphy* 15.5 ± 1.7	7 days	Baseline	Described as significant
	Fukushima[3]	*LVEF: Echocardiopgraphy* 9.4 ± 0.9 %	7 days	Baseline	P<0.05
	Huang[4]	*LVEF: Echocardiopgraphy* ~5 % ~12 % (magnetic targeting)	3 weeks	 PBS controls PBS controls	 P<0.05 P<0.05
	Huang[5]	*LVEF: Echocardiopgraphy* 10.7 ± 4.0 % 11.1 ± 3.7 %	4 weeks	 PBS controls Sham	 P<0.05 P<0.05
	Suzuki[6]	*LVEF: Pressure volume derived* 11.4 ± 6.7 %	3 weeks	 DMEM controls	 P<0.05
Large animals	Formigli[12]	*LVEF: Echocardiography* ~17 % (cells) ~20 % (cells + RLX)	1 month	 DMEM controls DMEM controls	 P<0.001 P<0.001
	Hagikura[13]	*LVEF: Pressure volume* 0.5 ± 3.8 % (cells) 9.7 ± 3.1 % (cells + VEGF)	4 weeks	 Saline controls Saline controls	 Described as not significant P<0.05
	Lu[17]	*LVEF: Echocardiopgraphy* ~5 % (cells + Adnull) ~11 % (cells + VEGF) ~27 % (cells + HGF) *LVEF: SPECT* ~8 % (cells + Adnull) ~10 % (cells + VEGF) ~22 % (cells + HGF)	4 weeks	 Baseline Baseline Baseline Baseline Baseline Baseline	 P<0.05 P<0.05 P<0.05 P<0.05 P<0.05 P<0.05
	Pogue[9]	*LVEF: Echocardiopgraphy* -28.5 %	2 years¥	 Baseline	 P<0.001
	Prifti[18]	*LVEF: Echocardiopgraphy* 12 ± 9.8 % *LVEF: SPECT* 13 ± 8.6 %	1 month	 Controls (no infusion procedure) Controls (no infusion procedure)	 P=0.001 P=0.001
	Sato[19]	*LVEF: Left ventriculography* Preserved, no data ~12 %	4 weeks	 Baseline DMEM controls	 No p-value P<0.01
	Sun[10]	*LVEF: Echocardiopgraphy* -0.1 ± 12.2 % (cells) -3.1 ± 12.9 % (cells + bFGF)	40 days	 Baseline Baseline	 Both described as not significant

Supplemental table 1. Continued

	Study	Change in LVEF (%) myocardial perfusion or CCS	Follow up timepoint	Versus	P- value
Large animals	Wang[11]	*LVEF: Echocardiopgraphy*			
		~ 7 % (cells)	4 weeks	Baseline	P=0.124
		11 % (cells + bFGF)		Baseline	P<0.01
		14.9 ± 3.8 % (cells)		Saline controls	P<0.01
		17.5 ± 3.3 % (cells + bFGF)		Saline controls	P<0.001
	Yokoyama[21]	*LVEF: Pressure volume derived*			
		~5 % (AMI)	4 weeks	Baseline	P<0.05
		~6 % (OMI)		Baseline	P<0.05
Clinical trials	Patel[22]	*LVEF: Left ventriculography*			
		6.0 % (niCMP)	12 months	Baseline	P=0.007
		8.1 % (iCMP)		Baseline	P=0.006
		not specified (niCMP)		Controls, no infusion procedure	P=0.954
		not specified (iCMP)		Controls, no infusion procedure	P=0.814
	Silva[23]	*LVEF: Radionuclide ventriculography*			
		0.4 ± 14.3 %	6 months	Baseline	P=0.88
	Tuma[24]	*LVEF: Echocardiopgraphy*			
		4.1 ± 7.4 % (100M cells)	12 months[$]	Baseline	P<0.05
		11.3 ± 6.9 % (200M cells)		Baseline	P<0.05
		13 ± 6.5 % (400M cells)		Baseline	P<0.05
	Tuma[25]	*CCS class*			
		1.4 ± 0.7		Baseline	P<0.001
		Myocardial perfusion: SPECT[£]			
		14.7 %	2 years[§]	Baseline	P=0.001
		LVEF: SPECT			
		4.3 %		Baseline	P=0.019

REFERENCES

1. Lascio G Di, Harmelin G, Targetti M, et al. Cellular retrograde cardiomyoplasty and relaxin therapy for postischemic myocardial repair in a rat model. *Tex Hear Inst J*. 2012;39(4):488-499.
2. Fukushima S, Varela-carver A, Coppen SR, et al. Direct Intramyocardial But Not Intracoronary Injection of Bone Marrow Cells Induces Ventricular Arrhythmias in a Rat Chronic Ischemic Heart Failure Model. *Circulation*. 2007;115:2254-2261. doi:10.1161/CIRCULATIONAHA.106.662577
3. Fukushima S, Coppen SR, Lee J, et al. Choice of Cell-Delivery Route for Skeletal Myoblast Transplantation for Treating Post-Infarction Chronic Heart Failure in Rat. *PLoS One*. 2008;3(8):e3071. doi:10.1371/journal.pone.0003071
4. Huang Z, Shen Y, Sun A, et al. Magnetic targeting enhances retrograde cell retention in a rat model of myocardial infarction. *Stem Cell Res Ther*. 2013;4:149. doi:doi.org/10.1186/scrt360.
5. Huang Z, Shen Y, Zhu H, et al. A New Coronary Retroinfusion Technique in the Rat Infarct Model: Transjugular Cardiac Vein Catheterization. *Exp Anim*. 2013;62(3):197-203. doi:10.1538/expanim.62.197
6. Suzuki K, Murtuza B, Fukushima S, et al. Targeted Cell Delivery Into Infarcted Rat Hearts by Retrograde Intracoronary Infusion: Distribution, Dynamics, and Influence on Cardiac Function. *Circulation*. 2004;110 (suppl:II-225-II-230. doi:10.1161/01.CIR.0000138191.11580.e3
7. Suzuki K, Murtuza B, Smolenski RT, Yacoub MH. Selective cell dissemination into the heart by retrograde intracoronary infusion in the rat. *Transplantation*. 2004;77(5):757-759. doi:10.1097/01.tp.0000114482.21064.8f
8. Zakharova L, Nural-guvener H, Feehery L, Popovic S, Nimlos J, Gaballa MA. Retrograde coronary vein infusion of cardiac explant – derived c-Kit 1 cells improves function in ischemic heart failure. *J Hear Lung Transplant*. 2014;33:644-653. doi:10.1016/j.healun.2014.03.006
9. Pogue B, Estrada AH, Sosa-Samper I, et al. Stem-cell therapy for dilated cardiomyopathy: a pilot study evaluating retrograde coronary venous delivery. *J Small Anim Pract*. 2013;54:361-366. doi:10.1111/jsap.12098
10. Sun Q-W, Zhen L, Wang Q, et al. Assessment of Retrograde Coronary Venous Infusion of Mesenchymal Stem Cells Combined with Basic Fibroblast Growth Factor in Canine Myocardial Infarction Using Strain Values Derived from Speckle-Tracking Echocardiography. *Ultrasound Med Biol*. 2016;42(1):272-281. doi:10.1016/j.ultrasmedbio.2015.09.010
11. Wang X, Zhen L, Miao H, et al. Concomitant Retrograde Coronary Venous Infusion of Basic Fibroblast Growth Factor Enhances Engraftment and Differentiation of Bone Marrow Mesenchymal Stem Cells for Cardiac Repair after Myocardial Infarction. *Theranostics*. 2015;5(9):995-1006. doi:10.7150/thno.11607
12. Formigli L, Perna A, Meacci E, et al. Paracrine effects of transplanted myoblasts and relaxin on post-infarction heart remodelling. *J Cell Mol Med*. 2007;11(5):1087-1100. doi:10.1111/j.1582-4934.2007.00111.x
13. Hagikura K, Fukuda N, Yokoyama S, et al. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. *Int J Cardiol*. 2010;142:56-64. doi:10.1016/j.ijcard.2008.12.108
14. Hong SJ, Hou D, Brinton TJ, et al. Intracoronary and Retrograde Coronary Venous Myocardial Delivery of Adipose-Derived Stem Cells in Swine Infarction Lead to Transient Myocardial Trapping with Predominant Pulmonary Redistribution. *Catheter Cardiovasc Interv*. 2014;83:E17-E25. doi:10.1002/ccd.24659
15. Hou D, Youssef EA, Brinton TJ, et al. Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary. *Circulation*. 2005;112(suppl I):I150-I156. doi:10.1161/CIRCULATIONAHA.104.526749
16. Kupatt C, Hinkel R, Lamparter M, et al. Retroinfusion of Embryonic Endothelial Progenitor Cells Attenuates Ischemia-Reperfusion Injury in Pigs. *Circulation*. 2005;112(9_supplement):I-117-I-122. doi:10.1161/CIRCULATIONAHA.104.524801
17. Lu F, Zhao X, Wu J, et al. MSCs transfected with hepatocyte growth factor or vascular endothelial growth factor improve cardiac function in the infarcted porcine heart by increasing angiogenesis and reducing fibrosis. *Int J Cardiol*. 2013;167:2524-2532. doi:10.1016/j.ijcard.2012.06.052
18. Prifti E, Di G, Harmelin G, et al. Cellular cardiomyoplasty into infracted swine's hearts by retrograde infusion through the venous coronary sinus: An experimental study. *Cardiovasc Revascularization Med*. 2016;17(4):262-271. doi:10.1016/j.carrev.2016.02.008
19. Sato T, Iso Y, Uyama T, et al. Coronary vein infusion of multipotent stromal cells from bone marrow preserves cardiac function in swine ischemic cardiomyopathy via enhanced neovascularization. *Lab Investig*. 2011;91(4):553-564. doi:10.1038/labinvest.2010.202
20. Vicario J, Piva J, Pierini A, et al. Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. 2003;3(2002):91-94. doi:10.1016/S1522-1865(03)00002-7
21. Yokoyama S, Fukuda N, Li Y, et al. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. *J Mol Cell Cardiol*. 2006;40(1):24-34. doi:10.1016/j.yjmcc.2005.06.008

22. Patel AN, Mittal S, Turan Go, et al. REVIVE Trial: Retrograde Delivery of Autologous Bone Marrow in Patients With Heart Failure. *Stem Cells Transl Med*. 2015;4(doi.org/10.5966/sctm.2015-0070):1-7.
23. Silva SA, Peixoto M, Soares AJS, et al. Autologous Bone-Marrow Mononuclear Cell Transplantation After Acute Myocardial Infarction: Comparison of Two Delivery Techniques. *Cell Transplant*. 2009;18:343-352. doi:10.3727/096368909788534951
24. Tuma J, Fernández-viña R, Carrasco A, et al. Safety and feasibility of percutaneous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina. *J Transl Med*. 2011;9(183). doi:10.1186/1479-5876-9-183
25. Tuma J, Carrasco A, Castillo J, et al. RESCUE-HF Trial: Retrograde Delivery of Allogeneic Umbilical Cord Lining Subepithelial Cells in Ptients with Heart Failure. *Cell Transplant*. 2015;25(9):1713-1721. doi:10.3727/096368915X690314
26. Vicario J, Campos C, Piva J, et al. Transcoronary sinus administration of autologous bone marrow in patients with chronic refractory stable angina Phase 1. *Cardiovasc Radiat Med*. 2004;5:71-76. doi:10.1016/j.carrad.2004.06.004
27. Vicario J, Campo C, Piva J, et al. One-year follow-up of transcoronary sinus administration of autologous bone marrow in patients with chronic refractory angina. *Cardiovasc Revascularization Med*. 2005;6(3):99-107. doi:10.1016/j.carrev.2005.08.002

CHAPTER 10

Lower retention after retrograde coronary venous infusion compared with intracoronary infusion of mesenchymal stromal cells in the infarcted porcine myocardium

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ABSTRACT

Background Commonly used strategies for cell delivery to the heart are intramyocardial injection and intracoronary (IC) infusion, both having their advantages and disadvantages. Therefore, alternative strategies, such as retrograde coronary venous infusion (RCVI), are explored. The aim of this confirmatory study was to compare cardiac cell retention between RCVI and IC infusion. As a secondary end point, the procedural safety of RCVI is assessed.

Methods Four weeks after myocardial infarction, 12 pigs were randomised to receive mesenchymal stromal cells, labelled with Indium-111, via RCVI (n=6) or IC infusion (n=6). Four hours after cell administration, nuclear imaging was performed to determine the number of cells retained in the heart both in vivo and ex vivo. Procedure-related safety measures were reported.

Results Cardiac cell retention is significantly lower after RCVI compared with IC infusion (in vivo: RCVI: median 2.89% vs IC: median 13.74%, p=0.002, ex vivo: RCVI: median 2.55% vs IC: median 39.40%, p=0.002). RCVI led to development of pericardial fluid and haematomas on the frontal wall of the heart in three cases. Coronary venous dissection after RCVI was seen in three pigs, of which one also developed pericardial fluid and a haematoma. IC infusion led to no flow in one pig.

Conclusion RCVI is significantly less efficient in delivering cells to the heart compared with IC infusion. RCVI led to more procedure-related safety issues than IC infusion, with multiple cases of venous dissection and development of haematomas and pericardial fluid collections.

INTRODUCTION

Cell therapy is suggested as a potential treatment option for ischaemic heart disease, yet only moderate improvement in cardiac function is achieved.[1,2] The delivery of cells to the myocardium is an important limitation of current cell injection methodologies.[3] The ideal strategy is safe, easy to perform and efficient in cell delivery. Intracoronary (IC) infusion and intramyocardial (IM) injection have been thoroughly tested.[4–7] Both techniques present with disadvantages such as the need for patent coronary arteries and the risk of embolisation leading to decreased blood flow in case of IC infusion.[8–10] The intramuscular injection procedure is time-consuming and requires specialised equipment in the catheterisation laboratory. Furthermore, rapid loss of cells via venous drainage is seen after IM injection.[11] Alternative delivery strategies could possibly overcome these drawbacks. Retrograde coronary venous infusion (RCVI) is less commonly applied, but could be a good alternative to IC infusion and IM injection. However, the available data on technical and safety aspects of RCVI are insufficient and incomplete. At present, there are not enough arguments to proceed with this technique in the clinical arena because well-designed confirmatory studies on retention rates and safety data are required to prove its value.[12]

With RCVI, cells are retrogradely infused in the coronary venous system, which is typically free of atherosclerotic disease, and therefore could potentially improve delivery to the target area compared with IC infusion. An important limitation of cardiac cell therapy is the retention of cells in the heart after delivery. IM injection and IC infusion show comparable retention rates of 10%–15%.[4,13,14] However, there are only limited data available on safety and the retention of cells in the heart after RCVI in large animal models and in the clinical setting. Currently, no direct comparison is available on cardiac cell retention after RCVI versus IC infusion in the setting of chronic myocardial ischaemia. In view of future clinical trials it is important to determine whether RCVI is a good alternative to IC infusion. Therefore, the aim of this confirmatory study is to compare the retention rates of radiolabelled mesenchymal stromal cells (MSCs) in the heart after RCVI and IC infusion and provide an estimate of safety of RCVI in a porcine model of chronic myocardial infarction (MI). We did not aim to provide data on cardiac repair because animals were terminated 4 hours after cell infusion to enable ex vivo scintigraphy of different organs.

METHODS

Ethical statement

All animals received care in compliance with the 'Guide for the Care and Use of Laboratory Animals', published by the National Institutes of Health (National Institutes

of Health publication 85–23, revised 1985). It was not possible to perform this experiment without animals due to the fact that the haemodynamics and biological nature of the heart and the whole body cannot be replicated in such a way that the results of this study would be translatable to the real situation. We minimised the number of animals used by performing a sample size calculation beforehand. Refinement was done by using proven techniques, performed by trained personnel. Furthermore, maximum effort was put into ensuring the best conditions for the animals in terms of housing, enrichment and analgesia.

Study design

MI was induced in 16 female Dutch Topigs pigs (Van Beek SPF varkensfokkerij B.V., Lelystad, The Netherlands). Pigs were selected as the preferred animal for this experiment because of the resemblance of the pig and human heart in terms of anatomy and haemodynamics. Animals that survived 4 weeks after MI (n=12) were randomised (1:1) to receive MSCs labelled with Indium-111 (In111) via RCVI (n=6) or IC infusion (n=6). Randomisation was performed using a closed envelope system. Nuclear imaging was carried out 4 hours after MSC delivery, after which the anaesthetised animals were euthanised by potassium chloride overdose. Nuclear imaging data were analysed by lab technicians blinded to the infusion procedure. The protocol of this study was registered on https://www. preclinicaltrials. eu/ (PCTE0000104) and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were followed for reporting. Heart rate, mean arterial pressure, left ventricular (LV) internal diameter at diastole and systole (LVIDd, LVIDs) were determined prior to MI (baseline) and directly prior to cell infusion.

Experimental outcomes

The primary end point of this study is retention of radiolabelled cells in the heart 4 hours after delivery. Cell retention was determined in vivo and ex vivo. In vivo analysis was performed by nuclear total body imaging of the live pig after which the percentage of total radioactive signal (counts) coming from the heart was divided by the total radioactive counts coming from the total body of the pig, including the bladder catheter. Because the heart is partially superimposed over the lungs during total body scanning, termination of the pigs and ex vivo scanning of individual organs (heart, lungs, kidneys, liver, spleen) was performed directly after in vivo scanning to check whether this superposition would influence the results of the total body imaging. The total radioactive signal (counts) coming from the heart was then divided by the sum of all radioactive counts coming from all aforementioned organs. The secondary end point is safety in terms of procedure-related complications such as occurrence of vessel dissections, flow obstruction during or after cell administration, development of pericardial effusion, and development of haematomas on the LV wall. Experimental set-up is shown in figure 1.

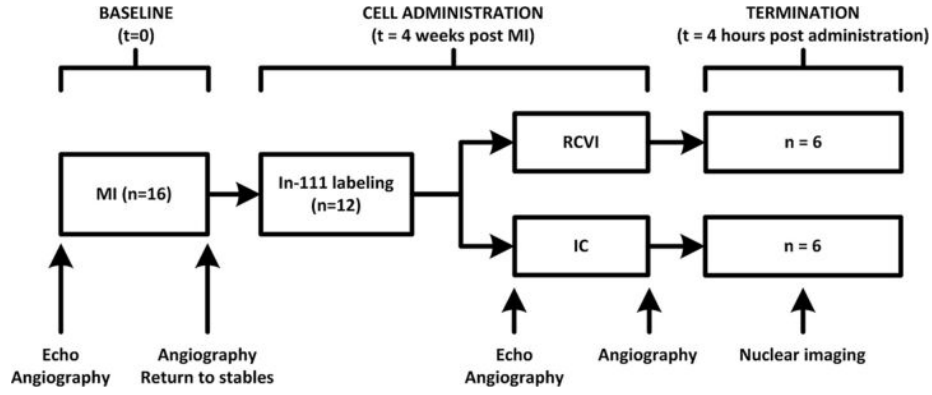

Figure 1. Experimental set-up. IC, intracoronary infusion; MI, myocardial infarction; n, number of animals; RCVI, retrograde coronary venous infusion; t, time point.

Experimental procedures

Anaesthesia and analgesia

Prior to MI induction, all animals received a Butrans 5 µg/h patch. Animals were pretreated with amiodarone (1200 mg/day, 7 days), clopidogrel (75 mg/day, 3 days) and carbasalate calcium (loaded with 320 mg, 1 day), which was continued until the end of the experiment (daily dose 80 mg). Premedication (ketamine 10–15 mg/ kg, midazolam 0.7 mg/kg and atropine 0.5 mg) was delivered intramuscularly. Anaesthesia was induced with thiopental sodium 4 mg/kg delivered through the ear vein. General anaesthesia and analgesia were maintained with a bolus of midazolam 10 mg and sufentanil 0.25 mg followed by intravenous delivery of midazolam 1 mg/ kg/h, sufentanil 10 µg/kg/h and pancuronium bromide 0.1 mg/kg/h. Animals received 300 mg amiadarone in 500 mL venofundin 6% infused in 30 min. Mechanical ventilation was performed using a mixture of O2 and air (1:2) with a tidal volume of 10 mL/kg with 12 breaths per minute. Animals received 5000 IU of heparin every 2 hours during the procedure.

Myocardial infarction

MI was induced percutaneously by a temporal (90 min) occlusion of the left anterior descending artery (LAD) using an angioplasty balloon. The preferred occlusion site was after diagonal branch 2, but the infarct site was determined per pig based on the anatomy of the coronary arteries (thickness and tract). In case of ventricular fibrillation (VF) or ventricular tachycardia without output, 200-joule shocks were delivered using an external defibrillator in order to restore sinus rhythm. Chest compressions were given between shocks to maintain circulation. In addition, amiadarone (maximum of 3 times 150 mg), adrenalin (0.1 mg) and/or atropine (0.5 mg) were administered. Arterial blood pressure, ECG and capnogram were monitored during the entire procedure.

MSC culture and In111 labelling

Allogeneic MSCs were isolated and cultured in Minimal Essential Media with alpha modifications (αMEM) (Invit- rogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum, 0.2 ng/mL vitamin C (Sigma- Aldrich, St. Louis, Missouri, USA), 1 ng/mL basic fibro- blast growth factor (Sigma-Aldrich, St. Louis, Missouri, USA) and 1% penicillin/streptomycin. The cells were incubated at 37°C and medium was changed every 3 days. Cells were cultured in a 75 cm2 flask and passaged when they reached confluence, until passage 2–3. MSCs were frozen in 10% dimethylsulfoxide and 90% culture medium. Characterisation of MSCs was performed as previously described.[15,16] Seven days prior to transplantation, MSCs were thawed, plated in flasks and grown to confluence, until passage 5–7. At the day of cell delivery, 107 MSCs were labelled with carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, Carlsbad, California, USA) dissolved in dimethyl sulfoxide (DMSO) (Sigma- Aldrich, St. Louis, Missouri, USA) to a concentration of 5 mM after which cells were trypsinised and subsequently labelled with a median of 36.3 (IQR 33.5–40.5) megabecquerel (MBq) of In111 at 37°C for 20 min. After incubation, cells were washed up to three times with Hank's balanced salt solution CaCl2 +MgCl2+ (Life Technologies Corp, Grand Island, New York, USA) to remove unbound label. Radiolabel uptake efficiency was measured with a dose calibrator. After labelling, cell viability was assessed via trypan blue (Sigma-Aldrich, St. Louis, Missouri, USA) counting. Before injection, MSCs were resuspended in 10 mL phosphate buffered saline pH 7.4 (Life Technologies Corp, Grand Island, New York, USA). The protocol on labelling of MSCs with In111 can be found at: https://www.protocols.io/view/labeling-of-porcine-mesenchymal-stromal-cells-mscs-mr9c596

Histochemistry

Directly after termination, representative myocardial tissue samples were collected from areas of the heart that showed activity during nuclear imaging and were snap frozen in liquid nitrogen. Tissue samples were cut with the Cryostar NX70 (ThermoFisher, Waltham, Massachusetts, USA) at 10 µM. The EVOS FL (ThermoFisher, Waltham, Massachusetts, USA) cell imaging system was used to check for CFSE positivity. Histological samples were subsequently fixed with acetone, permeabilised with 0,1% Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA) and blocked with 10% normal goat serum S-1000 (Vector Laboratories, Burlingame, California, USA). Monoclonal anti-α-actinin (sarcomeric) mouse antihuman (Sigma- Aldrich, St. Louis, Missouri, USA) (1:350) was used as the primary antibody followed by the secondary antibody goat antimouse-568 (1:350) (Invitrogen, Carlsbad, California, USA) and 1 µg/mL Hoechst 33 342 (Invitrogen, Carlsbad, California, USA). Samples were mounted with fluormount-G (ThermoFisher, Waltham, Massachusetts, USA). Imaging was performed with a confocal Leica SP8X microscope (Leica, Amsterdam, Netherlands).

Retrograde coronary venous infusion

Two different infusion catheters were used for RCVI. In case the coronary sinus (CS) was ≥5 mm in diameter a dedicated CS infusion catheter was used (Advance CS Infusion Catheter, Cook Medical, Bloomington, Indiana, USA). In case the diameter of the CS was <5 mm, an over-the-wire balloon catheter (Advance 35LP Low-Profile PTA Balloon Dilatation Catheter, Cook Medical, Bloomington, Indiana, USA) was used. Balloons were inflated at low pressure (maximum of 2 atmospheres) in the CS after which a venogram was made to ensure total occlusion of the CS. When total occlusion was observed, 2 mL of cell suspension followed by 8 mL of sodium chloride 0.9% was infused during 60 s. This procedure was performed a total of five times in order to infuse a total of 10 mL of cell suspension flushed with 40 mL of sodium chloride 0.9% in 5 min. Occlusion of the CS was maintained for 10 minutes after infusion to prevent washout of cells.

IC infusion

IC infusion was performed by placing an over-the-wire balloon (Emerge over-the-wire PTCA dilatation catheter, Boston Scientific Corp, Natick, Massachusetts, USA) of equivalent size to the LAD at the same site where occlusion was created during MI induction. After inflation of the balloon at low pressure, 3.3 mL of cell suspension was infused in 30–45 s. The balloon was deflated after 3 min to reinstate flow. After 3 min of flow, the procedure was repeated another two times to infuse a total of 10 mL of cell suspension.

Nuclear imaging and analysis

In vivo and ex vivo scintigraphy was performed 4 hours after MSC administration using a dual head gamma camera (Phillips Skylight). A whole-body scan was acquired at both 174 keV and 247 keV energy windows using the following imaging parameters: medium-energy general-purpose collimator and 512×1024 projection matrix. The retained activity in syringes was measured with a dose calibrator (Azbil Telstar Benelux). Both anterior and posterior images were captured for each total body scan (in vivo) and each individual organ (ex vivo). The number of counts used for analysis was based on the geometrical mean of the anterior and posterior counts. After in vivo scanning, regions of interest (ROIs) were placed over the major visceral organs and total body of the pig (figure 2), using manufacturer's software (JETStream workspace; Philips, Best, The Netherlands). The retention of In111-labelled cells in the heart was calculated as a ratio of the total radioactive signal (counts) coming from the heart divided by the total counts coming from the total body of the pig (including bladder catheter), after correction for anatomy. After ex vivo scanning of individual organs, the retention of In111-labelled cells in the heart was calculated as a ratio of the total radioactive signal (counts) coming from the heart divided by the

total counts coming from all individual organs combined. Data analysis was performed by two to three laboratory analysts per animal coming from a pool of four analysts, supervised by an expert analyst, all blinded for treatment allocation.

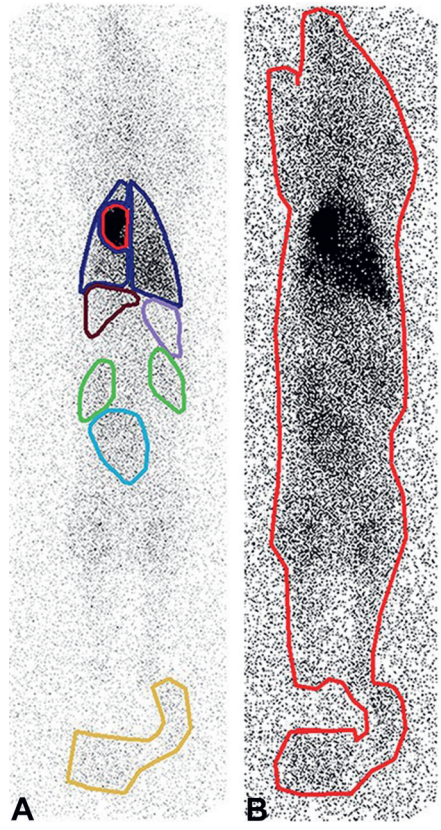

Figure 2. Total body scintigraphy with regions of interest (ROIs). (A) ROIs placed over visceral organs (heart in red, lungs in blue, kidneys in green, liver in brown, spleen in pink, bladder in light blue) and catheter bag in yellow. (B) ROI placed over total body of pig including catheter bag. Of note: both image A and image B are anterior captures. Both anterior and posterior images were captured for each animal and the number of counts used for analysis was based on the geometrical mean of the anterior and posterior counts.

Echocardiography

Transthoracic echocardiography (X5-1 probe, IE-33, Philips, Best, The Netherlands) was performed directly before MI induction and 4 weeks later, directly before MSC infusion. Chamber dimensions (LVIDd and LVIDs) were obtained in short-axis view at mid-papillary level. Analysis was performed in a blinded fashion by a trained physician.

Experimental animals

Sample size

A total number of 12 animals (median age and weight at time of MI: 20 weeks (IQR: 18–22) and 72 kilograms (IQR: 68–76), respectively) was allocated to receive MSCs via either RCVI (n=6) or IC (n=6) infusion. This sample size was predefined, and calculated for an α of 0.05, power of 80%, maximum SD of 4% and an expected maximum absolute difference in cell retention of 7.5%. Because 4 animals died during or after MI induction, a total of 16 animals had to be used to include 12 animals in the analysis.

Housing

Animals were housed in stables with up to two pigs in the same stable before MI. After MI, animals were housed in separate stables to minimise stress. Animals were still able to see, smell and hear each other through the grates that divide the stables. Straw was used for bedding and environmental enrichment was provided in the form of special rods that the animals could nibble on and play with. Welfare was assessed daily by animal caretakers.

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics V.25 (IBM, Armonk, New York, USA). Baseline characteristics and cell retention are presented as median with IQRs. Comparison of data between two groups was performed using Mann-Whitney U test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Procedural data

Ventricular fibrillationduring MI induction occurred in 13 out of 16 pigs, of which 2 died due to refractory VF. Another two pigs died in the stables due to acute heart failure or a heart rhythm disorder (day 4 and day 19) as a result of the MI. The remaining 12 pigs were randomised to RCVI (n=6) or IC infusion (n=6). No significant differences in heart rate, mean arterial pressure, LVIDd and LVIDs were seen between groups as seen in table 1A, although a trend was seen towards a larger LVIDs in pigs that were allocated to IC infusion both at baseline and at follow-up.

Cell viability and numbers

The median viability of MSCs after labelling with In111 was 66.8% (IQR: 62.1–72.4) in the IC group versus 53.6% (IQR: 49.8–73.8) in the RCVI group (p=0.418). The median total administered cells was 3.2 M (IQR: 3.2–3.7) in the IC group versus 2.8 M (IQR: 2.1–3.1) in the RCVI group (p=0.180) The median number of administered live cells was

Table 1. Heart rate (HR), mean arterial pressure (MAP), left ventricular internal diameter at diastole (LVIDd) and systole (LVIDs) before myocardial infarction (baseline) and directly prior to cell infusion.

1a. Parameter	Baseline			Prior to cell infusion		
	IC (n=6)	RCVI (n=6)	P-value	IC (n=6)	RCVI (n=6)	P-value
HR (beats/min)	71 [63 – 81]	72 [66 – 73]	0.937	73 [64 – 82]	69 [63 – 76]	0.589
MAP (mmHg)	74 [67 – 89]	70 [64 – 88]	0.699	79 [72 – 87]	75 [72 – 82]	0.699
LVIDd (mm)	49 [48 – 50]	47 [46 – 49]	0.342	58 [47 – 59]	58 [47 – 62]	0.985
LVIDs (mm)	35 [34 – 37]	32 [29 – 34]	0.063	45 [40 -51]	38 [37 – 38]	0.115
1b. Parameter	**IC (n=6)**	**RCVI (n=6)**	**P-value**			
Cell viability after labeling (%)	66.8 [62.1 – 72.4]	53.6 [49.8 – 73.8]	0.418			
Total administered cells (x 10^6)	3.2 [3.2 – 3.7]	2.8 [2.1 – 3.1]	0.180			
Total administered live cells (x 10^6)	2.4 [1.6 – 2.4]	1.6 [1.3 – 1.7]	0.167			

Cell viability, total administered cells, and total administered live cells. Values are depicted as median with interquartile ranges. IC, intracoronary; RCVI, retrograde coronary venous infusion.

2.4 M (IQR: 1.6–2.4) in the IC group versus 1.6 M (IQR: 1.3–1.7) in the RCVI group (p=0.167). Results are shown in table 1B.

Cell retention

In vivo analysis

A significant difference in MSC retention in the heart was seen between the RCVI and IC infusion groups with a median retention of 2.89% (IQR: 2.14–3.86) in the RCVI group versus 13.74% (IQR: 10.20–15.41) in the IC infusion group (p=0.002). No significant differences in cell retention were seen in lungs, kidneys, liver, spleen and bladder between RCVI and IC infusion, although a trend was seen towards higher retention of cells in the lungs after RCVI. Data are presented in table 2 and figure 3A,B.

Ex vivo analysis

In accordance with the in vivo results, a significant difference was seen in MSC retention in the heart between the RCVI and IC infusion groups after ex vivo analysis. The median retention was 2.55% (IQR: 1.86-3.16) in the RCVI group versus 39.40% (IQR: 38.54-44.64) in the IC group (p=0.002). Significant differences between RCVI and IC infusion were also seen for lung and liver retention (p=0.002 and p=0.04, respectively), with a significantly higher number of cells retained in the lungs after RCVI and a significantly higher number of cells retained in the liver after IC infusion. Data are represented in table 3 and figure 4.

Histological analysis

Histology shows CFSE-labelled cells in the heart in the areas that are active on the scintigraphy. As expected, very few CFSE-positive cells were found in the myocardial tissue samples from pigs belonging to the RCVI group because myocardial cell retention was low in these animals. In line with our expectations, CFSE-positive cells were more abundant in tissue samples from the IC infusion group. Representative histological images are presented in figure 5.

Table 2. *In vivo* analysis of activity in heart, lungs, kidneys, liver, spleen, and bladder as a percentage of total body activity. Values are depicted as median with interquartile ranges.

Organ	Median activity [interquartile range]	Median activity [interquartile range]	P-value
	RCVI (n=6)	IC (n=6)	
Heart	2.89 [2.14 – 3.86]	13.74 [10.20 – 15.41]	0.002
Lungs	35.45 [26.53 – 45.22]	22.07 [20.36 – 29.22]	0.132
Kidneys	1.39 [0.97 – 2.12]	2.32 [1.14 – 3.24]	0.240
Liver	2.76 [2.20 – 3.27]	2.95 [2.56 – 3.44]	0.310
Spleen	0.89 [0.61 – 1.08]	0.81 [0.77 – 1.05]	0.818
Bladder	0.96 [0.38 – 2.74]	0.88 [0.64 – 1.22]	0.937

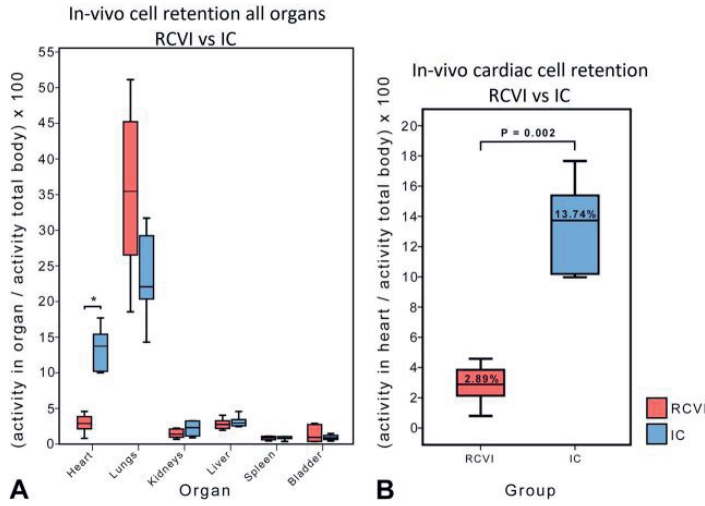

Figure 3. *In vivo* retention of cells in major organs presented as a percentage of total body activity. (A) *In vivo* analysis of activity in heart, lungs, kidneys, liver, spleen and bladder presented as a percentage of total body activity: retrograde coronary venous infusion (RCVI) versus intracoronary (IC) infusion. Only activity in the heart differed significantly between RCVI and IC infusion (*=p=0.002). (B) Magnification of figure 3A. Retention of mesenchymal stromal cells (MSCs) in the heart is significantly worse after RCVI compared with IC infusion. (p=0.002).

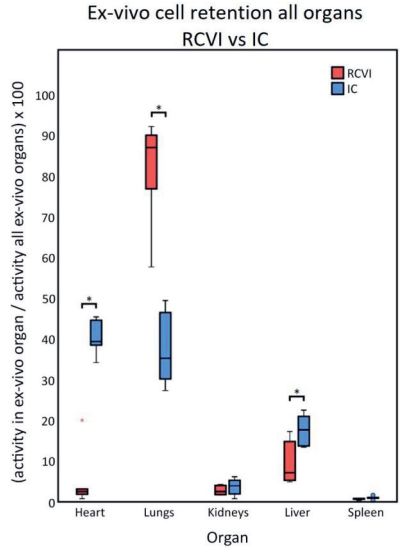

Figure 4. *Ex vivo* retention of cells in individual organs. *Ex vivo* analysis of activity in heart, lungs, kidneys, liver and spleen as a percentage of total activity from all individual organs combined. Activity in the heart, lungs and liver differed significantly between RCVI and IC infusion ($p<0.05$). IC, intracoronary; RCVI, retrograde coronary venous infusion.

Safety aspects

RCVI group

Dissection of the CS occurred in three out of six pigs at the site of the balloon catheter tip. Two animals with the largest dissection later showed a radioactive hotspot in the heart instead of a more disseminated activity pattern as would be expected in case of cell infusion. Cardiac cell retention in these two pigs was the highest of all RCVI pigs and well above the median of 2.89% with 3.86% and 4.59% (in vivo data), respectively. Three animals presented with a small-to-moderate, clear pericardial effusion and a haematoma of approximately 4 cm2 on the atrioventricular groove of the left ventricle (LV) at termination. Only one animal was free of dissection and development of haematoma and pericardial fluid. In this one animal, the occlusion of the CS was found to be compromised after the infusion was completed, possibly leading to direct drainage of cells into the right atrium. Nevertheless, the retention in this pig was 2.97% (in vivo data).

IC group

One animal in the IC group showed no flow directly after cell infusion, probably due to thrombus formation. Flow was restored after 5 min of angioplasty.

Table 3. *Ex vivo* analysis of activity in heart, lungs, kidneys, liver and spleen as a percentage of the total counts coming from all individually scanned organs combined. Values are depicted as median with interquartile ranges.

Organ	Median activity [interquartile range]	Median activity [interquartile range]	P-value
	RCVI (n=6)	IC (n=6)	
Heart	2.55 [1.86 – 3.16]	39.40 [38.54 – 44.64]	0.002
Lungs	87.10 [76.95 – 90.13]	35.32 [30.22 – 46.53]	0.002
Kidneys	2.53 [1.77 – 4.03]	3.95 [1.95 – 5.28]	0.394
Liver	7.17 [5.28 – 14.83]	17.71 [13.74 – 21.01]	0.041
Spleen	0.78 [0.64 – 0.84]	0.99 [0.89 – 1.08]	0.065

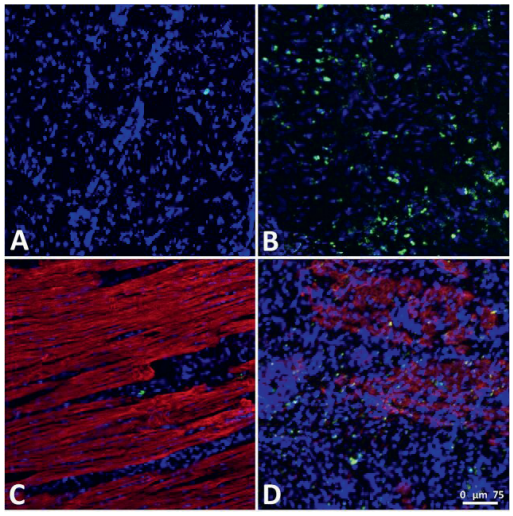

Figure 5. CFSE-positive cells in myocardial tissue samples. (A) Myocardial tissue sample after retrograde coronary venous infusion (RCVI), Hoechst staining and carboxyfluorescein succinimidyl ester (CFSE) signal. (B) Myocardial tissue sample after intracoronary (IC) infusion, Hoechst staining and CFSE signal. (C) Myocardial tissue sample after RCVI, Hoechst and α-actinin staining and CFSE signal. (D) Myocardial tissue sample after IC infusion, Hoechst and α-actinin staining, and CFSE signal. (Blue = Hoechst signal, Red = α-actinin signal, Green=CFSE signal).

DISCUSSION

Cell retention

The purpose of this study was to compare cardiac cell retention after RCVI and IC infusion and assess safety of RCVI. It was not possible to generate results on cardiac repair because the study design required termination of animals 4 hours after cell administration. To our knowledge, this is the first confirmatory study that directly compared retention between RCVI and IC infusion in a chronic MI pig model. We showed that RCVI of MSCs is inferior to IC infusion in terms of cardiac cell retention with RCVI showing a mean retention of 2.89% vs 13.74% with IC infusion (in vivo data). One can imagine that higher cell retention in the heart equals a greater effect of cell therapy, making IC infusion preferable over RCVI. We chose to infuse the same number of cells in both the IC infusion group and the RCVI group in order to make sure that the results are comparable. Because retention of cells in the heart is calculated as a percentage of the total administered cell dose in case of in vivo analysis, it is probable that a higher cell dose would not result in a higher retention rate. However, it is known that infusion of larger volumes of cells (30×106–50×106) via the IC route can result in a higher index of microcirculatory resistance.[17,18]

Currently, there is no evidence that larger cell volumes infused via the retrograde route would impair venous flow. This could implicate that more cells can be infused with RCVI, making up for the lower retention. The retention rates that we observed for IC infusion are comparable to results of other studies.[4,13,14] Three pig studies with small sample sizes (n=5, n=6 and n=7) and only one clinical trial (n=9) reported retention rates after RCVI in a model of acute MI. However, no data on cell retention in a chronic

ischaemia model in large animals are available. Retention of cells, measured as radioactive positive signals coming from the heart, was low in these four trials, ranging from 3% to 8% of total injected activity, corresponding with our results.[13,14,17,19] A possible explanation for the low retention of cells with RCVI is that cells are maintained in the CS after infusion but are directly flushed into the right atrium after abrogation of the CS occlusion and reinstitution of flow. This could explain the higher retention of cells in the lungs of RCVI-treated animals. It is also possible that the cells are not adequately pushed though the microvascular bed as is the case with IC infusion, possibly effecting cell retention. Additionally, a low retention with RCVI could occur due to the existence of aberrant (and/or collateral) veins draining directly in the right atrium, effectively negating the blockade of the CS. An experienced cardiologist analysed the fluoroscopy images made during cell infusion in this study and found anatomical variations of the coronary veins strongly suggesting the presence of aberrant venous drainage in three out of six RCVI-treated animals. We could not find a relation between possible aberrant venous drainage and cell retention in these pigs, possibly due to the small number of pigs and other factors present such as CS dissection and pericardial effusion.

Differences between in vivo and ex vivo data

With in vivo analysis we only found a significant difference in cell retention in the heart between RCVI and IC infusion, while we find a significant difference in cardiac, pulmonary and hepatic retention after ex vivo analysis. Also, a different magnitude of retention is seen between in vivo and ex vivo measurements.

To explain these differences, it is important to understand that cell retention in organs is calculated differently for the in vivo analysis and ex vivo analysis. In case of in vivo analysis, cell retention in a certain organ (numerator) is calculated as a fraction of total body activity (denominator). In case of ex vivo analysis, cell retention in a certain organ (numerator) is calculated as a fraction of total ex vivo organ activity (denominator). The difference in denominator between in vivo and ex vivo analysis means that in vivo and ex vivo data cannot be directly compared with each other. However, both analyses are relevant. In vivo data show the percentage of total administered cell dose that is retained in the heart. However, in vivo analysis has a few shortcomings. With in vivo analysis, ROIs are drawn around individual organs to determine the amount of radioactive counts coming from these organs. In this study, the ROIs were determined by experienced technicians and were accurately defined. However, there is always a margin of error with ROI definition for total body scans. When few cells are retained in an organ, the radioactive signal coming from this organ is low, making the contours of this organ difficult to discern from the surrounding tissue. This makes ROI definition more difficult in organs such as liver, spleen and kidneys, as seen in figure 2. Cardiac and pulmonary borders are usually easier to define, because of the higher retention in the lungs and heart. In case of low cardiac retention, as is the case after RCVI, the

cardiac border is still easy to define because of the large difference of signal between the heart and surrounding lung tissue. A second drawback of in vivo total body imaging is overprojection of organs such as the heart and lungs. This could lead to overestimation of signal coming from the heart. Excision of organs followed by ex vivo scanning ensures that only counts coming from the individual organ are identified, overcoming errors caused by ROI definition and superposition of organs such as the heart and lungs. Thus, differences in significance of pulmonary and hepatic retentions between in vivo and ex vivo imaging can be explained by the difference in the way that retention is calculated, ROI definition and superposition of organs. However, total body scanning is the best option to determine the number of cells retained in the heart as a percentage of the total administered cell dose. With ex vivo analysis, the retention of cells in the heart cannot be expressed as a percentage of the total administered cells, because part of the activity and thus the cells are distributed outside of the organs, for instance in muscles and blood pool. For this reason, we decided to incorporate both in vivo and ex vivo data in this study. Both methods show that cell retention is significantly lower in the RCVI group compared with the IC infusion group.

Safety aspects of RCVI

RCVI was associated with multiple safety issues in this study. We found pericardial fluid and haematoma development on the atrioventricular groove of the LV in three pigs and occurrence of CS dissection in three pigs, of which one also showed a haematoma and pericardial fluid at termination. Only one animal in the RCVI group was free of adverse events. It is striking that in this specific animal, the occlusion of the CS appeared to be incomplete after infusion. We do not know at which time point during the infusion procedure the occlusion was compromised.

In one animal, overinflation of balloon of the advance CS infusion catheter (>2 atmospheres) could have been the cause of development of a CS dissection, haematoma on the atrioventricular groove and pericardial fluid collection.

The most likely explanation for the development of pericardial fluid and haematoma is a sudden rise in pressure in the coronary venous system during infusion even though we infused cells slowly at 10 mL/min. Significant contrast blushing was seen on the fluoroscopy images made after infusion, supporting this hypothesis. We identified 10 studies that used RCVI for cell delivery in pigs. [14,17,19–26] The median infused volume in these studies was 15 mL (IQR: 10–25 mL), with two studies infusing a higher volume of 40 mL[26] and 250 mL.[19] The study that infused 40 mL did so during 4 hours, making it likely that no pressure or volume overload could develop.[26] However, the study that infused 250 mL did so during 10 min, making both the infused volume and infusion rate higher than in our study.[19] Unfortunately, it is unclear if the CS was occluded during infusion in these two trials, so it is not possible to make a statement on pressure or volume overload in these cases. Three other trials infused cells at a much higher rate

and did not report development of pericardial fluid and haematomas.[14,17,22] However, the infused volume was only 10 mL in these three trials.

It is unfortunate that the majority of the RCVI pig studies reported did not state anything on procedural safety. The studies that do mention absence of arrhythmias and microvascular obstruction, but nothing on occurrence of dissection of the CS or development of haematomas or pericardial fluid. It is also possible that pericardial fluid collection and haematoma formation were related to CS injury in some of the cases. Contrary to RCVI studies, development of haematomas on the atrioventricular groove, pericardial fluid collections and damage to the CS have been reported in the field of cardiac surgery and have been related to traumatic catheter insertion, overinflation of the balloon in the CS and elevated CS infusion pressure during retrograde cardioplegia.[27–30] With retrograde cardioplegia, the CS is accessed with a balloon-catheter to occlude the CS and subsequently infuse fluid to arrest the heart and protect the myocardium. This procedure is in a way comparable to RCVI. Injury to the CS, such as CS perforation or rupture, was reported to occur in 0.6% to 0.06% of the patients that underwent retrograde cardioplegia, essentially proving safety of this technique.[27,31] A possible explanation for the high number of adverse events in the RCVI group in this study compared with an event rate of only 0.6% to 0.06% in human cases could be the difference in anatomy of the CS between humans and pigs. Contrary to humans, the hemiazygos vein drains in the CS in pigs. This leaves less room for balloon positioning in pigs, increasing the chance to perforate the CS with the catheter tip due to the small operating area. Clinical trials that have used RCVI did not report safety issues beside a rise in cardiac enzymes in some cases.[13,32–36]

The occurrence of CS dissections did not appear to have a negative effect on cell retention in the heart. On the contrary, the two pigs with the largest dissection showed the highest retention rates of all six RCVI pigs. It is likely that the infused cells collected between the wall layers of the dissected area, effectively trapping the cells and preventing them from washing out. IC infusion was associated with less safety issues with one animal showing no reflow directly after cell infusion, which could be restored within 5 min. Decreased blood flow after IC infusion is a known drawback and has been attributed to coronary embolisms leading to microvascular plugging in the past.[8–10]

FUTURE IMPLICATIONS

Here, we found that retention rates with both RCVI and IC infusion are low (<14%), which may hamper the effectiveness of cell therapy. Therefore, alternative approaches to increase cell retention and survival are being investigated. These include the use of carrier materials such as nanomatrix gels, microspheres and cell sheets or patches,[37–39] and pretreatment of grafted cells or target tissues, for instance by overexpressing prosurvival genes to increase survival of grafted cells in a hostile environment.[40,41]

Conclusion

Cardiac cell retention after RCVI is significantly lower compared with IC infusion. Our results confirm previous research comparing retention of cells after RCVI with IC infusion in the setting of acute MI.

Furthermore, RCVI presented with more safety issues than IC infusion. Taking both efficiency and safety into account, IC infusion is the preferred method of cell delivery between the two.

Strengths and limitations of the study

- To our knowledge, this is the first confirmatory study performed on cell retention after retrograde coronary venous infusion versus intracoronary infusion in a porcine model of chronic myocardial infarction.
- Adequate steps were taken to limit the risk of bias: the primary end point was prespecified; sample size was calculated beforehand to ensure adequate power of the study and prevent unnecessary use of animals.
- The study was performed in a randomised manner and outcome assessment was performed by blinded investigators.
- Radiolabelling with In^{111} made it possible to quantify cell retention in a very precise way.
- Precise determination of cell retention in the heart on total body images of pigs is challenging due to overprojection of the lungs and heart. This means counts coming from areas of the lungs that are positioned over the heart are attributed to the heart, leading to a slightly higher cell retention in the heart than was actually the case. Ex vivo measurements of cell retention were performed to overcome this drawback of in vivo imaging.

Footnotes

This article has received OSF badges for Open Data, Open Materials and Preregistration.

REFERENCES

1. Fisher SA, Zhang H, Doree C, Mathur A, Martin-Rendon E. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev*. 2015;(9). doi:10.1002/14651858.CD006536.pub4
2. Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. *Cochrane Database Syst Rev 2016*. 2016;(12). doi:10.1136/heartjnl-2017-311684
3. Feyen DAM, Gaetani R, Doevendans PA, Sluijter JPG. Stem cell-based therapy: Improving myocardial cell delivery. *Adv Drug Deliv Rev*. 2016;106(Pt A):104-115. doi:10.1016/j.addr.2016.04.023
4. Van Der Spoel TI, Vrijsen KR, Koudstaal S, et al. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of chronic ischemic heart disease: A randomized study on efficiency using nuclear imaging. *Circulation*. 2011;124(21). http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L70619647.
5. Kandala J, Upadhyay G a, Pokushalov E, Wu S, Drachman DE, Singh JP. Meta-analysis of stem cell therapy in chronic ischemic cardiomyopathy. *Am J Cardiol*. 2013;112(2):217-225. doi:10.1016/j.amjcard.2013.03.021
6. Sadat K, Ather S, Heo J, Iskandrian AE, Aljaroudi W, Hage FG. The effect of bone marrow mononuclear stem cell therapy on left ventricular function and myocardial perfusion . *J Nucl Cardiol* . 2014;21(2):351-367. doi:http://dx.doi.org/10.1007/s12350-013-9846-4
7. Afzal MR, Samanta A, Shah ZI, et al. Adult Bone Marrow Cell Therapy for Ischemic Heart Disease: Evidence and Insights from Randomized Controlled Trials. *Circ Res*. 2015;117(1):558-575. doi:10.1161/CIRCRESAHA.114.304792
8. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet*. 2004;363(9411):P783-784. doi:10.1016/S0140-6736(04)15695-X
9. Suzuki K, Brand NJ, Smolenski RT, Jayakumar J, Murtuza B, Yacoub MH. Development of a novel method for cell transplantation through the coronary artery. *Circulation*. 2000;102(10 suppl 3):III359-64. doi:10.1161/01.cir.102.suppl_3.iii-359
10. Freyman T, Polin G, Osman H, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J*. 2006;27:1114-1122. doi:10.1093/eurheartj/ehi818
11. van den Akker F, Feyen DAM, van den Hoogen P, et al. Intramyocardial stem cell injection: go(ne) with the flow. *Eur Hear*. 2017;38(3):184-186. doi:10.1093/eurheartj/ehw056
12. Gathier WA, Ginkel DJ Van, van der Naald M, van Slochteren FJ, Doevendans PA, Chamuleau SAJ. Retrograde Coronary Venous Infusion as a Delivery Strategy in Regenerative Cardiac Therapy: an Overview of Preclinical and Clinical Data Canadian Cardiovascular Society scale. *J Cardiovasc Transl Res*. 2018;11:173-181. doi:10.1007/s12265-018-9785-1
13. Silva SA, Peixoto M, Soares AJS, et al. Autologous Bone-Marrow Mononuclear Cell Transplantation After Acute Myocardial Infarction: Comparison of Two Delivery Techniques. *Cell Transplant*. 2009;18:343-352. doi:10.3727/096368909788534951
14. Hou D, Youssef EA, Brinton TJ, et al. Radiolabeled Cell Distribution After Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary. *Circulation*. 2005;112(suppl I):I150-I156. doi:10.1161/CIRCULATIONAHA.104.526749
15. Noort WA, Oerlemans MI, Rozemuller H, et al. Human versus porcine mesenchymal stromal cells: phenotype, differentiation potential, immunomodulation and cardiac improvement after transplantation. *J Cell Mol Med*. 2012;16(8):1827-1839. doi:10.1111/j.1582-4934.2011.01455.x
16. Feyen DAM, van den Akker F, Noort W, Chamuleau SAJ, Doevendans PA, Sluijter JPG. Isolation of Pig Bone Marrow-Derived Mesenchymal Stem Cells. In: Gnecchi M, ed. *Mesenchymal Stem Cells: Methods and Protocols*. New York, NY: Springer New York; 2016:225-232. doi:10.1007/978-1-4939-3584-0_12
17. Hong SJ, Hou D, Brinton TJ, et al. Intracoronary and Retrograde Coronary Venous Myocardial Delivery of Adipose-Derived Stem Cells in Swine Infarction Lead to Transient Myocardial Trapping with Predominant Pulmonary Redistribution. *Catheter Cardiovasc Interv*. 2014;83:E17-E25. doi:10.1002/ccd.24659
18. Fiarresga A, Mata MF, Cavaco-Gonçalves S, et al. Intracoronary delivery of human mesenchymal/stromal stem cells: Insights from coronary microcirculation invasive assessment in a swine model. *PLoS One*. 2015;10(10):e0139870. doi:10.1371/journal.pone.0139870
19. Kupatt C, Hinkel R, Lamparter M, et al. Retroinfusion of Embryonic Endothelial Progenitor Cells Attenuates Ischemia-Reperfusion Injury in Pigs. *Circulation*. 2005;112(9_supplement):I-117-I-122. doi:10.1161/CIRCULATIONAHA.104.524801
20. Formigli L, Perna A, Meacci E, et al. Paracrine effects of transplanted myoblasts and relaxin on post-infarction heart remodelling. *J Cell Mol Med*. 2007;11(5):1087-1100. doi:10.1111/j.1582-4934.2007.00111.x
21. Hagikura K, Fukuda N, Yokoyama S, et al. Low invasive angiogenic therapy for myocardial infarction by retrograde transplantation of mononuclear cells expressing the VEGF gene. *Int J Cardiol*. 2010;142:56-64.

doi:10.1016/j.ijcard.2008.12.108
22. Lu F, Zhao X, Wu J, et al. MSCs transfected with hepatocyte growth factor or vascular endothelial growth factor improve cardiac function in the infarcted porcine heart by increasing angiogenesis and reducing fibrosis. *Int J Cardiol*. 2013;167:2524-2532. doi:10.1016/j.ijcard.2012.06.052
23. Prifti E, Di G, Harmelin G, et al. Cellular cardiomyoplasty into infracted swine's hearts by retrograde infusion through the venous coronary sinus: An experimental study. *Cardiovasc Revascularization Med*. 2016;17(4):262-271. doi:10.1016/j.carrev.2016.02.008
24. Sato T, Iso Y, Uyama T, et al. Coronary vein infusion of multipotent stromal cells from bone marrow preserves cardiac function in swine ischemic cardiomyopathy via enhanced neovascularization. *Lab Investig*. 2011;91(4):553-564. doi:10.1038/labinvest.2010.202
25. Vicario J, Piva J, Pierini A, et al. Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. 2003;3(2002):91-94. doi:10.1016/S1522-1865(03)00002-7
26. Yokoyama S, Fukuda N, Li Y, et al. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. *J Mol Cell Cardiol*. 2006;40(1):24-34. doi:10.1016/j.yjmcc.2005.06.008
27. Kaul TK, Fields BL, Jones CR. Iaterogenic injuries during retrograde delivery of cardioplegia. *Cardiovasc Surg*. 2000;8(5):400-403. doi:10.1177/096721090000800513
28. Kurusz M, Girouard MK, Brown PS. Coronary sinus rupture with retrograde cardioplegia. *Perfusion*. 2002;17(1):77-80. doi:10.1191/0267659102pf534cr
29. Krishnan S, Papworth DP, Farivar RS, Ueda K. Identification of Coronary Sinus Injury by Transesophageal Echocardiography During Placement of a Retrograde Cardioplegia Catheter for Minimally Invasive Cardiac Surgery. *Anesth Analg*. 2013;116(3). https://journals.lww.com/anesthesia-analgesia/Fulltext/2013/03000/Identification_of_Coronary_Sinus_Injury_by.10.aspx.
30. Aigner C, Wolner E, Mohl W. Management of central coronary sinus ruptures using the pericardial patch repair technique. *Ann Thorac Surg*. 2006;81(4):1275-1278. doi:10.1016/j.athoracsur.2005.11.037
31. Menasché P, Subayi JB, Piwnica A. Retrograde coronary sinus cardioplegia for aortic valve operations: A clinical report on 500 patients. *Ann Thorac Surg*. 1990;49(4):556-564. doi:10.1016/0003-4975(90)90301-L
32. Patel AN, Mittal S, Turan Go, et al. REVIVE Trial: Retrograde Delivery of Autologous Bone Marrow in Patients With Heart Failure. *Stem Cells Transl Med*. 2015;4(doi.org/10.5966/sctm.2015-0070):1-7.
33. Tuma J, Carrasco A, Castillo J, et al. RESCUE-HF Trial: Retrograde Delivery of Allogeneic Umbilical Cord Lining Subepithelial Cells in Ptients with Heart Failure. *Cell Transplant*. 2015;25(9):1713-1721. doi:10.3727/096368915X690314
34. Tuma J, Fernández-viña R, Carrasco A, et al. Safety and feasibility of percutaneous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina. *J Transl Med*. 2011;9(183). doi:10.1186/1479-5876-9-183
35. Vicario J, Campos C, Piva J, et al. Transcoronary sinus administration of autologous bone marrow in patients with chronic refractory stable angina Phase 1. *Cardiovasc Radiat Med*. 2004;5:71-76. doi:10.1016/j.carrad.2004.06.004
36. Vicario J, Campo C, Piva J, et al. One-year follow-up of transcoronary sinus administration of autologous bone marrow in patients with chronic refractory angina. *Cardiovasc Revascularization Med*. 2005;6(3):99-107. doi:10.1016/j.carrev.2005.08.002
37. Gaetani R, Feyen DA, Verhage V, et al. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. *Biomaterials*. 2015;61:339-348. doi:10.1016/j.biomaterials.2015.05.005
38. Madonna R, Petrov L, Teberino MA, et al. Transplantation of adipose tissue mesenchymal cells conjugated with VEGF-releasing microcarriers promotes repair in murine myocardial infarction. *Cardiovasc Res*. 2015;108:39-49. doi:10.1093/cvr/cvv197
39. Ban K, Park H-J, Kim S, et al. Cell Therapy with Embryonic Stem Encapsulated in Injectable Nanomatrix Gel Enhances Cell Engraftment and Promotes Cardiac Repair. *ACS Nano*. 2014;8(10):10815-10825.
40. Don CW, Murry CE. Improving survival and efficacy of pluripotent stem cell-derived cardiac grafts. *J Cell Mol Med*. 2013;17(11):1355-1362. doi:10.1111/jcmm.12147
41. Samper E, Diez-Juan A, Montero JA, Sepúlveda P. Cardiac Cell Therapy: Boosting Mesenchymal Stem Cells Effects. *Stem Cell Rev Reports*. 2013;9(3):266-280. doi:10.1007/s12015-012-9353-z

CHAPTER 11

Report of unexpected findings after cardiac stem cell injections in a preclinical model

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ABSTRACT

Introduction Cardiac regenerative therapy is a proposed therapy for ischemic heart disease. So far efficacy has been low and this might partly be explained by low cardiac cell retention. In this study we aimed to investigate if cardiac cell retention improves using ureido-pyrimidinone units (UPy-gel) as a cell carrier.

Methods We used an ischemia-reperfusion model. Pigs were randomized to intramyocardial injections with mesenchymal stromal cells (MSC) labelled with both Indium-111 and a fluorescent tracer in either PBS or in the UPy-gel. After 4 hours, a total body scintigraphy was performed to determine the cardiac cell retention and histology was obtained.

Results In the first 4 pigs, we noticed focused areas of radio activity (hotspots) outside the heart in both the control and UPy-gel arm, and decided to interrupt the study. At histology we confirmed one hotspots to be located in a lymph node. No satisfactory explanation for these, potentially harmful, hotspots was found.

Conclusion This study was interrupted due to unexpected extra-cardiac hotspots. Although we do not have a conclusive explanation for these findings, we find that sharing these results is important for future research. We recommend to use total body imaging in future retention studies to confirm of reject the occurrence of extra-cardiac cell accumulation after intramyocardial cell injection and discover the pathophysiology and its clinical implications.

INTRODUCTION

Cardiac cell therapy has been a promising therapy to repair the damaged heart. However, efficacy has been low in preclinical and clinical trials[1], [2]. One possible explanation for the observed low efficacy could be inefficient cell delivery. We previously showed that cardiac retention after intracoronary infusion or intramyocardial injection of bone marrow derived mesenchymal stromal cells (MSC) is limited to 10-15%[3], [4]. Additionally, we showed that retrograde coronary venous infusion does not improve cardiac retention[4]. In this study we aim to test if delivery with a cell carrier improves cardiac retention. Here we use a pH-switchable hydrogel based on ureido-pyrimidinone units telechelically coupled to poly(ethylene glycol) (UPy-gel)[5]. This hydrogelator is in the liquid state at basic conditions and turns into a gel state at a lower, i.e. neutral or acidic, pH. We aimed to show increased cardiac retention when injecting MSCs combined with UPy-gel, compared to MSCs in phosphate-buffered saline (PBS) in a confirmatory pig study. We found extra-cardiac focused areas of high intensity signal (hotspots) implying extra-cardiac accumulation of cells in the first pig and confirmed this in the following 3 pigs. The hotspots were observed in both study arms. This finding was unexpected and has potential harmful clinical consequences. Therefore we decided to interrupt and de-blind this study. Here we share our unexpected findings, discuss possible explanations and provide recommendations for future research.

METHODS

Ethical statement

All experiments were performed in compliance with the "*Guide for the Care and Use of Laboratory Animals*", published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985). The protocol was approved by the Animal Experiments Committee of the Utrecht University (AVD115002015257) and registered at www.preclinicaltrials.eu (PCTE0000105). Protocols of comparable experiments are available online[3], [4], [6], [7].

Animals and housing

Female Yorkshire pigs (van Beek, SPF varkensfokkerij B.V. Lelystad) of approximately 70 kg were used in these experiments. Animals were housed in stables embedded with straw and enriched with rods. Animal welfare was assessed on a daily base by animal caretakers.

Study design

Myocardial infarction was induced at baseline. After 4 weeks, all surviving pigs were

randomized to intramyocardial injections of mesenchymal stromal cells (MSC), radioactively labeled with Indium^{111} and fluorescently-labeled with carboxyfluorescein succinimidyl ester (CSFE), in either a solution of 1) PBS or in 2) UPy-gel (figure 1). If animals reached an human endpoint (severe immobility, severe dyspnea or cyanosis, wound infection) they were euthanized and excluded. There were no additional inclusion criteria. According to sample size calculations, 14 pigs were needed to show a 6% increase in cardiac cell retention. The alpha was set on 0.05, beta on 0.20, the standard deviation on 3 and we expected 20% of the animal to drop-out due to fatal rhythm disorders during or shortly after infarct induction. We used block randomization, generated by a computer-generated random number sequence. Animals were randomized in a one-to-one ratio. All procedures were performed by the same researchers (cell culture (KN), catheter handling (MN), cell labeling and syringe control (TB)). The researcher handling the catheter was blinded for treatment allocation. Scintigraphy analyses, including drawing the regions of interest in the scintigraphy images, were performed by the same two technicians and supervised by the same nuclear medicine physician (JB), all of them were blinded for treatment allocation.

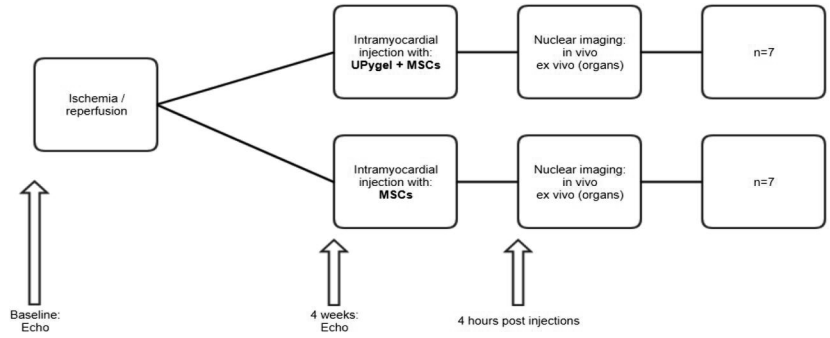


Figure 1. Study design. Ischemia/reperfusion was induced by a 90 minute occlusion of the Left Anterior Descending artery with a balloon via a percutaneous procedure. Four weeks after ischemia-reperfusion, intramyocardial injections were performed. Four hours after injections in vivo total body scintigraphy was performed, and the pigs were sacrificed for ex vivo scintigraphy of the organs and histology.

Anesthesia and analgesia

All animals were treated with amiodarone (1200 mg/day, 7 days), clopidogrel (75 mg, 3 days) and carbasalate calcium (320 mg, 1 day) prior to the myocardial infarction. Animals were anesthetized in the supine position with intramuscular ketamine (10-15 mg/kg), midazolam (0.7 mg/kg) and atropine (0.5 mg) and intravenous thiopental sodium (4 mg/kg), midazolam (10 mg) and sufentanil (0.25 mg). A bolus of amiodarone (300 mg in 30 minutes) was administered intravenously. During the procedure the animals received midazolam (1mg/kg/h), sufentanil 10 µg/kg/h) and pancuronium

bromide (0.1 mg/kg/h). Heparin (5000 IU) was given every 2 hours. All animals received a butrans patch (5 µg/h). Animals were ventilated with a mixture of dioxygen (O_2) and air (1:2) with a tidal volume of 10 ml/kg with 12 breaths per minute. Carbasalate calcium was continued (80 mg/day) until euthanasia.

Ischemia-reperfusion model

Animals were monitored during the entire procedure via continuous electrocardiogram, arterial pressure and capnogram. First the left coronary system was visualized via a coronary angiography. The myocardial infarction (MI) was induced by a 90-minute occlusion of the left anterior descending artery (LAD) using an angioplasty balloon. The balloon position was based on the coronary anatomy, the preferred position was after the second diagonal branch. In case of ventricular fibrillation or ventricular tachycardia without output, an electrical shock of 200 joules was delivered using an external defibrillator. Additionally, chest compressions were given and animals received amiodarone (150 mg, max 3 times), adrenaline (0.1 mg) and/or atropine (0.5) mg.

Cell culture and labeling

For this experiment we used allogeneic mesenchymal stromal cells (MSCs). These were isolated from the sternum and cultured as described earlier[8]. Cells (1×10^7) from passage 5-7 were used for transplantation after staining with carboxyfluorescein succinimidyl ester (CSFE) (Invitrogen, Carlsbad, California, USA) on the day of the transplantation. Cells were labelled with 30 MBq In^{111} by incubation at 37°C for 20 minutes and washed with Hank's balanced salt solution (Life Technologies Corp, Grand Island, New York, USA) to remove excess unbound In^{111} as described before[3].

Hydrogel specifications

The UPy-hydrogelater (SyMO-Chem BV, Eindhoven, the Netherlands) was prepared as described before[5], [9], [10]. In short, the UPy-hydrogelator was dissolved at 5 weight percentage (wt%) in phosphate buffered saline (PBS) pH 11.7 and temperature of 70 °C using a magnetic stirrer. After dissolving, the solution reaches a pH of 9.5. The solution was then cooled down. The cells were then pipetted into the solution and stirred for 10 minutes to reach uniform distribution.

Intramyocardial cell injection

An electromechanical map of the left ventricle was obtained using the NOGA system (Biosense Webster, Cordis, Johnson & Johnson, USA). Cells were injected in the myocardial border zone as previously defined, using the MYOSTAR® injection catheter (Biosense Webster, Cordis, Johnson & Johnson, USA)[11]. Per injection approximately 0.3 mL was injected, 10-12 injections were performed per pig. Needle depth was set at 5-7 mm. The cells were injected slowly, approximately 30 seconds per injection, and the injection needle was left in situ for an additional 10 seconds to avoid leakage.

Nuclear imaging and analysis

A scintigraphy scan, using a dual head gamma camera (Philips NM SkyLight) was performed after 4 hours to determine cell retention in the heart and other organs of interest (liver, spleen, kidneys, lung, and bladder) (figure 1). First, an in vivo total body scan was performed at 174 keV and 247 keV energy windows. After euthanizing the animal, the organs of interest were excised and scanned. Anterior and posterior images were captured for the total body scan and the ex-vivo scan of the organs. The number of counts was based on the geometrical mean of the anterior and posterior counts. Cell retention was measured by the number of counts in the region of interest as a percentage of total body activity. Analysis were performed directly after each experiments by a team blinded to treatment allocation.

RESULTS

We performed experiments with 4 out of 14 pigs according to protocol, with an experienced team and did not encounter any obvious technical issues. After analyses of our first results we found focused areas of radio-activity (hotspots) outside the heart (figure 2). These hotspots were distributed throughout the body, including the abdomen, head and extremities. We did not expect to find any hotspots outside the target organs, and suggested this can compromise the value of this study. We decided to interrupt and de-blind the study after 4 pigs to investigate a reasonable explanation for the origin of these hotspots. Since we could not find a satisfying explanation and could not rule out potential harm of these hotspots, we decided to stop the study. Ethical considerations regarding use of animal and resources also contributed to this decision.

Hotspots

Two authors (TB and MN) discussed the scintigraphy images and rated areas of increased signal intensity as hotspots by visual inspection. Quantification of signal intensity over background in the hotspots did not occur. In the UPy-gel group we identified a total of 11 hotspots (8 and 3), compared to 3 hotspots in the PBS group (2 and 1). We tried identifying the exact location of the hotspots by obduction and with use of the scintigraphy scan. We traced one of the hotspots to a lymph node. However not all hotspots were traceable with this strategy. Histology confirmed CSFE-labelled MSCs in the retrieved hotpot (figure 3). Unfortunately, we could not perform additional imaging (i.e. computed tomography scan) within this study.

Cardiac retention

Whole body scintigraphy revealed that cardiac retention was low in both groups. Retention in the heart was 4.3% and 5.3% in the UPy-gel group compared to 3.4% and 4.0% in the PBS group (table 1). Cells accumulated in lungs, liver, kidney and spleen.

Table 1. Cell retention in the target organs, measured as number of counts as percentage of number counts in the total body

	Heart	Lungs	Kidneys	Liver	Spleen
Pig 1 (UPy)	4.3%	17.2%	2.7%	8.2%	1.6%
Pig 2 (PBS)	3.4%	18.8%	3.2%	9.5%	0.7%
Pig 3 (PBS)	4.0%	23.1%	2.9%	4.2%	1.1%
Pig 4 (UPy)	5.3%	20.4%	2.8%	4.2%	1.0%

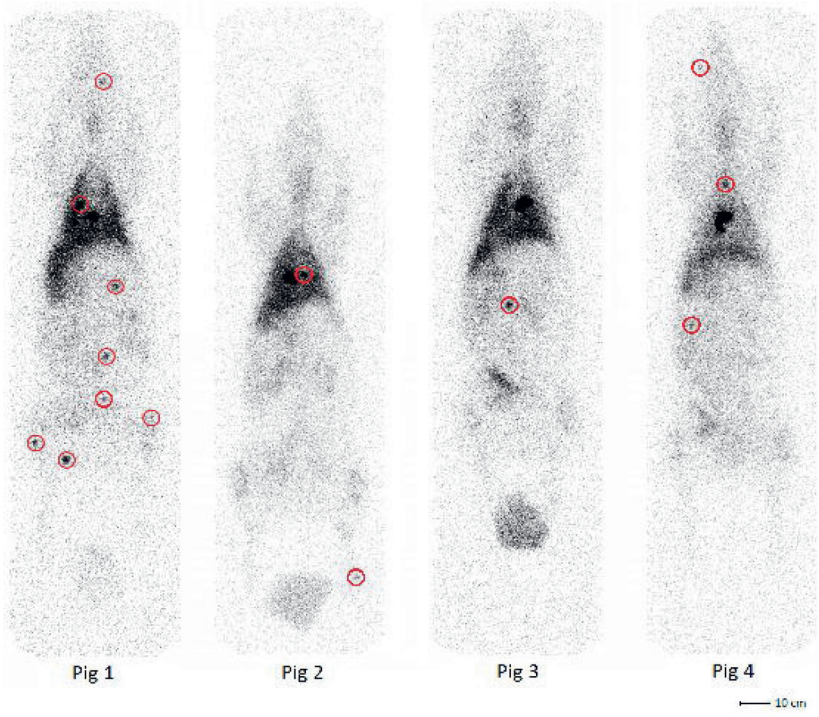


Figure 2. Total body (including urine catheter) scintigraphy scan images 4 hours after injection. Pig 1 and pig 4 were randomized to UPy-gel injections, pig 2 and pig 3 were injected with cells in PBS. The hotspots are marked with red circles.

Tracing of UPy-gel

We hypothesized that the UPy-gel would turn into a gel state immediately after injection and thus remain in the heart as previously shown[5], [10], [12]. We further hypothesized that the UPy-gel might have remained in the heart and only the radio-active labeled cells were distributed throughout these hotspots. We therefore performed an additional, post-hoc, in vivo experiment (n=1) to investigate whether hotspots contain UPy-gel. UPy-gel (5 wt%, pH 9.5) in combination with UPy-DOTA-Gadolinium (UPy-DOTA), which is traceable with magnetic resonance imaging (MRI), was injected in

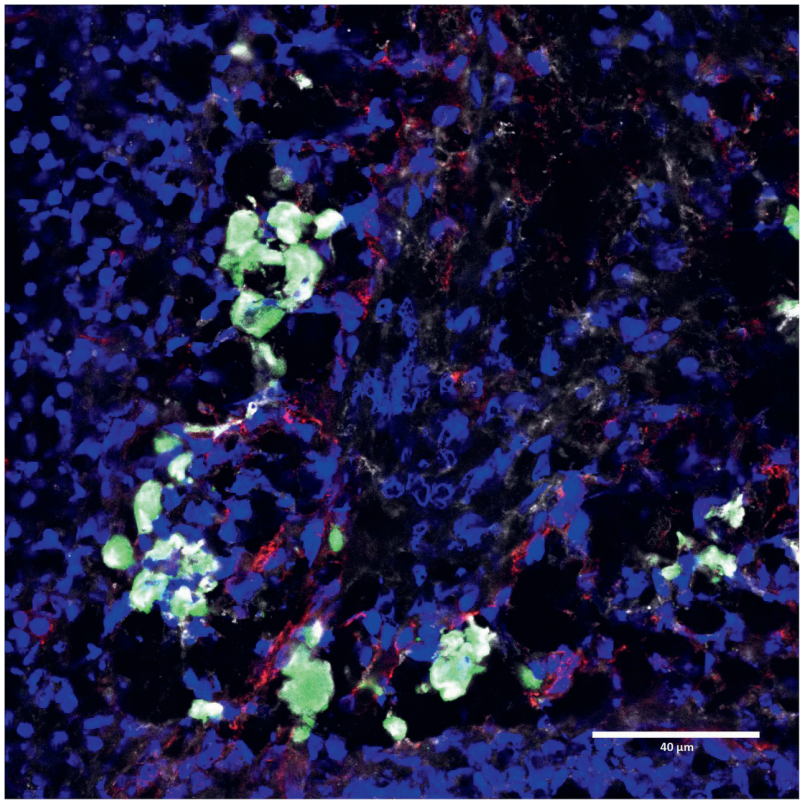


Figure 3. Histology performed on one hotspot (lymph node): Green: CSFE labeled injected MSCs. Red: CD31 endothelial vascular cells. Blue: Hoechst nuclei. Gray: Ly6G immune cells.

combination with radioactive labeled MSCs via intramyocardial injections, using the same number of cells and injection method as the original experiment[12]. Scintigraphy showed 4 intra-cardiac hotspots and 1 extra-cardiac hotspot in the mediastinum (figure 4A). An MRI of the heart confirmed the intra-cardiac hotspots contained UPy-gel. No additional imaging techniques or imaging of the extra-cardiac hotspot were performed in this experiment.

DISCUSSION

With this study we aimed to show increased cardiac retention of cells using a cell carrier in an animal model. We found extra-cardiac hotspots in the first 4 out of 14 pigs, in both the PBS and the UPy-gel group. Additionally, the cardiac retention in these four pigs was lower than expected based on previous experiments using the same protocols.

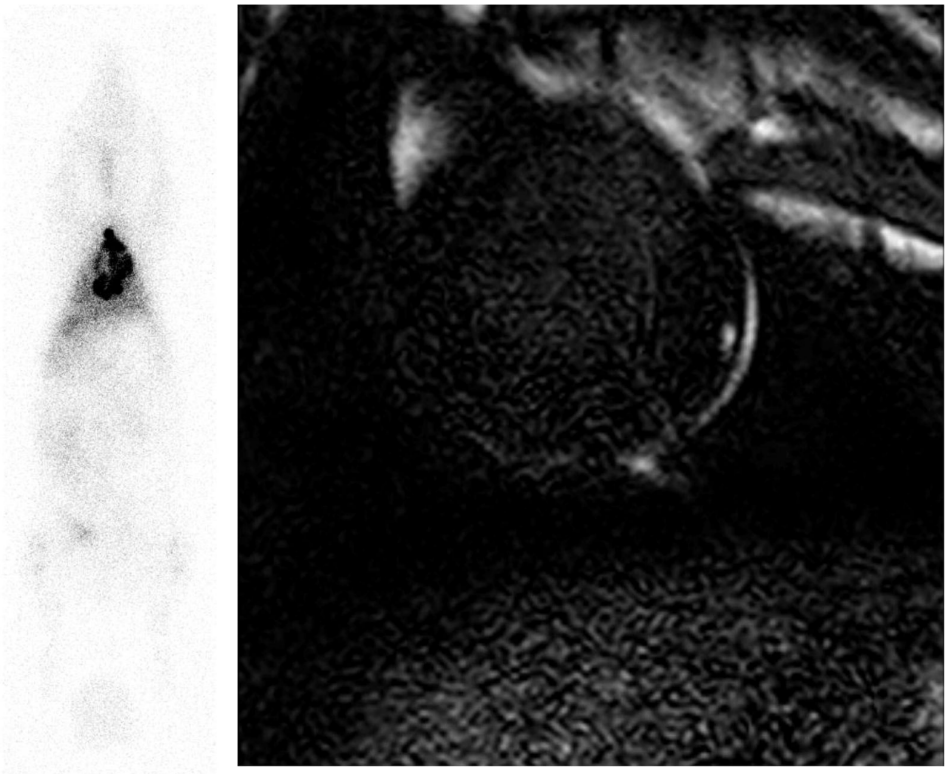


Figure 4A (left). Scintigraphy image of post-hoc experiment with 1 pig using UPy-DOTA.
Figure 4B (right). Short axis 3D viability scan with SENSE of post-hoc experiment with 1 pig using UPy-DOTA.

We could not find a satisfactory explanation for these findings and propose these results potentially compromise the value of this study. Therefore we decided to interrupt this study. Here we share our unexpected findings, not only because we find sharing (unexpected) results contributes to transparent research, but we also propose these findings demand further research to confirm the safety of intramyocardial cell injections in this model.

Extra-cardiac hotspots

Tracing of cells after cardiac transplantation has been performed in several animal studies and a little number of clinical studies. Based on these previous studies, we know that cardiac retention is low and most cells can be traced back in the lungs, intestine, kidney, bladder and liver [3], [13]–[16]. We expected to find diffusely distributed radio-activity outside the heart. Surprisingly, in the present study we found focused areas of radio-activity outside the heart (hotspots). Four potential explanations were considered: arterial embolisms, role of the hydrogel, venous-lymphatic spill, or

technical issues. First, the cells could have formed clots in the myocardium and leak back in the left ventricle (or pushed out of the myocardium by cardiac contraction) through the injection site, causing potential harmful arterial embolisms. We could not rule out arterial obstructions in this study as we did not perform CT-angiography. Importantly, in clinical studies over 2600 people received cardiac cell transplantation, of which over 200 patients received percutaneous intramyocardial cell injections. In these studies cell therapy seems to be safe and did not show a major risk of embolisms[17]. Second, we considered the hydrogel to contribute to these hotspots. We found hotspots in the study arm without the use of this hydrogel. We re-analyzed data of our previous retention study with intramyocardial injections of mesenchymal stromal cells in PBS with a comparable study protocol, but without the use of a hydrogel carrier[3]. Although this was not reported specifically, in hindsight hotspots were also visible. Taken together, we propose that is it is unlikely that the hydrogel plays a role in the formation of hotspots. Third, we hypothesized that the cells could have entered the venous system of the heart. Involvement of the lymphatic system is suggested to explain the prominently right-sided distribution of cells[18]. Possibly, the lymphatic system could then play a role in formation of hotspots, as we confirmed one extracardiac hotspot to be located in a lymph node. A clinical study that traced cells and performed total body imaging after intracoronary infusions, which is expected to have comparable venous drainage, did not show any extra-cardiac hotspots and could not provide evidence of involvement of the lymphatic system[15]. The fourth explanation could be technical issues. We have a team of skilled technicians and researchers with abundant expertise in translational studies for cardiac regeneration. Experiments are conducted according to strict protocols[6], [7]. With these measures we limited the risk of a procedural flaw. Hotspots were, when looking back at previous work, only found in studies with intramyocardial injections. We considered the possibility of a technical failure of these injection catheters. High pressure is used to inject the product through the catheters, that potentially could have led to failure (e.g. damaged lumen or damaged injection needle). However, we exclude such technical issue since we checked and flushed all catheters after the procedures and did not find any problem/inconsistency.

Three additional studies were found that performed percutaneous intramyocardial cell injections and performed total body imaging (table 2)[13], [14], [19]. All three studies were performed in pigs and used the same MYOSTAR® catheter to perform cell injections. Collantes et al applied positron emission tomography/computed tomography (PET-CT), allowing 3D visualisation of all tissues[14]. This study describes high radioactivity concentrations in mediastinal lymph nodes. Perin et al used a reporter gene, which passes on to daughter cells during proliferation, and performed repetitive imaging over time. They described involvement of the lymphatic system around the heart and cervical region [19]. It should be noted that the distribution of the hotspots

seems to be different in our study, as not all hotspots in our study are located in the mediastinum. Nevertheless, this supports one of our theories that the lymphatic system plays a role. Interestingly, Lyngbæk et al did not report extra-cardiac hotspots[13]. A CT-angiography to rule out arterial embolisms was not performed in any of these studies.

Relatively lower cardiac retention

We observed in these 4 pigs that the cardiac retention is limited (3-5%), both in our control and UPy-gel group, and lower compared to previous work[3], [4], [13], [14]. Clearly, this study was not completed and no definite conclusions can be drawn about cardiac retention. We did not find a clear explanation for the assumed lower cardiac retention. The risk of insufficient internal study validity (because previous results were not reproduced in our control group) contributed to the discussion to interrupt this study.

Conclusion

This study was initially designed to show an increased cardiac retention with the use of a hydrogel, but was interrupted due to unexpected findings. We found extra-cardiac hotspots and a lower cardiac retention in our control group as expected. Although we do not have a conclusive explanation for these findings, we find that sharing these results are important for future research and contributes to transparency. Clinical trials did not show safety issues related to intramyocardial cell injections, but only a limited number of studies performed total body imaging and therefore extra-cardiac hotspots could have been missed. The limited number of studies that did perform total body imaging are all preclinical studies and have conflicting results. Most studies showed involvement of the lymphatic system, but the distribution of cell accumulation seems to differ from our current findings. Further research should confirm or exclude the occurrence of extra-cardiac hotspots after intramyocardial cell injection and provide a better understanding of its pathophysiology and clinical implications, before continuing research to optimize cell retention with carriers. We encourage researchers to include total body imaging in future research in this field.

Table 2. Comparison of studies on in vivo cell tracking, all studies are performed in pig models. I/R = ischemie/reperfusion, PET-CT= positron emission tomography-computed tomography.

	Present study	van der Spoel[3]	Lyngbæk[13]	Collantes[14]	Perin[19]
Porcine model	I/R	I/R	Healthy	I/R	I/R
Cells	Mesenchymal stromal cells	Mesenchymal stromal cells	Mesenchymal stromal cells	Cardiac stem/progenitor cells	Mesenchymal stromal cells
Cell donor	Allogeneic	Allogeneic	Xenogeneic (human)	Allogeneic	Autologous
Number of cells	1 x 10^7	1 x 10^7	1.5 to 3.3 x 10^6	50 × 10^6	1 x 10^8
Label used	$Indium^{111}$	$Indium^{111}$	$Indium^{111}$	18F-FDG/GFP	sr39HSV1-tk gene
Volume injected	10-12 injections, 0.3 ml per injection	10-12 injections, 0.3 ml per injection	10 injections, 0.3 ml per injection	30 injections 0.3 ml per injection	3 injections, 0.1 ml per inection
Imaging technique	Scintigraphy	Scintigraphy	Scintigraphy	PET-CT	[18F]FEAU PET/CT
Timing of imaging	4 hours after injections	4 hours after injections	0.5 hour after injection	4 hours after injections	4 hours to 5 months after injection
Hotspots outside target organs	Yes	Yes	No	Yes	Yes
Explanation for hotspots	One in lymph node, other unconfirmed	No	Not applicable	Mediastinal lymph nodes	Periaortic lymphatic structures, coronary trunks, cervical lymph nodes.

REFERENCES

[1] S. J. Jansen of Lorkeers *et al.*, "Xenotransplantation of Human Cardiomyocyte Progenitor Cells Does Not Improve Cardiac Function in a Porcine Model of Chronic Ischemic Heart Failure. Results from a Randomized, Blinded, Placebo Controlled Trial.," *PLoS One*, vol. 10, no. 12, p. e0143953, Jan. 2015.

[2] M. Gyöngyösi *et al.*, "Meta-Analysis of Cell-based CaRdiac stUdiEs (ACCRUE) in patients with acute myocardial infarction based on individual patient data.," *Circ. Res.*, vol. 116, no. 8, pp. 1346–60, Apr. 2015.

[3] T. I. G. van der Spoel *et al.*, "Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischaemic cardiomyopathy: a study on delivery efficiency," *J. Cell. Mol. Med.*, vol. 16, no. 11, pp. 2768–76, Nov. 2012.

[4] W. A. Gathier *et al.*, "Lower retention after retrograde coronary venous infusion compared with intracoronary infusion of mesenchymal stromal cells in the infarcted porcine myocardium," *BMJ Open Sci.*, vol. 3, no. 1, p. e000006, 2019.

[5] M. M. Bastings *et al.*, "A fast pH-switchable and self-healing supramolecular hydrogel carrier for guided, local catheter injection in the infarcted myocardium," *Adv Heal. Mater*, vol. 3, no. 1, pp. 70–78, 2014.

[6] S. Koudstaal *et al.*, "Myocardial infarction and functional outcome assessment in pigs," *J Vis Exp*, no. 86, 2014.

[7] A. C. Pape *et al.*, "An Injectable and Drug-loaded Supramolecular Hydrogel for Local Catheter Injection into the Pig Heart," *J Vis Exp*, no. 100, p. e52450, 2015.

[8] D. A. M. Feyen, F. van den Akker, W. Noort, S. A. J. Chamuleau, P. A. Doevendans, and J. P. G. Sluijter, "Isolation of Pig Bone Marrow-Derived Mesenchymal Stem Cells," in *Mesenchymal Stem Cells: Methods and Protocols*, M. Gnecchi, Ed. New York, NY: Springer New York, 2016, pp. 225–232.

[9] S. Koudstaal *et al.*, "Sustained delivery of insulin-like growth factor-1/hepatocyte growth factor stimulates endogenous cardiac repair in the chronic infarcted pig heart," *J Cardiovasc Transl Res*, vol. 7, no. 2, pp. 232–241, 2014.

[10] M. J. G. Schotman *et al.*, "In Vivo Retention Quantification of Supramolecular Hydrogels Engineered for Cardiac Delivery," *Adv. Healthc. Mater.*, vol. 10, p. 2001987, 2021.

[11] T. I. G. van der Spoel *et al.*, "Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease.," *Cardiovasc. Res.*, vol. 91, no. 4, pp. 649–58, Sep. 2011.

[12] M. H. Bakker *et al.*, "MRI Visualization of Injectable Ureidopyrimidinone Hydrogelators by Supramolecular Contrast Agent Labeling," *Adv. Healthc. Mater.*, vol. 7, no. 11, p. 1701139, Jun. 2018.

[13] S. Lyngbæk *et al.*, "Serial in vivo imaging of the porcine heart after human mesenchymal stromal cells," *Int J Cardiovasc Imaging*, vol. 26, pp. 273–284, 2010.

[14] M. Collantes *et al.*, "Non-invasive in vivo imaging of cardiac stem/progenitor cell biodistribution and retention after intracoronary and intramyocardial delivery in a swine model of chronic ischemia reperfusion injury," *J. Transl. Med.*, vol. 15, p. 56, 2017.

[15] M. Hofmann *et al.*, "Monitoring of bone marrow cell homing into the infarcted human myocardium.," *Circulation*, vol. 111, no. 17, pp. 2198–202, May 2005.

[16] W. Hudson, M. C. Collins, D. deFreitas, Y. S. Sun, B. Muller-Borer, and A. P. Kypson, "Beating and Arrested Intramyocardial Injections Are Associated with Significant Mechanical Loss: Implications for Cardiac Cell Transplantation," *J. Surg. Res.*, vol. 142, no. 2, pp. 263–267, Oct. 2007.

[17] M. R. Afzal *et al.*, "Adult bone marrow cell therapy for ischemic heart disease: Evidence and insights from randomized controlled trials," *Circ. Res.*, vol. 117, no. 6, pp. 558–575, 2015.

[18] D. Hou *et al.*, "Radiolabeled Cell Distribution After Intramyocardial , Intracoronary, and Interstitial Retrograde Coronary," *Circulation*, vol. 112, no. 9 suppl, pp. I150–I156, 2005.

[19] E. C. Perin *et al.*, "Imaging long-term fate of intramyocardially implanted mesenchymal stem cells in a porcine myocardial infarction model," *PLoS One*, vol. 6, no. 9, 2011.

CHAPTER 12

Summary and discussion

SUMMARY

Medical research aims to find new treatments for human diseases. Despite researchers dedication and integrity, researchers preconceived views can influence their judgement, causing suboptimal research and limiting the value of the results. In addition, research is influenced by availability of funding and the availability of publications and possibilities to publish.

In the first part of this thesis we discuss several vulnerabilities in research design, conduct and reporting in animal studies that hamper translation to clinical therapies. We then provide several solutions to enhance the value of translational research. In the second part of this thesis we focus on research in cardiac repair. We also performed several animal studies in cardiac repair and aimed to implement these proposed solutions in our own research. These two themes are further introduced in **chapter 1**.

Part I - Enhancing quality in translational research

In the first part of this thesis we review vulnerabilities in the design, conduct and reporting of animal studies and propose several ways to improve scientific rigour and reproducibility in preclinical research. In **chapter 2** we explain how sharing study protocols before the start of a study (preregistration) can improve translational research. Preregistration increases research transparency by providing an unbiased overview of animal studies and by sharing of planned outcome measures. Furthermore, preregistration can raise the standards of measurements to reduce the risk of bias in animal studies. In addition, preregistration can help to avoid unintended duplication of animal studies. To facilitate this we developed the first study register dedicated to animal studies, www.preclinicaltrials.eu. **Chapter 3** illustrates that meta-research of animal studies is a specialized research discipline requiring methodology development tailored to the statistical characteristics of animal study data. We detected a common mistake in preclinical meta-analyses with respect to asymmetry testing of funnel plots to assess publication bias. In preclinical research these are often plotted by using the standardized mean difference (SMD) against the standard error. We noticed that these funnel plots are distorted in their assessment of publication bias. We were able to replicate this error with empirical datasets and computer simulations and suggested more reliable alternatives, by using the normalized mean difference or plotting the SMD against a sample size-based precision estimate, to optimize future research. In **chapter 4** we show the extent of publication bias in preclinical research. For this purpose we traced animal study protocols in one research centre over time to calculate the publication rate of animal studies. We found that only 60% of performed animal studies are shared via publications and only 26% of total animals used was reported in these publications. More specifically, 34% of animals used for published experiments were reported within these publications. That means that for 2/3 of the animals within

published studies no data are available. The researchers involved in these experiments stated that non-significance of the results was one of the major reasons not to publish. We also showed the magnitude of time-lag bias, with an average time between acceptance of a study protocol and publication of results of 30.7 months. In **chapter 5** we evaluate the first years of www.preclinicaltrials.eu and describe the growing international support for preregistration of animal studies after the launch of this platform. Despite this support, the number of registered protocols is still low, showing that overall the scientific community has not yet fully embraced this strategy. In **chapter 6** we focus on the attitude of animal researchers towards the concept of preregistration. We first established six major issues that are, according to animal research, hampering translational research. These include flawed study designs, incomplete reporting and publication bias and irreproducibility. We then showed that animal researchers acknowledge that preregistration can improve internal study validity, increases transparency and reproducibility, and reduces the unnecessary repetition of animal studies. However, animal researchers reported that they struggle with a high administrative burden and high pressure to succeed, limiting their enthusiasm for yet another obligation. Also, researchers express their concerns about the current research climate that does not seem to allow researchers to share preliminary ideas without the fear of being scooped.

Part II - Optimizing delivery techniques for cardiac repair

In the second part of this thesis we focus on cardiac repair, and we demonstrate how to implement our recommendations from part I of this thesis into practice. In **chapter 7** we provide the rationale for cardiac repair and show that so far beneficial effects in clinical setting are limited. Several strategies were suggested to improve efficacy, including use of more potential cell types, use of other cell sources and improving cell retention. In **chapter 8**, together with the Cochrane Collaboration, we perform a systematic review and meta-analysis and show that in a clinical setting therapy with autologous bone marrow-derived cells does not reduce mortality or morbidity in patients with acute myocardial infarctions. In the next chapters we focus on optimizing delivery techniques to improve cardiac repair. One proposed strategy to improve cardiac cell retention is the use of retrograde coronary venous infusions (RCVI). In **chapter 9** we performed a systematic review, of both preclinical and clinical studies, to provide an overview of current evidence on RCVI. Moreover, we aimed to gather more details on techniques and procedures. We found there is wide variety in techniques, procedures, and results and no conclusive recommendation on safety and efficacy could be made. In **chapter 10** we performed a rigorous large animal study to test safety and efficacy of RCVI compared to intracoronary infusions. We showed that cardiac retention after RCVI was lower compared to intracoronary infusions. Moreover, RCVI caused pericardial fluid and haematomas, jeopardizing patient safety. We

therefore advised not to advance RCVI as a treatment strategy. We did not register this study before the start of the study (as no dedicated platform was available yet), but we did publish the study protocol on www.preclinicaltrials.eu before the study results were published. In **chapter 11** we aimed to show increased cardiac retention using a cell carrier. We designed a randomised, controlled and blinded large animal study to trace radioactively labelled cells with a full body scintigraphy. We found unexpected and potentially dangerous extra-cardiac focused areas of radioactivity ("hotspots") in the first pig. We repeated the experiment, confirming these unexpected findings. We decided to interrupt the study as we could not guarantee safety of the procedure. In this chapter we share our limited results and explore possible explanations for these findings. The protocol of this study has been available on www.preclinicaltrials.eu before the results were shared.

In conclusion, in this thesis we showed vulnerabilities in the design, conduct and reporting of animal studies We provide clear recommendations for robust studies and transparency to improve translational research. We developed the first online register dedicated to preregistration of animal studies to facilitate preclinical preregistration, contributing to increased transparency and more robust research. Despite growing consensus for the need to enhance research quality in translational research and researchers' dedication and integrity, it remains difficult for individual researchers to adhere to these recommendations.

GENERAL DISCUSSION

The prevalence of heart failure remains high and current treatment strategies are insufficient. Cardiac repair is a research field aiming to answer this unmet clinical need. The first experiences in this research field are approximately two decades old and developments from preclinical research have advanced to the clinical arena quickly ever since. However, key findings could not be replicated and clinical successes have been limited so far[1]. We now know that the field was disturbed by scientific misconduct and fraudulent reports[2,3]. This has put cardioreparative research in urgent need for more transparency and experimental rigour, not only to increase rigor of results, but also to regain trust.

In this thesis we explore vulnerabilities in design, conduct and reporting of animal studies, learning from experiences in cardiac repair. We provide several solutions to enhance quality in translational research and share our own experiences in preclinical research. We implemented our proposed solutions to improve our own research, and reflect on the difficulties that we experienced.

Part I - Enhancing quality in translational research

Robust results

Studies should be designed so that the observed results represent the outcome in the population that is studied. However, animal studies are often subject to a high risk of bias, causing less robust results. One strategy to improve robustness was proposed in **chapter 2:** preclinical multicentre studies. This design embeds reproducibility within the set-up, as results are confirmed in different centres and are therefore less prone for interlaboratory variation. In addition multicentre studies stimulate teamwork and collaboration between different centres of expertise. Study designs, also when performed in single-centre setting, can be further optimized to reduce the risk of bias. Randomization and blinding should be considered to prevent selection, performance and detection bias. Preregistration of study hypotheses and outcome measurements prevent bias due to HARKing (hypothesizing after results are known) and selective outcome reporting. Appropriate sample size calculations ensure that studies are adequately powered to reject or accept the null hypothesis. Analyses of data which are at a higher risk of interpretation bias could be centralized to reduce variability in interpretation.

Multi-centre studies could especially be relevant for final confirmation of efficacy of promising novel interventions before entering the clinical arena[4]. Several preclinical multi-centre studies were described in the last decade[5–7]. A recent systematic review showed that twelve multi-centre preclinical studies were published to date, of which the majority in the last 5 years[8]. This not only showed feasibility of multicentre studies, but also confirmed a lower risk of bias and higher completeness of reporting compared

to typical single-centred studies[8]. Importantly, the multicentre studies provided clear recommendations for future studies, including advancing interventions to clinical trials, or pausing of ongoing clinical trials. However, the number of studies is still low, possibly due to additional costs related to such a project and these types of studies are difficult to finance via common funds[9].

Transparency and complete reporting

Complete and open reporting of study findings is necessary to properly inform the field on new developments. This allows the entire research community (and others) to use the information (for example for reproducing results and to perform meta-analyses), but it also allows the field to learn from experiences and to evaluate studies. Open discussion, reflection and debate are crucial to improve quality and researchers should be open to feedback to strengthen our work and advance biomedical research. Being transparent facilitates feedback.

Systematic reviews and meta-analyses of animal studies played a key role in reflection on translational research. Besides a rigorous appraisal of available data, they can be used to evaluate the quality of included primary studies, assess publication bias and inform on future trial design. Preclinical meta-analyses showed that internal study validity is often limited in primary studies and publication bias is frequent in preclinical research. Preclinical meta-analyses differ fundamentally from clinical meta-analyses, and therefore they need to be tailored to the purposes and specifications of animal studies[10]. In **chapter 3** we describe an error seen in preclinical meta-analysis. The Cochrane Collaboration has acknowledged this specific error and implemented it in their Handbook for Systematic Reviews of Interventions[11]. We provided two alternative methods, which were implemented in multiple meta-analyses afterwards[12,13].

Researchers are mostly dependent on publications in medical journals to be informed on other's work. Another way to share research findings is via oral or poster presentations on conferences, which usually is accompanied by publication of an abstract. However, it is difficult to use these sources for developing new studies, because the abstract data is often limited and additional information from presentations is not publicly shared and therefore cannot be referenced to[14]. Eventually, only half of studies shared via conferences are eventually published as full-texts[15]. Consequently, researchers often remain uninformed on research if the involved researchers do not pursue publication, or if journals reject their work.

Publication bias, the phenomenon that statistically significant and beneficial results are more likely to be published, and time-lag bias, where "positive" results are published quicker, limit the availability of data and influence future research, as this will be based on biased data. Additionally, not sharing study findings prevents constructive feedback on "non-significant" or "failed" experiments and can cause unnecessary repetition of experiments. Although repeating experiments might be valuable to test reproducibility

and to confirm essential results before moving forward, repeating experiments because one is simply unaware it has already been performed elsewhere is a source of research waste which should be prevented. Examples to decrease publication bias are withholding part of the funding upon publication, stimulating preregistration by requirement of study protocols for publication, and offering of sufficient publication possibilities[16–18].

In **chapter 4** we show that in one centre results from approximately one third of performed studies are not shared. Involved researchers state that non-significance of the results is one of the major reasons not to publish. Furthermore, within published studies, data from two third of the animals used are not shared, which could indicate high attrition going unreported, or selective reporting of outcomes. Not sharing of these data restricts colleagues to interpret and learn from these results. Besides, there are ethical arguments to share data from all animals used. A study performed in Germany showed a comparable publication rate, showing that these rates are likely to be representative for animal research in general[19]. In 2020, the journal Science dedicated a news item to our findings based on an interview. In this article they extrapolate the numbers to the global use of animals for scientific purposes and state that "millions of animals may be missing form scientific studies" (appendix 3).

For the complete understanding and interpretation of animal experiments, researchers are dependent on the reporting of authors. Their reports, often peer-reviewed, are limited in details on procedures, planned outcomes measures and statistical analysis and measures to reduce the risk of bias. One example aiming to enhance reporting of preclinical research are reporting guidelines. The ARRIVE guidelines were originally a set of recommendations on 20 items to guide researchers and peer reviewers in reporting[38]. Although these guidelines were endorsed by many journals, the compliance remained low[39]. As a results, the ARRIVE guidelines were revised and updated in 2020, including 10 essential items that should be met and described as a minimum requirement[40]. This example shows the gap between optimal research and feasibility within the scientific community and illustrates the lengthy process with continuous evaluation to change the system. In addition, all relevant study data are rarely accessible.

Transparency in research can be stimulated in several ways. Researchers can disclose study protocols and publish all study results within a reasonable time after start of the study. Data can be made accessible via data repositories (e.g. figshare, zenodo) or as a preprint (e.g. biorxiv or metarxiv). Conference abstracts could require more study details and oral presentations could be made accessible via conference websites. Funders could require and publish reports on the invested funds. Comparably, institutions can require and publish reports from affiliated researchers. Journals editors could require more study details, including raw data, and compare published results

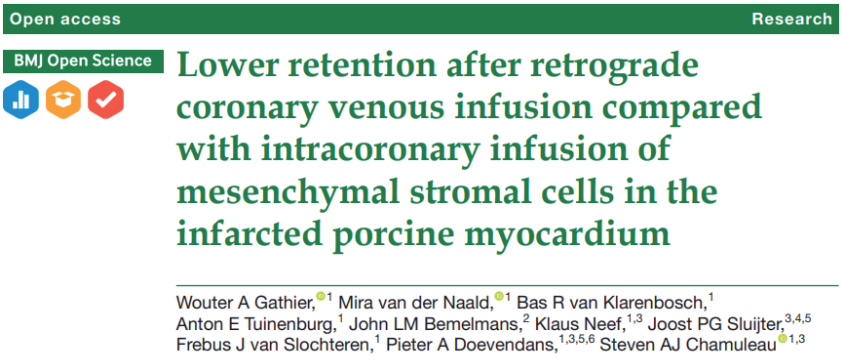
with planned outcome measures. Besides more complete information, data should also be available for everyone and free of costs.

Preregistration

Preregistration of animal study protocols limits translational failure and enhances transparency. Preregistration ensures all studies are accessible and findable, also those that remain unpublished otherwise. Preregistration includes specification on study details to optimize internal study validity, contributing to more reliable research. Furthermore, preregistrations prevents unnecessary repetition of animal studies. It was shown that preregistered studies provide more study details, but strict guidance in the specificity of study details is required to make preregistration effective[36,37]. Clinical trial registries are often searched in systematic reviews and meta-analyses to provide a complete overview of available evidence, illustrated by inclusion of clinical trial registries in the Cochrane Central Register of Controlled Trials. In addition, clinical trial registries gives us insights in the level of reporting bias, but there is now evidence registries are always checked by journal editors to compare planned and reported outcome measures[35].

To facilitate preregistration of animal studies we developed the first platform dedicated to preregister animal studies: www.preclinicaltrials.eu (figure 1). With this register we aim to set an example and intensify the discussion on preclinical preregistration. We succeeded in this endeavour, as in 2018 Dutch politicians filed a motion encouraging the use of www.preclinicaltrials.eu, which was supported by the entire Dutch House of Representatives[20]. In addition, the former Dutch Minister Carola Schouten (Ministry of Agriculture, Nature and Food Quality) expressed her support for preregistration of animal studies and preclinicaltrials.eu and funded preclinicaltrials.eu[21]. The societally relevance of this discussion was underlined by an interview in a Dutch newspaper of record, where the relevance of preregistration for the current credibility crisis in translational research is appreciated (appendix 4). Additionally Dutch funders are promoting the use of www.preclinicaltrials.eu and local institutions are endorsing preclinical preregistration and planning to mandate preregistration of confirmatory animal studies over the coming years[22]. Several international institutes

Figure 1. QR code to the online video explaining the importance of preregistration - Preclinicaltrials.eu (https://www.youtube.com/watch?v=xYjLvDBTsV4).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Lower retention after retrograde coronary venous infusion compared with intracoronary infusion of mesenchymal stromal cells in the infarcted porcine myocardium

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Figure 2. Badges awarded by the *British Medical Journal: Open Science* journal for our article (chapter 9). The three badges are displayed and apply to the following 1) open data, 2) open materials and 3) preregistered.

are encouraging preclinical preregistration and preclinicaltrials.eu was rewarded several prizes by international institutions or initiaves[23,24]. Journals are increasingly considering preclinical preregistration, for example by applying badges on your paper as a reward for preregistration (figure 2)[25,26]. In 2019 Nature focused on animal study registries in their journal (appendix 5). In this feature several experts in the field express their support for the initiative and difficulties around implementation of preregistration are discussed.

In **chapter 5** we show that despite the growing international support for preclinical preregistration, overall the scientific community currently has not yet embraced preregistration of preclinical studies and the number of registered protocols is still low. These low numbers are not specific for preclinical research. A survey among researchers from all academic institutions in the Netherlands showed that 43% of researchers preregister their work[27]. Another study showed that, even within the community of research integrity, only 19% of the studies is preregistered[28]. Currently, there are little incentives for preregistration of animal studies[29]. As discussed before, some journals provide badges if a study is preregistered and especially Dutch funders and institutes are encouraging preregistration. However, the most important stakeholders for preclinical preregistration, those that that have to actually preregister their study, are researchers involved in animal studies. In **chapter 6** we investigated their attitude towards preregistration and tried to unravel the resistance among animal researchers to preregistration. We showed that animal researchers agree that preregistration improves internal study validity, increases transparency and reproducibility and reduces the unnecessary repetition of animal studies. However, awareness seems low and researchers state they will need a 'stick rather than a carrot' before they preregister

studies. Therefore it seems a logical step that, akin to the clinical arena, medical journals take the lead and require preregistration for publication in their journal.

For clinical trials, preregistration was introduced in the 1980s, clinicaltrials.gov was launched in 2000 and the International Committee of Medical Journal Editors (ICMJE) required registration of clinical trials in 2005.

Due to the high number of available registers for clinical trials the World Health Organization provides an International Clinical Trials Registry Platform search portal, allowing access to all protocols registered on any of the eighteen connected registers. As part of this programme, the World Health Organization has defined international standards for clinical trial registries to ensure a baseline level of data quality of included registries[30]. Registration of clinical study protocols is often mandatory by law and required by medical journals for publication. Researchers are also mandated by law to report results on clinical trials[18,31,32]. It seems that all trials are now being (pre) registered, but it took several decades for this to happen, showing the lengthy process of changing the system. Also the reporting of results is still limited[33,34].

Even though the number of registered protocols is still limited, there are currently three platforms available for preregistration of animal studies. Preclinicaltrials.eu was the first registry dedicated to animal studies, quickly followed by the Animal Study Registry. In addition, the Open Science Framework provides a platform for preregistration, although this platform and adherent database is not limited to animal studies. Although it might seem controversial to have three different platforms available when preregistration numbers are still low, this might have benefits as well. First, the existence of different platforms can help in the development of registries in the starting phase. For this purpose the three registries have defined common standards for preclinical preregistration[41]. Second, these three databases are provided by different type of stakeholders and this might broaden the support for preregistration. Preclinicaltrials.eu was established by researchers involved in animal research, the open science framework by researchers from all types of research and the animal study registry is developed by a funding agency. Third, it is unlikely that one platform can manage all animal studies performed worldwide. This way the workload for registries is better divided, especially if preregistration becomes more established. If more animal studies are preregistered and searching of related databases becomes more valuable, it would be relevant to have one search engine that can search through all relevant registries, comparable to clinical trials registries.

Part II - Experiences in cardiac repair

In the second part of the thesis we focus on translational research in cardiac repair. We provide an overview of cardioreparative research and the disappointing clinical effects so far. We then focus on animal studies to address a specific issue in the field of cardiac repair; optimizing delivery techniques to increase cardiac retention.

In **chapter 7** we provide the rationale for reparative therapy and show that beneficial effects are limited after the first two decades of reparative therapy for the heart. In **chapter 8** we show that autologous bone marrow-derived cell therapy in patients suffering from acute myocardial infarction is not an effective treatment strategy. Although a moderate effect on left ventricular ejection fraction was seen, this seems clinical irrelevant, as it does not translate into clinical benefits for patients nor a reduced mortality. Although autologous bone marrow derived cell therapy might be more effective in other patients groups (ischemic cardiomyopathy and refractory angina pectoris), we would recommend to move forward to alternative treatment strategies. Other cell types and sources, like mesenchymal stem cells (MSCs), more purified cell populations and adipose-tissue derived cells are being evaluated as more potent cells. Use of allogeneic cells, instead of autologous cells, could reduce donor variability and the influence of patient comorbidities[43]. Another hypothesis is that endogenous stem cells can be stimulated to repair the heart itself[44]. Several cell-free therapeutics, like exosomes and microRNAs, are being evaluated to test potency of stimulating endogenous repair[45,46]. In this thesis we focus on optimizing of delivery techniques to increase cardiac retention and improve cardiac repair.

Current delivery techniques include intracoronary infusion, intramyocardial injections and epicardial injections. These techniques have limited effect as only 10-15% of transplanted cells remain in the heart[47]. In **chapter 9 to chapter 11** we performed animal studies and a systematic review on animal studies focusing on improving delivery techniques. In **chapter 9** we showed that several (preclinical and clinical) studies were performed to investigate cardiac retention after retrograde coronary venous infusions (RCVI), including more than 300 participants to test cardiac retention. Nevertheless, data regarding safety and technical issues were lacking in most of the studies and only limited safety issues were discussed in the publications. Following these results, in **chapter 10** we designed a randomised, controlled, large animal study to test safety and efficacy of RCVI compared to intracoronary infusions. We found severe safety issues, jeopardizing this procedure. Therefore, we advise not to use RCVI in future research and instead focus on alternative strategies like (repeated) intracoronary infusions, of which safety is proven. Furthermore, it may be efficient to use smart biomaterials to increase retention. Therefore, in **chapter 11** we aimed to show increased cardiac retention in a pig study by using a cell carrier. We traced radio-actively labelled cells with a full body scintigraphy. We found extra-cardiac focused areas of radio-activity ("hotspots") in the first pig. This was an unexpected finding and no immediate satisfying explanation was found, but an important cause like arterial embolisms could not be ruled out. We decided to interrupt the study as there was a potential dangerous unexpected finding. Additionally, the retention rate in the control arm was not comparable with findings from previous studies, threatening internal study validity. Despite our commitment for more transparent research, the study

protocol was only shared after the experiments were performed (preclinicaltrials.eu was not available at the start of this study) and the study results are subjected to time-lag bias. We did register our protocol on preclinicaltrials.eu (PCTE0000105) shortly after the experiments were performed and mentioned the study was interrupted, allowing researchers to find the study protocol. The best explanation why it took us 4 years to publish the results of this study is that we were struggling with the lack of a proper explanation for the findings, and we presumed that journals would not be interested in these results. After being rejected in a peer-reviewed journal we decided to share the data on Bioarxiv. Although we are not aware of any research that was (partly) based on our results, the manuscript was downloaded over 100 times in the first half year after publication.

Final remarks

With this thesis we show the importance of robustness, openness and transparency to improve research. We learned from our experiences in cardiac repair, but vulnerabilities in study design, conduct and reporting are also seen in other fields of research. We proposed several solutions to improve the value of research. Importantly, we developed a platform to facilitate preclinical preregistration and put preclinical preregistration on the agenda. We believe it is time for the scientific community to take responsibility and move towards even more robust preclinical research. Medical research aims to find new treatment for human diseases, but improving research should also be part of the agenda. Based on the ongoing developments we are hopeful this thesis will contribute to a sustained change in scientific research. We encourage the scientific community to be more transparent and contribute to improving science by acting responsibly.

REFERENCES

1. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004;428(6983):664-668. doi:10.1038/nature02446
2. Oransky I, Marcus A. Harvard and the Brigham call for more than 30 retractions of cardiac stem cell research. *Stat*. 2018;10(14):1-7. https://www.statnews.com/2018/10/14/harvard-brigham-retractions-stem-cell/.
3. Chien KR, Frisén J, Fritsche-Danielson R, Melton DA, Murry CE, Weissman IL. Regenerating the field of cardiovascular cell therapy. *Nat Biotechnol*. 2019;37(3):232-237. doi:10.1038/s41587-019-0042-1
4. Mogil JS, Macleod MR. No publication without confirmation. *Nature*. 2017;542:409-411.
5. Rossello X, Rodriguez-Sinovas A, Vilahur G, et al. CIBER-CLAP (CIBERCV Cardioprotection Large Animal Platform): A multicenter preclinical network for testing reproducibility in cardiovascular interventions. *Sci Rep*. 2019;9(1):1-11. doi:10.1038/s41598-019-56613-6
6. Lefer DJ, Bolli R. Development of an NIH consortium for preclinicAl AssESsment of CARdioprotective therapies (CAESAR): a paradigm shift in studies of infarct size limitation. *J Cardiovasc Pharmacol Ther*. 2011;16(3-4):332-339. doi:10.1177/1074248411414155
7. Howells DW, Sena ES, Macleod MR. Bringing rigour to translational medicine. *Nat Rev Neurol*. 2014;10(1):37-43. doi:10.1038/nrneurol.2013.232
8. Hunniford VT, Grudniewicz A, Fergusson DA, Grigor E, Lansdell C, Lalu MM. Multicenter preclinical studies as an innovative method to enhance translation: A systematic review of published studies. *bioRxiv*. 2019;March 27:591289. doi:10.1101/591289
9. Bolli R. CAESAR's legacy: a new era of rigor in preclinical studies of cardioprotection. *Basic Res Cardiol*. 2021;116(33). doi:10.1007/s00395-021-00874-8
10. Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: A practical guide. *J Neurosci Methods*. 2014;221:92-102. doi:10.1016/j.jneumeth.2013.09.010
11. Higgins J, Thomas J, Chandler J, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). *Cochrane*. 2022. www.training.cochrane.org/handbook.
12. Rivero-Santana A, Perestelo-Perez L, Alvarez-Perez Y, et al. Stepped care for the treatment of depression: a systematic review and meta-analysis. *J Affect Disord*. 2021;294:391-409. doi:10.1016/j.jad.2021.07.008
13. Friis Berntsen C, Rootwelt P, Dahm AEA. Bias in animal studies of estrogen effects on cardiovascular disease: A systematic review and meta-analysis. *Res Pract Thromb Haemost*. 2021;5(4). doi:10.1002/rth2.12507
14. Dundar Y, Dodd S, Dickson R, Walley T, Haycox A, Williamson PR. Comparison of conference abstracts and presentations with full-text articles in the health technology assessments of rapidly evolving technologies. *Health Technol Assess (Rockv)*. 2006;10(5). doi:10.3310/hta10050
15. Lensen S, Jordan V, Showell M, et al. Non-publication and publication bias in reproductive medicine: A cohort analysis. *Hum Reprod*. 2017;32(8):1658-1666. doi:10.1093/humrep/dex236
16. Turner S, Wright D, Maeso R, Cook A, Milne R. Publication rate for funded studies from a major UK health research funder: a cohort study. *BMJ Open*. 2013;3(5):e002521. doi:10.1136/bmjopen-2012-002521
17. The World Health Organization (WHO). *Joint Statement on Public Disclosure of Results from Clinical Trials*.; 2017. https://www.who.int/news/item/18-05-2017-joint-statement-on-registration.
18. De Angelis C, Drazen JM, Frizelle FA, et al. Clinical Trial Registration: A Statement from the International Committee of Medical Journal Editors. *N Engl J Med*. 2004;351(12):1250-1251.
19. Wieschowski S, Biernot S, Deutsch S, Glage S, Strech D. Publication rates in animal research. Extent and characteristics of published and non- published animal studies followed up at two German university medical centres. *PLoS One*. 2019;14(11):e022375. doi:10.1371/journal.pone.0223758
20. De Groot, Dik-Faber, Von Martels. *Motie van Het Lid De Groot c.s. over Het Registreren van Alle Individuele Dierproeven*.; 2018:ISSN 0921-7371. https://www.tweedekamer.nl/kamerstukken/.
21. Schouten C. *Jaarregistratie, Moties En Toezeggingen Dierproeven*.; 2019:19081770. https://www.rijksoverheid.nl/binaries/rijksoverheid/documenten/kamerstukken/2019/04/05/kamerbrief-over-jaarregistratie-moties-en-toezeggingen-dierproeven/kamerbrief-over-jaarregistratie-moties-en-toezeggingen-dierproeven.pdf.
22. Kimmelman J, Mogil JS, Dirnagl U. Distinguishing between Exploratory and Confirmatory Preclinical Research Will Improve Translation. *PLoS Biol*. 2014;12(5):e1001863. doi:10.1371/journal.pbio.1001863
23. Advisory committee to the Director (National Institute of Health). ACD working group on enhancing rigor, transparency, and translatability in animal research - final report. *acd.od.nih.gov*. 2021. https://acd.od.nih.gov/documents/presentations/06112021_ACD_WorkingGroup_FinalReport.pdf.
24. National Health and Medical Research Council (NHMRC). *Best Practice Methodology in the Use of Animals for Scientific Purposes*. www.nhmrc.gov.au/guidelines-publications/ea20.
25. Journal of Neuroscience Research. Preregistered badge Journal of Neuroscience Research. https://onlinelibrary.wiley.com/page/journal/10974547/homepage/open_research_badges.html.

26. BMJ Open Science. Preregistered badge BMJ Open Science. https://openscience.bmj.com/pages/policies/#Ethics.
27. Gopalakrishna G, Wicherts JM, Vink G, et al. Prevalence of responsible research practices among academics in the Netherlands. *MetaArXiv*. 2021:1-27. doi:10.31222/osf.io/xsn94
28. Sijtsma K, Emons WHM, Steneck NH, Bouter LM. Steps toward preregistration of research on research integrity. *Res Integr Peer Rev*. 2021;6(1):5. doi:10.1186/s41073-021-00108-4
29. Heinl C, Chmielewska J, Olevska A, Grune B, Schönfelder G, Bert B. Rethinking the incentive system in science: animal study registries. *EMBO Rep*. 2020;21(1):2-5. doi:10.15252/embr.201949709
30. World Health Organization. *International Standards for Clinical Trial Registries - Version 3.0*.; 2018. https://www.who.int/publications/i/item/international-standards-for-clinical-trial-registers.
31. Department of Health and Human Services NI of H. *Clinical Trials Registration and Results Information Submission; Final Rule*. Vol 81.; 2013:64981-65157. https://www.gpo.gov/fdsys/pkg/FR-2016-09-21/pdf/2016-22129.pdf.
32. World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. *Bull World Health Organ*. 2013;79(4):373. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.
33. Kaplan RM, Irvin VL. Likelihood of null effects of large NHLBI clinical trials has increased over time. *PLoS One*. 2015;10(8):e0132382. doi:10.1371/journal.pone.0132382
34. Devito NJ, Bacon S, Goldacre B. Compliance with legal requirement to report clinical trial results on ClinicalTrials.gov: a cohort study. *Lancet*. 2020;395:361-369. doi:10.1016/S0140-6736(19)33220-9
35. Goldacre B, Drysdale H, Powell-Smith A, et al. *The COMPare Trials Project*.; 2016.
36. Bakker M, Veldkamp CLS, Van Assen MALM, et al. Ensuring the quality and specificity of preregistrations. *PLoS Biol*. 2020;18(12):1-18. doi:10.1371/journal.pbio.3000937
37. Bakker M, Veldkamp CLS, van den Akker OR, et al. Recommendations in pre-registrations and internal review board proposals promote formal power analyses but do not increase sample size. *PLoS One*. 2020;15(7 July):1-15. doi:10.1371/journal.pone.0236079
38. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol*. 2010;8(6):e1000412. doi:10.1371/journal.pbio.1000412
39. Hair K, Macleod MR, Sena ES. A randomised controlled trial of an Intervention to Improve Compliance with the ARRIVE guidelines (IICARus). *Res Integr Peer Rev*. 2019;4(12):1-17.
40. Percie Du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *BMJ Open Sci*. 2020;4(1):e100115. doi:10.1136/bmjos-2020-100115
41. Heinl C, Scholman-végh AMD, Mellor D, et al. Declaration of common standards for the preregistration of animal research – Speeding up the scientific progress. *PNAS Nexus*. 2022;pgac016. doi:10.1093/pnasnexus/pgac016
42. Mathur A, Arnold R, Assmus B, et al. The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: rationale and design of the BAMI trial. *Eur J Heart Fail*. 2017;19(11):1545-1550. doi:10.1002/ejhf.829
43. Kastrup J, Haack-Sorensen M, Juhl M, et al. Cryopreserved Off-the-Shelf Allogeneic Adipose-Derived Stromal Cells for Therapy in Patients with Ischemic Heart Disease and Heart Failure-A Safety Study. *Stem Cells Transl Med*. 2017;6(11):1963-1971. doi:https://dx.doi.org/10.1002/sctm.17-0040
44. Ellison GM, Nadal-Ginard B, Torella D. Optimizing Cardiac Repair and Regeneration Through Activation of the Endogenous Cardiac Stem Cell Compartment. *J Cardiovasc Transl Res*. 2012;5(5):667-677. doi:10.1007/s12265-012-9384-5
45. Barile L, Moccetti T, Marbán E, Vassalli G. Roles of exosomes in cardioprotection. *Eur Heart J*. 2017;38(18):1372-1379. doi:10.1093/eurheartj/ehw304
46. Madonna R, Van Laake LW, Davidson SM, et al. Position Paper of the European Society of Cardiology Working Group Cellular Biology of the Heart: Cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure. *Eur Heart J*. 2016;37(23):1789-1798. doi:10.1093/eurheartj/ehw113
47. Van Der Spoel T, Vrijsen K V, Nijsen F, et al. Transendocardial cell injection is not superior compared with intracoronary infusion in a porcine model of chronic ischemic heart disease: A randomized study imaging labelled cells. *Eur Hear J*. 2011;32((Van Der Spoel T.; Cramer M.J.M.; Doevendans P.A.; Van Belle E.; Chamuleau S.A.J.) University Medical Center Utrecht, Department of Cardiology, Utrecht, Netherlands):819. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L70536134.

APPENDIX

NEDERLANDSE SAMENVATTING

Medisch onderzoek heeft als doel het vinden van nieuwe behandelingen voor humane ziektes. Ondanks de toewijding en integriteit van onderzoekers, kunnen onderzoekers beïnvloed worden door hun ideeën (bias), wat kan leiden tot suboptimaal onderzoek en de waarde van de resultaten kan beperken. Ook wordt onderzoek beïnvloedt door financiering, de beschikbaarheid van publicaties en de mogelijkheden om te publiceren. In het eerste deel van dit proefschrift bediscussiëren we verschillenden kwetsbaarheden in het ontwerp, uitvoeren en rapporteren van dierstudies, die translatie van nieuwe therapieën naar de kliniek kunnen hinderen. Vervolgens dragen we verscheidene oplossingen aan om de waarde van translationeel onderzoek te versterken. In het tweede deel van dit proefschrift gaan we dieper in op onderzoek in cardiaal herstel. In dit kader hebben we dierstudies uitgevoerd, waarbij we hebben gepoogd de voorgestelde oplossingen uit het eerste deel van dit proefschrift te implementeren. Deze twee thema's worden verder geïntroduceerd in **hoofdstuk 1**.

Deel I – Verbeteren van kwaliteit in translationeel onderzoek

Robuuste onderzoeksresultaten

Translationeel onderzoek, waarbinnen dierstudies een belangrijke rol kunnen spelen, beoogt nieuwe therapieën te vertalen vanuit preklinisch werk naar een klinische therapie. Studies zouden zo opgezet moeten worden dat de geobserveerde resultaten een afspiegeling zijn van de uitkomst in de populatie die wordt onderzocht. In het eerste deel van dit proefschrift beschrijven we verschillende kwetsbaarheden in het ontwerp, uitvoeren en rapporteren van dierstudies die translationeel onderzoek hinderen. Vervolgens bespreken we meerdere oplossingen om robuustheid en reproduceerbaarheid in preklinisch onderzoek te verbeteren en bias in preklinisch onderzoek te verminderen.

In **hoofdstuk 2** beschrijven we meerdere mogelijkheden om robuustheid in onderzoek te vergroten. Een strategie hiervoor is het uitvoeren van preklinische multicenter studies. Hierbij wordt door de opzet van de studie reproduceerbaarheid gewaarborgd, waarbij resultaten worden bevestigd in verschillende centra en ze daarom minder afhankelijk zijn van de verschillen binnen de laboratoria. Bovendien wordt in deze setting samenwerking gestimuleerd tussen verschillende expertisecentra. De studie opzet, zowel in multicenter alsook in single-center studies, kan verder worden geoptimaliseerd door het risico op bias te beperken. Randomisatie en blinderen dienen hiervoor overwogen worden om zodoende selectie, performance en detectie bias te voorkomen. Preregistratie van de studie hypothese en beoogde uitkomstmaten vermindert bias door *HARKing* (hypothesizing after results are known; ofwel hypothese vormen nadat de resultaten bekend zijn) en het selectief rapporteren van uitkomsten.

Daarnaast is het van belang om een studie adequaat te poweren zodat de hypothese terecht geaccepteerd of verworpen kan worden, hiervoor is een toereikende berekening van de steekproefomvang (sample size berekening) nodig. Het analyseren van data die gevoelig zijn voor interpretatiebias kunnen worden gecentraliseerd om de variabiliteit in interpretatie te beperken. Preregistratie vergroot transparantie door het verstrekken van een volledig overzicht van dierstudies (inclusief niet gepubliceerde studies) en door het delen van geplande uitkomstmaten. Bovendien kan preregistratie bijdragen aan het reduceren van bias, doordat maatregelen als blinderen en randomiseren worden aangeprezen. Daarnaast kan preregistratie helpen om onbedoelde duplicatie van dierstudies te voorkomen. Om preregistratie te faciliteren hebben we www.preclinicaltrials.eu ontwikkeld, het eerste online platform toegewijd aan preregistratie van dierstudies.

In **hoofdstuk 3** laten we het belang zien van op maat gemaakte methoden en statistiek voor preklinische meta-analyses. We detecteerden een gebruikelijke misvatting in preklinische meta-analyses met betrekking tot het testen voor publicatiebias met zogenaamde *funnel plots*. In preklinisch onderzoek wordt hiervoor vaak de *standardized mean difference* (SMD) geplot tegen de *standard error*. Wij merkten op dat deze *funnel plots* vertekend zijn in hun schatting van publicatie bias. We hebben deze systemische vertekening aan te tonen met empirische datasets en computer stimulaties. Om toekomstig onderzoek te optimaliseren suggereerden we twee alternatieve methoden, namelijk het gebruik van de *normalized mean difference* of het gebruik van een andere precisiemaat. De *Cochrane Collaboration* erkent deze systemische vertekening en heeft deze en onze aanbevelingen overgenomen in hun *Handbook for systematic Reviews of Interventions*.

Transparant en compleet rapporteren

Onderzoekers zijn met name afhankelijk van publicaties in medische tijdschriften om geïnformeerd te worden over andermans werk. Een andere manier om bevindingen te delen is via orale of poster presentaties op congressen, wat vaak gepaard gaat met een publicatie van een samenvatting. Het is echter moeilijk om deze bronnen te gebruiken om nieuwe studies te ontwikkelen, want de data is vaak beperkt, aanvullende informatie van presentaties wordt niet publiekelijk gedeeld en er kan niet naar gerefereerd worden. Slechts de helft van de studies die wordt gedeeld op congressen wordt uiteindelijk als *full-tekst* artikel gepubliceerd. Daarom blijft het onderzoekersveld vaak onwetend over onderzoek als er geen publicatie wordt geambieerd door de betrokken onderzoekers of als hun werk niet wordt geaccepteerd door medische tijdschriften. Publicatie bias is het fenomeen waarbij statistisch significante en gunstige resultaten meer kans hebben om gepubliceerd te worden en time-lag bias het fenomeen waarbij deze “positieve” resultaten eerder gepubliceerd worden; waardoor

de beschikbaarheid van data beperkt is en toekomstig onderzoek beïnvloed kan worden. Bovendien voorkomt het niet delen van resultaten constructieve feedback op "niet significante" of "mislukte" experimenten en kan het leiden tot het onnodig herhalen van experimenten. Hoewel het herhalen van experimenten waardevol kan zijn om reproduceerbaarheid aan te tonen en om essentiële resultaten te bevestigen alvorens ermee verder te gaan, is het herhalen van experimenten omdat iemand simpelweg niet op de hoogte is dat een onderzoek elders reeds is uitgevoerd een verspilling die voorkomen zou moeten worden.

In **hoofdstuk 4** tonen we de omvang van non-publicatie in preklinisch onderzoek. Hiervoor hebben we in een onderzoekscentrum studie protocollen getraceerd naar publicaties om het percentage gepubliceerde dierstudies te berekenen. We ontdekten dat slechts 60% van de uitgevoerde dierstudies wordt gepubliceerd en dat slechts 26% van het totale aantal gebruikte dieren wordt gerapporteerd in publicaties. Meer specifiek wordt slechts 34% van de gebruikte dieren in wel gepubliceerde studies gerapporteerd. Dat betekent dat binnen de gepubliceerde studies, slechts data van twee derde van de gebruikte dieren wordt gerapporteerd. Dit zou kunnen duiden op een hoog (niet-gerapporteerd) uitvalspercentage of het selectief rapporteren van uitkomsten. De betrokken onderzoekers gaven in een aanvullende enquête aan dat één van de belangrijkste redenen om niet te publiceren was dat de resultaten niet significant waren. Het niet delen van data beperkt collega's om de studie adequaat te interpreteren en te leren van de resultaten. Daarnaast zijn er ethische argumenten om het delen van alle data van gebruikte dieren te delen. Ook toonden we aan dat er een vertraging op publicaties is, waarbij het in gemiddeld 30.7 maanden duurde voordat goedgekeurde studie protocollen werden gepubliceerd. In 2020 heeft het medische tijdschrift *Science* een nieuwsbericht gewijd aan onze bevindingen, gebaseerd op een interview. In dit artikel extrapoleren ze onze cijfers naar het globale gebruik van proefdieren voor wetenschappelijk onderzoek en concluderen ze dat er mogelijk miljoenen dieren missen in wetenschappelijke studies (appendix 3).

Transparantie in onderzoek kan op verscheidene manieren worden gestimuleerd. Onderzoekers kunnen studie protocollen openbaar maken en alle studie resultaten delen binnen een afzienbare periode na de start van de studie. Data kan toegankelijk worden gemaakt middels *data repositories* (zoals figshare, zenodo) of als *preprint* (bijvoorbeeld via bioarxiv of metarxiv). Samenvattingen van congressen zouden meer details kunnen vereisen en orale presentaties zouden toegankelijk kunnen worden gemaakt via congres websites. Financiers zouden rapporten over de geleverde bijdrage kunnen vereisen en deze kunnen publiceren. Ook onderzoeksinstituten kunnen dergelijke rapporten verlangen en publiceren. Redacteuren van medische tijdschriften kunnen meer studie details verlangen, inclusief ruwe data, en toezien dat de te publiceren resultaten vergeleken worden met de geplande uitkomstmaten. Naast meer

complete informatie moet data ook voor iedereen beschikbaar zijn zonder aanvullende kosten.

Preregistratie

Preregistratie van dierstudie protocollen vergroot translatie en transparantie. Preregistratie waarborgt dat alle studies toegankelijk en vindbaar zijn, ook die studies die anders niet gepubliceerd worden. Preregistratie bevat specificaties over de studie opzet om zo de interne studie validiteit te optimaliseren en draagt daarom bij aan meer betrouwbaar onderzoek. Bovendien voorkomt preregistratie het onnodig herhalen van dierstudies. Platforms voor preregistratie van klinische studies, zoals clinicaltrials.gov, worden vaak meegenomen voor systematische reviews om een compleet overzicht van beschikbare *evidence* te maken. Om preregistratie van dierstudies mogelijk te maken hebben we het eerste platform toegewijd aan preregistratie van dierstudies opgezet: www.preclinicaltrials.eu. Met dit platform beogen we een voorbeeld te geven en de discussie omtrent preregistratie van dierproeven te intensiveren. Hierin zijn we geslaagd, aangezien in 2018 Nederlandse politici een motie hebben ingediend om het gebruik van www.preclinicaltirals.eu aan te moedigen. Deze motie werd unaniem gesteund door de Tweede Kamer. Vervolgens heeft de voormalig Minister Carola Schouten (Landbouw, Natuur en Voedselkwaliteit) haar steun voor preregistratie van dierstudies en www.preclinicaltrials.eu geuit en hier ook financiële steun aan gegeven. Het maatschappelijk belang van deze discussie werd onderschreven door een interview in een gerenommeerd krant, te weten het *NRC Handelsblad*, waar de relevantie van preregistratie voor de huidige geloofwaardigheidscrisis werd benadrukt (appendix 4). Nederlandse financiers promoten het gebruik van www.preclinicaltrials.eu en enkele instituten omarmen preregistratie van dierstudies en plannen om het verplicht te maken voor bepaalde studies in de komende jaren. Ook wereldwijd is er aandacht voor preregistratie van dierstudies, waarbij preclinicatrials.eu meerdere internationale prijzen heeft mogen ontvangen. Medische tijdschriften steunen preregistratie in toenemende mate, bijvoorbeeld door het toepassen van specifieke badges op publicaties die zijn gepreregistreerd. In 2019 heeft het medische tijdschrift *Nature* een artikel gepubliceerd over preregistratie (appendix 5). Hierin beschrijven verschillende experts hun steun voor preregistratie en worden moeilijkheden omtrent implementatie van preregistratie bediscussieerd.

In **hoofdstuk 5** evalueren we de eerste jaren van www.preclinicaltrials.eu en beschrijven de groeiende internationale steun voor preregistratie van dierstudies na de lancering van dit platform. Ondanks deze steun blijft het aantal geregistreerde protocollen laag, wat aantoont dat de wetenschappelijke gemeenschap deze strategie nog niet volledig omarmd heeft. Uiteindelijk is de belangrijkste stakeholder om preregistratie te implementeren de onderzoekers die de dierproeven uitvoeren. In **hoofdstuk 6**

onderzoeken we de houding van onderzoekers betrokken zijn in dierstudies over het concept preregistratie. We hebben zes grote problemen vastgesteld die volgens deze onderzoekers translationeel onderzoek hinderen. Dit betreft onder andere gebrekkige studie opzet, incompleet rapporteren van data en publicatie bias en het onvermogen om onderzoeksresultaten te reproduceren. De onderzoekers erkennen dat preregistratie interne studie validiteit kan verbeteren, transparantie en reproduceerbaarheid kan vergroten en het onnodig herhalen van dierstudies kan verminderen. Echter, de hoge administratieve last en hoge druk om te slagen wordt aangedragen als bezorgdheid voor preregistratie. Ook uiten de onderzoekers hun zorgen over het huidige onderzoeksklimaat waarin onderzoekers ogenschijnlijk niet in staat zijn hun prille ideeën te delen, uit angst dat iemand anders het idee oppikt, er eerder over publiceert en de erkenning krijgt. Het lijkt een logische stap dat, in vergelijking met het klinische veld, medische tijdschriften het voortouw nemen en preregistratie verplicht stellen om publicatie in hun blad mogelijk te maken.

Deel II – Optimaliseren van toedieningsmethoden voor cardiaal herstel

In het tweede deel van dit proefschrift focussen we op cardiaal herstel en demonsteren we hoe de aanbevelingen van deel I van dit proefschrift in praktijk kunnen worden gebracht. In **hoofdstuk 7** beschrijven we de rationale voor cardiaal herstel en de tot nu toe beperkte gunstige effecten in de kliniek. Er zijn meerdere suggesties om de werkzaamheid van stamceltherapie te vergroten, waaronder het gebruik van meer potente celtypes, het gebruik van ander cel bronnen en het verbeteren van cel retentie.

In **hoofdstuk 8** voeren we, samen met de Cochrane Collaboration, een systematische review en meta-analyse uit en tonen dat in patiënten met een acuut myocardinfarct, infusies met autologe, uit het beenmerg afkomstige cellen niet leidt tot een reductie in mortaliteit of morbiditeit. Hoewel er een minimaal effect op linker ventrikel ejectiefractie gezien werd, lijkt dit klinisch niet relevant, getuige het ook niet lijdt tot subjectieve verbetering voor de patiënt of objectieve verandering in de mortaliteit. Hoewel autologe, van beenmerg afkomstige cellen mogelijk meer effectief zijn in andere patiënten populaties (bijvoorbeeld ischemische cardiomyopathie of refractaire angina pectoris), bevelen wij aan om naar alternatieve behandelstrategieën te kijken. Andere celtypes, zoals mesenchymale stamcellen (MSCs), of van vetweefsel afkomstige stamcellen worden geëvalueerd als meer potente cellen. Het gebruik van allogene cellen van gezonde donoren kan heft effect van co-morbiditeit en variatie in kwaliteit wegnemen. Een andere hypothese is dat endogene stamcellen gestimuleerd kunnen worden om het hart te repareren. Hiertoe worden bepaalde cel-vrije therapieën nu geëvalueerd, zoals exosomen en microRNA. In dit proefschrift focussen we op het optimaliseren van toedieningsmethoden om cardiale retentie te vergroten en daarmee mogelijk cardiaal herstel te bewerkstelligen.

Huidige toedieningsmethoden betreffen vaak intracoronaire infusies, intramocardiale injecties en epicardiale injecties. Deze technieken hebben een beperkt effect, waar slechts 10-15% van de getransplanteerde cellen in het hart achterblijven.

In de **hoofdstukken 9 t/m 11** onderzoeken we of de toedieningsmethoden verbeterd kunnen worden om cardiale cel retentie te vergroten. Een voorgestelde methode hiervoor is het gebruik van retrograde infusies in de sinus coronarius. In **hoofdstuk 9** voeren we een systematische review uit, met zowel preklinische als klinische studies, om een overzicht te creëren over het huidige bewijs voor deze retrograde infusies en meer details te vergaren over gebruikte technieken en procedures. We ontdekten dat er veel variatie is in gebruikte technieken, procedures en resultaten en dat er geen overtuigende aanbevelingen over veiligheid en werkzaamheid kunnen worden getrokken uit deze studies. In **hoofdstuk 10** onderzochten we de veiligheid en werkzaamheid van retrograde infusies in een dierstudie met grote proefdieren. We toonden aan dat cardiale retentie na retrograde infusies via de sinus coronarius lager was in vergelijking met intracoronaire infusies. Bovendien was er vaker sprake van pericardeffusie en hematomen met een potentieel gevaar voor de patiënt. Daarom was ons advies om deze techniek niet naar een volgend onderzoekstadium te bevorderen. Deze studie zelf is niet gepreregistreerd, omdat er nog geen toegewijd platform beschikbaar was bij de start van de studie. We hebben het studie protocol wel gedeeld op www.preclinicaltrials.eu zodra het kon en zodoende was het protocol wel vindbaar voordat de studie resultaten werden gepubliceerd. In **hoofdstuk 11** hebben we onderzocht of een celdrager cardiale retentie kan vergroten. We ontwierpen een gerandomiseerde, gecontroleerde en geblindeerde dierstudie met grote proefdieren, waarbij we radioactief gelabelde cellen traceerden middels een scintigrafie van het gehele lichaam. We ontdekten onverwachte en potentieel gevaarlijke extracardiale haarden met radioactiviteit (“hotspots”) in het eerste varken. We herhaalden het experiment waarbij deze onverwachte bevinding bevestigd werd. We konden geen verklaring voor deze hotspots bevestigen en een gevaarlijke oorzaak zoals arteriële embolieën konden we niet uitsluiten. Omdat we de veiligheid van de procedure niet konden garanderen hebben we er voor gekozen om de studie te onderbreken. Ook viel op dat de retentie in de controle arm niet overeenkwam met eerdere bevindingen, waardoor de interne studie validiteit niet gewaarborgd was. In dit hoofdstuk delen we onze beperkte resultaten en exploreren mogelijke verklaringen voor onze bevindingen. Ondanks onze inzet voor meer transparant onderzoek, hebben we het studie protocol van deze studie pas gedeeld nadat de experimenten al waren uitgevoerd (preclinicaltrials.eu was nog niet beschikbaar ten tijde van de start van de studie) en zijn de studie resultaten onderworpen aan flinke time-leg bias. We hebben ons protocol geregistreerd op preclinicaltrials.eu (PCTE0000105) kort nadat de experimenten waren uitgevoerd, waardoor ons onderzoek wel vindbaar werd, en hebben daar aangegeven

dat de studie is stopgezet. De beste verklaring waarom we er 4 jaar voor nodig hebben gehad om de resultaten van de studie te delen is dat we zelf worstelde met het gebrek aan een goede verklaring voor de onverwachte resultaten. Daarbij hebben we aangenomen dat medische tijdschrijften niet geïnteresseerd waren in onze resultaten. Nadat ons manuscript was afgekeurd voor publicatie door een medisch tijdschrift hebben we er voor gekozen de data te delen op Bioarxiv.

Conclusie

Concluderend tonen we in dit proefschrift het belang van robuustheid, openheid en transparantie om de wetenschap te verbeteren. We hebben geleerd van onze ervaringen in cardiaal herstel, maar zwakheden in het ontwerp, uitvoeren en rapporteren van dierstudies worden ook gezien in andere onderzoeksvelden. We verstrekken duidelijke aanbevelingen voor meer robuuste studies en het vergroten van transparantie, om translationeel onderzoek te verbeteren. We ontwikkelden het eerste online register toegewijd aan de preregistratie van dierstudies om preregistratie van dierstudies te faciliteren en de discussie over preregistratie te intensiveren. Daarmee leverden we een bijdrage aan meer transparant en robuust onderzoek. Het blijft voor individuele onderzoekers moeilijk om deze aanbevelingen op te volgen, ondanks de toewijding en integriteit van onderzoekers en de groeiende behoefte om de kwaliteit in translationeel onderzoek te verbeteren. Wij geloven dat het tijd is voor de wetenschappelijke gemeenschap om hun verantwoordelijkheid te nemen naar nog robuuster preklinisch onderzoek. Medisch onderzoek heeft het doel om nieuwe therapieën te ontwikkelen, maar het verbeteren van onderzoek zou ook op de agenda moeten staan. Wij moedigen de wetenschappelijke gemeenschap aan om meer transparant te zijn en bij te dragen aan verbeterde wetenschap door verantwoord te handelen.

LIST OF PUBLICATIONS

1. Preregistration of animal research protocols: development and 3-year overview of preclinicaltrials.eu. Van der Naald M, Chamuleau SAJ, Menon JML, de Leeuw W, de Haan JJ, Duncker DJ, Wever KE. BMJ Open Sci. 2022 Mar 16;6(1):e100259. doi: 10.1136/bmjos-2021-100259. eCollection 2022. PMID: 35372701

2. Report of unexpected findings after cardiac stem cell injections in a preclinical model. Van der Naald M, van den Broek HT, Bemelmans JLM, Neef K, Bakker MH, Dankers PYW, Kraaijeveld AO, Chamuleau SAJ. Bioarxiv, 2021. https://doi.org/10.1101/2021.10.26.465939

3. A 3-year evaluation of preclinicaltrials.eu reveals room for improvement in preregistration of animal studies. Van der Naald M, Chamuleau SAJ, Menon JML, de Leeuw W, de Haan JJ, Duncker DJ, Wever KE. PLoS Biol, 2021 Sep 9;19(9):e3001397. doi: 10.1371/journal.pbio.3001397. eCollection 2021 Sep. PMID: 34499640

4. Publication rate in preclinical research: a plea for preregistration. Van der Naald M, Wenker S, Doevendans PA, Wever KE, Chamuleau SAJ. BMJ Open Science 2020. Aug 27;4(1):e100051. doi: 10.1136/bmjos-2019-100051. eCollection 2020. PMID: 35047690

5. Lower Retention after Retrograde Coronary Venous Infusion Compared to Intracoronary Infusion of Mesenchymal Stromal Cells in the Infarcted Porcine Myocardium. Gathier WA, van der Naald M, van Klarenbosch BR, Tuinenburg AE, Bemelmans JLM, Neef K, Sluijter JPG, van Slochteren FJ, Doevendans PA, Chamuleau SAJ. BMJ Open Science 2019;3:e000006.

6. Validation of a novel stand-alone software tool for image guided cardiac catheter therapy. Van Es R, van den Broek HT, van der Naald M, de Jong L, Nieuwenhuis E, Kraaijeveld AO, Doevendans PA, Chamuleau SA, van Slochteren FJ. Int J Cardiovasc Imaging. 2019 Feb;35(2):225-235.

7. Fracture of the sesamoid bone of the thumb: a case report and summary on literature. Van der Naald M, van der Naald N, van der Velde D, Schuurman A. JBJS Case Connector 2019 Jan-Mar;9(1):e10.

8. Retrograde Coronary Venous Infusion as a Delivery Strategy in Regenerative Cardiac Therapy. Gathier WA, van Ginker DJ, van der Naald M, van Slochteren FJ, Doevendans PA, Chamuleau SA. J Cardiovasc Transl Res. 2018; 11(3): 173-181

9. Translational research in cardiovascular repair: a call for a paradigm shift. Chamuleau SAJ*, van der Naald M*, Climent AM, Kraaijeveld A, Wever, KE, Duncker DJ, Fernández- Avilés Francisco, Bolli R. Circulation Research. 2018;122:310-318

10. Een online register voor preregistratie van dierstudies: www.preclinicaltrials.eu Nicoline Smit, Kimberley E Wever, Dirk J Duncker, Steven Chamuleau, Mira van der Naald. Biotechniek aug 2018, nummer 57/4

11. Standardized mean differences cause funnel plot distortion in publication bias assessments: the plot thickens. Zwetsloot PP, van der Naald M, Sena EA, Howells DW, Inthout J, de Groot JAH, Chamuleau SAJ, Macleod MR, Wever KE. eLife 2017 6 Article Number e24260

12. Stamceltherapie voor het beschadigde hart. Van der Naald M*, Tseng CCS*, Doevendans PA, Chamuleau SAJ. Ned Tijdschr Hematol 2016;13:95-100

13. Surgery and inhibitor development in hemophilia A: a systematic review. Eckhardt CL, van der Bom JG, van der Naald M, Peters M, Kamphuisen PW, Fijnvandraat K. J Thromb Haemost. 2011 Oct;9(10):1948-58.

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COMMENT IN SCIENCE

Millions of animals may be missing from scientific studies
Analysis at Dutch university suggests researchers are not reporting a large number of animal experiments

14 OCT 2020 BY DALMEET SINGH CHAWLA (doi: 10.1126/science.abf2669)

Most animals used in biomedical experiments are not accounted for in published papers, a first-of-itskind study suggests. The analysis found that only one-quarter of more than 5500 lab animals used over a 2-year period at one university in the Netherlands ended up being mentioned in a scientific paper afterward. The researchers believe the pattern could be similar at institutions around the world, resulting in potentially millions of animals disappearing from scientific studies.

"I think it's just outrageous that we have such a low rate of results published for the number of animals used," says Michael Schlüssel, a medical statistician at the University of Oxford who was not involved in the study. "If we only look for groundbreaking research, the evidence base won't be solid," he adds. And that could impact studies that may confirm or refute the benefits of certain drugs or medical interventions.

Scientists have long suspected that a considerable share of animal studies doesn't get published. That could be because the results aren't deemed interesting enough, or the study didn't find anything noteworthy. But many academics argue that such "negative" results are important and worth publishing, and that failing to do so constitutes publication bias.

Yet getting a handle on this problem has been hard because it's difficult to track how many animals scientists use—and what happens with them. Researchers usually list such details in applications for ethical approval, but those often remain confidential. For the new study, researchers asked scientists at three University Medical Center Utrecht (UMCU) departments for permission to review the study protocols they had filed with an animal ethics committee in 2008 and 2009. (They picked those years in part to be completely sure that the scientists had plenty of time to conduct and report the studies.) Then the team—led by Mira van der Naald, a doctoral student at UMCU—searched the medical literature for papers resulting from the work.

Of the approved studies, 46% were published as a full-text paper; if conference abstracts—short summaries of a talk or poster presented at a scientific meeting—were counted as well, 60% ended up being published. Yet out of the 5590 animals used in the studies, only 1471 were acknowledged in published papers and abstracts, the team reports in BMJ Open Science. Small animals, including mice, rats, and rabbits—which made up 90% of the total—were most often missing in action: Only 23% of them showed up in publications, versus 52% of sheep, dogs, and pigs.

The researchers also surveyed the scientists involved to find out why so many animals were missing. The most common reasons they gave were that the studies didn't achieve statistical significance, a controversial but commonly used threshold for publication; that the data were part of a pilot project; and that there were technical issues with the animal models.

But none of these is a valid excuse to not publish your findings in the scientific record, says study coauthor Kimberley Wever, a metascientist at Radboud University Medical Center. "All animal studies should be published, and all studies are valuable for the research community." Not publishing all research means other scientists may waste time, effort, and money redoing studies that have previously failed, Wever says. She adds that the trend likely holds up at institutions around the world and hopes other researchers will conduct similar studies.

"It's a very big issue," agrees Anita Bandrowski, an information scientist at the University of California, San Diego, who has created software that automatically scans published papers for details such as the sex of animals used in studies.

Van der Naald and her colleagues launched a potential remedy for the problem in 2018: preclinicaltrials.eu, the first online registry dedicated to animal research. (A similar registry, animalstudyregistry.org, was recently set up by the German Centre for the Protection of Laboratory Animals.) In these databases, researchers can share methodologies, protocols, and hypotheses before carrying out their experiments—a process called preregistration that has been gaining traction in the academic community in recent years.

The Dutch government has said it is sympathetic to the idea. But despite a 2018 motion in support of registration passed by the Dutch House of Representatives, the government has not made it compulsory yet.

NRC INTERVIEW

Proefdier belandt vaak niet in artikel - NRC 22 oktober 2020

Door onze redacteur - Gemma Venhuizen

Amsterdam. Verreweg de meeste proefdieren die voor biomedisch onderzoek worden gebruikt, belanden nooit in een wetenschappelijke publicatie. Dat concluderen onderzoekers van het UMC Utrecht en het Nijmeegse Radboud UMC in BMJ Open Science, op basis van Nederlandse gegevens. Bijna driekwart van de gebruikte proefdieren blijft onvermeld en zelfs bij onderzoek dat wél gepubliceerd wordt, worden niet alle gebruikte dieren in publicaties vermeld.

De Nederlandse onderzoekers inventariseerden van 67 proefdierstudies die in 2008 of 2009 bij het UMC Utrecht werden aangemeld hoeveel er uiteindelijk tot een wetenschappelijke publicatie leidden: dat waren er 31 (46 procent). Als conference abstracts (samenvattingen voor presentaties op congressen) ook werden meegerekend, dan lag het totale aantal op 40 (60 procent).

Daarmee raakt het artikel aan een veelbesproken punt: publicatiebias, het verschijnsel dat vooral studies worden gepubliceerd met positieve resultaten. Een studie die aantoont dat een behandeling werkt heeft meer kans gepubliceerd te worden dan een onderzoek dat geen effect vindt. Dat leidt tot discussie, omdat veel wetenschappers vinden dat zulke negatieve uitkomsten net zo goed naar buiten moeten worden gebracht.

Juist binnen het biomedisch onderzoek raakt die publicatiebias aan een ander heikel punt: het gebruik van proefdieren. Ook daarover is al jaren discussie en er wordt onderzocht hoe er met minder proefdieren kan worden gewerkt.

Wetenschappers die proefdieren willen gebruiken moeten daarvoor een aanvraag indienen bij een ethische commissie, waarin ze aangeven hoeveel dieren ze willen gebruiken en wat ze ermee doen. Dergelijke aanvragen zijn vertrouwelijk, maar de auteurs van de huidige publicatie kregen toestemming om de gegevens in te zien als per aanvraag ten minste één van de betrokken onderzoekers akkoord ging.

Zodoende kwamen ze uit bij de 67 aangemelde studies, die in totaal van 5.590 proefdieren gebruikmaakten. Daarvan werden uiteindelijk 1.471 dieren beschreven in de 40 publicaties en presentaties. In die 40 studies waren in totaal 4.402 proefdieren gebruikt.

Vooral kleine proefdieren (muizen, ratten en konijnen) komen er vaak bekaaid vanaf: in de publicaties werd gemiddeld maar zo'n 30 procent van deze dieren vermeld. Bij proeven met grotere dieren (varkens, honden, schapen) wordt iets meer dan 70 procent van de dieren vermeld. „We hebben niet in detail onderzocht waarom die dieren onvermeld zijn gebleven", vertellen auteurs Mira van der Naald (UMC Utrecht) en Kimberley Wever (Radboud Universiteit) aan de telefoon. „We wilden vooral

inventariseren hoeveel proefdierstudies uiteindelijk gepubliceerd werden."

Na hun analyse van de proefdieraantallen stuurden Van der Naald, Wever en hun collega's de onderzoekers van de 67 studies een vragenlijst, waarin ze onder meer vroegen wat de reden was dat een onderzoek niet werd gepubliceerd. Veelal ging het om het ontbreken van statistisch relevante positieve resultaten, of betrof het een verkennende pilotstudie. Maar ook dergelijke studies zijn waardevol voor de wetenschap, zegt Wever. "Neutrale data of pilotstudies hoeven geen reden te vormen om een onderzoek niet te publiceren."

Hoewel het huidige onderzoek alleen op de Nederlandse publicaties ingaat, vermoeden de auteurs dat in het buitenland soortgelijke situaties spelen. Wereldwijd zou het om miljoenen 'verdwenen' proefdieren kunnen gaan. Van der Naald: „Elk land kent zijn eigen richtlijnen".

Volgens de auteurs is het belangrijk om proefdieronderzoek wereldwijd transparanter te maken. Daartoe hebben ze in 2018 zelf een website gelanceerd, preclinicaltrials.eu. Daarop kunnen onderzoekers hun eigen proefdieronderzoek registreren voordat het is uitgevoerd. Wever: „De early adopters hebben zich al aangemeld, en we hopen dat steeds meer biomedici zich zullen aansluiten."

Volgens Frans Stafleu, universitair docent dierethiek aan de Universiteit Utrecht, haakt het onderzoek in op een huidige geloofwaardigheidscrisis in de wetenschap. „Het blijkt dat de meeste dierproeven niet goed te 'vertalen' zijn naar de mensen en één van de oorzaken daarvan is bias. Wetenschappers denken vaak dat ze boven elke vorm van bias verheven zijn, maar dat is dus niet zo. Zo'n preregistratie kan dan een goede stok achter de deur zijn om niet af te wijken van het oorspronkelijke plan. De auteurs van dit artikel laten dat mooi zien."

COMMENT IN NATURE

ANIMAL REGISTRIES AIM TO REDUCE BIAS

Some advocates are betting that documenting experimental plans online will improve animal research, but uptake has been slow.

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Millions of mice and rats are used in research each year. But one-third to one-half of animal experiments are never published, and of those that are, many are too poorly conducted to be reliable. Advocates for better animal research and reproducibility are promoting a strategy established in other fields to counter publication bias, improve investigations and increase transparency: study registries.

Registries ask researchers to detail their hypotheses, experimental strategy and analytical plans before studies begin. The intention is to prevent teams from simply cherry-picking significant or desirable findings and to supply the scientific community with a way of learning about experiments that would otherwise go unpublished.

The best-known registry, clinicaltrials.gov, has logged more than 300,000 human clinical trials since it launched in 2000, amid outrage over drug companies burying unfavourable clinical-trial results. Regulatory authorities around the world now require registration for drugs and devices approved for market, and medical journals require it for publication.

The Open Science Framework is an example of a voluntary registration system. Researchers, mainly psychologists and social scientists, input or 'preregister' research plans before starting a project, which they can keep private, or 'embargoed', for up to four years. More than 30,500 preregistrations have been entered since 2012, but few of these involve animals.

The first registry specifically set up for animal studies, preclinicaltrials.eu, was launched in April 2018. Registry co-founder Mira van der Naald and her colleagues at the University Medical Center Utrecht in the Netherlands were carrying out systematic reviews in cardiac regenerative medicine, and found themselves frustrated by the consistently poor quality of preclinical evidence. They felt a dedicated registry would help, and were surprised that none existed. "We thought, 'Hey, let's just start it. We're not getting anywhere just talking about it'."

Unknown to them, Germany's centre for the protection of laboratory animals, Bf3R in Berlin, had taken on a similar project. Animalstudyregistry.org launched in January. Together, the two registries have only a few dozen entries.

The registries use templates specifically designed for animal experiments, with fields for species as well as several experimental design parameters described in a set of reporting guidelines known as Animal Research: Reporting of In Vivo Experiments,

or ARRIVE. (In 2017, M.B. served on a working group to update these guidelines, which ask authors to state whether they have preregistered their experiment.) Curators at both registries review entries and can ask for more detail. Registration is open to researchers worldwide.

A tough sell

Malcolm Macleod, a stroke researcher at the University of Edinburgh, UK, who has documented research quality and bias in preclinical work, says that for journal editors and peer reviewers, registration can boost a study's credibility. "Registries and preregistration are pretty essential in terms of being able to demonstrate the rigour with which the research was done, and to reassure research users that you answered the questions that you set out to answer," he says.

But convincing researchers to use animal-study registries could prove to be a tough sell, he says. "We are going to ask a group of researchers who have not had any experience with this at all to suddenly change what they do." Researchers are used to communicating their work as a final manuscript that describes experiments and findings as if everything went to plan, notes physiologist Kieron Rooney, a registry advocate at the University of Sydney, Australia. "You don't see any battle scars of my project, where I had to change direction."

Scientists largely agree that registration would yield communal advantages by reducing cherrypicking, publication bias and duplication, says Daniel Strech, a bioethicist at Charité Medical University in Berlin who studies animal researchers' attitudes to study registration. But they also worry about individual disadvantages such as increased administrative burden, the possibility of having their ideas stolen and being targeted by animal-rights activists (S. Wieschowski et al. PLoS Biol. 14, e2000391; 2016). "They think, on average, animal registries will have no impact on efficiencies," Strech says.
Researchers who have submitted protocols to their animal-ethics committees or funding agencies can simply paste relevant portions into the registry, upload supporting files (animalstudyregistry.org) or provide URLs (preclinicaltrials.eu). Finalized registrations are time-stamped, but researchers can add annotations to explain deviations from the plan, or to flag that further experiments have been done. The registries also provide secure embargo periods.

Still unclear, however, is which types of study should be registered. Bf3R head Gilbert Schönfelder encourages researchers to log any study requiring approval from an institute's ethical advisory board. This helps to advance the ethical aim that any experiment using animals should increase the overall level of knowledge. Bioethicist Jonathan Kimmelman at McGill University in Montreal, Canada, counters that the push should be for researchers doing preclinical trials — highly structured studies that serve as the basis for deciding whether to test a drug in people.

To Macleod, the optimal registration process would target confirmatory studies that set out to test (rather than generate) hypotheses and require no more than half an hour to complete, even if that means omitting some details. If researchers documented half a dozen items including the hypothesis, experimental intervention, primary outcome and how it will be measured, and statistical parameters, "you deal with 95% of the problems that arise". It would also increase the number of entries for those studies in which registration is most advantageous, he says. "If people get 90% of the benefits for ten minutes, I think that would be much more likely to happen than getting 100% of the benefits for two hours."

Also worth logging are animal housing and handling details, says Adrian Smith, secretary of Norecopa, an organization in Oslo that aims to improve and reduce the use of animals in research. Isolating mice or picking them up by the tail can strongly impact certain types of study, he notes. "It is unthinkable to try and solve the reproducibility crisis without also attending to these 'nonmathematical' factors."

Broad participation and fully described experiments are key, says Deborah Zarin, who from 2005 to 2018 directed clinicaltrials.gov. Yet it could prove difficult to get researchers to provide sufficient detail in their registrations to really know whether they are cherry-picking results, she warns. Also, the fewer researchers who participate in a registry, the less valuable it will be for helping others to identify collaborators, or to know whether anyone else has tried to address similar questions. And separate, uncoordinated registries will make searching for particular kinds of study inefficient, further undermining their use.

Even incomplete registries could promote "good researcher hygiene" that would improve individual studies, says Kimmelman. Still, the availability of a registry is just one piece of the puzzle, says Manoj Lalu, an anaesthesiologist at Ottawa Hospital Research Institute, who is working to improve translational research. Many researchers do not understand why techniques to reduce bias are necessary or how they should be implemented. This means that even if they do register a study, they might do so inaccurately. Thus, registries must be combined with educational resources, he says. Incentives are also essential, adds Roberta Scherer, who studies clinical-trial methodology at Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland. "If researchers go to the site, they may become educated, but they have to get there first." Funders, journals and institutions will have to require or reward registration for it to become common practice, she predicts.

Rooney says that a better strategy would be to show that registries can benefit researchers by helping them to find collaborators or determine whether and how to repeat studies other researchers have tried. "We have to say we want this not because we want to make science difficult, but because we want to fix some issues," he says. "Give it a few years, and it just becomes part of the process."

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CURRICULUM VITAE

Mira van der Naald was born on August 26th, 1988 in Amsterdam, to Wytze van der Naald and Astrid van de Ven. She grew up in the city together with her older brother Niels. In 2005 she graduated from the Montessori Lyceum Amsterdam and first started studying biomedical sciences at the University of Amsterdam. After one year she switched to medicine at the Academic Medical Centre in Amsterdam, where she became interested in cardiology. She did her research rotation at the department of vascular medicine which grew her interest for science and led to her first scientific publication. In 2014 she obtained a position as a PhD candidate at the cardiology department of the University Medical Centre Utrecht. Apart from this dissertation, she was involved in the development of www.preclinicaltrials.eu, an international platform for preregistration of animal studies. Mira has been in the steering committee of this platform since 2017. In November 2022 she will start her residence in cardiology at the University Medical Centre Utrecht.