Trends in Cardiovascular Medicine 32 (2022) 127–135

Contents lists available at ScienceDirect

Trends in Cardiovascular Medicine

journal homepage: www.elsevier.com/locate/tcm

Translational insights from single-cell technologies across the cardiovascular disease continuum

Irene V. van Blokland[a,b], Hilde E. Groot[a], Lude H. Franke[b], Monique G.P. van der Wijst[b,1], Pim van der Harst[b,c,*]

[a] Department of Cardiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, the Netherlands
[b] Department of Genetics, University of Groningen, University Medical Center Groningen, Oncode Institute, Groningen, the Netherlands
[c] Department of Cardiology, University Medical Centre Utrecht, Utrecht, the Netherlands

ARTICLE INFO

Key words:
ScRNA-seq
scATAC-seq
coronary artery disease
myocardial infarction
heart failure
inflammation

ABSTRACT

Cardiovascular disease is the leading cause of death worldwide. The societal health burden it represents can be reduced by taking preventive measures and developing more effective therapies. Reaching these goals, however, requires a better understanding of the pathophysiological processes leading to and occurring in the diseased heart. In the last 5 years, several biological advances applying single-cell technologies have enabled researchers to study cardiovascular diseases with unprecedented resolution. This has produced many new insights into how specific cell types change their gene expression level, activation status and potential cellular interactions with the development of cardiovascular disease, but a comprehensive overview of the clinical implications of these findings is lacking. In this review, we summarize and discuss these recent advances and the promise of single-cell technologies from a translational perspective across the cardiovascular disease continuum, covering both animal and human studies, and explore the future directions of the field.

© 2021 The Authors. Published by Elsevier Inc.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

Nonstandard abbreviations and acronyms

ApoE$^{-/-}$, apolipoprotein E knockout; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CAD, coronary artery disease; CANTOS, Canakinumab Anti-Inflammatory Thrombosis Outcome Study; CCR2, C-C chemokine receptor 2; CF, cardiac fibroblasts; CKAP4, cytoskeleton-associated protein 4; CM, cardiomyocyte; CVD, cardiovascular disease; CyTOF, cytometry by time-of-flight; EC3, endothelial cells cluster 3; eQTL, expression quantitative trait loci; FACS, fluorescence-activated cell sorting; F-Trans, fibroblast-transitory; F-Wnt-X, fibroblast-Wnt expressing; HF, heart failure; IL-1β, interleukin 1β; iPSCs, induced pluripotent stem-cells; iPSC-CFs, iPSC-cardiac fibroblasts; LAD, left anterior descending; *Ldlr*$^{-/-}$, low-density lipoprotein receptor knockout; MI, myocardial infarction; NPR1, natriuretic peptide receptor-1; RUNX1, runt-related transcription factor 1; Sca1, Stem Cell Antigen 1; sc/snRNA-seq, single-cell/single-nucleus RNA sequencing; scATAC-seq, single-cell Assay for Transposase-Accessible Chromatin sequencing; TREM2^{hi}, Triggering Receptor Expressed on Myeloid cells 2; VSC, vascular stem cells; VSMCs, vascular smooth muscle cells; Wif1, WNT inhibitory factor 1

Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in Western society [1]. CVD has a wide spectrum of severity, ranging from coronary artery disease (CAD) and myocardial infarction (MI) all the way to ischemic heart failure (ischemic HF) [2]. Across this spectrum, various cell types play important roles in the disease, and these include not only cells located in and around the heart (cardiomyocytes, fibroblasts and adipose cells) but also circulatory immune cells or vascular smooth muscle cells (VSMCs) [3,4]. Understanding the contributions of these cells across the CVD spectrum can help improve treatment and even prevent CVD in the future. For example, the recent Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) trial highlighted the importance of targeting non-cardiac

* Corresponding author at: Department of Cardiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, the Netherlands.
E-mail addresses: i.v.van.blokland@umcg.nl (I.V. van Blokland), p.van.der.harst@umcg.nl (P. van der Harst).
[1] Shared last author.

https://doi.org/10.1016/j.tcm.2021.02.009
1050-1738/© 2021 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

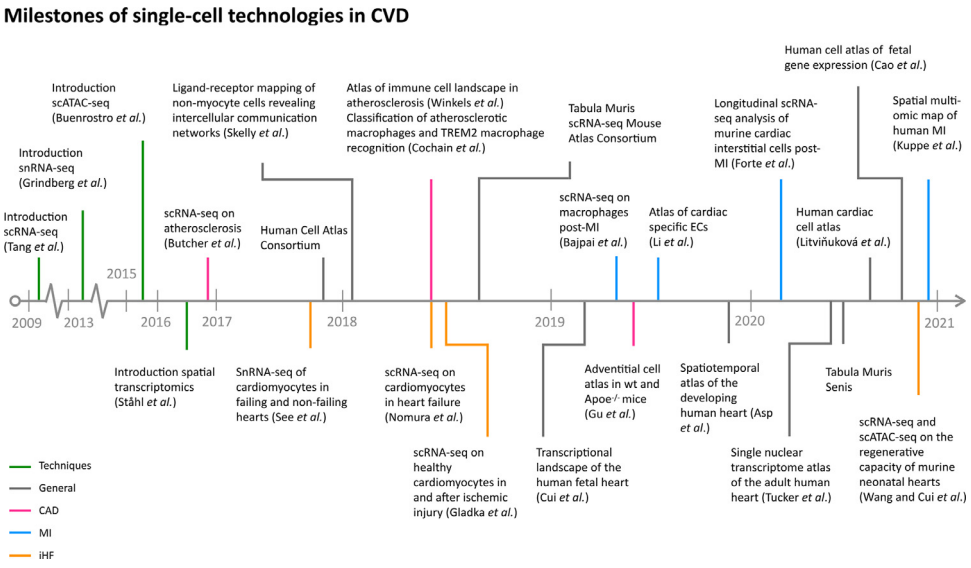

Fig. 1. Timeline of single-cell technology milestones that have helped increase our understanding the CVD continuum. *TREM2*: Triggering Receptor Expressed on Myeloid cells 2, *MI*: Myocardial infarction, *ECs*: Endothelial cells, *wt*: Wild type, *CVPs*: Cardiovascular progenitor cells, *HF*: Heart failure.

cells as an effective route to reducing morbidity; inhibiting immune cell interleukin-1β (IL-1β) signaling in patients with previous MI using the monoclonal antibody Canakinumab led to a significantly lower cardiovascular re-event rate [5].

Over the past five years, researchers have gained a new experimental toolbox to dissect the role and interactions of individual cell types in health and disease. Single-cell techniques such as single-cell RNA sequencing and single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq) now enable systematic and unbiased mapping of potentially every single cell in the body [6,7]. Previously, the characterization of cell types and cell subtypes was either limited in resolution or biased by previous knowledge (i.e. limited to a small set of marker proteins used in techniques as fluorescence-activated cell sorting (FACS) or cytometry by time-of-flight (CyTOF) [8–10]). Single-cell technologies have since the emergence in 2009 become more widely adopted as their scale and cost have improved tremendously [11]. Methodological improvements like combinatorial indexing [12,13] in combination with sample multiplexing using natural genetic barcodes [14] or strategies like hashtagging [15] now allow cost-efficient processing of up to 100,000 s of cells in a single experimental run.

Single-cell technologies can be used to address a variety of questions, from uncovering the involvement of cell types in CVD to determining how gene expression and its regulation become dysregulated in disease [11,16,17]. However, to study CVD at the single cell–level, we first need to understand the healthy heart and its normal variation within the population (i.e. due to genetics, environment, aging). In the last 5 years, various consortia have been established that focus on creating such reference maps in healthy mice (Tabula Muris) [18–20] and humans (Human Cell Atlas [7,17] (Box 1, Fig. 1, Table 1). With the aid of these healthy reference maps, it is now becoming easier to define disease states of, for example, the heart or the immune system, and we discuss several ongoing efforts that are relevant for CVD in more detail in this review [19–35].

Here, we summarize how the application of single-cell technologies has led to new disease insights across the CVD continuum on both the cellular- and transcriptional-level. More specifically, we discuss the literature in three sections that cover the entire disease spectrum with increasing severity, from CAD to MI to ischemic HF. Finally, we discuss the advances and promise of single-cell work from a translational perspective.

Coronary artery disease

The most common form of CAD is atherosclerosis, where plaque formation in the coronary artery eventually limits blood flow and thereby the supply of nutrients and oxygen to cardiomyocytes. Coronary atherosclerosis is considered to be an inflammatory disease, involving a plethora of inflammatory processes in the artery wall, and is associated with both pro- and anti-inflammatory cytokines and leukocytes [36,37]. Single-cell technologies provide valuable tools to identify which cell types drive plaque formation and progression, which facilitate the development of novel therapeutic strategies to halt or even reverse plaque formation [38,39].

The most important mouse models to study atherosclerosis are single- or double-knockouts of the apolipoprotein E (*ApoE-/-*) gene and the low-density lipoprotein receptor (*Ldlr-/-*) gene (Fig. 1) [34,37,39–44]. Winkels et al. used *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice to study atherosclerosis by harvesting cells from the whole thoracic and abdominal aorta (Fig. 1). The cellular fraction consisted of cells from the atherosclerotic lesion, aortic adventitia and media. They used scRNA-seq analysis to trace cells belonging to the atherosclerotic plaques and identified macrophages, T-cells and monocytes as the main constituents [37]. To translate their observations to human pathophysiology, Winkels et al. applied CyTOF to leukocytes from human carotid endarterectomy tissue specimens, which revealed that the cellular composition of human atherosclerotic plaques is similar to that of mice. Finally, to provide evidence for the clinical relevance of their findings, they associated immune cell type frequencies with the rate of ischemic events post-endarterectomy. Interestingly, this showed that macrophages were associated with a higher risk of ischemic events, whereas memory T-cells were associated with a lower risk. Altogether, the Winkels et al. study showed that combining data from surface markers and the expression of genes can be a powerful tool to further understand atherosclerosis and might provide information for risk stratification [37].

Subsequent scRNA-seq data from Cochain et al., who profiled leukocytes during different stages of atherosclerosis using the same mouse models as Winkels et al., identified monocytes and dendritic cells in intermediate to advanced lesions and a novel set of TREM2^{hi} (expressing triggering receptor expressed on myeloid cells 2) macrophages that was only present in advanced atherosclerosis (Figs. 1, 2) [34]. Using immuno-

Table 1
Summary of main studies applying single-cell RNA sequencing to cardiovascular disease.

Study	Study design	Study population/tissue	Outcome measure	Main findings
Bajpai et al. (2019)	Animal research	Cardiomyocytes of mouse strains *Rosa26-td, Rosa26-YFP, Flt3-Cre,CX3CR1^{ertCre}, CCR2^{GFP}, CD169-DTR, CCR2-DTR, Myd88^{flox}, LysM-Cre, Zbtb46^{GFP}* and *Tnnt2-DTR*	Infarct expansion, LV systolic dysfunction, LV dilatation and atherosclerotic plaque progression	Identification of one monocyte cluster and 7 clusters of macrophages of which a set of cardiac-resident CCR2^{+} macrophages that are responsible for recruitment of monocytes to injured myocardium which can serve as a therapeutic target post-MI. Tissue-resident CCR2^{-} macrophages oppose the recruitment.
Cochain et al. (2018)	Animal research	Mouse aortas of chow and western diet fed (11 w) *Ldr*$^{-/-}$ mice and western diet (12 w) of *Apoe*$^{-/-}$ mice	Aortic macrophage heterogeneity in atherosclerosis	Identification of arterial leukocytes in atherosclerosis revealing three macrophage populations of which one previously unrecognized atherosclerosis-associated subset expressing *TREM2*.
Depuydt et al., (2020)	Human research	Advanced human atherosclerotic plaques	Cell type classification	Full cellular composition of human carotid plaques with atherosclerotic plaque cell type classification and cell communication.
Dobnikar et al. (2018)	Animal research	Mouse aortas *of Myh11-CreERt2/Confetti/Apoe*$^{-/-}$ mice	Molecular differences underlying clonal expansion in disease	Characterization of Sca1 as hallmark of phenotypic switching of VSMCs towards an inflammatory phenotype in atherosclerosis.
Farbehi et al. (2019)	Animal research	Cardiac interstitial cells of *Pdgfra*$^{trm11(EGFP)Sor}$ *PDGFRa*$^{GFP/+}$ mouse models	Understanding of non-CM fraction in response to injury	Identification of 9 cellular lineages and 24 cellular populations of which the novel cellular fibroblast subset (F-Wnt-X) expressing the gene *Wif1*.
Flores et al. (2020)	Animal research	Mouse aortas of *Apoe*$^{-/-}$ mice on a *C57BL/6* background	Expression of inflammatory genes	The use of nanoparticles to promote the clearance of apoptotic cells in atherosclerosis and decrease inflammation.
Forte et al. (2020)	Animal research	Interstitial cardiac cells of mice of nine different inbred strains	Cardiac interstitial gene expression response to acute and chronic phase after MI	Characterization of cardiac interstitial cells over time post-MI, with identification of cardiac fibroblasts that transition early on in response to injury which serve as a critical risk for cardiac rupture and pathological remodeling post-MI.
Gladka et al. (2018)	Animal research	CMs of mice on a *C57BL/6J* background having undergone ischemia reperfusion or control	Novel insights in fibroblast activation	Identification of cardiac subpopulations and the previously undescribed CKAP4 marker for activated fibroblasts in the injured heart.
Kuppe et al., (2020)	Human research	Transplanted adult hearts 2–5d, 3 months and 12years post-MI and non-transplanted donor control heart	Gene-regulatory programs involved post-MI	Characterization and visualization of cellular zones of injury, repair and remodeling post-MI. Identification of RUNX1 as driver of cardiac fibrosis post-MI in the differentiation of cardiac fibroblasts to myofibroblasts.
Skelly et al. (2018)	Animal research	Cardiac non-myocyte cells of *C57BL/6J* mice	Intercellular communication	Profiling of non-myocyte cells in the adult murine heart and characterization of intercellular communication networks by ligand–receptor analysis.
Tang et al. (2020)	Animal research	Mouse femoral arteries of lines *Sca1-CreER, Sca1-CreER;R26-tdTomato, CD45- Dre* and *Pdgfrb-LSL-Dre* mice	Artery repair	Identification of Sca1^{+} VSCs in the adventitial layer of artery walls that produce new smooth muscle cells after vessel injury.
Wang et al. (2020)	Human tissue analysis	Primary human CMs isolated from the ventricles and atria of HF patients with dilated cardiomyopathy and coronary heart disease and healthy controls	Cardiac function	Recognition of five major groups of cells and 35 subclusters, of which endothelial cell cluster 3 is an important mediator in the pathophysiology of coronary heart failure.
Wang and Cui et al. (2020)	Animal research	Pups from timed-pregnant ICR/CD-1 mice (Charles River Laboratories)	Injury response	Identification of gene regulatory networks and crosstalk of cell populations during regeneration and identification of CLCF1 as promoter of cardiomyocyte proliferation in neonatal murine hearts.
Winkels et al. (2018)	Animal research	Mouse aortas of chow and western diet fed *Apoe*$^{-/-}$ and *Ldr*$^{-/-}$ mice	Immune cell characterization in atherosclerosis	Identification of arterial leukocytes in atherosclerosis, with the main components being macrophages, T-cells and monocytes. Also identified heterogenous clusters of arterial leukocytes behaving differentially in healthy and atherosclerotic arteries.
Zhang *et al.* (2019)	Human stem cell research	iPSC-CFs and iPSC-CMs	Cardiac fibrosis and drug screening	Generation of iPSC-CFs through knowledge gained on scRNA-seq data of mouse models, supports the possibility of generating patient-specific iPSC-CFs to test effectiveness of drugs *in vitro*. Identification of the anti-fibrotic ANP/BNP-NPR1 pathway that can serve useful in anti-fibrotic therapy.

AECs: arterial endothelial cells, *CCR2*: C-C chemokine receptor 2, *cHF*: coronary HF, *CMs*: cardiomyocytes, *CKAP4*: cytoskeleton-associated protein, *CVPs*: cardiovascular progenitor cells, *dHF*: dilated HF, *ECs*: endothelial cells, *hESCs*: human embryonic stem cells, *hPSCs*: human pluripotent stem cells, *LV*: left ventricle, *iPSC-CF*: human induced pluripotent stem cell-derived cardiac fibroblasts, *iPSC-CMs*: human induced pluripotent stem cell-derived cardiac myocytes, *TREM2*: Triggering Receptor Expressed on Myeloid cells 2, *VSMCs*: vascular smooth muscle cells.

Box 1
Resources for single-cell and spatial transcriptomic atlases in healthy tissue and CVD.

	Healthy	Diseased
Mouse	**Adult** - Tabula Muris (Schaum et al.) - Tabula Muris Senis (Almanzar et al.) - Mouse cell atlas by microcell-sequencing (Han et al.) - Chromatin accessibility single-cell atlas (Cusanovich et al.)	**Fetal** - Atlas of regenerative hearts after injury (Wang and Cui et al.) **Adult** - Atlas of immune cell repertoire in atherosclerosis (Winkels et al., Cochain et al.) - Atlas of non-CM cells post-MI (Farbehi et al.) - Atlas of cardiac interstitial cells post-MI (Forte et al.) - Atlas of cardiac cells post-injury (Gladka et al.)
Human	**Fetal** - Transcriptional landscape of the human fetal heart (Cui et al.) - DESCARTES: A human cell atlas of fetal gene expression (Cao et al.) - Spatiotemporal atlas of the developing human heart (Asp et al.) **Adult** - Single-nuclear transcriptome atlas of the human heart (Tucker et al.) - Adult human cardiac cell atlas (Litviňuková et al.)	**Adult** - Atherosclerotic plaque transcriptome atlas (Depuydt et al.) - Spatial multi-omic map of MI (Kuppe et al.) - Transcriptome atlas of ischemic HF (Wang et al., Nat Cell Biol 2020)

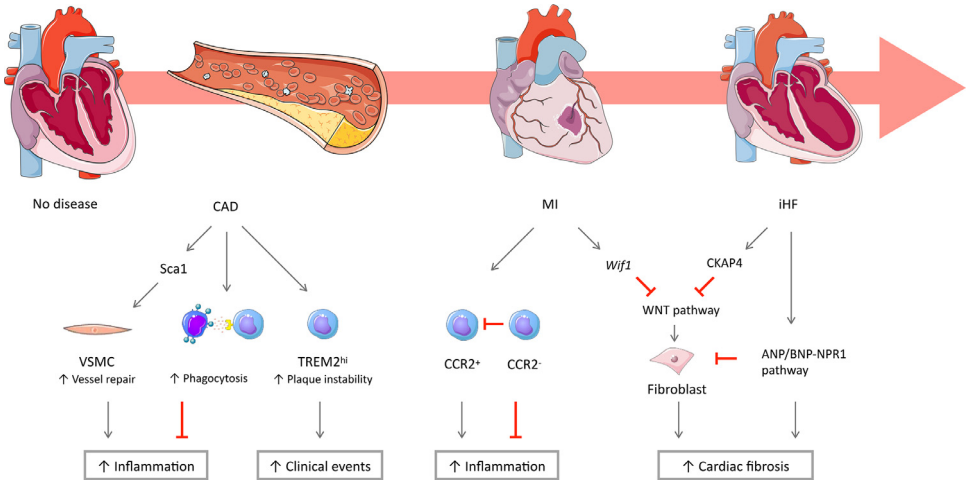

Fig. 2. Overview of novel insights in the pathophysiology across the CVD continuum, identified through the use of single-cell technologies. In CAD, a Sca1 expressing SMC population gives rise to VSMCs facilitating repair, inflammatory monocytes show upregulation of CD47 which diminishes their ability to phagocyte apoptotic cells and the presence of TREM2 macrophages in advanced atherosclerotic lesions led to more plaque instability. In MI, tissue resident CCR2^{+} macrophages mediate neutrophil recruitment and depletion of CCR2^{-} led to higher levels of CCR2^{+} macrophages post-MI and cardiac fibroblasts show involvement in the WNT pathway. In ischemic HF, the fibroblast population expressing CKAP4 is thought to inhibit the WNT pathway and the ANP/BNP-NPR1 stimulates fibrosis. *ANP/BNP-NPR1 pathway*: Atrial natriuretic peptide/brain natriuretic peptide and natriuretic peptide receptor-1, *CAD*: coronary artery disease, *CCR2*: C–C chemokine receptor 2, *CKAP4*: cytoskeleton-associated protein 4, *ECs*: endothelial cells, *iHF*: ischemic heart failure, *MI*: myocardial infarction, *Sca1*: multipotent progenitor marker, *TREM2*: Triggering Receptor Expressed on Myeloid cells 2, *VSMCs*: vascular smooth muscle cells, *Wif1*: WNT inhibitory factor 1.

histochemistry on human endarterectomy carotid artery tissue samples of high-grade (>70%) carotid artery stenosis, they confirmed the presence of TREM2^{hi} macrophages in human samples [34]. Reassuringly, a recent study in advanced human carotid atherosclerotic plaques showed that mouse TREM2^{hi} macrophages resemble foamy TREM2^{+} macrophages in humans [22], indicating that the pathophysiology mimicked in mice captures important biological processes of human atherosclerosis [22]. The TREM2^{hi} macrophages are of interest in the pathophysiology because they generate micro-calcifications in plaques and are associated with plaque instability in mice [45,46]. The level of TREM2^{hi} macrophages could therefore be a potential biomarker to predict the stage of atherosclerosis and the risk of clinical events.

Another hallmark of the pathophysiology occurring during atherosclerosis is the recruitment of inflammatory monocytes. These inflammatory monocytes show upregulation of the antiphagocytic signaling molecule CD47 [47]. When macrophages bind to CD47 on inflammatory monocytes, their ability to phagocytose apoptotic cells is suppressed [48]. This could be the underlying mechanism for the TREM2^{hi} macrophages recently recognized by Cochain et al., which exhibited suppressed the phagocytotic function associated with monocyte infiltration [34]. As a result of the inability to phagocyte, monocytes and macrophages accumulate in the plaque core, become apoptotic and can cause plaque rupture and thrombus formation [49]. Clearing dead cells from the necrotic atherosclerotic core could therefore be a strategy to reduce the risk of plaque rupture. Flores et al. successfully intro-

duced such a strategy, using nanoparticles to target an antiphagocytic pathway and thereby restoring phagocytosis of apoptotic cells in the atherosclerotic lesions of *ApoE*$^{-/-}$ mice (Fig. 2) [48]. ScRNA-seq analysis of aortic cells treated with the nanoparticles showed reduced cytokine-dependent vascular inflammation. The advantage of this more localized approach to suppressing lesional inflammation, as compared to the systemic anti-inflammatory inhibition using interleukin 1β (IL-1β) applied in the CANTOS trial [5], is that it minimizes severe side effects such as the immunosuppression leading to fatal infections seen in the CANTOS trial [5,48]. Although it is still early days for this approach, restoring phagocytosis of apoptotic cells could be the first step towards a targeted therapy that effectively clears out those cells that contribute to the inflammation and instability of atherosclerotic lesions.

Along with specific immune cells, specific VSMCs also accumulate during the early stages of plaque progression [50–53]. ScRNA-seq analysis of VSMCs harvested from the medial aortic layer of *ApoE*$^{-/-}$ and healthy mice revealed a new subset of VSMCs that express the multipotent progenitor marker Stem Cell Antigen 1 (Sca1), which was upregulated in atherosclerotic plaques (Fig. 2) [51]. This upregulation initiated the phenotypic switching of VSMCs towards a macrophage-like phenotype [54] and stimulated expression of genes associated with wound healing and the migration of more VSMCs through recruitment [51]. To find the origin of the VSMC recruitment, Tang et al. applied scRNA-seq to cells from the femoral artery of mouse models. This revealed that Sca1 was mainly expressed by adventitial cells and upregulation of pathways regulating differentiation, proliferation and migration of smooth muscle cells (SMCs) [55]. To investigate whether Sca1+ cells give rise to SMCs, the authors used a fluorescent-labeled arterial anastomosis model exposed to severe vessel injury to confirm that adventitial stromal Sca1+ cells initiate a repair response upon injury, giving rise to VSMCs in the medial layer [55]. Interestingly, this study further revealed that Sca1+ cells do not contribute to VSMCs upon homeostasis and wire injury. Pre-existing SMCs may facilitate repair under normal conditions and wire injury, while adverse injury that disrupts the lamina of the adventitia may facilitate migration of Sca1+ stem cells, giving rise to VSMCs in the medial layer [55]. The phenotypic switching of VSMCs could be an important contributing factor in the progression of the atherosclerotic plaque. The extent of Sca1 upregulation may thus serve as a biomarker of disease progression. Further study on the clinical impact of Sca1 in CVD will determine whether it is a suitable drug target.

Myocardial infarction

When CAD progresses, the occlusion of the coronary lumen will reach a state in which insufficient oxygen and nutrients reach the heart muscle. Eventually, occlusion can be complete when a thrombus is formed at the damaged endothelium, resulting in an acute MI. In this stage, a cascade of inflammatory pathways is activated that trigger cardiac repair and remodeling [56]. The size of the MI depends on the location of the occlusion and probably also on the severity of the inflammatory response. This excessive remodeling may lead to non-contracting scar tissue that reduces the heartś pump function and can lead to clinical signs and symptoms of HF [5,56–59]. Studying the immune system in MI is therefore important for understand how best to balance the inflammatory processes in order to induce repair while preventing excessive remodeling, thereby potentially identifying targets for therapy that deter MI progression to ischemic HF.

Post-MI, leukocytes are recruited from circulation and extend ischemia in the heart [56,60–62]. Based on previous knowledge, several potential drug targets have already been identified that may alleviate morbidity post-MI. For example, the pro-inflammatory cytokines IL-1β and IL-6 have been recognized as potential targets because they are upregulated post-MI and blocking of IL-1β leads to a lower re-event rate of cardiovascular events [5,56]. Moreover, the excessive inflammation, which can lead to scar tissue formation, can be reduced by inhibiting the C-C chemokine receptor 2 (CCR2) on macrophages [63–66]. Tissue-resident CCR2^{+} macrophages mediate neutrophil recruitment into the myocardium, so inhibiting CCR2 could reduce this process [67]. Despite this growing body of knowledge, it remains unclear how individual cell types are involved in a MI. Below, we discuss how new insights into the inflammatory and fibrotic response post-MI are obtained using various single-cell analysis techniques (Fig. 1).

To study the differentiation and recruitment of cells, Bajpai et al. recently performed scRNA-seq analysis on isolated monocytes and macrophages in mouse models 4 days post an MI induced through occlusion of the left anterior descending coronary artery (LAD) (Fig. 1, 2) [65]. Cell clustering based on expression markers revealed one group of monocytes and seven different groups of macrophages [65]. Interestingly, depletion of CCR2^{+} macrophages prior to induction of MI led to lower numbers of monocytes being recruited to the heart [65]. In contrast, depletion of the CCR2^{-} macrophages resulted in increased numbers of CCR2^{+} macrophages in the heart. This work confirmed the heterogeneity of macrophages in the heart post-MI and specifically how CCR2^{+} and CCR2^{-} macrophages mediate the inflammatory response. By combining the single-cell expression levels of ligands and receptors of these cell types, future work could help to further elucidate the cellular interactions that underlie the inflammatory process following an MI [68]. This may lead to the development of drugs that target specific post-MI ligand–receptor interactions between cells, moving towards the field of precision medicine.

As a main component of the heart, cardiac interstitial cells are an important cell type to study when looking at the impact of the fibrotic and inflammatory response after MI. Farbehi et al. recently applied scRNA-seq analysis to cardiac interstitial cells of the ventricles and interventricular septum of mice 3 and 7 days after induction of a MI through ligation of the LAD and of healthy control mice [35]. They identified nine major cell types and 24 subtypes consisting of fibroblasts/ myofibroblasts, endothelial cells, mural cells, macrophages, dendritic cells, glial cells, B-cells, T-cells and natural killer cells [35]. Within the fibroblast subset, they further identified two novel populations, Fibroblast-Wnt expressing (F-Wnt-X) and Fibroblast-transitory (F-Trans), present in both MI-induced and control mice [35]. Differential gene expression analysis on F-Wnt-X cells showed upregulation of WNT inhibitory factor 1 (*Wif1*), which has been suggested to inhibit cardiac fibrosis while being essential in cardiac repair post-MI, whereas the F-Trans population appeared to be an intermediate fibroblast state proliferating towards F-Wnt-X fibroblasts [35,69,70]. To visualize the location of these F-Wnt-X cells, they performed immunofluorescence on the WIF1 protein in mouse hearts. This revealed expression in the infarct border zone 3 days post-MI but not 1 or 7 days post-MI. These results contrast with those from an earlier study on WIF1 expression post-MI using FACS sorting and bulk RNA-seq, in which elevated levels were already observed 1 day after a MI [71]. This difference may be explained by the immunochemistry method underestimating the number of cells expressing WIF1. Whether WIF1 expression occurs in the acute phase post-MI in response to myocardial injury remains to be further elucidated. Recently, Forte et al. generated a longitudinal single-cell dataset of cardiac interstitial cells in response to cardiac injury [21] and identified that the WNT-X fibroblasts originate from endocardial-derived fibroblasts and are present at homeostasis throughout injury. Using longitudinal single-cell data, they revealed that cardiac injury induces phenotypic switching of epicardial-derived fibroblasts to myofibroblasts, a population that does not express *Wif1*,

which serves as a critical risk for cardiac rupture and pathological remodeling [21]. The additional resolution gained by this study highlights how longitudinal single-cell data is a valuable tool to gain new insights into CVD pathophysiology [21].

Ischemic heart failure

Ischemic HF has been suggested to result from the inflammatory response after a MI and is characterized by systolic dysfunction and reduced cardiac output [26,56,58,72,73]. Ischemic HF occurs due to an imbalance in oxygen supply and demand in the cardiac muscle, where cardiac repair and remodeling are ongoing processes [26,56,58,72,73]. Although the prognosis for ischemic HF has improved in the past few years, additional therapies are still needed [74]. Animal and human studies using single-cell technologies are now advancing our understanding of ischemic HF (Fig. 1).

Fibrotic remodeling is a major component leading to the development of ischemic HF. The activated fibroblasts involved in this remodeling can generate scar tissue, which reduces myocardial contractility [56]. Gladka et al. performed scRNA-seq analysis on ischemic myocardial cells from mouse models of ischemic HF after ligation of the LAD and identified a novel fibroblast population that is activated after ischemic injury and actively expresses cytoskeleton-associated protein 4 (CKAP4) (Fig. 1, 2) [26]. Earlier studies had suggested that CKAP4 may act through inhibition of WNT signaling, as was previously shown in pancreatic and lung cancers. Notably, this is the same pathway through which the *Wif1* gene inhibited monocyte activation and initiation of repair by activated fibroblasts after ischemic injury [71,75]. These studies hint at a more general role for WNT signaling in tissue repair post cardiac event and provide interesting leads for the development of novel drug targets.

To make this next step towards clinical translation, we need to study cardiac fibroblasts (CFs) in response to various conditions and drugs. However, this advance is currently hampered by the unknown translatability of study outcomes from murine models to human pathophysiology. Furthermore, the availability of primary human CFs is limited because they require isolation from ventricles of the human adult heart. Alternatively, stem cells could be differentiated to CFs as an *in vitro* model, but this has been challenging to date. Using the knowledge gained from the scRNA-seq mouse atlas Tabula Muris (**Box 1 – resources**), Zhang et al. characterized fibroblast-specific genes that were potentially also specific for human fibroblasts (Fig. 1) [20,76]. These were then successfully used to develop a protocol to differentiate human induced pluripotent stem-cells (iPSCs) towards iPSC-cardiac fibroblasts (iPSC-CFs). Having generated iPSC-CFs that closely resemble primary CFs, the authors could study cardiac fibrosis in human cells. Further analysis of Tabula Muris allowed them to study intercellular communication between CFs and other cardiac cells, revealing the previously undescribed expression of natriuretic peptide receptor-1 (NPR1) on CFs and its interaction with two hypertrophic cardiomyocyte proteins: atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (Fig. 2). Interaction of these proteins with cardiomyocytes and CFs led to suppressed CF activation and fibrosis. Zhang et al. used this knowledge to inhibit fibrosis by treating the iPSC-CMs and iPSC-CFs with the anti-fibrotic drug sacubitril, which inhibits the ANP/BNP-degrading enzyme neprilysin [77]. This result supports earlier studies that demonstrated the potential of the ANP/BNP-NPR1 pathway as a target in anti-fibrotic treatment in ischemic HF [76]. Furthermore, the iPSC-CF protocol enables the generation of patient-specific iPSC-CFs *in vitro* which can be useful for personalized drug testing. Testing anti-fibrotic drugs on patient-specific iPSC-CFs would allow us to test their effectiveness for patients with ischemic HF [76].

Application of scRNA-seq to ischemic HF in human subjects has only just begun because it is challenging to isolate adult CMs. These cells are large and fragile, which makes it difficult to perform scRNA-seq analysis on them [24]. To overcome the challenges associated with cell dissociation, or cells being too large for microfluidic scRNA-seq technologies, single-nuclear RNA-sequencing (snRNA-seq) has been developed. With snRNA-seq the gene expression of single nuclei instead of cells is being analyzed [78]. Recently, Wang et al. successfully isolated CMs and performed scRNA-seq analysis on primary human CMs isolated from the ventricles and atria of HF patients with dilated cardiomyopathy and coronary heart disease and of healthy controls [24,79]. The authors identified five major groups of cells, consisting of cardiomyocytes, endothelial cells, fibroblasts, macrophages and smooth muscle cells, and 36 minor cellular groups. Nine of the minor cellular groups were upregulated in coronary HF, and six were downregulated, compared to healthy controls. Correlation of ligands known to be released by the minor cellular groups revealed that the endothelial cell cluster 3 (EC3, ACKR1$^+$) was downregulated in coronary HF. Cell–cell interaction maps generated by correlating ligands to receptors further revealed EC3 to interact with most other cell types, and this high number of cell–cell interactions indicates that these cells are likely an important mediator in the pathophysiology of ischemic HF [24].

To expand the knowledge on ischemic HF and identify new potential therapeutic targets, Wang and Cui et al. approached ischemic HF from a different perspective [33]. Combining scRNA-seq and single-cell open chromatin (scATAC-seq) data of murine neonatal regenerative and non-regenerative hearts, they gained a comprehensive overview of the regenerative properties of murine hearts [33]. Up to post-natal day 7, the murine neonatal heart is known to have regenerative properties following injury that are lost in adult hearts [80]. Furthermore, a snRNA-seq cell transcriptome atlas of the human developmental heart was recently published (**Box 1**) [28]. Combining the single-cell techniques, Wang and Cui et al. unraveled pathways involved in regeneration of neonatal murine hearts that could potentially be targeted in ischemic HF to induce cardiac repair formation. Furthermore, they identified cardiotrophin-like cytokine factor 1 (CLCF1) secreted by neonatal macrophages as a potential activator of cardiomyocyte proliferation *in vitro*, thereby highlighting that further studies should be done in vivo and in adulthood to study the therapeutic potential of this protein in ischemic HF [33]. The work by Wang and Cui et al. has given us new insights in ischemic HF and shows that we are currently just beginning to understand the pathophysiology of ischemic HF on single-cell gene expression level and to identify potential therapeutic targets.

Discussion

In this review, we have summarized the advances and promises of recent applications of single-cell techniques in various diseases across the CVD continuum, from a clinical perspective. Across the CVD continuum, cardiac and immune cells play a key role, with different cellular subsets being involved at different stages (Fig. 2). Various subsets of macrophages play important roles in the pathophysiology of the CVD continuum, where the phagocytotic ability of macrophages is reduced in advanced atherosclerotic lesions, and apoptotic cells are insufficiently cleared as a result. This leads to plaque progression and a higher risk of clinical events. After MI, activated CCR2$^+$ macrophages are major mediators of the inflammatory response, where depletion of this group leads to a decrease in the number of type I IFN macrophages, which have been associated with excessive left ventricular remodeling [81]. CCR2$^+$macrophages would therefore be an interesting target group to minimize negative clinical outcomes after MI. At the end of the CVD contin-

uum, application of single-cell technologies in ischemic HF have led to the identification of the ANP/BNP-NPR1 pathway as a potential target in anti-fibrotic treatment. Furthermore, a subset of endothelial cells has been recognized to exhibit a cardioprotective role by maintaining cardiac contractility, and this subset could also serve as a potential target. Altogether, single-cell technologies have shown that we can study the CVD continuum at a higher resolution than before, which yields new insights into ways to target inflammation across the CVD continuum.

To extend our knowledge of cardiac cells across the CVD continuum, future studies on the full cardiac cellular composition should be performed on single-cell transcriptomic level. To date, it has remained challenging to isolate living cardiac cells for scRNA-seq analysis because cardiomyocytes are highly prone to damage when collected [24,82,83]. Gladka et al. resolved this by enzymatically dispersing cardiac tissue and separating the cells using FACS to capture 1000 heart cells for use in scRNA-seq analysis [26]. This technique appeared promising, but the cellular viability remained low [26,82]. By combining retrograde perfusion of the heart via the aorta, known as Langendorff perfusion, with dissociation of the ventricles and manual micro-pipetting of viable cardiomyocytes, Nomura et al. obtained living cells from mouse models and human samples [82,84]. This technique yielded high levels of living cardiomyocytes and was recently successfully applied to scRNA-seq analysis of fetal hearts (19–22 weeks of gestation) [85]. A challenge encountered when studying diseased hearts is that, upon ischemic injury and in heart failure, cardiomyocytes have often undergone multinucleation stages [86,87]. Using snRNA-seq, one could consider every nucleus as an individual observation and compare these instead of cells [82,88]. See et al. applied this approach using snRNA-seq to both human and mouse hearts and successfully uncovered sub-populations of cardiomyocytes and key nodal regulatory long intergenic non-coding RNAs that could be targeted for cardiac repair [88]. Altogether, these advances reveal novel targets for treatment of the CVD continuum, from MI to ischemic HF, that would not have been found using conventional bulk analysis.

Challenges in translation between animal and human scRNA-seq studies

In this review, we have seen that the majority of single-cell studies on the CVD continuum have used mouse models. Although animal models can be useful in mimicking disease across the CVD spectrum and in obtaining single cells for analysis, it remains challenging to recapitulate human pathophysiological complexity and diversity in mouse models [56]. Importantly, the human contexts of genetic profile, age, sex, ethnicity and comorbidities such as diabetes are lost (at least to a certain extent). Gene expression profiles in mice could therefore identify genes involved in CVD, but the interactions of these genes with other genes in the human body remain obscure. For single-cell studies across the CVD continuum to progress, future studies should focus on making the translation to humans, for example by studying the replicability of findings in mice in humans.

Future directions

CVD are caused by a multitude of genetic and environmental risk factors. In this review, we have highlighted CVD-associated cell types, genes and proteins, but many more players remain to be discovered. The complex interplay between each of these factors causes disease, but it remains difficult to distinguish the changes that are the cause versus the consequence of the disease. Single-cell data can provide additional insights here by mapping the downstream expression effects (expression quantitative trait loci, eQTL) of genetic variants that are associated with CVD [16,17]. By providing the cellular contexts in which the downstream effects of genetic variants take place, single-cell data could yield new insights into pathways activated specifically in CVD, which could then serve as potential therapeutic targets [16,17]. Furthermore, mapping cell–cell communication using knowledge from known receptor–ligand interaction databases is an important step in gaining a comprehensive understanding of cellular physiology and pathophysiology [89,90]. Skelly et al. recently performed such ligand–receptor pair analysis on scRNA-seq data of non-cardiomyocyte cells in healthy mouse hearts, which revealed cardiac intercellular communication networks for the first time [91]. Even though the addition of such intercellular communication adds a valuable layer of information to our current understanding of cellular functioning, none of the single-cell CVD studies to date have taken into account the spatial component. With the recent availability of spatial transcriptomics, it is now possible to explore this missing link between spatial and transcriptomic data at the single-cell level [30,92]. A helpful online resource of 3D spatiotemporal information about the developing healthy heart, based on spatial and scRNA-seq data, recently became available as an online tool and can be used as a starting point for exploring this spatiotemporal link in the context of CVD (https://hdca-sweden.scilifelab.se/a-study-on-human-heart-development/) (**Box 1**) [30].

So far, studies have characterized cell types and provided insights into the inflammatory process post-MI, but spatial information concerning the location and interaction between cell types remained to be elucidated. Kuppe et al. (*Preprint*) combined three techniques (snRNA-seq, scATAC-seq and spatial transcriptomics) to generate a comprehensive, spatially resolved map of MI that characterizes and visualizes distinct zones of injury, repair and remodeling post-MI [23]. They also identified post-MI cardiac fibrosis to be driven by differentiation of fibroblasts to myofibroblasts, in line with the findings by Forte et al., and to be regulated by the transcription factor runt-related transcription factor 1 (RUNX1) [21,23]. An integrative analysis of snRNA-seq with scATAC-seq data revealed myofibroblast differentiation to be driven by the TGFβ signaling pathway, which is regulated through interaction of RUNX1 with the downstream transcription factor SMAD1. The transcription factor RUNX1 activates the TGFβ pathway driving cardiac fibrosis and could aid as potential therapeutic target post-MI [23]. The Kuppe et al. study exemplifies how integration of single-cell and spatial transcriptomics data increases the resolution of our understanding of MI. With this extra dimension, we expect to gain more detailed insights into the role of cell–cell communication between diseased and nearby healthy tissue that could open up new approaches for treatment strategies across the CVD continuum.

Conclusion

We have discussed the advances and promises of recent applications of single-cell technologies across the CVD continuum from a translational perspective. We find that single-cell analysis has led to the development of cellular atlases and the identification of subsets of cells involved in CVD. Furthermore, we have discussed new insights into the role of immunological cells in CVD and the direction of future research in the field. In sum, we present recently emerged tools and discuss new insights that can be used in developing therapeutic strategies to minimize progression across the CVD continuum.

1) This material is the authors' own original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

5) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.

6) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

Funding

LF is supported by the Dutch Organisation for Scientific Research (NWO) Corona Fast-Track grant (440.20.001), an Oncode Senior Investigator grant, a grant from the European Research Council (ERC Starting Grant agreement number 637640 ImmRisk) and an NWO Vidi grant (917.14.374). MW is supported by a NWO Veni grant (192.029).

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

Acknowledgements to figures: Images used for the figures were downloaded from https://smart.servier.com/. Original images were used, no changes were made. The license for using the images is Creative Commons License, Attribution 3.0 Unported License.

Author contributions

IVB and PH contributed to the conception, IVB performed the search strategy and selection of articles, IVB wrote the manuscript with critical input from HG, LF, MW and PH. The manuscript is not submitted elsewhere or under consideration for publication. All authors have read and agreed to the submission for publication.

References

[1] Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the potential to reduce the global burden of Atherothrombotic disease. Circ Res 2016;118(4):535–46.
[2] Dzau VJ, Antman EM, Black HR, Hayes DL, Manson JE, Plutzky J, et al. The Cardiovascular disease continuum validated : clinical evidence of improved patient outcomes part I : Pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). Circulation 2006;114(25):2850–70. doi:10.1161/CIRCULATIONAHA.106.655688.
[3] Swirski FK, Nahrendorf M. Cardioimmunology: the immune system in cardiac homeostasis and disease. Nat Rev Immunol 2018;18(12):733–44. doi:10.1038/s41577-018-0065-8.
[4] Groot HE, van Blokland IV, Lipsic E, Karper JC, van der Harst P. Leukocyte profiles across the cardiovascular disease continuum: a population-based cohort study. J Mol Cell Cardiol 2020;138(December 2019):158–64.
[5] Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with Canakinumab for Atherosclerotic disease. N Engl J Med 2017;377(12):1119–31. NEJMoa1707914.
[6] Buenrostro JD, Wu B, Litzenburger UM, Ruff D, Gonzales ML, Snyder MP, et al. Single-cell chromatin accessibility reveals principles of regulatory variation. Nature 2015;523(7561):486–90.
[7] Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, Birney E, et al. The human cell atlas. Elife 2017;6:1–30.
[8] Gadalla R, Noamani B, MacLeod BL, Dickson RJ, Guo M, Xu W, et al. Validation of CyTOF against flow cytometry for immunological studies and monitoring of human cancer clinical trials. Front Oncol 2019;9(May):1–13.
[9] Palit S, Heuser C, De Almeida GP, Theis FJ, Zielinski CE. Meeting the challenges of high-dimensional single-cell data analysis in immunology. Front Immunol 2019;10(July):1–12.
[10] Ornatsky O, Bandura D, Baranov V, Nitz M, Winnik MA, Tanner S. Highly multiparametric analysis by mass cytometry. J Immunol Methods 2010;361(1–2):1–20.
[11] Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, Xu N, et al. mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods 2009;6(5):377–82.
[12] Datlinger P, Rendeiro AF, Boenke T, Krausgruber T, Barreca D, Bock C. Ultra-high throughput single-cell RNA sequencing by combinatorial fluidic indexing. bioRxiv 2019:1–27. doi:10.1101/2019.12.17.879304.
[13] Lareau CA, Duarte FM, Chew JG, Kartha VK, Burkett ZD, Kohlway AS, et al. Droplet-based combinatorial indexing for massive-scale single-cell chromatin accessibility. Nat Biotechnol 2019;37(8):916–24. doi:10.1038/s41587-019-0147-6.
[14] Kang HM, Subramaniam M, Targ S, Nguyen M, Maliskova L, McCarthy E, et al. Multiplexed droplet single-cell RNA-sequencing using natural genetic variation. Nat Biotechnol 2018;36(1):89–94.
[15] Stoeckius M, Zheng S, Houck-Loomis B, Hao S, Yeung BZ, Mauck WM, et al. Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. Genome Biol 2018;19(1):1–12.
[16] Van Der Wijst MGP, Brugge H, De Vries DH, Deelen P, Swertz MA, Franke L. Single-cell RNA sequencing identifies celltype-specific cis-eQTLs and co-expression QTLs. Nat Genet 2018;50(4):493–7.
[17] van der Wijst MG, de Vries DH, Groot HE, Trynka G, Hon C-C, Bonder M-J, et al. The single-cell eQTLGen consortium. Elife 2020;9:1–21.
[18] Schaum N, Lehallier B, Hahn O, Pálovics R, Hosseinzadeh S, Lee SE, et al. Ageing hallmarks exhibit organ-specific temporal signatures. Nature 2020;583(July):596–602.
[19] Almanzar N, Antony J, Baghel AS, Bakerman I, Bansal I, Barres BA, et al. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. Nature 2020;583(7817):590–5.
[20] Schaum N, Karkanias J, Neff NF, May AP, Quake SR, Wyss-Coray T, et al. Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris: the Tabula Muris Consortium. Nature 2018;562(7727):367–72.
[21] Forte E, Skelly DA, Chen M, Daigle S, Morelli KA, Hon O, et al. Dynamic interstitial cell response during myocardial infarction predicts resilience to rupture in genetically diverse mice. Cell Rep 2020;30(9):3149–63. doi:10.1016/j.celrep.2020.02.008.
[22] Depuydt M, Prange KHM, Slenders L, Elbersen D, Boltjes A, de Jager SCA, et al. Microanatomy of advanced human atherosclerotic plaques through single-cell transcriptomics. Atherosclerosis 2019;287(2019):e5. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L2002467239.
[23] Kuppe C, Ramirez Flores RO, Li Z, Hannani M, Tanevski J, Halder M, et al. Spatial multi-omic map of human myocardial infarction. bioRxiv 2020. doi:10.1101/2020.12.08.411686.
[24] Wang L, Yu P, Zhou B, Song J, Li Z, Zhang M, et al. Single-cell reconstruction of the adult human heart during heart failure and recovery reveals the cellular landscape underlying cardiac function. Nat Cell Biol 2020;22(1):108–19.
[25] Winkels H, Ehinger E, Vassallo M, Buscher K, Dinh HQ, Kobiyama K, et al. Atlas of the immune cell repertoire in mouse atherosclerosis defined by single-cell RNA-sequencing and mass cytometry. Circ Res 2018;122(12):1675–88.
[26] Gladka MM, Molenaar B, De Ruiter H, Van Der Elst S, Tsui H, Versteeg D, et al. Single-cell sequencing of the healthy and diseased heart reveals cytoskeleton-associated protein 4 as a new modulator of fibroblasts activation. Circulation 2018;138(2):166–80.
[27] Rajewsky N, Almouzni G, Gorski SA, Aerts S, Amit I, Bertero MG, et al. LifeTime and improving European healthcare through cell-based interceptive medicine. Nature 2020;587(7834):377–86.
[28] Cui Y, Zheng Y, Liu X, Yan L, Fan X, Yong J, et al. Single-cell transcriptome analysis maps the developmental track of the human heart. Cell Rep 2019;26(7):1934–50. doi:10.1016/j.celrep.2019.01.079.
[29] Cao J, O'Day DR, Pliner HA, Kingsley PD, Deng M, Daza RM, et al. A human cell atlas of fetal gene expression. Science 2020;370(6518):7721.
[30] Asp M, Giacomello S, Larsson L, Wu C, Fürth D, Qian X, et al. A spatiotemporal organ-wide gene expression and cell atlas of the developing human heart. Cell. 2019;179(7):1647–60.
[31] Litviňuková M, Talavera-López C, Maatz H, Reichart D, Worth CL, Lindberg EL, et al. Cells of the adult human heart. Nature 2020;588(7838):466–72.
[32] Tucker NR, Chaffin M, Fleming SJ, Hall AW, Parsons VA, Bedi KC, et al. Transcriptional and cellular diversity of the human heart. Circulation 2020;142(5):466–82.
[33] Wang Z, Cui M, Shah AM, Tan W, Liu N, Bassel-Duby R, et al. Cell-type-specific gene regulatory networks underlying murine neonatal heart regeneration at single-cell resolution. Cell Rep 2020;33(10):108472. doi:10.1016/j.celrep.2020.108472.
[34] Cochain C, Vafadarnejad E, Arampatzi P, Pelisek J, Winkels H, Ley K, et al. Single-cell RNA-seq reveals the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis. Circ Res 2018;122(12):1661–74.
[35] Farbehi N, Patrick R, Dorison A, Xaymardan M, Janbandhu V, Wystub-Lis K, et al. Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. Elife 2019;8:1–39.
[36] Otsuka F, Joner M, Prati F, Virmani R, Narula J. Clinical classification of plaque morphology in coronary disease. Nat Rev Cardiol 2014;11(7):379–89.
[37] Winkels H, Ehinger E, Vassallo M, Buscher K, Dinh HQ, Kobiyama K, et al. Atlas of the immune cell repertoire in mouse atherosclerosis defined by single-cell RNA-sequencing and mass cytometry. Circ Res 2018;122(12):1675–88.
[38] Winkels H, Ehinger E, Ghosheh Y, Wolf D, Ley K. Atherosclerosis in the single–cell era. Curr Opin Lipidol 2018;29(5):389–96.
[39] Rahman K, Vengrenyuk Y, Ramsey SA, Vila NR, Girgis NM, Liu J, et al. Inflammatory Ly6C hi monocytes and their conversion to M2 macrophages drive atherosclerosis regression find the latest version : inflammatory Ly6C hi monocytes and their conversion to M2 macrophages drive atherosclerosis regression. J Clin Invest 2017;127(8):2904–15.

[40] Gu W, Ni Z, Tan YQ, Deng J, Zhang SJ, Lv ZC, et al. Adventitial cell Atlas of wt (Wild Type) and ApoE (Apolipoprotein E)-deficient mice defined by single-cell RNA sequencing. Arterioscler Thromb Vasc Biol 2019;39(6):1055–71.

[41] Kalluri AS, Vellarikkal SK, Edelman ER, Nguyen L, Subramanian A, Ellinor PT, et al. Single-cell analysis of the normal mouse aorta reveals functionally distinct endothelial cell populations. Circulation 2019;140(2):147–63.

[42] Kim K, Shim D, Lee JS, Zaitsev K, Williams JW, Kim KW, et al. Transcriptome analysis reveals nonfoamy rather than foamy plaque macrophages are proinflammatory in atherosclerotic murine models. Circ Res 2018;123(10):1127–42.

[43] Zadelaar S, Kleemann R, Verschuren L, De Vries-Van Der Weij J, Van Der Hoorn J, Princen HM, et al. Mouse models for atherosclerosis and pharmaceutical modifiers. Arterioscler Thromb Vasc Biol 2007;27(8):1706–21.

[44] Wang X, Huang R, Zhang L, Li S, Luo J, Gu Y, et al. A severe atherosclerosis mouse model on the resistant NOD background. DMM Dis Model Mech 2018;11(10):1–10.

[45] Otsuka F, Sakakura K, Yahagi K. Has Our Understanding of calcification in human coronary atherosclerosis progressed? 2014;34(4):724–36.

[46] Nakahara T, Dweck MR, Narula N, Pisapia D, Narula J, Strauss HW. Coronary artery calcification: from mechanism to molecular imaging. JACC Cardiovasc Imaging 2017;10(5):582–93.

[47] Kojima Y, Volkmer J-P, McKenna K, Leeper NJ. CD47 blocking antibodies restore phagocytosis and prevent atherosclerosis. Nature 2016;536(7614):86–90.

[48] Flores AM, Hosseini-Nassab N, Jarr KU, Ye J, Zhu X, Wirka R, et al. Pro-efferocytic nanoparticles are specifically taken up by lesional macrophages and prevent atherosclerosis. Nat Nanotechnol 2020;15(2):154–61.

[49] Martinet W, Coornaert I, Puylaert P, De Meyer GRY. Macrophage death as a pharmacological target in atherosclerosis. Front Pharmacol 2019;10(APR):1–18.

[50] Shanahan CM, Weissberg PL. Smooth muscle cell heterogeneity: patterns of gene expression in vascular smooth muscle cells *in vitro* and in vivo. Arterioscler Thromb Vasc Biol 1998;18(3):333–8.

[51] Dobnikar L, Taylor AL, Chappell J, Oldach P, Harman JL, Oerton E, et al. Disease-relevant transcriptional signatures identified in individual smooth muscle cells from healthy mouse vessels. Nat Commun 2018;9(1):4567.

[52] Wirka RC, Wagh D, Paik DT, Pjanic M, Nguyen T, Miller CL, et al. Atheroprotective roles of smooth muscle cell phenotypic modulation and the TCF21 disease gene as revealed by single-cell analysis. Nat Med 2019;25(8):1280–9.

[53] Liu M, Gomez D. Smooth muscle cell phenotypic diversity. Arterioscler Thromb Vasc Biol 2019;39(9):1715–23.

[54] Wolf MP, Hunziker P. Atherosclerosis: insights into vascular pathobiology and outlook to novel treatments. J Cardiovasc Transl Res 2020;13(5):744–57.

[55] Tang J, Wang H, Huang X, Zhang L, Gao D, Zhou B. Arterial Sca1 + vascular stem cells generate de novo smooth muscle for artery repair and regeneration article arterial Sca1 + vascular stem cells generate De Novo smooth muscle for artery repair and regeneration. Stem Cell 2020;26(1):81–96.

[56] Frangogiannis NG. The inflammatory response in myocardial injury, repair and remodeling. Nat Rev Cardiol 2014;11(5):255–65.

[57] Groot HE, Al Ali L, van der Horst ICC, Schurer RAJ, van der Werf HW, Lipsic E, et al. Plasma interleukin 6 levels are associated with cardiac function after ST-elevation myocardial infarction. Clin Res Cardiol 2018;0(0):0.

[58] Hartman MHT, Groot HE, Leach IM, Karper JC, van der Harst P. Translational overview of cytokine inhibition in acute myocardial infarction and chronic heart failure. Trends Cardiovasc Med 2018;28(6):369–79.

[59] Groot HE, Karper JC, Lipsic E, van Veldhuisen DJ, van der Horst ICC, van der Harst P. High-sensitivity C-reactive protein and long term reperfusion success of primary percutaneous intervention in ST-elevation myocardial infarction. Int J Cardiol 2017;248:51–6.

[60] Entman ML, Youker K, Shoji T, Kukielka G, Shappell SB, Taylor AA, et al. Neutrophil induced oxidative injury of cardiac myocytes: a compartmented system requiring CD11b/CD18-ICAM-1 adherence. J Clin Invest 1992;90(4):1335–45.

[61] Yamazaki T, Seko Y, Tamatani T, Miyasaka M, Yagita H, Okumura K, et al. Expression of intercellular adhesion molecule-1 in rat heart with ischemia/reperfusion and limitation of infarct size by treatment with antibodies against cell adhesion molecules. Am J Pathol 1993;143(2):410–18.

[62] Christia P, Frangogiannis NG. Targeting inflammatory pathways in myocardial infarction. Eur J Clin Invest 2013;43(9):986–95.

[63] Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interf Cytokine Res 2009;29(6):313–25.

[64] Frantz S, Nahrendorf M. Cardiac macrophages and their role in ischaemic heart disease. Cardiovasc Res 2014;102(2):240–8.

[65] Bajpai G, Bredemeyer A, Li W, Zaitsev K, Koenig AL, Lokshina I, et al. Tissue resident CCR2- and CCR2+ cardiac macrophages differentially orchestrate monocyte recruitment and fate specification following myocardial injury. Circ Res 2019;124(2):263–78.

[66] Hayasaki T, Kaikita K, Okuma T, Yamamoto E, Kuziel WA, Ogawa H, et al. CC chemokine receptor-2 deficiency attenuates oxidative stress and infarct size caused by myocardial ischemia-reperfusion in mice. Circ J 2006;70(3):342–51.

[67] Li W, Hsiao H-M, Higashikubo R, Saunders BT, Bharat A, Goldstein DR, et al. Heart-resident CCR2+ macrophages promote neutrophil extravasation through TLR9/MyD88/CXCL5 signaling. JCI Insight 2016;1(12):1–14.

[68] Efremova M, Vento-Tormo M, Teichmann SA, Vento-Tormo R. CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. Nat Protoc 2020;15(4):1484–506. doi:10.1038/s41596-020-0292-x.

[69] Foulquier S, Daskalopoulos EP, Lluri G, Hermans KCM, Deb A, Blankesteijn WM. WNT signaling in cardiac and vascular disease. Pharmacol Rev 2018;70(1):68–141.

[70] Palevski D, Levin-Kotler LP, Kain D, Naftali-Shani N, Landa N, Ben-Mordechai T, et al. Loss of macrophage Wnt secretion improves remodeling and function after myocardial infarction in mice. J Am Heart Assoc 2017;6(1):1–18.

[71] Meyer IS, Jungmann A, Dieterich C, Zhang M, Lasitschka F, Werkmeister S, et al. The cardiac microenvironment uses non-canonical WNT signaling to activate monocytes after myocardial infarction. EMBO Mol Med 2017;9(9):1279–93.

[72] Tarone G, Balligand JL, Bauersachs J, Clerk A, De Windt L, Heymans S, et al. Targeting myocardial remodelling to develop novel therapies for heart failure: a position paper from the Working Group on Myocardial Function of the European Society of Cardiology. Eur J Heart Fail 2014;16(5):494–508.

[73] Van Berlo JH, Maillet M, Molkentin JD. Signaling effectors underlying pathologic growth and remodeling of the heart. J Clin Invest 2013;123(1):37–45.

[74] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2016;37(27). 2129–2200m.

[75] Kikuchi A, Fumoto K, Kimura H. The Dickkopf1-cytoskeleton-associated protein 4 axis creates a novel signalling pathway and may represent a molecular target for cancer therapy. Br J Pharmacol 2017;174(24):4651–65.

[76] Zhang H, Tian L, Shen M, Tu C, Wu H, Gu M, et al. Generation of quiescent cardiac fibroblasts from human induced pluripotent stem cells for *in vitro* modeling of cardiac fibrosis. Circ Res 2019;125(5):552–66.

[77] Mogensen UM, Køber L, Kristensen SL, Jhund PS, Gong J, Lefkowitz MP, et al. The effects of Sacubitril/Valsartan on coronary outcomes in PARADIGM-HF. Am Heart J 2017;188:35–41.

[78] Grindberg R V, Yee-Greenbaum JL, McConnell MJ, Novotny M, O'Shaughnessy AL, Lambert GM, et al. RNA-sequencing from single nuclei. Proc Natl Acad Sci U S A. 2013;110(49):19802–7.

[79] Guo GR, Chen L, Rao M, Chen K, Song JP, Hu SS. A modified method for isolation of human cardiomyocytes to model cardiac diseases. J Transl Med 2018;16(1):1–9. doi:10.1186/s12967-018-1649-6.

[80] Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. Science 2011;331(6020):1078–80.

[81] King KR, Aguirre AD, Ye YX, Sun Y, Roh JD, Ng RP, et al. IRF3 and type i interferons fuel a fatal response to myocardial infarction. Nat Med 2017;23(12):1481–7.

[82] Ackers-Johnson M, Tan WLW, Foo RS-Y. Following hearts, one cell at a time: recent applications of single-cell RNA sequencing to the understanding of heart disease. Nat Commun 2018;9(1):4434.

[83] Ackers-Johnson M, Li PY, Holmes AP, O'Brien SM, Pavlovic D, Foo RS. A simplified, Langendorff-free method for concomitant isolation of viable cardiac myocytes and nonmyocytes from the adult mouse heart. Circ Res 2016;119(8):909–20.

[84] Nomura S, Satoh M, Fujita T, Higo T, Sumida T, Ko T, et al. Cardiomyocyte gene programs encoding morphological and functional signatures in cardiac hypertrophy and failure. Nat Commun 2018;9(1):1–17.

[85] Suryawanshi H, Clancy R, Morozov P, Halushka MK, Buyon JP, Tuschl T. Cell atlas of the foetal human heart and implications for autoimmune-mediated congenital heart block. Cardiovasc Res 2020;116(8):1446–57.

[86] Liu Z, Yue S, Chen X, Kubin T, Braun T. Regulation of cardiomyocyte polyploidy and multinucleation by cyclinG1. Circ Res 2010;106(9):1498–506.

[87] Meckert PC, Rivello HG, Vigliano C, González P, Favaloro R, Laguens R. Endomitosis and polyploidization of myocardial cells in the periphery of human acute myocardial infarction. Cardiovasc Res 2005;67(1):116–23.

[88] See K, Tan WLW, Lim EH, Tiang Z, Lee LT, Li PYQ, et al. Single cardiomyocyte nuclear transcriptomes reveal a lincRNA-regulated de-differentiation and cell cycle stress-response in vivo. Nat Commun 2017;8(1):1–13.

[89] Paik DT, Cho S, Tian L, Chang HY, Wu JC. Single-cell RNA sequencing in cardiovascular development, disease and medicine. Nat Rev Cardiol 2020;17(8):457–73. doi:10.1038/s41569-020-0359-y.

[90] Ramilowski JA, Goldberg T, Harshbarger J, Kloppman E, Lizio M, Satagopam VP, et al. A draft network of ligand-receptor-mediated multicellular signalling in human. Nat Commun 2015;6:7866.

[91] Skelly DA, Squiers GT, McLellan MA, Bolisetty MT, Robson P, Rosenthal NA, et al. Single-cell transcriptional profiling reveals cellular diversity and intercommunication in the mouse heart. Cell Rep 2018;22(3):600–10. doi:10.1016/j.celrep.2017.12.072.

[92] Ståhl PL, Salmén F, Vickovic S, Lundmark A, Navarro JF, Magnusson J, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science 2016;353(6294):78–82.