Clinical aspects of glucocorticoid and selective glucocorticoid receptor modulator therapy in rheumatoid arthritis

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Clinical aspects of glucocorticoid and selective glucocorticoid receptor modulator therapy in rheumatoid arthritis

Klinische aspecten van glucocorticoïd- en selectieve glucorticoïdreceptor-modulatortherapie bij reumatoïde artritis

(met een samenvatting in het Nederlands)

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About the cover

The cover depicts the balance between efficacy and safety of glucocorticoids and selective glucocorticoid receptor modulators. It also portrays the balance between work and personal life, effort and relaxation, grieving and finding joy after a loss, and growing up in a Western society while maintaining Afghan cultural values.

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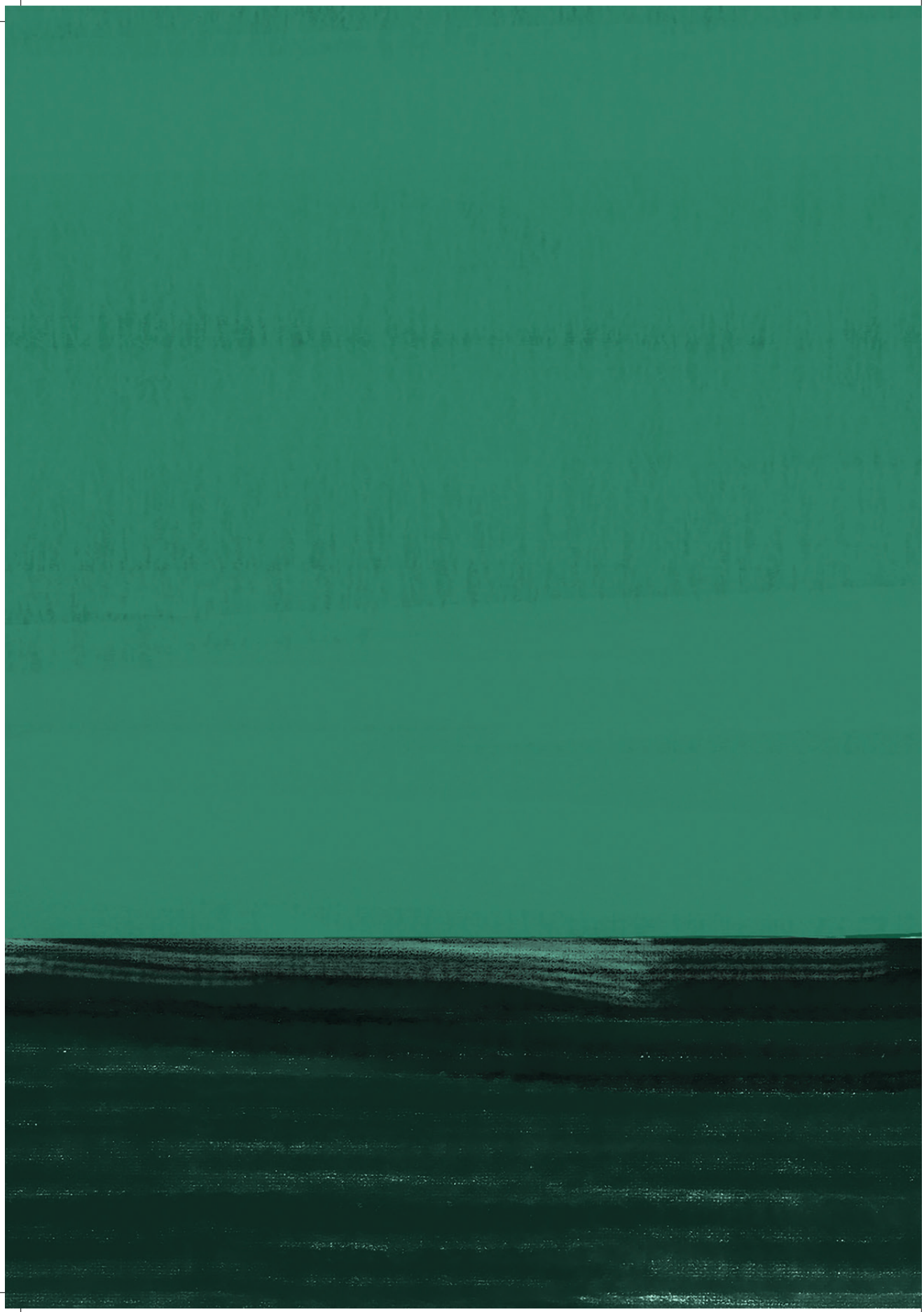
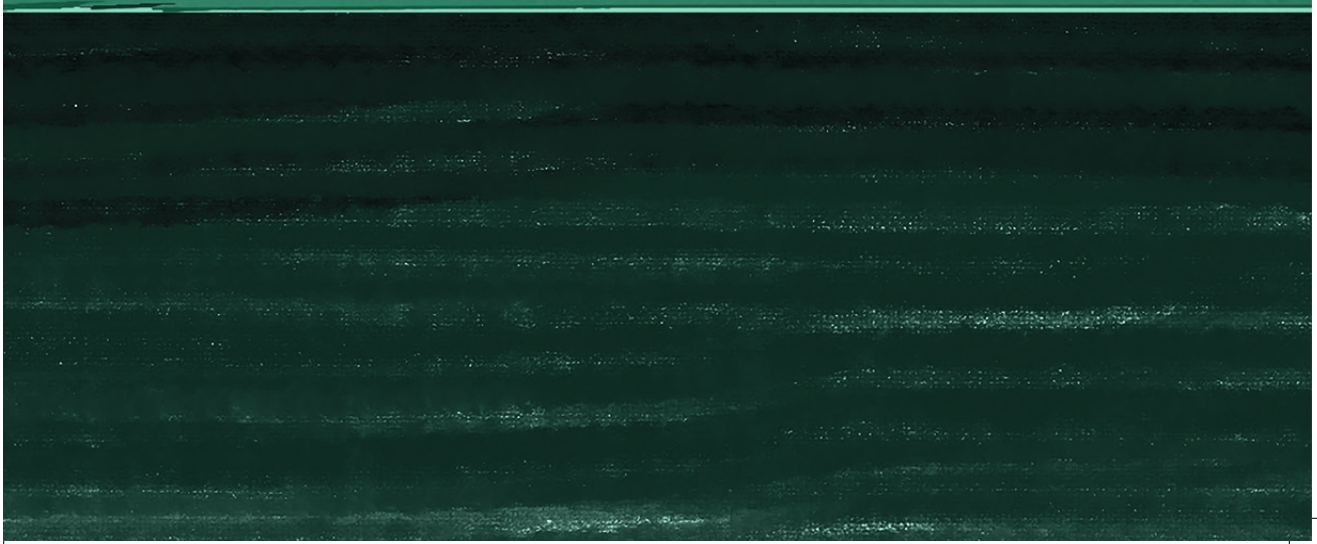


1.

General introduction, aims and outline of this thesis



Rheumatoid arthritis: treatment options and use of glucocorticoids

Rheumatoid arthritis (RA) is a common multisystem chronic inflammatory disease leading to joint involvement and functional disability.[1] Also, extra-articular manifestations may occur, such as skin manifestations, ocular involvement, cardiovascular and pulmonary manifestations.[2] Treatment of RA is necessary to reduce and prevent disability and comorbidities and should be focussed on disease activity, and consider safety and patient-related factors such as comorbidities. The 2019 updated European Alliance of Associations for Rheumatology (EULAR) RA management recommendations indicate initiation of therapy with disease modifying anti-rheumatic drugs (DMARDs) as soon as the diagnosis of RA has been established. The aim of the therapy should be achieving the target of sustained remission or low disease activity. In the first initiated treatment strategy, methotrexate (MTX), a conventional synthetic DMARD (csDMARD), is the hallmark of the disease management. When treatment target is not achieved with the first csDMARDs strategy, adding a biologic DMARD (bDMARD) or targeted synthetic DMARD should be contemplated.[3]

When initiating or changing csDMARDs, additional short-term usage of glucocorticoids (GCs) should be considered because of the lag time (one to several weeks) between starting a csDMARD and these exerting a clinical effect. GCs have been used in RA treatment for more than seven decades and have proven good efficacy in reducing inflammation via their immunomodulatory properties.[4] However, GCs have adverse effects depending on dosage and duration of GC-therapy; the quest for an optimal use of GCs in RA is still ongoing.[5] Despite the fact that GCs have been used for a long time, there is still much unknown about their exact modes of action. An explanation is that GCs have many pleiotropic effects, affecting approximately 20% of the human genome and thus affecting many pathways.[6, 7]

There are several GC drugs, and GCs can be administrated orally, intramuscularly, intravenously, and intra-articularly, and in different dosages: low-dose (<7.5 mg/day prednisone-equivalent), medium-dose (7.5 to 100 mg/day) and high dose (≥100 mg daily).[8] Short-term high dosed intravenous administration is often used to treat RA-patients with high (extra-)articular involvement, showing good efficacy, but due to the high dosage, it has an increased risk of adverse effects.[9] Intra-articular GC administration is predominantly used in patients who have one or few joints involved with active RA. The effect of oral low to medium dose GCs in reducing disease activity in RA patients has been well-described. Besides reducing RA activity, they have been shown to have DMARD-properties, i.e., inhibiting the progression of joint damage.[10-15]

Long-term GC use is common, with between 30% to 60% of the RA patients using it for long-term and with only 35% of patients being able to discontinue its use.[16, 17] One of the reasons for this high rate of long-term use is that tapering of GCs is often associated with flare up of the disease. Factors associated with unsuccessful tapering of GCs and flare ups are a high disease activity prior to treatment, female gender, high dose and long duration of GC treatment.[18] Smokers have a higher need of DMARDs and smoking negatively affects clinical response of several DMARDs, but it is not known yet if, and how it might affect the efficacy of GCs.[19]

Mode of action of GCs

GCs have multiple mechanisms of action and powerful anti-inflammatory immunomodulatory effects: GCs cause inhibition of leucocyte traffic and prevent access of leucocytes to the site of inflammation; they dysregulate the function of leucocytes, endothelial cells and fibroblasts; GCs also interfere with humoral immune system by inhibition of production and action of humoral factors.[20, 21] More in detail, GCs cause a lowering of the number of circulating monocytes and macrophages. They inhibit the synthesis of pro-inflammatory cytokines such as interleukin (IL)-6, tumour necrosis factor (TNF)-α and of prostaglandins.[22] GCs also affect T cells by lowering the number of circulating T cells and most importantly, by lowering the production and action of pro-inflammatory cytokine IL-2. They also lower the number of basophil and eosinophil granulocytes. GCs also influence the endothelial cells by lowering vessel permeability, expression of adhesion molecules and production of IL-1.[6] Furthermore, GCs lower proliferation of fibroblasts and production of fibronectin.[20]

There are four different mechanism by which GCs are capable of these anti-inflammatory and immunomodulatory effects: cytosolic glucocorticoid receptor (cGCR)-mediated classical genomic effects, cGCR-mediated non-genomic effects, membrane-bound GCR (mGCR) mediated non-genomic effects and non-specific non-genomic effect, see Figure 1.[21-24] Here we will focus more on the cGCR-mediated effects. During the cGCR-mediated classical genomic process, GCs can up-or downregulate specific regulatory proteins. This occurs by binding of the GC molecule to the cGCR, which causes an activated GC/GCR multiprotein complex. This complex binds to GC-response elements, which are specific DNA-binding sites.[25] This can lead to so called transactivation, a process in which upregulation of the synthesis of specific proteins occurs.[26] Another process is transrepression: the GC/GCR complex interferes with the transcription of certain factors, such as activator-protein-1 and nuclear factor-kappa B.[27, 28] This causes downregulation of synthesis of proinflammatory

cytokines, such as IL-1, IL-6 and TNF-α. A clinical example of transrepression is the limiting effect of GCs on radiological progression in RA patients.[29] Joint damage in RA is caused by multiple factors and pro-inflammatory cytokines play an important role in that process. These cytokines lead to stimulation of osteoblasts and T cells causing a cascade which leads to a higher number of activated osteoclasts. These osteoclasts cause bone resorption and erosions which can be seen in RA patients.[30]

The cGCR-mediated non-genomic effects of GCs are thought to be caused by a release of proteins from the cGCR complex, leading to swift effects in minutes.[31]

As described above, many cytokines are involved in the effects of GCs; therefore, the multi-biomarker disease activity (MBDA) score which exists of 12 biomarkers, might predict the clinical response to GCs in RA patients.[32]

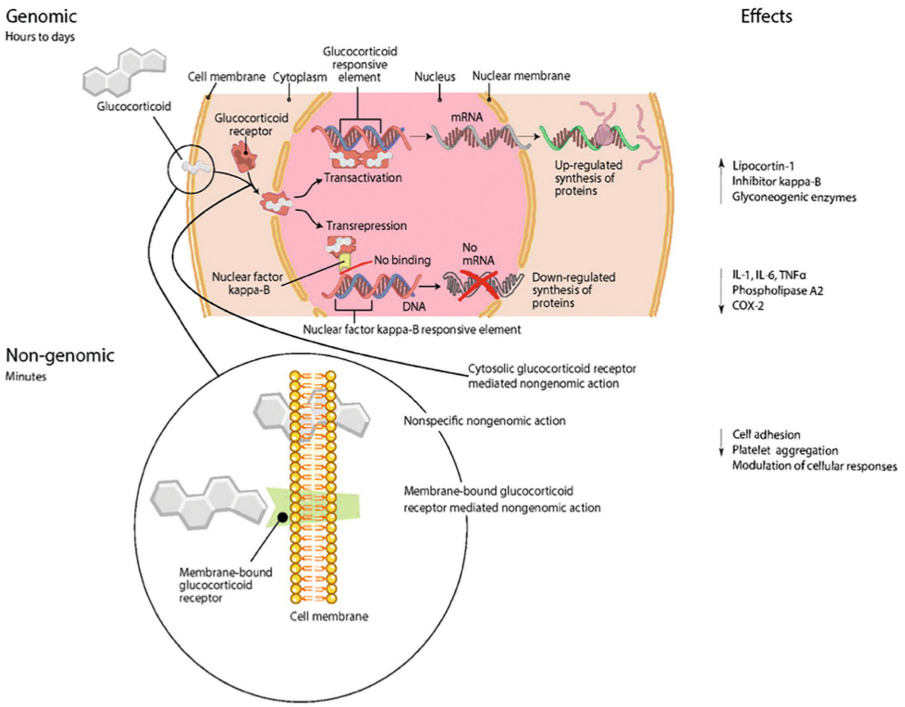


Figure 1. Schematic overview of the multiple mechanisms of action of glucocorticoids (GCs) and some of their effects.

GCs exert their main actions through the cytosolic GC receptors (GCRs), which are present in almost all tissues. These genomic effects require passing of GC molecules through the cell membrane, binding to the cytosolic GCR to form monomer or dimer complexes. This is followed by either the classic cytosolic GCR-mediated genomic effects or by cytosolic GCR-mediated nongenomic effects. In the classic cytosolic GCR-mediated genomic mechanism the monomer or dimer complexes migrate into the nucleus where they finally influence gene expression and protein synthesis via transactivation or transrepression. Dimers of the complex GC-GR bind to GC-responsive elements in DNA, and lead to increased synthesis (transactivation) of certain regulatory proteins, mainly those responsible for unwanted metabolic effects of GCs. Monomers of the GC-GR complex inhibit nuclear transcriptional factors, such as nuclear factor kappa-B, resulting in downregulation (transrepression) of (predominantly proinflammatory) protein synthesis. Other non-genomic effects occur because of GCs interacting with cell membranes either specifically, via membrane bound GCRs or via nonspecific interactions. IL: interleukin; TNF: tumour necrosis factor; COX: cyclooxygenase. Adapted from Huisman AM et al. and Stahn C et al.[33, 34] With permission from Vereniging Nederlands Tijdschrift voor Geneeskunde and Elsevier.

Safety of GCs

Safety concerns regarding the use of GCs are limiting their long-term use. GC-related adverse effects are dose-related but even low-dose long-term GC-use has been shown to cause an increased rate of infections, bone loss and clinical vertebral fractures,[35, 36] and to be associated with increased risk of cardiovascular, cerebrovascular events, cataract, diabetes and death. [37] Due to the safety aspects of conventional GCs, the search for a treatment option with a better benefit/risk balance continues. The main therapeutic and adverse effects of GCs are shown in Figure 2.

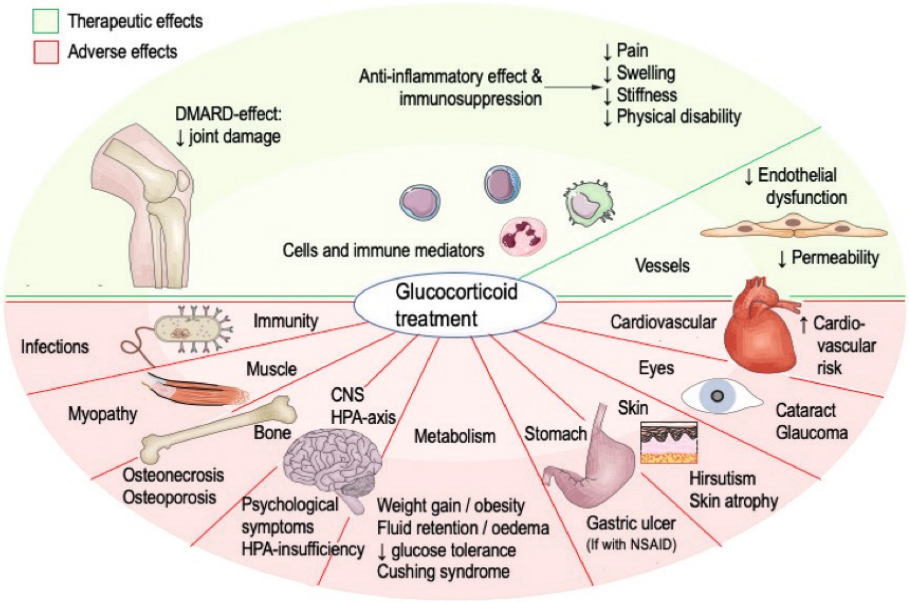


Figure 2. Overview of the therapeutic and adverse effect of GCs.

DMARD: disease-modifying antirheumatic drug; RA: rheumatoid arthritis; CV: cardiovascular; NSAIDs: non-steroidal anti-inflammatory drugs; CNS: central nervous system; HPA: hypothalamic-pituitary-adrenal. Adapted from Hoes JN et al.[38] With permission from Nature Publishing Group.

A common hypothesis is that transrepression is the main mechanism behind the anti-inflammatory and immunosuppressive properties of GCs (wanted effects), while transactivation was thought to be responsible for the unwanted, adverse effects of GCs.[39] An example of a common adverse effect which is thought to be driven by transactivation is GC-induced hyperglycaemia. This is caused by transactivation of an important enzyme (hepatic nuclear factor

3) in the gluconeogenesis pathway.[40, 41]. Other clinically important adverse effects of GCs such as skin atrophy and osteoporosis are thought to be at least partially caused by transactivation.[42] However, it is an oversimplification to attribute all beneficial effects of GCs to transrepression and the adverse effects to transactivation. For example, the increased risk of (opportunistic) infections is due to the immunosuppressive effects of GCs, which are predominantly due to transrepression.[43] For example, GCs stimulate IL-10 secretion by macrophages and Th2 cells, leading to suppressed immune responses. GCs have shown to reactivate for example cytomegalovirus, by NF-kB mediated activation. Furthermore, also transactivation can lead to anti-inflammatory functions, by upregulation of for example GC-induced leucine zipper (GILZ, which inhibits NFkB and AP-1) and IL-10 secretion by macrophages and Th2 cells.[44-51]

GC-related osteoporosis is caused by both transrepression and transactivation. For example, the mRNA levels of receptor activator of NF-κB ligand (RANKL), which stimulates the number of activated osteoclasts, are increased by transactivation, but osteoprotegerin (OPG) and protein levels are decreased by transrepression. This leads to an enhanced RANKL/OPG ratio and therefore more bone resorption. Interestingly, by reducing RA-disease activity, GCs also have a bone protecting mode of action; their net effect on bone is negative, however.[39, 52, 53]

Possible alternatives for conventional GCs

Over the course of years, a few possible alternatives for conventional GCs have been investigated, such as modified release (MR) formulations, liposomal GCs, nitro-steroids and selective glucocorticoid receptor modulators (SGRMs). MR formulations were developed because RA has a diurnal pattern of disease activity, due to circadian variations in the HPA axis and inflammatory cytokines during the night.[54, 55] MR prednisone reduced morning stiffness of the joints more potently than conventional prednisone with no differences in adverse effects.[56] Liposomal GCs are small nanoparticles containing GCs. These were initially aimed for intra-articular injection and later for systemic use, targeting sites of inflammation, where the permeability of the blood vessels is increased, in very high concentrations and thus leading to a higher efficacy. Due to their encapsulation within liposomes, it was hypothesized that they would cause less systemic adverse effects. The clinical efficacy and safety of intravenously administrated liposomal prednisolone versus intramuscular methylprednisolone was assessed in treating RA flares in a recent randomised clinical trial (RCT). Treatment with liposomal pegylated prednisolone (Nanocort) showed better EULAR response at week one. Adverse events were reportedly similar in the two treatment groups, but more frequent hypersensitivity reactions were reported in the liposomal prednisolone group.[57]

Posttranscriptional modification of conventional GCs by nitric oxide (NO) was thought to lead to a better efficacy and safety profile. In an animal study, the anti-inflammatory effects of GCs were improved in a NO-releasing prednisolone derivative, compared to conventional prednisolone and osteoclast activity was not enhanced by this nitro-steroid, leading to less bone resorption. Clinical human studies investigating nitro-steroids are still lacking.[43]

SGRMS are selective GR ligands that aim to predominantly exert GCs therapeutic action by initiating transrepression (anti-inflammatory) and reducing transactivation (cellular metabolism). It is hypothesized that by this selective, dissociative working mechanism, their anti-inflammatory effects would be similar to conventional GCs but with less adverse effects, leading to a better efficacy/safety balance. Thus far, only one phase-2 RCT investigating the efficacy and safety of a dissociated agonist, fosdagrocorat, has been published.[58] Fosdagrocorat 10 mg had similar efficacy to prednisone 10 mg and reported AE were also similar for fosdagrocorat and prednisone, i.e., reported adverse effects were not less frequent in the dissociated agonist group as would have been expected based on their dissociative working mechanism.

Aims and outline of this thesis

The aims of this thesis were to: 1) unravel several underexplored clinical issues relating to GC therapy for RA and 2) evaluate the effectiveness and safety of SGRMs.

In **Chapter 2,** we describe the results of a follow-up study of a trial on the effects of an MTX plus prednisone strategy compared to MTX plus placebo in early RA patients on radiographic outcome and onset of GC-related adverse events. The effect of GC therapy on the need of initiation of the first bDMARD was also investigated.

Although concomitant GC therapy in RCTs investigating the efficacy and safety outcomes of bDMARDs is remarkably common, little is known on how these GCs affect the efficacy and safety outcomes of the investigated bDMARDs. In **Chapter 3**, we describe the results of our analyses of data of four double-blind RA RCTs with in total four tocilizumab (TCZ), one adalimumab (ADA) and two MTX monotherapy arms, including patients using background GCs.

To potentially improve the use of GC therapy in clinical practice of RA patients, we set up three studies in which we tried to identify possible predictors for clinical response in individual RA patients using GC therapy. A possible predictor we assessed was the MBDA, as described in **Chapter 4**. Furthermore, we investigated

if current smoking, which is known to negatively affect clinical response of several DMARDs[19], also might be predictive of a less beneficial clinical response of GC therapy; these results are outlined in **Chapter 5.**

SGRMs could have a better efficacy/safety balance than conventional GCs. **Chapter 6** describes what is known about the efficacy and safety of SGRMs in comparison to conventional GCs in arthritis, using clinical and pre-clinical studies available. To actually investigate the clinical efficacy and safety of a SGRM, in **Chapter 7** a phase-2a randomised, double-blind, parallel-group, multicentre RCT is described, in which the efficacy/safety balance of a non-steroidal SGRM (AZD9567) was compared to that of prednisolone in early active RA patients.

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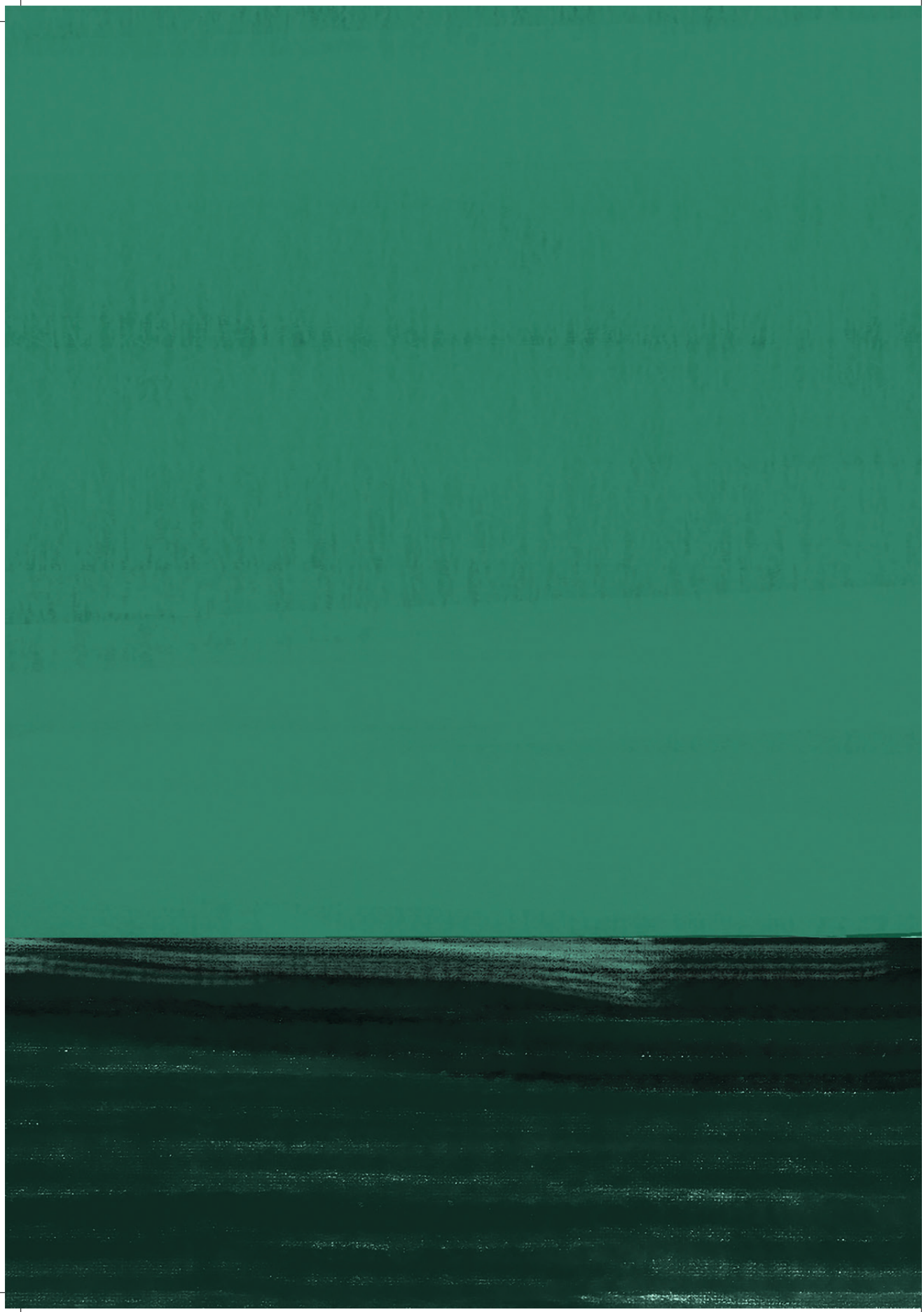

2.

Long-term outcome is better when a methotrexate-based treatment strategy is combined with 10mg prednisone daily. Follow-up after the second Computer Assisted Management in Early Rheumatoid Arthritis trial.

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Abstract

Objectives. In the second Computer Assisted Management in Early Rheumatoid Arthritis trial, patients had started with methotrexate and 10mg prednisone (MTX+pred) or placebo (MTX+plac). After the trial, prednisone was tapered and stopped, if possible. The objective was to compare, during post-trial follow-up between the 2 former strategy groups, initiation of the first biological (b) DMARD, radiographic outcome and onset of glucocorticoid (GC) related comorbidities.

Methods. Data on prednisone and bDMARD use and onset of GC-related comorbidities was collected retrospectively. Sharp/van der Heijde scoring was performed. Data were analysed using Fisher's exact and Mann-Whitney U tests.

Results. Of 218 patients post-trial follow-up data was available. Maximum follow-up time was 11.8 years. Fewer patients initiated a first bDMARD in the former MTX+pred compared to the former MTX+plac strategy group: 31% vs 50%, p=0.003. At 2 years post-trial follow-up, the median erosion score was significantly lower in the former MTX+pred versus former MTX+plac strategy group: 0 (range 0-0) versus 0 (0-2), p=0.002. No significant differences between the former strategy groups in onset of GC-related comorbidities during post-trial follow-up were found.

Conclusion. Addition of 10mg prednisone daily to an MTX-based treatment strategy in early RA results in a lower initiation rate of a first bDMARD and significantly better radiographic outcomes, yet does not result in more GC-related comorbidities.

Introduction

The introduction of biological disease modifying anti-rheumatic drugs (b) DMARDs in the treatment of rheumatoid arthritis (RA) has in general led to better disease control and improved functional ability and quality of life. [1] Disadvantages, are higher cost and risk of severe infections of bDMARDs compared to conventional synthetic (cs)DMARDs.[2-3] In about one third of patients with RA, bDMARD use does not result in sufficient clinical improvement. [4] Therefore, it is important to optimize treatment strategies based on csDMARDs before the next step, to adding a bDMARD, is taken. In this way, initiation of a bDMARD may be delayed or even prevented.

In the second Computer Assisted Management in Early Rheumatoid Arthritis trial (CAMERA-II), patients initiated a methotrexate (MTX)-based treatment strategy, with 10mg prednisone (MTX+pred) or with placebo (MTX+plac) daily. Addition of 10mg prednisone resulted in significantly faster reduction of disease activity, less erosive joint damage after 2 years and less frequent initiation of TNF-inhibitor treatment,[5] showing the potential of effective disease control by csDMARDs (especially with glucocorticoids (GCs)) for a large proportion of patients and of cost-savings by reduced bDMARD initiation.

In this study, we wondered whether the beneficial effects of adding 10mg prednisone to an MTX-based treatment strategy during the CAMERA-II trial persisted during post-trial follow-up. Hypothetically, the need of initiating a bDMARD in the former MTX+pred strategy group could be increased (rebound), since after the end of the trial it was the strategy to taper and stop the prednisone therapy. To investigate this, we examined initiation of the first bDMARD among patients who had participated in CAMERA-II. In addition, we investigated whether the benefit regarding radiographic progression persisted during post-trial follow-up. Lastly, we aimed to gain insight into the long-term GC-related comorbidities after the CAMERA-II trial, as there is a paucity of systematically collected data concerning long-term adverse-effects of medium dose GC-use,[6-7] in contrast to those of high dose GC-use.[8]

Methods

In the two year double blind randomised placebo-controlled CAMERA-II trial, DMARD naive patients with early RA were randomised to initiate treatment with either MTX+pred, with a stable dosage of 10mg of prednisone daily during the whole trial period, or MTX+plac. It was a tight-controlled and treat-to-target study, aiming for remission. Depending on disease activity, subsequent treatment steps were taken, including ultimately addition of the TNF-inhibitor adalimumab.[9] In

post-trial follow-up, patients were treated by their rheumatologist according to good clinical care. In the former MTX+pred group, the aim was to taper prednisone, if possible.

We retrospectively collected from medical charts data on prednisone and bDMARD use, on mortality and on onset of GC-related comorbidities during post-trial follow-up. Radiographs of hands and feet were scored with Sharp/van der Heijde scoring (SHS).[10] Since yearly radiographs were not present in all patients during post-trial follow-up, we arbitrarily decided to restrict these analyses to up to 2 years of post-trial follow-up, with 72% of radiographs available. For other analyses, post-trial follow-up data up to 11 years was used. Discontinuation of prednisone use in the former MTX+pred strategy group was visualized using a Kaplan Meijer survival curve, as was bDMARD initiation in both former strategy groups, using Cox's proportional hazard regression analysis for testing.

To avoid reporting bias by beliefs in safety of prednisone among rheumatologists as well as patients during the open, post-trial follow-up, we chose to only investigate GC-related comorbidities, based on literature review and expert opinion, for which treatment was initiated. This is more objective than investigating all negative events of which the scoring would be rather subjective and of which it would be hard to discriminate whether caused by RA or GC-use. Dichotomous data was tested with Fisher's exact tests and continuous data with Mann Whitney U tests. Erosion scores were visualized with a cumulative probability plot. For data analyses, IBM SPSS Statistics, Version 22.0 (Armonk, NY: IBM Corp.) was used.

The institutional review boards of the participating centres confirmed that the Medical Research Involving Human Subjects Act (WMO) was not applicable to this study.

Results

Post-trial follow-up data was available for 218 of the 236 patients of CAMERA-II; 18 patients were no longer followed for various reasons, e.g. change of hospital. Of these 218 patients, at start of CAMERA-II, characteristics between the randomised groups were similar (Table 1).

Table 1. Baseline characteristics at start of CAMERA-II trial of the groups presently investigated

	Former MTX+pred strategy group (N=107)	Former MTX+plac strategy group (N=111)
Female, gender, number (%)	64 (60)	68 (61)
RF positive, number (%)	61 (57)	75 (68)
Age in years, mean (SD)	55 (14)	53 (13)
VAS-GH, 0-100 mm (worst), median (range)	57 (0-100)	56 (0-99)
28TJC, median (range)	12 (0-25)	8 (0-26)
28SJC, median (range)	12 (2-26)	9 (0-24)
ESR, mm/h^{1st}, median (range)	33 (2-118)	32 (2-129)
DAS28, median (range)	5.8 (2.8-8.3)	5.5 (3.3-7.9)

MTX: methotrexate; pred: prednisone; plac: placebo; RF: rheumatoid factor; SD: standard deviation, VAS-GH: visual analogue scale for global health; 28TJC: 28 joints tender joint count; 28SJC: 28 joints swollen joint count; ESR: erythrocyte sedimentation rate; mm/h: millimetre/hour; DAS28: Disease Activity Score assessing 28 joints.

The median post-trial follow-up time in the former MTX+pred strategy group was 6.7 years (range 0.1-10.1; interquartile range (IQR) 5.2-8.3) versus 6.6 (range 0.3–11.8; IQR 5.2-8.0) in the former MTX+plac group, p=0.71. Half of the patients in the former MTX+pred strategy group had discontinued prednisone one-year post-trial, and 79% at the end of post-trial follow-up (supplementary figure 1). During the post-trial follow-up, significantly fewer patients initiated a first bDMARD in the former MTX+pred strategy group compared to the former MTX+plac group (31% versus 50%, respectively, p=0.003). Also, during the combined study and post-trial follow-up period, fewer patients had initiated a first bDMARD in the (former) MTX+pred strategy group compared to the (former) MTX+plac strategy group (figure 1, hazard ratio (HR) 0.46, 95% confidence interval (CI) 0.30-0.72; p=0.001).

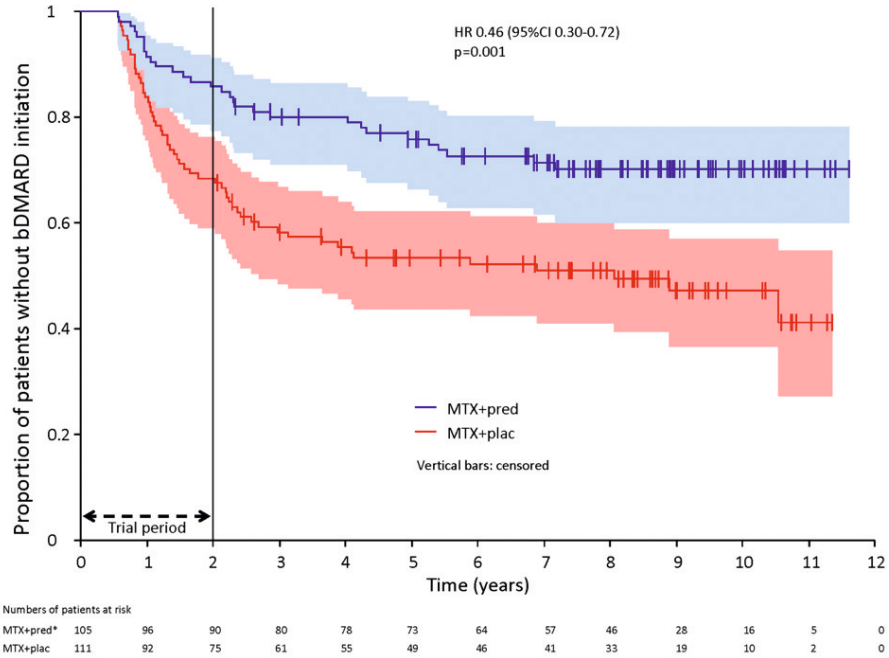

Figure 1. Initiation of a first bDMARD in (former) strategy groups during the trial and post-trial follow-up.

*Survival curve with 95% confidence interval, showing the proportion of patients who did not initiate a first bDMARD. Both the trial and follow-up period are depicted. Vertical black line indicates end of the two-year trial period. The numbers of patients at risk are shown beneath the graph with yearly intervals. *missing: n=2. 95% CI, 95% confidence interval; bDMARD, biological disease modifying anti-rheumatic drug; HR, hazard ratio; MTX, methotrexate; plac, placebo, pred: prednisone.*

The median SHS of hands and feet at 2 years post-trial follow-up was not significantly different between the two groups (former MTX+pred median 0 (IQR 0-0), former MTX+plac (IQR 0-1.5); p=0.271). Two years post-trial, 83% of patients was erosion free in the former MTX+pred strategy group vs 62% in the former MTX+plac strategy group, p=0.16 (supplementary figure 2). The median erosion score at 2 years post-trial was 0 (IQR 0-0) in the former MTX+pred strategy group vs 0 (0-2) in the former MTX+plac strategy group, p=0.002.

Table 2. Incidence of new onset comorbidity, and mortality in post-trial follow-up

New onset comorbidity n (%)	Former MTX+pred strategy group(N=107)	Former MTX+plac strategy group (N=111)	P-value
≥1 comorbidity	36 (34)	36 (32)	1.00
Hypertension	4 (4)	10 (9)	0.11
Cardiovascular disease	13 (12)	8 (7)	0.27
Diabetes mellitus type 2	3 (3)	2 (2)	0.68
Peptic ulcer	1 (1)	0 (0)	0.49
Cataract	8 (8)	7 (6)	0.80
Glaucoma	0 (0)	1 (1)	1.00
Serious infection	10 (9)	14 (13)	0.49
Osteonecrosis	0 (0)	0 (0)	-
Fracture	11 (10)	14 (13)	0.55
Mortality*	10 (9)	6 (5)	0.33

MTX: methotrexate; pred: prednisone; plac: placebo; hypertension for which treatment was initiated, diabetes mellitus type 2 for which treatment was initiated, serious infection for which hospitalization was needed. Cardiovascular disease was: angina pectoris, myocardial infarction, transient ischemic attack, cerebral vascular accident and peripheral vascular disease (e.g. claudication).
**In the former MTX+pred group 5 patients died of a malignancy (pancreas, urothelial and prostate, lung, cerebral metastases of unknown origin, B-cell lymphoma); in the former MTX+plac group 3 patients died of a carcinoma (lung, colon, urothelial). One patient in the former MTX+pred group died due to a subdural haematoma. In both groups the cause of death could not be retrieved in 3 cases.*

The incidence of long-term GC-related comorbidities during post-trial follow-up was not significantly different between the former strategy groups (table 2), although there were some numerical differences: more cardiovascular comorbidities in the former MTX+pred than in the former MTX+plac strategy group (n=13 vs n=8) and a higher mortality rate (n=10 vs n=6).The most frequent cause of death in both groups was malignancy (see Table 2 for details). In either group, the cause of death could not be retrieved in 3 cases.

Discussion

We found that during the post-trial follow-up of CAMERA-II, fewer patients initiated a first bDMARD in the former MTX+pred than in the former MTX+plac strategy group, despite less initiation of a bDMARD in the former MTX+pred strategy group during the trial and tapering and stopping of prednisone, after the trial. Furthermore, compared to the former MTX+plac strategy group, in the former MTX+pred strategy group still less erosive damage was present during post-trial follow-up and no significantly increased incidence of long-term post-trial GC-related comorbidities.

Our results show no rebound of initiation of a first bDMARD during post-trial follow-up in the former MTX+pred strategy group. These show that long-term bDMARD initiation can be reduced by a tight-control and treat-to-target strategy including prednisone. Importantly, the lower rate of bDMARD initiation in the former MTX+pred strategy group did not negatively affect post-trial radiographic outcome at 2 years. An explanation of these favourable findings could be the DMARD properties of prednisone during the window of opportunity period, since the MTX schemes in both strategy groups during the whole trial, so at least during the window of opportunity period, often interpreted as the first 3-6 months, were identical.

In comparison, in the 56 weeks COBRA trial treatment with either sulfasalazine or a combination of sulfasalazine, MTX and prednisone was given. A follow-up study was done up to 11 years, in which bDMARD initiation and incidence of new onset comorbidities were comparable between the groups.[11] In the Better Anti-Rheumatic PharmacOTherapy (BARFOT) study, patients with early RA received 7.5mg prednisone daily in addition to csDMARDs alone. The long-term risk of ischemic cardiovascular events was higher in the prednisone group, with a trend towards reduced survival.[12] Our finding of a higher number of cardiovascular comorbidities and mortality even in early RA patients in the former MTX+pred strategy group, although not statistically significant, could be clinically relevant, since this result is in line with findings of the BARFOT study.

On the basis of our own findings and the current body of evidence on GC schemes in early RA, we propose initiation of treatment in early RA-patients with an MTX-based tight control and treat-to-target strategy, in combination with a rapidly remission inducing agent. From the viewpoint of cost, glucocorticoids should be preferred over bDMARDs as rapidly remission inducing agent. Our finding of initiation of bDMARDs in 41% of the early RA patients in the MTX+plac strategy group during the 2-year CAMERA-II trial is similar to that of another study in which 47% of patients with early RA on subcutaneous MTX initiated a

bDMARD during an average follow-up of 1.8 (standard deviation 1.6) years.[13] Ours is, however, the first study that investigated a possible rebound increased initiation of a first bDMARD after tapering and stopping of prednisone.

As in the post-trial follow-up period the controlled situation was lost and treatment was open to the rheumatologists, long-term outcomes in our study may have been influenced by the use of different anti-rheumatic drugs. However, our study provides real-life data on daily clinical practice. There may have been reporting bias in onset of comorbidities between the former strategy groups, and some data was missing, but we do not expect that this would have affected the main results. In conclusion, our results indicate that initiation of treat-to target therapy with MTX and 10mg prednisone daily in early RA results in a persistently lower initiation rate of a first bDMARD and significantly better radiographic outcomes. This was not clearly associated with increased incidence of long-term GC-related comorbidities.

Conflicts of interest

No conflicts of interest were reported.

Authors' contributors

All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; drafted the work or revised it critically for important intellectual content.

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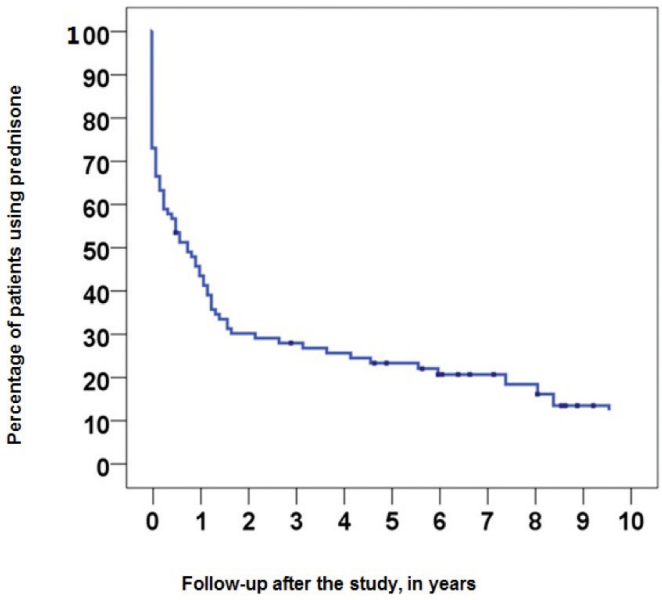
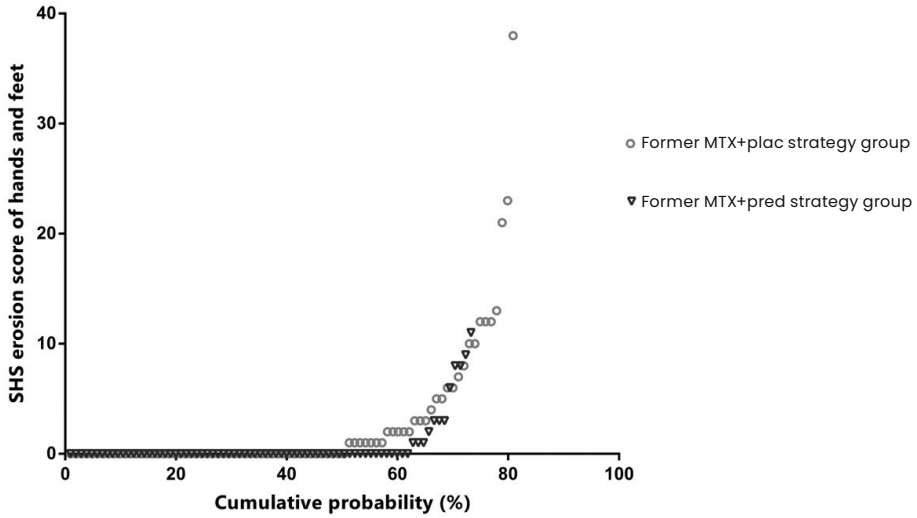
Supplementary Files



Supplementary Figure 1. Discontinuation of prednisone use in the former MTX+pred strategy group during post-trial follow-up.

Survival curve showing percentage of patients still using prednisone in the former MTX+pred strategy group during the two year post-trial follow-up. Post-trial follow-up durations were different for individual patients; for that reason, data on differences in proportion of patients using glucocorticoids between both former strategy groups at the end of post-trial follow-up are not shown. In the former MTX+plac treatment strategy group during post-trial follow-up, n=34 (31%) patients initiated and used at least temporarily glucocorticoid therapy.



Supplementary Figure 2. Erosion score at two years post-trial follow-up in former strategy groups.

Former MTX+plac strategy group: n=82
Former MTX+pred strategy group: n=77
Cumulative probability: cumulative percentage of patients with a score less than or equal to that specific score.
SHS: Sharp/van der Heijde Score

Appendix

eLetter to the Editor: "Discussion of Methotrexate Dosage"

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We thank Safy *et al.* [1] for their recent article discussing clinical outcomes in early treatment of rheumatoid arthritis with methotrexate and 10 mg daily of prednisolone versus methotrexate alone. This was a post-trial follow-up of the CAMERA II trial, which monitored for radiographic evidence of disease progression, use of biologic disease-modifying antirheumatic drugs (DMARDs) and incidence of glucocorticoid comorbidities. We appreciate the work that went into the review of up to 11 years worth of data; however, we feel there are outstanding issues worth discussion. It was noted with interest that despite the careful collection of data for the follow-up analysis, there was no description of methotrexate dosage in either study group. Are we to assume dosages were comparable between the two groups? If so, what were the median doses of methotrexate? Previous research has shown improved clinical outcomes from using intensive methotrexate treatment strategies with rapid dose increase [2] as compared with lower induction doses and slower titration regimes. For this reason, information about the methotrexate dosed must be available prior to conclusions about the additional benefit of steroid being drawn from the data presented. As this study collected data over an 11-year period, it should

be acknowledged that trends in methotrexate prescribing has significantly changed over this time period. Current European League Against Rheumatism (EULAR) guidelines advise the use of methotrexate in doses up to 25–30 mg per week.[3]

This study had a number of merits, which we read with interest. This is the first study to examine potential rebound of disease activity following weaning of prednisolone and commencement of bDMARDs. Although Safy et al demonstrated lower use of bDMARDs in the patient population studied, we would question if these findings were affected by the close monitoring of disease activity using a treat to target approach as opposed to the use of prednisolone.

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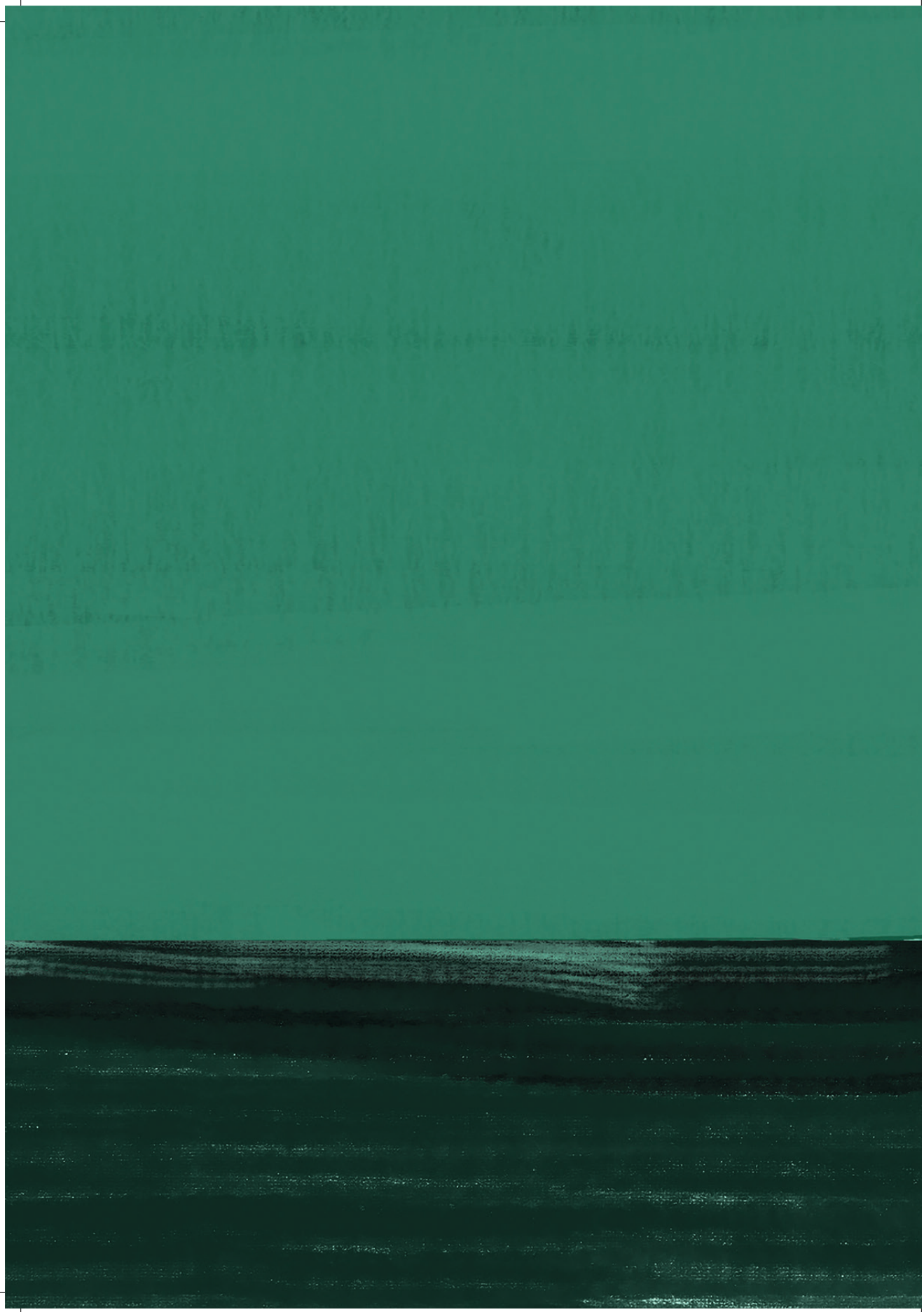

2a.

Response to the eLetter to the Editor regarding "Discussion of Methotrexate Dosage"

M.Safy[1], J.W.G. Jacobs[1], N.D. IJff[1], J.W.J. Bijlsma[1], J.M. van Laar[1], M.J.H. de Hair[1], on behalf of the Society for Rheumatology Research Utrecht (SRU)

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In their letter to the editor 'Discussion of Methotrexate Dosage', Maguire *et al.* [1] raised three issues regarding our recent paper.[2] We appreciate their interest in our study and will address these issues here.

First, regarding the methotrexate dosage, we reiterate what we have discussed in the discussion section of our paper: 'As in the post-trial follow-up period, the controlled situation was lost and treatment was open to the rheumatologists, long-term outcomes in our study may have been influenced by the use of different antirheumatic drugs'. We did not systematically record the methotrexate dosages during the post-trial follow-up period after the Computer Assisted Management in Early Rheumatoid Arthritis (CAMERA)-II trial. However, since all patients were treated to target during the post-trial follow-up period, we see no convincing argument to assume that this lack would disqualify our findings.

Next, Maguire et al raised the issue that it should be acknowledged that trends in methotrexate prescribing have significantly changed over the 11-year study period and that current European League Against Rheumatism (EULAR) guidelines advise the use of methotrexate in doses up to 25–30 mg per week. Importantly, already in our first CAMERA trial, which was published in 2007 and conceived several years before, the maximum dose of 30 mg methotrexate per week was applied.[3] Also in CAMERA-II and its post-trial follow-up, we applied the maximum dose of 30 mg,[4] which is still recommended in the newest EULAR guidelines.[5] Finally, Maguire et al questioned if the lower use of biological disease-modifying antirheumatic drugs (bDMARDs) observed in the former methotrexate and prednisone compared with the methotrexate and placebo treatment strategy group was affected by the close monitoring of disease activity utilising a treat to target approach as opposed to the use of prednisone.

Of course, a tight control regime applying the full range of dosing of methotrexate and of other conventional synthetic DMARDs could be bDMARD sparing, compared with less strict regimes.

However, in the CAMERA-II trial, both treatment strategy groups were tightly controlled. In the post-trial follow-up period, all patients were treated to target; so, the difference in outcome between the two groups can only be ascribed to the only difference between the two groups, which is the use of prednisone or placebo during the study period, tapered off and stopped in most patients during post-trial follow-up.

Conflicts of interest

No conflicts of interest were reported.

Authors’ contributors

All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; drafted the work or revised it critically for important intellectual content.

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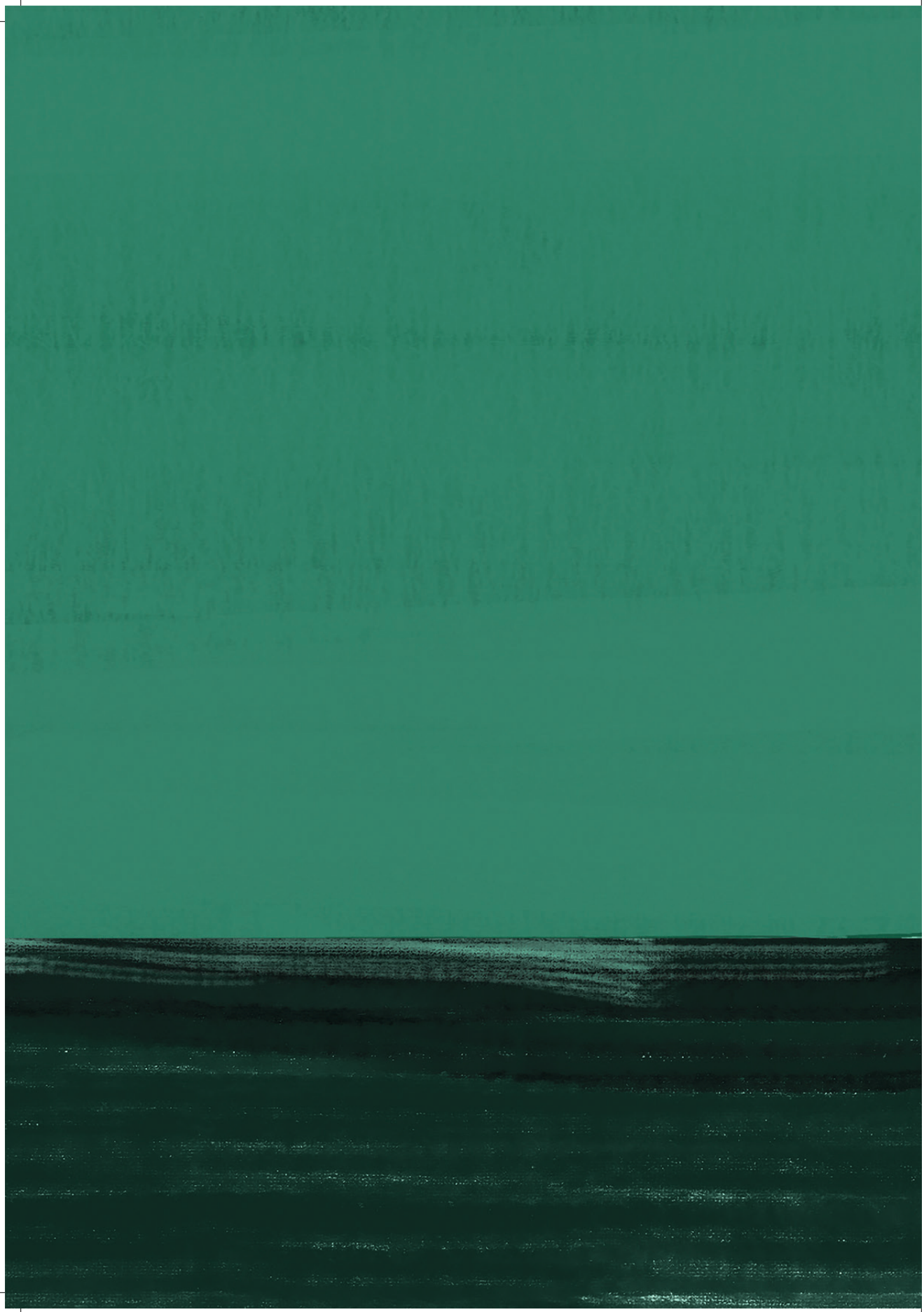

3.

Effect on efficacy and safety trial outcomes of also enrolling patients on ongoing glucocorticoid therapy in rheumatoid arthritis clinical trials of tocilizumab or adalimumab or methotrexate monotherapy

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Abstract

Background. In rheumatoid arthritis (RA) trials, inclusion of patients on background treatment with glucocorticoids (GC) might impact efficacy and safety outcomes.

Objectives. To determine if inclusion of patients on background GC-use influenced efficacy and safety outcomes of RA randomised clinical trials on initiation of tocilizumab (TCZ) or adalimumab (ADA) or methotrexate (MTX) monotherapy.

Methods. Data of 4 double-blind RA RCTs (AMBITION, ACT-RAY, ADACTA and FUNCTION) with in total 4 TCZ, 1 ADA and 2 MTX monotherapy arms were analysed. Analyses of covariance of changes from baseline to week 24 in efficacy endpoints and radiographic progression up to week 104 were performed, correcting for relevant covariates. Incidence rates of serious adverse events (SAEs) were assessed.

Results. No statistically significant differences were found in efficacy parameters between background GC-users and non-GC-users, except for less radiographic progression associated with GC-usage in one MTX arm. SAE rates were not statistically significantly different between GC-users and non-GC-users in the treatment arms.

Conclusion. No effect of including patients on background GC treatment on efficacy and safety trial outcomes was found, with the exception of reduced radiological joint damage in one MTX arm.

Introduction

The efficacy and safety of low to moderate dose glucocorticoids (GC) have been established in numerous randomised controlled trials (RCTs) in early rheumatoid arthritis (RA).[1-5] GC-use in early RA is endorsed by current European League against Rheumatism (EULAR) recommendations; low dose GC treatment is generally applied in many patients with active RA despite treatment with disease modifying anti-rheumatic drugs (DMARDs).[6] For RCTs, patients on a stable background low-dose GC therapy are generally not excluded: of RA patients included in RCTs, 38-64% used GC at baseline when initiating infliximab (IFX) or tocilizumab (TCZ).[7] RA patients on background GC-use had reduced radiographic progression of joint damage in placebo arms of IFX trials.[7] Inclusion of RA patients on background GC-use may improve efficacy outcomes of trials, because GCs reduce RA signs and symptoms.[1-5] On the other hand, patients on stable background GC treatment may have more refractory RA and thus may show less clinical improvement in a trial. Background GC treatment might negatively affect the safety in DMARD trials.[8]

The potential effects of RA patients on GC background use in RA studies including the use of biologics so far has only been reported in an open label trial programme with TCZ,[9] but has not yet been evaluated in the context of rigorously controlled RCTs. Therefore, the aim of this study was to establish whether inclusion of RA patients on stable background oral GC-use influenced efficacy and safety outcomes in RCTs on initiation of TCZ, adalimumab (ADA), or methotrexate (MTX) monotherapy for RA in a rigorously controlled RCT setting.

Methods

In this post-hoc study, we analysed data of individual RA patients from 4 double-blind RCTs on initiation of TCZ, ADA and/or MTX monotherapy: AMBITION, ACT-RAY, ADACTA and FUNCTION.[10-13] Study participants were MTX-naïve,[10,13] or MTX intolerant,[12] or had an inadequate response to MTX.[11,12] Furthermore, patients were all biological DMARD (bDMARD)-naive or, in the case of AMBITION, were either bDMARD-naive, or had discontinued bDMARDs, but were not bDMARD nor MTX irresponsive. FUNCTION excluded patients with an RA duration >2 years. Other selection criteria of these RCTs were similar. GC-use at inclusion (background GC-use) was allowed, if dose was stable for ≥4 to 6 weeks prior to randomisation and continued unchanged during the first 24 weeks of the trial. We selected as efficacy endpoints clinical disease activity index (CDAI), a disease activity score assessing 28 joints without acute phase reactant, because of the direct biologic effects of tocilizumab on the reduction of acute-phase reactant levels,[14] American College of Rheumatology 50 (ACR50) response as well as the patient

reported outcomes (PRO's) "functional assessment of chronic illness therapy" fatigue subscale (FACIT-F) and mental and physical component summaries (MCS; PCS) of the "36-item short-form health survey (SF-36)".

Statistical analyses

Per trial arm we used analyses of covariance (ANCOVA) to estimate differences between GC-users and non-GC-users in changes from baseline to week 24 in efficacy endpoints, CDAI and radiographic progression (modified Total Sharp Score (mTSS) or Genant Modified Sharp Score (GSS)), corrected for relevant covariates, see Supplementary file.

In addition, unadjusted CDAI scores over time for GC-users versus non-GC-users in TCZ, ADA and MTX monotherapy arms were plotted.

Differences in incidence rates of serious adverse events (SAEs) as a group and serious infections by GC-use were tested by comparing exact Poisson 95%CI for the rates. P-values <0.05 were considered statistically significant. All were intent to treat analyses and all statistical tests were two-sided and were performed with SAS version 9.4.

Results

Data from a total of 1,750 RA patients were used for analyses, except for radiographic data and patient-reported outcomes, which were only available from FUNCTION and ACT-RAY (n=855) and AMBITION, FUNCTION, ADACTA (n=1474), respectively. The numbers of GC-users vs. non-GC-users were for TCZ arms 484 vs. 533, for MTX arms 242 vs. 329 and for the ADA arm 92 vs. 70, respectively. Baseline characteristics were mostly similar between background GC-users and non-GC-users in each treatment arm for each study (Supplementary Table). Baseline mean (SD) GC dosage in mg/day prednisone equivalents was low for all RCTs: 7.4 (2.7) for AMBITION, 7.5 (2.4) for FUNCTION, 6.7 (2.5) for ACT-RAY and 6.4 (2.7) for ADACTA.

Efficacy

The adjusted differences with 95% confidence intervals (95%CI) of CDAI change at week 24 between background GC-users and non-GC-users in TCZ monotherapy arms of AMBITION, ACT-RAY, ADACTA and FUNCTION were small with values of -1.4 (-4.8, 2.1), 1.2 (-4.0, 6.3), -4.2 (-9.7, 1.4) and 0.8 (-2.5, 4.1), respectively (Table

Table 1. Efficacy outcome measures of included GC-users versus non-GC-users per initiated monotherapy

	AMBITION				FUNCTION			
	TCZ Mono		MTX Mono		TCZ Mono		MTX Mono	
	GC-users (n=137)	Non-GC-users (n=149)	GC-users (n=133)	Non-GC-users (n=151)	GC-users (n=118)	Non-GC-users (n=174)	GC-users (n=109)	Non-GC-users (n=178)
CDAI change‡								
LSM	-26.5	-25.1	-21.8	-20.9	-26.7	-27.5	-24.1	-22.3
LSM difference (95% CI)	-1.4 (-4.8;2.1)		-0.9 (-5.5;3.6)		0.8 (-2.5;4.1)		-1.7 (-6.0;2.5)	
CDAI remission⁺								
n (%)	18 (13.1)	14 (9.4)	9 (6.8)	7 (4.6)	20 (16.9)	40 (23.0)	16 (14.7)	22 (12.4)
OR (95% CI)	1.2 (0.5;2.6)		1.4 (0.5;4.2)		0.7 (0.4;1.3)		1.31 (0.6;2.7)	
ACR50⁺								
n (%)	60 (43.8)	66 (44.3)	45 (33.8)	50 (33.1)	52 (44.1)	87 (50.0)	44 (40.4)	80 (44.9)
OR (95% CI)	0.8 (0.5;1.3)		0.9 (0.5;1.5)		0.8 (0.5;1.3)		0.8 (0.5;1.3)	
FACIT-F score*								
LSM	9.4	9.7	7.7	6.9	9.7	9.3	6.5	10.3
LSM difference (95% CI)	-0.3 (-2.5;1.9)		0.9 (-1.6;3.4)		0.3 (-2.2;2.9)		-3.8 (-6.3;-1.2)	
PCS of SF-36*								
LSM	10.3	10.7	9.1	8.3	10.3	12.1	8.7	9.7
LSM difference (95% CI)	-0.4 (-2.6;1.8)		0.8 (-1.2;2.8)		-1.8 (-4.0;0.4)		-0.9 (-3.3;1.4)	

Table 1. Continued.

	AMBITION				FUNCTION			
MCS of SF-36*								
LSM	7.5	8.1	5.5	5.8	9.3	9.7	4.0	6.2
LSM difference (95% CI)	-0.6 (-4.0;2.8)		-0.3 (-2.8;2.3)		-0.3 (-3.1;2.5)		-2.2 (-4.9;0.6)	

	ADACTA				ACT-RAY	
	TCZ Mono		ADA Mono		TCZ Mono	
	GC-users (n=89)	Non-GC-users (n=74)	GC-users (n=92)	Non-GC-users (n=70)	GC-users (n=140)	Non-GC- users (n=136)
CDAI change‡						
LSM	-26.3	-22.1	-19.1	-23.5	-25.4	-26.5
LSM difference (95% CI)	-4.2 (-9.7;1.4)		4.3 (-4.0;12.6)		1.2 (-4.0;6.3)	
CDAI remission⁺						
n (%)	18 (20.2)	10 (13.5)	9 (9.8)	6 (8.6)	11 (7.9)	10 (7.4)
OR (95% CI)	1.6 (0.7;4.3)		3.1 (0.5;19.0)		1.0 (0.4;2.6)	
ACR50⁺						
n (%)	48 (53.9)	29 (39.2)	25 (27.2)	20 (28.6)	59 (42.1)	52 (38.2)
OR (95% CI)	1.8 (0.9;3.7)		1.1 (0.5;2.2)		1.3 (0.8;2.1)	
FACIT-F score*						
LSM	11.8	11.0	10.0	11.3	-	-
LSM difference (95% CI)	0.7 (-3.7;5.2)		-1.3 (-4.9;2.3)		-	-

PCS of SF-36*						
LSM	10.5	11.2	8.8	8.2	-	-
LSM difference (95% CI)	-0.8 (-4.0;2.4)		0.6 (-2.4;3.5)		-	-
MCS of SF-36*						
LSM	10.5	10.3	5.2	7.4	-	-
LSM difference (95% CI)	0.2 (-4.4;4.8)		-2.3 (-6.0;1.5)		-	-

The results shown are changes from baseline to week 24 (for CDAI/DAS28/FACIT-F score/PCS/MCS) or scores at week 24 (CDAI remission/ACR50).
‡*Analyses of covariance models for CDAI and DAS28 all included baseline GC use (yes/no), and baseline covariates CDAI/DAS28, region, sex, and RA duration. For some models, age, race (Asian, black, white, Native American/Pacific islander, other), and rheumatoid factor (RF) positivity (ADA arm only) are included. Most of the models included interaction terms among these covariates or with GC use. Included interactions with GC use are with region, sex, race and RF positivity.*
⁺*Logistic regression models all included baseline GC use (yes/no) and covariate age. All but 1 included baseline CDAI/DAS28. All but 2 models included region and gender. 2 models included the interaction of age with GC use. Other interactions were age with CDAI, gender with DAS28 and age with RA duration.*
**Analyses of covariance models for patient reported outcomes (PROs) for AMBITION/FUNCTION/ADACTA were performed and all included baseline GC use (yes/no) and baseline covariate FACIT/PCS/MCS score. Other covariates included are age, gender, region, C-reactive protein (CRP), health assessment questionnaire disability index (HAQ-DI), RA duration and baseline DAS28. Most of the models included interaction terms among these covariates or with GC use. 5 models included the interaction of baseline score (FACIT/PCS/MCS) with GC use. Other interactions with GC use were with region, age, gender, CRP and HAQ-DI.*
GC: glucocorticoid; 95% CI: 95% confidence interval; TCZ: tocilizumab; MTX: methotrexate; ADA: adalimumab; LSM: least squares means; CDAI: clinical disease activity index; OR: Odds Ratio; ACR50: American College of Rheumatology ≥50% improvement; FACIT-F: functional assessment of chronic illness therapy fatigue subscale; SF-36:36-item short-form health survey; PCS: physical component summary; MCS: mental component summary.

1). Similarly, differences in CDAI change to 24 weeks were small and 95%CI for the mean differences between GC-users and non-GC-users in ADA and MTX arms included 0, indicating non-significance. The Figure shows the CDAI scores over time for GC-users versus non-GC-users in TCZ, ADA and MTX monotherapy arms. Differences in CDAI remission rates and ACR50 response rates at 24 weeks between GC-users and non-users were also small and 95%CI of odds ratios included 1 in all arms, indicating non-significance (Table 1). Repeated measures analyses up to week 24 showed similar changes in CDAI between GC-users and non-GC-users in the TCZ arms. Analyses of PRO's showed no statistically significant differences between GC-users and non-users, see Table 1.

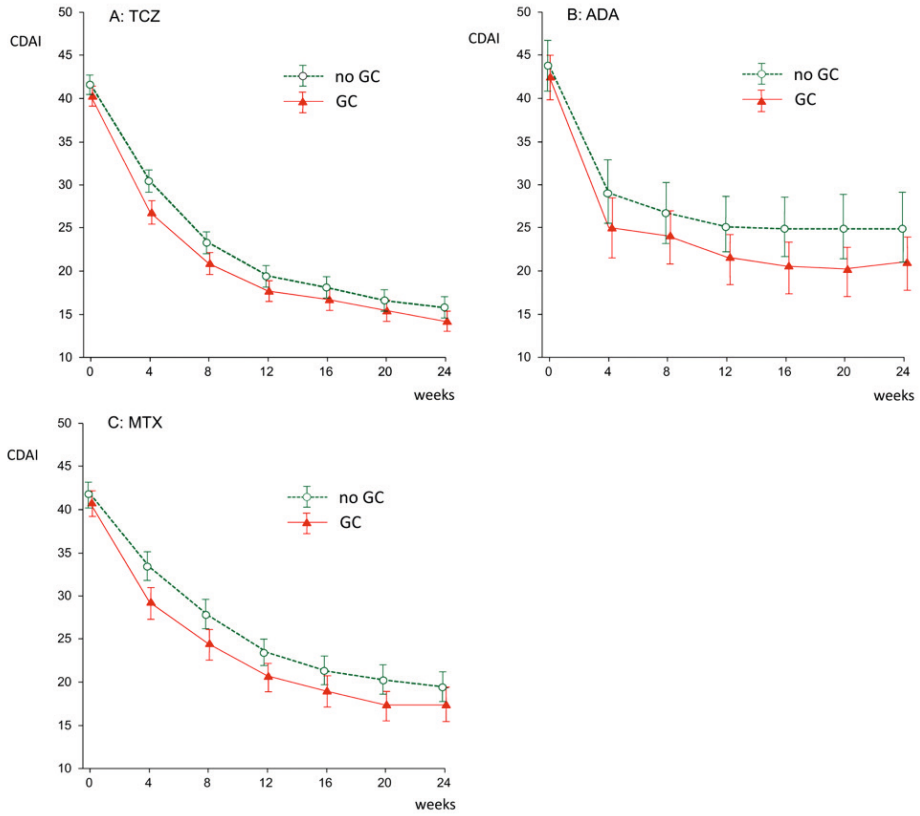

Figure. CDAI scores of GC users and non-GC users in TCZ, ADA and MTX monotherapy arms over time.

Unadjusted CDAI scores are plotted. CDAI: clinical disease activity index, range 0-76, interpretation 2.9–10 reflects low, 10.1–22 moderate and 22.1–76 high disease activity; mean with 95% confidence interval. For tocilizumab (TCZ) 4 trial arms, n=533 with no glucocorticoid background use (no GC) and 484 with glucocorticoid background use (GC). For adalimumab (ADA) 1 trial arm, no GC n=70, GC n=92. For methotrexate 2 trial arms, no GC n=242, GC n=242. Intent to treat analyses.

Adjusted differences with 95% CI in radiographic change between GC-users and non-GC-users in the TCZ arm of FUNCTION or ACT-RAY were similar and not statistically significant: in FUNCTION, the adjusted difference in mTSS at week 52 was 0.18 (-0.28, 0.64), at week 104, 0.32 (-0.73, 1.36); ACT-RAY: adjusted difference in GSS at week 52 0.5 (-0.0, 1.1), week 104 0.70 (-0.30, 1.60). However, in the MTX arm of the FUNCTION trial, adjusted differences in mTSS change from baseline to week 52 and week 104 between GC-users and non-GC-users were modest but statistically significant: -1.16 (-2.21, -0.12) and -1.60 (-3.12, -0.08), respectively, indicating in this trial arm less progression of radiological joint damage in background GC-users versus non-GC users.

Safety

The SAE rate among GC-users and non-GC-users in the TCZ arms was equal (16 vs. 16 per 100 patient-years (PYs), as shown in Table 2. SAE rate was not statistically significantly different for GC users versus non-GC-users in the MTX arms (16 vs. 9 per 100 PYs). In the ADA arm, GC-users had not statistically significantly different SAE rates nor serious infections rates compared to non-GC-users: 37 vs. 13 per 100 PYs and 12 vs. 5 per 100 PYs, respectively.

Table 2. SAE and serious infection rate of included GC-users and non-GC-users for initiated TCZ, MTX and ADA monotherapy

	TCZ arms		MTX arms		ADA arm	
	GC-users n=484	Non-GC-users n=533	GC-users n=242	Non-GC-users n=329	GC-users n=92	Non-GC-users n=70
All SAEs						
N (%)	30 (6)	34 (6)	13 (5)	9 (3)	12 (13)	4 (6)
Rate per 100 PY (95% CI)	16 (11, 22)	16 (11, 21)	16 (10, 26)	9 (5, 15)	37 (22, 59)	13 (4, 31)
Serious infections						
N (%)	12 (3)	9 (2)	3 (1)	2 (1)	4 (4)	1 (1)
Rate per 100 PY (95% CI)	6 (3, 10)	4 (2, 7)	3 (1, 8)	1 (0, 5)	12 (5, 27)	5 (1, 19)

SAE: serious adverse event; GC: glucocorticoid; TCZ: tocilizumab; MTX: methotrexate; ADA: adalimumab; PY: patient-years; 95% CI: 95% confidence interval.

Discussion

No effect of including RA patients on background GC use on efficacy, including PRO's, was found for initiating TCZ, ADA and MTX monotherapy, nor for TCZ on radiographic progression. Less radiographic progression was observed for patients on background GC, initiating MTX monotherapy in the FUNCTION trial,[13] an early RA trial conducted among MTX-naïve patients. This finding is in line with results of the Computer Assisted Management in Early Rheumatoid Arthritis trial-II (CAMERA-II),[4] and the Better Anti-Rheumatic PharmacOTherapy (BARFOT) study,[15] which showed less radiographic progression in early RA patients treated with MTX plus GC compared to MTX monotherapy. The finding that there was some progression of radiographic damage in the MTX monotherapy groups in these previous studies [4,15] and our study, as well as in placebo-IFX arms of 2 pooled IFX trials,[7] but no significant progression in the TCZ groups (data not shown), could explain that no joint sparing effect of GC was found if used concomitantly with TCZ.

Studies on the effect on outcomes of including RA-patients on background GC therapy in bDMARD RCTs are scarce. In an open label study,[9] efficacy benefits of TCZ were similar between RA patients with and without previous and continued oral GC treatment, with generally similar safety profiles, corroborating our results. In 6 tofacitinib trials, background GC use did not affect clinical or radiographic efficacy.[16]

In our study, SAE rates and serious infection rates were not statistically significantly different between GC-users and non-GC-users, initiating TCZ, ADA or MTX monotherapy.

Our study has some limitations. We analysed clinical data a period up to maximally 24weeks, based on the available trial data. Our research does not answer the question whether initiation of ADA or TCZ together with GC therapy would modify outcome when compared to initiation of ADA or TCZ without GC therapy; this would necessitate randomisation for GC.

In conclusion: no effect of including patients on background GC treatment on efficacy and safety outcomes of trials, initiating TCZ or ADA or MTX monotherapy, was found, with the exception of reduced radiological joint damage in one MTX arm in an early RA population. These findings support inclusion of RA-patients, who are on a low-moderate and stable GC dose, in RCTs, as is common practice.

Conflicts of interest

MSK received a student grant from AstraZeneca. AstraZeneca was not involved in this study. APS, XT, YL and JD are employees of F Hoffmann-La Roche. MJHdH is an employee of Novartis Pharma BV. Novartis Pharma BV was not involved in this study. MDE is an employee of Everest Clinical Research. JWJB reported grants and fees form Roche, AbbVie, Bristol-Myers, Squibb, Merck Sharp & Dohme, Pfizer and UCB. JMvL received fees from Arthrogen, MSD, Pfizer, Eli Lilly and BMS and research grants from Astra Zeneca and RocheGenentech. JWGJ and PMJW report no competing interests.

Authors' contributors

MSK, JWGJ, APS and JWJB contributed to the study design. MSK, APS, YL, MDE and JWGJ contributed to data collection. All authors had full access to the study data, contributed to data analysis, data interpretation, writing and review of the manuscript.

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Supplementary file

Supplementary methods: statistical analyses

Per trial arm we used analyses of covariance (ANCOVA) to estimate differences between included GC-users and non-GC-users in changes from baseline to week 24 in efficacy endpoints, correcting for relevant covariates. These were selected from the variables region, sex, and baseline age, RA duration, HAQ-DI, CRP, and disease activity score assessing 28 joints (DAS28) by a stepwise procedure, considering main effects and 2-way interactions, using a p-value criterion of 0.15; interactions were only allowed if the corresponding main effects were selected. Logistic regression analyses were used to assess associations between GC-use and CDAI remission as well as ACR50 response at week 24, including relevant covariates. Repeated measures analyses of CDAI including data of all post-baseline visits up to week 24 using the same covariates as well as time (as fixed effect and as random effect), were also performed. In addition, radiographic progression (modified Total Sharp Score (mTSS) or Genant Modified Sharp Score (GSS)) was analysed up to week 104 using data from ACT-RAY [11] and FUNCTION.[13] Covariates which had a statistically significant effect ($p<0.05$) upon each outcome were retained in each model.

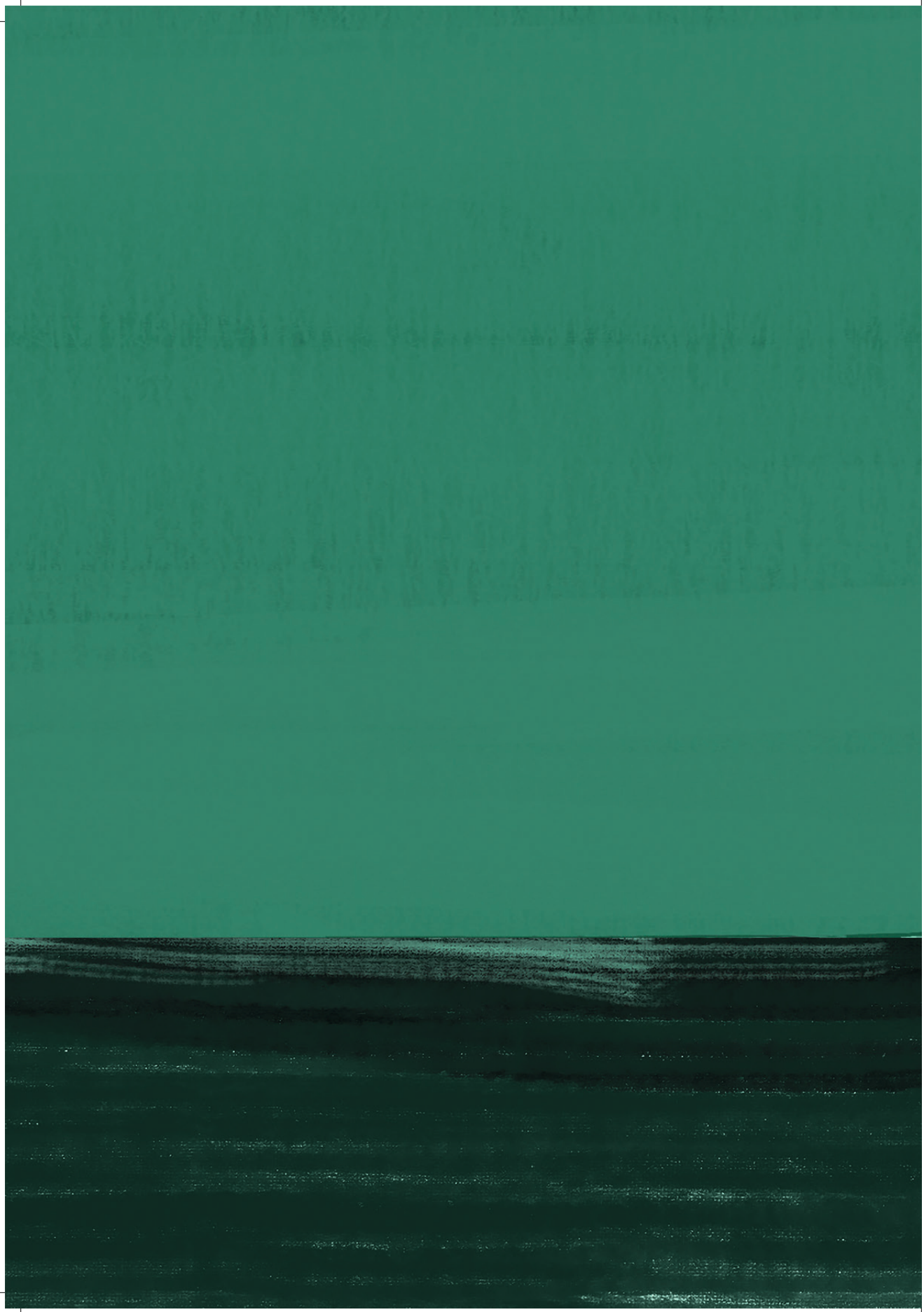
Supplementary Table. Baseline RA characteristics of included GC-users versus non-GC-users per monotherapy trial-arm

		GC-users			Non-GC-users		
		TCZ mono	MTX mono	ADA mono	TCZ mono	MTX mono	ADA mono
Duration of RA (years), mean (SD)	AMBITION	6.8 (7.9)	6.0 (6.5)	-	6.1 (7.9)	6.4 (8.8)	-
	FUNCTION	0.5 (0.4)	0.4 (0.5)	-	0.5 (0.5)	0.4 (0.5)	-
	ACT-RAY	8.2 (8.9)	-	-	8.4 (7.8)	-	-
	ADACTA	7.2 (7.2)	-	5.9 (6.8)	7.4 (9.0)	-	6.7 (7.2)
Prior anti-TNF usage, n(%)	AMBITION	9 (6.6)	12 (9.0)	-	14 (9.4)	12 (8.0)	-
	FUNCTION	0	0	-	0	0	-
	ACT-RAY	1 (0.7)	-	-	0	-	-
	ADACTA	1 (1.1)	-	1 (1.1)	1 (1.4)	-	0
Oral prednisone equivalents dosage (mg/day), mean (SD)	AMBITION	7.4 (2.7)	7.1 (2.4)	-	-	-	-
	FUNCTION	7.5 (2.4)	7.4 (2.4)	-	-	-	-
	ACT-RAY	6.7 (2.5)	-	-	-	-	-
	ADACTA	6.4 (2.7)	-	6.4 (2.7)	-	-	-

Supplementary Table. Continued.

		GC-users			Non-GC-users		
		TCZ mono	MTX mono	ADA mono	TCZ mono	MTX mono	ADA mono
RF positivity, n (%)	AMBITION	106 (77.4)	103 (77.4)	-	107 (71.8)	109 (82.2)	-
	FUNCTION	108 (91.5)	95 (87.2)	-	154 (89.0)	159 (89.3)	-
	ACT-RAY	12 (44.4)	-	68 (73.9)	29 (78.4)	-	-
	ADACTA	71 (79.8)	-		51 (68.9)		51 (72.9)
DAS28, mean (SD)	AMBITION	6.6 (1.0)	6.8 (0.9)	-	6.9 (1.0)	6.8 (0.9)	-
	FUNCTION	6.7 (1.0)	6.4 (1.0)	-	6.7 (1.0)	6.7 (1.0)	-
	ACT-RAY	6.4 (1.0)	-	-	6.4 (1.0)	-	-
	ADACTA	6.6 (0.9)	-	6.7 (0.9)	6.9 (0.9)	-	6.9 (9.2)
CDAI, mean (SD)	AMBITION	41.5 (13.5)	43.0 (11.9)	-	44.0 (12.5)	43.5 (12.2)	-
	FUNCTION	40.9 (12.7)	38.1 (13.1)	-	41.0 (13.7)	40.3 (13.8)	-
	ACT-RAY	39.0 (13.6)	-	42.5 (13.0)	39.4 (12.2)	-	43.8 (12.2)
	ADACTA	39.6 (12.8)	-		42.3 (11.6)		
HAQ-DI, mean (SD)	AMBITION	1.6 (0.7)	1.6 (0.6)	-	1.6 (0.6)	1.5 (0.6)	-
	FUNCTION	1.6 (0.6)	1.5 (0.7)	-	1.6 (0.7)	1.5 (0.7)	-
	ACT-RAY	1.5 (0.6)	-	-	1.4 (0.6)	-	-
	ADACTA	1.6 (0.6)	-	4.7 (0.6)	1.7 (0.6)	-	1.7 (0.6)

RA: rheumatoid arthritis; GC: glucocorticoid; SD: standard deviation; TCZ: tocilizumab; MTX: methotrexate, ADA: adalimumab; TNF: tumour necrosis factor; RF: rheumatoid factor; DAS28: disease activity score assessing 28 joints: ESR: erythrocyte sedimentation rate; CDAI: clinical disease activity index; HAQ-DI: healthy assessment questionnaire disability index.



4.

The multi-biomarker disease activity test for assessing response to treatment strategies using methotrexate with or without prednisone in the CAMERA-II Trial

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Abstract

Objectives. The CAMERA-II trial compared two tight-control, treat-to-target strategies, initiating methotrexate with prednisone (MTX+pred) or MTX with placebo (MTX+plac), in patients with early rheumatoid arthritis (RA). The multi-biomarker disease activity (MBDA) blood test objectively measures RA disease activity with a score of 1-100. In CAMERA-II, response profiles of the MBDA score, its individual biomarkers and DAS28-ESR were assessed.

Methods. We evaluated 92 patients from CAMERA-II who had clinical data and serum for MBDA testing at baseline and ≥1 timepoint from months 1, 2, 3, 4, 5, 6, 9 or 12. Changes (Δ) from baseline for DAS28-ESR and MBDA score and comparisons of ΔDAS28-ESR and ΔMBDA score over time for patients treated with the MTX+pred strategy versus the MTX+plac strategy, were tested for significance with t-tests. Changes in biomarker concentration from baseline to months 1-5 were tested with Wilcoxon signed rank test and tested for difference between treatment arms by Mann-Whitney U test.

Results. MBDA score and DAS28 showed similar response profiles, with gradual declines over the first 6 months in the MTX+plac group, and faster improvement during month 1, followed by gradual improvement. The 12 MBDA biomarkers could be grouped into 4 categories of response profile with significant responses observed for 4 biomarkers during the MTX+plac strategy and 9 biomarkers during the MTX+pred strategy.

Conclusions. MBDA tracked treatment response in CAMERA-II similarly to DAS28. More individual MBDA biomarkers tracked treatment response to MTX+pred than to MTX+plac. Four response profiles could be observed.

Introduction

Rheumatoid arthritis (RA) is a chronic disease of inflammation in synovial joints, resulting in joint damage, physical disability and decreased life span. RA affects approximately 0.5–1.0% of adults in industrialized countries.[1-2] As treatment options for RA have improved, it has become the goal of therapy to achieve remission as rapidly as possible.[3-5] Current guidelines recommend early initiation of methotrexate (MTX) as the anchor disease modifying anti-rheumatic drug (DMARD)[6-7] Tight control with treat-to-target strategies, preferably including MTX [8-10] have been shown to provide better outcomes than the contemporaneous standard practices.[11-16] In treat-to-target strategies, RA disease activity is quantitatively assessed at regular intervals and, based on pre-specified criteria for treatment response, treatment is adjusted to expeditiously achieve a target of low disease activity or remission.[17]

Treat-to-target or tight control strategies require that physicians assess RA disease activity quantitatively. Measures based on physical examination and history, including joint counts and patient global assessment, are subjective and variable between observers. The routine inflammatory response measures of RA disease activity, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), have the shortcoming that they are frequently in the normal range for patients with active RA and are not specific for the disease.[18-19] Studies with magnetic resonance imaging or ultrasound have demonstrated that, even when clinically-based criteria for remission are met, joint inflammation is often demonstrable and progressive damage can be ongoing.[20-21] Thus, there is a need for objective measures that are more sensitive to joint inflammation and more accurately predict progressive joint damage than current clinical assessment tools.

The multi-biomarker disease activity (MBDA) blood test measures 12 biomarkers relevant to the pathophysiology of RA to provide an objective measure of RA disease activity. It uses a validated algorithm to combine the biomarker concentrations to generate an integer score on a scale of 1 to 100.[22-25] The MBDA score correlates with the 28-joint disease activity score using CRP (DAS28-CRP) and other clinical measures of RA disease activity, and change in MBDA score correlates with change in DAS28-CRP.[26] In a study of patients with stablished RA receiving ongoing treatment with DMARDs, MBDA score was more strongly associated with radiographic progression than DAS28-CRP, and among patients in DAS28-CRP remission, progression was more frequent among those with a high MBDA score.[27] Similar analyses of patients from SWEFOT, a trial of tight control strategies for patients with early RA, found that baseline MBDA score was more strongly associated with radiographic progression than DAS28-ESR

or CRP.[28] Analyses of the MBDA score were mostly cross-sectional. No study yet evaluated the MBDA response longitudinally at multiple, monthly time points to MTX-based treatment strategies with or without prednisone, such as were applied in the Computer Assisted Management in Early RA Trial-II (CAMERA-II) [29]. In the present sub-study of CAMERA-II, the two strategy arms were compared longitudinally at monthly intervals to determine if the response profiles differed between the MBDA score and DAS28, or among the 12 individual biomarkers of the MBDA score.

Methods

CAMERA-II clinical study procedures and summary of results

The design, intervention and main analyses of the CAMERA-II study are reported in detail elsewhere.[29] To summarize, CAMERA-II was a 2-year, prospective, randomised, placebo-controlled, double-blind multicentre tight control and treat-to- target (remission) strategy trial among patients with early RA (<1 year since diagnosis). Patients were 18 years or older and naïve to DMARD therapy, including glucocorticoids.

At study baseline, all patients initiated a monthly step-up strategy using oral MTX, at a starting dosage of 10 mg per week, and were randomised to also receive either oral prednisone, 10 mg per day, or placebo. Rheumatologists assessed each patient monthly and a computer program indicated whether the patient had achieved response (>20% improvement) compared with the previous visit. If response was not sufficient and remission had not been achieved, MTX dosage was increased by 5 mg per week until the patient had achieved remission (swollen joint count (SJC) =0 and ≥2 of the following criteria: tender joint count (TJC) ≤3, visual analogue scale (VAS) score ≤20 mm and ESR ≤20 mm/hr). At the maximum (30 mg per week) or maximum tolerable MTX dosage, if a step-up in treatment was indicated, MTX was administered at the same dosage subcutaneously. As the next step, cyclosporine was added to the regimen. However, shortly after start of the trial, cyclosporine was replaced with adalimumab.[29] All patients received folic acid, calcium carbonate with vitamin D and a bisphosphonate.

The medical ethics committee of the University Medical Center Utrecht approved the study. All patients provided written informed consent before entering the study. Onset of efficacy was more rapid in the MTX+pred strategy group, and at 2 years, the MTX+pred strategy group had achieved a greater reduction in disease activity, as measured with the DAS28, and had less progression of erosive joint damage, fewer adverse effects, and less frequent need for additional biological (b) DMARD treatment.[18]

The MBDA score

The development and validation of the MBDA score are reported in detail elsewhere [24, 25]. In short, 130 candidate biomarkers were tested in feasibility studies, of which 12 were selected for final algorithm development and validation. The biomarker selection and algorithm were optimized to maximize the strength of the association of the MBDA score with DAS28-CRP in a cohort of patients on diverse treatments [25]. Concentrations of these 12 MBDA protein biomarkers (CRP, epidermal growth factor, interleukin (IL) 6, leptin, matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 3 (MMP-3), resistin, serum amyloid A (SAA), tumour necrosis factor receptor type I (TNF-RI), vascular cell adhesion molecule 1 (VCAM-1), vascular endothelial growth factor A (VEGF-A), and cartilage glycoprotein 39 (YKL-40)) were measured by multiplex immunoassay using the Meso Scale Discovery MULTI-ARRAY® platform. Biomarker concentrations were combined in the validated MBDA algorithm to generate the MBDA score, an integer from 1 to 100, for which the established categories of disease activity are low (< 30), moderate (30– 44), and high (> 44) [24]. Biomarker measurement and MBDA score calculation were performed in the CLIA-certified laboratory of Crescendo Bioscience, Inc., South San Francisco, CA, USA using the same instrument, reagents and algorithm as for the Vectra® DA test, which is commercially available in the United States.

Multiple biomarker-based disease activity assessment in CAMERA-II

MBDA biomarkers were evaluated in serum samples obtained at baseline and at 1, 2, 3, 4, 5, 6, 9 and 12 months. Numbers of samples available for the present study varied between time points, based on patient compliance and the volume of available sample. Of 104 patients in CAMERA-II for whom baseline sera were available for MBDA testing, MBDA scores and DAS28-ESR were analysed for the 92 who had at least one MBDA test result for months 1, 2, 3, 4, 5, 6, 9 or 12. For this 92-patient cohort, the average number of post-baseline tests per patient was 3.7.

Statistical Analyses

To evaluate changes from baseline for DAS28 and MBDA score and comparisons of change in DAS28 or MBDA score over time between patients treated with the MTX+pred or MTX+plac strategy, a t test was performed for each time point evaluated. Association between change from baseline to 12 months for DAS28 and MBDA score was assessed using Spearman's rank correlation. Concentrations of individual biomarkers were analysed for the subset of 51 patients who had an MBDA test at baseline and at least one time point from months 1 to 5, to focus on the initial biomarker responses to treatment and exclude possible effects from exposure to cyclosporine or adalimumab. The average number of postbaseline tests per patient was 3.3 in this subset.

Biomarker concentrations were analysed after base-10 logarithm (log10) transformation, to approximate a normal distribution. The changes from baseline in log10 biomarker concentrations were assessed for months 1–5 for each treatment arm by Wilcoxon signed rank test and compared between treatment arms by Mann-Whitney U tests. The means of the changes were calculated as averages of individual changes in log10 values, and standard error (SE) values were determined accordingly. For presentation in graphs, each mean change (D) was back transformed by raising 10 to the D power, thus reversing the log10 transformation to generate a fractional value, relative to baseline, on a linear scale. Thus, any time point demonstrating no change from baseline was represented on the graph with a value of 1.0, and for example, a 20% reduction from baseline was represented with a value of 0.8. Response profiles are the courses of changes from baseline for the MTX+plac and MTX+pred strategy arms. For the individual biomarkers, profile categories were defined, dependent on their response to MTX+plac, and their response to concomitant prednisone, i.e., the difference in response to MTX+plac and MTX+pred. This was based on visual inspection of curves representing change from baseline in biomarker concentration for each treatment strategy arm and on p values for changes from baseline and for the difference between treatment strategy arms. The software package R 2.15.1 (www.rproject.org) was used for the analyses. No clinical or biomarker data were imputed. A p value of < 0.05 was considered statistically significant. No adjustments were made for multiple testing.

Results

Baseline characteristics

Baseline characteristics of the 92 patients analysed to month 12 were similar between treatment arms (Table 1). Characteristics were similar between these 92 patients and the subset of 51 patients for whom individual biomarkers were analysed to month 5 (data not shown), and the 236 patients of the full CAMERA-II population, except for joint counts and CRP, which tended to be lower in the present study.[18]

Table 1. Patient characteristics at baseline

	All patients N=92	MTX+plac n=50	MTX+pred n=42
Sex, % female	59	56	62
Age	57 (47–65)	54 (46–65)	58 (47–67)
Smoking, as number of cigarettes per day	0 (0–5)	0 (0–0.5)	0 (0–5)
RF status, % positive	63	70	56
HAQ score, 0-3	1.1 (0.63–1.6)	1.2 (0.63–1.6)	1.1 (0.63–1.5)
General health VAS, 0-10	5.0 (2.7–6.7)	5.1 (3.6–6.6)	4.7 (2.2–6.7)
TJC28	10 (6–17)	9.5 (6–13)	12 (5–18)
SJC28	11 (7–15)	11 (7–15)	11 (6–15)
ESR mm/hr	31 (19–44)	29 (19–43)	31 (18–45)
CRP mg/L	16 (2.7–41)	16 (5.5–42)	16 (1.9–37)
DAS28-ESR	5.6 (4.9–6.6)	5.6 (5–6.3)	5.6 (4.1–6.9)
MBDA score, 1-100	51 (39–71)	54 (40–72)	49 (40–70)

No statistically significant differences in baseline characteristic between MTX+plac and MTX+pred groups. Values are median (interquartile range) or percentage RF, rheumatoid factor (RF status was available for 82 of 92, 43 of 50, and 39 of 42 patients, respectively); MTX, methotrexate; HAQ, health assessment questionnaire; VAS, visual analogue scale general health; TJC28, tender joint count assessing 28 joints; SJC28, swollen joint count assessing 28 joints; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, 28-joint-based disease activity score; MBDA, multi-biomarker disease activity; higher scores of HAQ, VAS, and MBDA score reflect worse scores; MTX+plac, the methotrexate and placebo strategy; MTX+pred the methotrexate and prednisone strategy.

Clinical, MBDA and biomarker responses to therapy

Reductions in DAS28-ESR and MBDA score had similar profiles of change from baseline over time, with more rapid and greater initial responses observed for patients treated with the MTX+pred strategy, compared with the MTX+plac strategy (Table 2, Figure 1). For the 59 patients with data at baseline and 12 months, the changes from baseline to 12 months for DAS28-ESR and MBDA score were significantly correlated, both overall ($r=0.56$, $p<0.001$) and within each treatment arm: MTX+pred ($n=28$, $r=0.57$, $p=0.002$); MTX+plac ($n=31$, $r=0.57$, $p=0.001$).

Table 2. Mean changes from baseline for DAS28-ESR and MBDA score during treatment with a tight-control strategy using MTX+plac or MTX+pred

	DAS28-ESR				MBDA score			
	MTX+plac		MTX+pred		MTX+plac		MTX+pred	
Timepoint (month)	n	Mean Change	n	Mean Change	n	Mean Change	n	Mean Change
1	16	-0.3 *P* = 0.243	11	-1.9 *P* < 0.001	18	-3 *P* = 0.265	14	-12 *P* = 0.013
2	15	-0.7 *P* = 0.021	11	-2.4 *P* < 0.001	17	-3 *P* = 0.251	14	-11 *P* = 0.02
3	22	-1.3 *P* < 0.001	13	-3.0 *P* < 0.001	25	-5 *P* = 0.093	17	-15 *P* = 0.002
4	13	-1.8 *P* = 0.001	10	-3.9 *P* < 0.001	17	-9 *P* = 0.029	14	-19 *P* = 0.003
5	15	-2.2 *P* < 0.001	10	-4.2 *P* < 0.001	18	-12 *P* = 0.006	12	-20 *P* = 0.003
6	18	-2.8 *P* < 0.001	12	-3.0 *P* = 0.001	29	-20 *P* < 0.001	19	-16 *P* = 0.001
9	17	-2.7 *P* < 0.001	12	-3.2 *P* = 0.001	24	-24 *P* < 0.001	17	-20 *P* = 0.001
12	31	-2.8 *P* < 0.001	28	-3.1 *P* < 0.001	44	-20 *P* < 0.001	37	-16 *P* < 0.001

Each n value indicates number of patients from the study cohort (total N = 92) with available data at that time-point. P values are for changes from baseline by t test DAS28 28-joint-based disease activity score, MBDA multi-biomarker disease activity, MTX+plac the methotrexate and placebo strategy, MTX+pred the methotrexate and prednisone strategy.

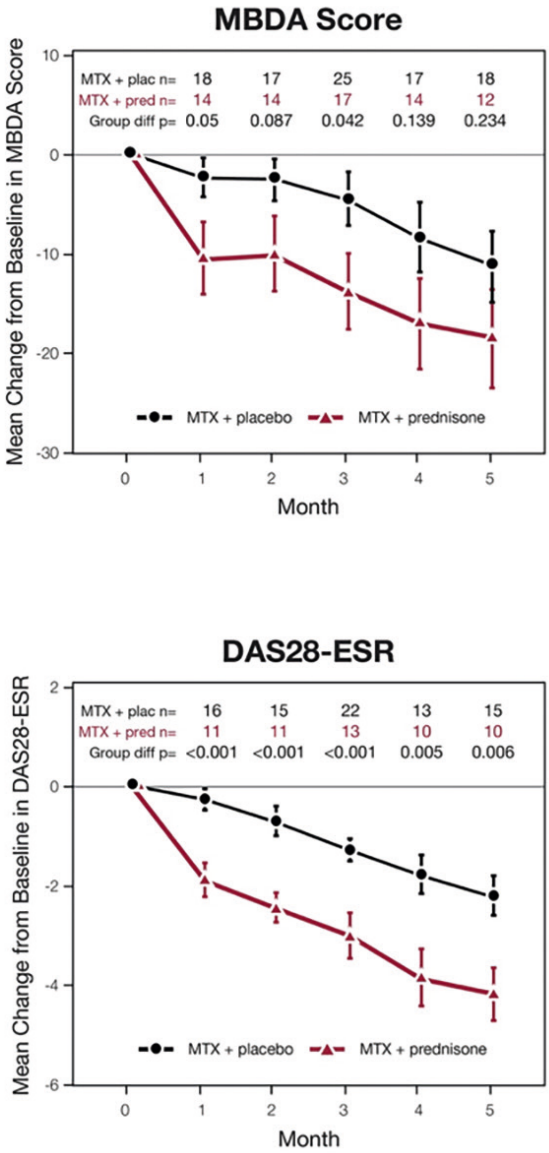

Figure 1. Mean (SE) changes from baseline in MBDA score and DAS28 for each strategy arm of the CAMERA-II study.

Mean (SE) changes from baseline in MBDA score and DAS28 for each strategy arm of the CAMERA-II study are shown over first 5 months at monthly assessments, each prior to dosing with MTX and placebo (MTX+plac) or MTX and prednisone (MTX+pred) at that time-point. P values of t tests for comparison between strategy arms (Group diff). Patient numbers are shown for each time-point in each strategy arm.

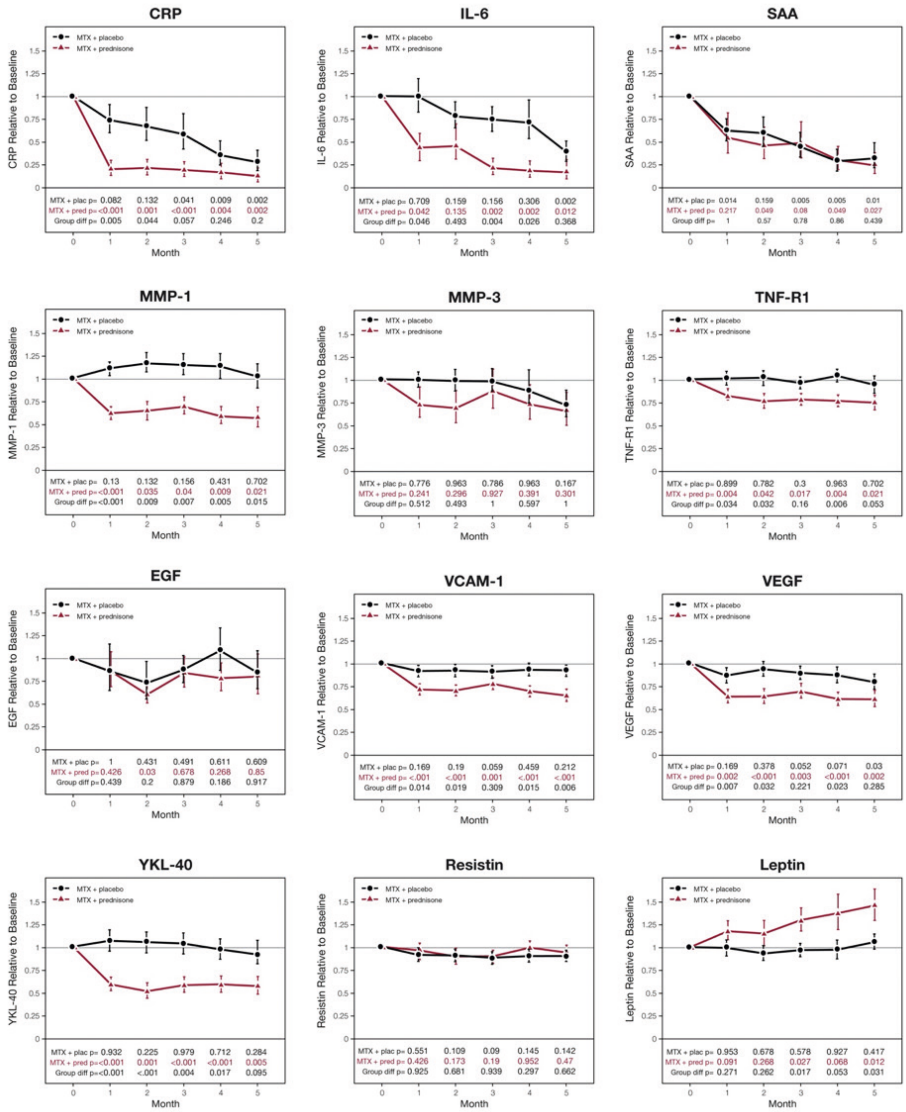

Figure 2. Mean (SE) changes in concentrations of MBDA biomarkers over the first 5 months of the CAMERA-II study.

Mean (SE) changes in concentrations of MBDA biomarkers over the first 5 months of the CAMERA-II study Each measurement was performed in serum obtained prior to dosing with MTX and placebo (MTX+plac) or MTX and prednisone (MTX+pred) at that time-point. Means and standard errors were calculated using log10-transformed values of the biomarker concentrations and, for graphic display, were then converted to fractional values relative to baseline on a linear scale (see the "Methods" section). Patient numbers for each time-point in each strategy arm are the same as in the MBDA panel of Fig. 1. Four profiles of biomarker response categories can be observed: (1) statistically significant

gradual reduction in biomarker concentration with the MTX+plac strategy versus a more rapid and statistically significantly greater reduction in biomarker concentration with the MTX+pred strategy (CRP, IL-6, VEGF); (2) little (but not statistically significant, except for EGF only at month 2 in MTX+pred), or no response to either treatment, with no significant difference between strategies (EGF, MMP-3, resistin); 3) little (but not statistically significant) or no biomarker response with the MTX+plac strategy versus a more rapid and statistically significantly greater biomarker response with the MTX+pred strategy (decrease: MMP-1, VCAM-1, TNF-R1, YKL-40; increase: leptin); and (4) significant gradual reduction in biomarker concentration over time with the MTX+plac strategy and the MTX+pred strategy, with no statistically significant difference between the two arms (SAA).

Most individual MBDA biomarkers showed statistically significant changes over time, and for eight biomarkers, these changes differed between treatment arms (Figure 2). Treatment with MTX+plac induced a statistically significant decline in concentration for 4 of the biomarkers: CRP, IL-6, SAA, and VEGF. Treatment with MTX+pred significantly decreased the concentrations of these 4 biomarkers and also MMP-1, TNF-R1, VCAM-1, and YKL-40. No sustained, significant decrease was observed with either treatment for MMP-3, EGF, or resistin. Leptin concentrations were unaffected by treatment with MTX+plac, but they increased with MTX+pred.

Discussion

The present study is the first to present the MBDA score at multiple, consecutive monthly time points following initiation of MTX-based treatment strategies, with or without prednisone. This frequency of testing allowed us to demonstrate that the added benefit from prednisone was almost entirely achieved within the first month, both clinically and in terms of biomarker measurements. Since prednisone provides symptomatic benefit rapidly after the first dose, physiological effects of prednisone in CAMERA-II probably started before the first postbaseline assessment at 1 month. The response of CRP, which is a component of the MBDA score, can precede clinical response with biologics [30, 31]. We found that the MBDA score declined steadily over the first 6 months of treatment with MTX+plac. For patients who received MTX+pred, the initial MBDA response was markedly greater than for those in the MTX+plac arm and was followed by a gradual, continued decline in MBDA score that approximately paralleled that of the MTX+plac arm. This profile of MBDA response resembled the clinical response, as assessed with DAS28. These findings are consistent with the fact that the MBDA score was validated based on its correlation with DAS28-CRP, DAS28, and other clinically based measures of disease activity [24, 25]. Similarly, change in MBDA score correlated here with change in DAS28 in both treatment arms, which is consistent with previous analyses of changes in MBDA score, DAS28-CRP, and DAS28 [25, 26]. For both DAS28 and MBDA score, responses during the second 6 months of treatment were similar between treatment arms and relatively stable, consistent with overall results of the study

[18]. While the response profiles for MBDA score and DAS28 appeared to be similar in this study, the changes from baseline and the differences between treatment arms were statistically significant at more time points for DAS28 than for the MBDA score. In addition, the DAS28 responses had narrower confidence intervals (Figure 2). These differences between MBDA score and DAS28 may be reflections of the tight control strategy in CAMERA-II, where treatment adjustments were based on the DAS28 component measures. An opposite result might have been obtained if, instead, the MBDA score had been used for dictating the tight-control strategy. Such results are not currently available. The similarity of the CAMERA-II response profiles for DAS28 and MBDA score, which are both composite measures, led us to examine whether the individual biomarkers of the MBDA test also exhibited similar patterns of change over time. We found that, while some biomarkers had similar response profiles to that of the MBDA score, considerable variability was observed among the 12 biomarkers. Leptin concentrations were unaffected by treatment with MTX+plac, but unlike any of the other biomarkers, they increased with MTX+pred. This result is consistent with findings in patients treated with glucocorticoids alone [32, 33]. By contrast, leptin concentrations have been reported to not change significantly from baseline during treatment with an anti-TNF agent and concomitant MTX, with or without a concomitant glucocorticoid [34, 35]. For SAA, a significant response was seen with MTX alone, but prednisone provided no additional effect. This category profile was unique to SAA and contrasts with that of CRP and IL-6, even though CRP and SAA are both acute phase proteins of which the production is driven by IL-6 [36]. The basis for this lack of prednisone effect on SAA is uncertain. Evidence that SAA is a more sensitive indicator of inflammation than CRP [37], and that glucocorticoids can increase the production of SAA outside of the liver [38, 39], suggests that the basis for our SAA finding may be multifactorial. The most conclusive findings in this study came from comparing the biomarkers in terms of their response profiles, i.e., the patterns observed by viewing the two treatment arms in tandem for each biomarker. The 12 MBDA biomarkers could be grouped into 4 categories based on their response profile to the MTX+pred and MTX+plac strategies. Whether or not a biomarker responded to MTX+plac, a greater response was usually observed with the addition of prednisone, as seen with CRP, IL-6, and VEGF, which decreased in both arms but to a greater degree in the MTX+pred arm. Another category profile was seen with MMP-1, VCAM-1, TNF-R1, YKL-40, and leptin, which did not seem to respond to MTX+plac, but did respond to MTX+pred, thus to prednisone. These two profiles suggest that prednisone affects a broader spectrum of immunosuppressive mechanisms than MTX. A limitation of this study is that it was a post hoc analysis of 92 of the 236 patients of CAMERA-II. Although the patients studied here were selected on the basis of availability of serum samples, their baseline data were similar between randomization arms and, overall, to those of the full CAMERA-II population.

Moreover, although sample size at individual time-points was small and it varied across time-points, statistical significance was achieved for all single-biomarker values that were interpreted as being changed from baseline or as being different between the two arms. Subset analyses were not performed, due to the limited sample size. Given that patient numbers were identical for the 12 biomarkers, the distinctiveness of the 4 categories of biomarker response profile suggests that they reflect true biological differences. The results obtained here are hypothesis-generating and suggest that a larger study is warranted for confirmation and further exploration.

Conclusions

In summary, during the first year of the CAMERA-II trial, the MBDA score and DAS28 were similar in their detection of response to treatment strategies initiating MTX with placebo or MTX and prednisone. Like the DAS28, the MBDA score demonstrated a more rapid and greater early response to MTX with prednisone compared with MTX with placebo. Analysis of the 12 MBDA biomarkers showed that more biomarkers responded to MTX with prednisone than to MTX with placebo, with 4 distinct categories of response profile observed.

Conflicts of interest

EHS was an employee of Crescendo Bioscience, Inc. MSK received a research grant from AstraZeneca. AstraZeneca was not involved in this study. MJHdH is an employee of Novartis Pharma BV. Novartis Pharma BV was not involved in this study. JWJB reported grants and fees from Roche, AbbVie, BristolMyers, Squibb, Merck Sharp & Dohme, Pfizer, and UCB. FPJGL is co-founder, shareholder, and co-director of ArthroSave BV and is consultant for Synerkine Pharma BV. ArthroSave and Synerkine were not involved in this study. MSJ, JWGJ, JT, and PMJW report no competing interests.

Authors' contributors

MSJ, MSK, MJHdH, JWJB, EHS, FPJG L, and JWGJ contributed to the study design. MSJ, MSK, EHS, and JWGJ contributed to the data collection. All authors wrote the manuscript.

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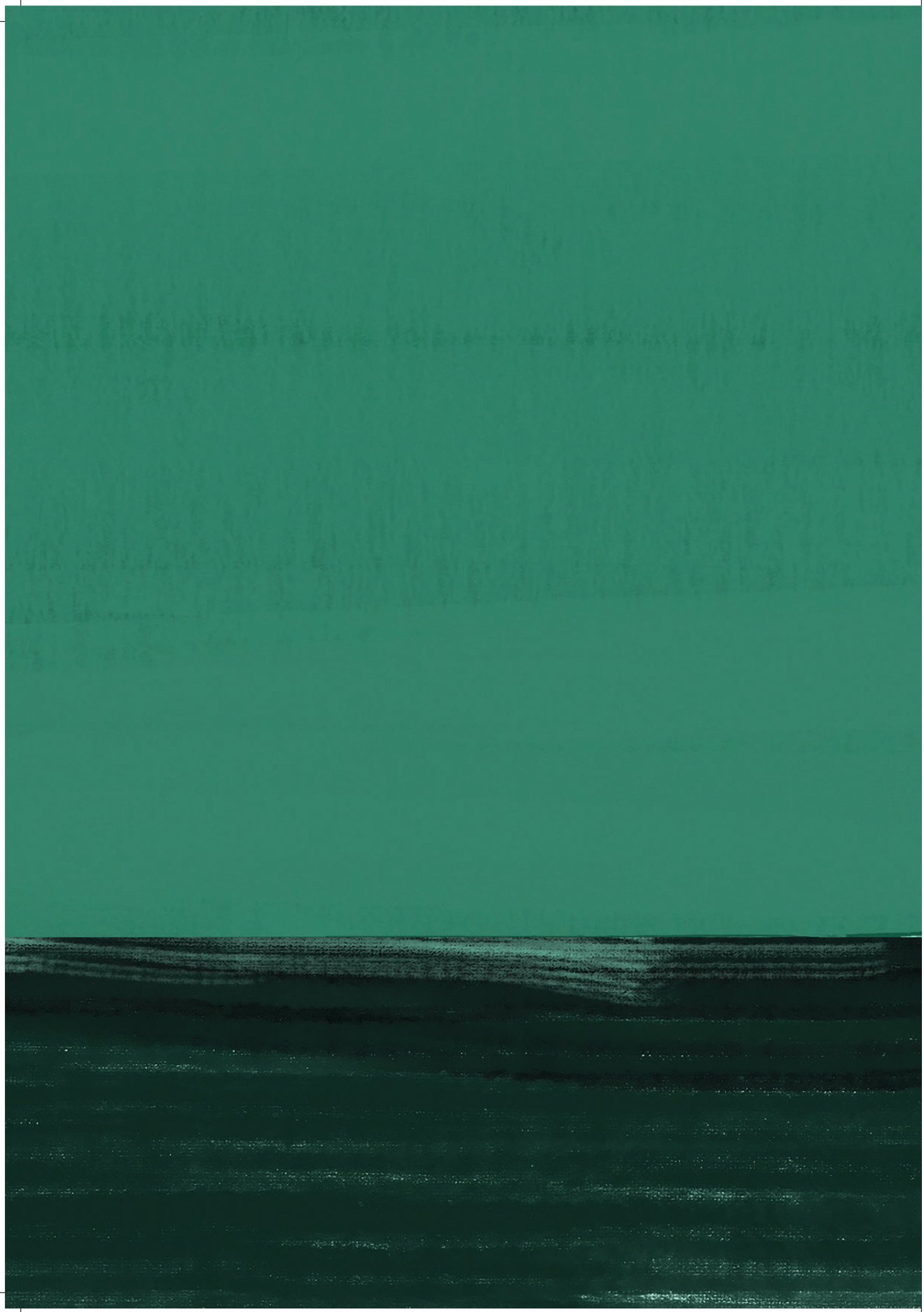

5.

Current smoking negatively affects the response to methotrexate in RA in a dose-responsive way, independently of concomitant prednisone use

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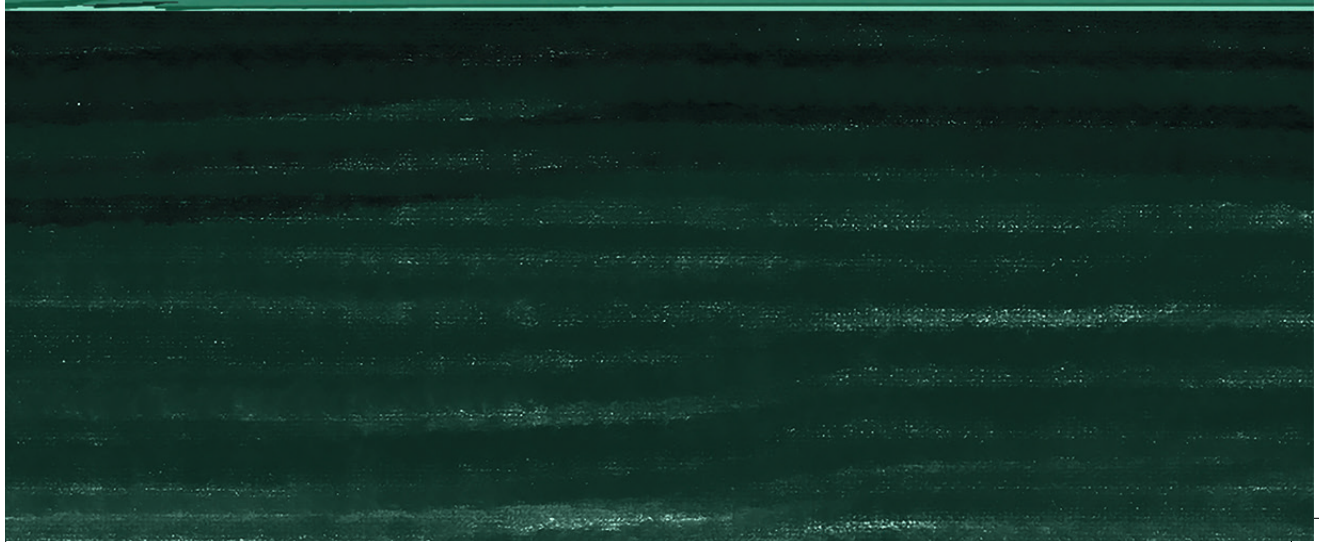

Abstract

Objective. Current smoking reduces clinical response to several disease-modifying antirheumatic drugs (DMARDs). It is unknown if this is also the case for prednisone. We aimed to determine whether current smoking affects the clinical response to concomitant prednisone in a methotrexate (MTX)-based treatment strategy.

Methods. In the CAMERA-II trial, early rheumatoid arthritis (RA) patients initiated an MTX-based strategy and were randomised to concomitant prednisone (MTX+pred) or placebo (MTX+plac) for 24 months. Linear mixed modelling was performed with disease activity score assessing 28 joints (DAS28) as dependent variable and strategy group and current smoking status as independent variables, correcting for relevant covariates. The interaction between current smoking and strategy was tested to find out whether the impact of current smoking on clinical response was different between the strategy groups with prednisone or placebo.

Results. Current smoking was significantly associated with higher DAS28 over time (mean difference with non-smokers 0.57 (95% confidence interval 0.22 to 0.92), $p<0.01$). This association was not different between the strategy groups with prednisone or placebo ($p=0.73$). This negative effect of current smoking on DAS28 was dose dependent.

Conclusion. Current smoking in early RA patients significantly reduces the clinical effect of an MTX-based strategy, independent of whether concomitant prednisone is used. This effect is dose dependent.

Introduction

Smoking is a known risk factor for the development of rheumatoid arthritis (RA),[1,2] and has been negatively associated with clinical response to several disease modifying anti-rheumatic drugs (DMARDs).[3-6] For example, in the SweFot study and the U-Act-Early trial, current smoking negatively predicted the likelihood of response to methotrexate (MTX) in early RA.[4] In the U-Act-Early trial, this association was dose-dependent.[4] Smoking was also found to be negatively associated with clinical response to rituximab and anti-TNF treatment.[5,6] As far as we know, the association of smoking and clinical response to glucocorticoids (GCs) has never been studied, whereas concomitant GC treatment in RA is common. Especially early RA patients at initiation of MTX treatment often receive concomitant GCs,[7] to further reduce disease activity and radiographic progression and improve functional ability.[8,9]. For example, in the second Computer Assisted Management in Early Rheumatoid Arthritis trial (CAMERA-II), disease activity improved faster and erosive joint damage was significantly less in the MTX strategy group with prednisone, compared to in the MTX strategy group with placebo, showing benefit of concomitant prednisone.[9] However, it has been found that approximately 30% of patients has a reduced or absent clinical response to GCs.[10,11] This might be associated with smoking, similarly to the negative effects of smoking on efficacy of other DMARDs. The primary objective of this study was to determine if the added clinical benefit of prednisone, when used concomitantly with MTX, is reduced among smokers.

Methods

We used data of the CAMERA-II trial (International Standard Randomised Controlled Trial Number: ISRCTN 70365169) in which 236 early RA patients were randomised to a treatment strategy initiating MTX and prednisone 10 mg/day (MTX+pred) or MTX and placebo (MTX+plac). In short, MTX treatment was started at 10mg/week and a tight control and treat-to-target strategy was followed with patient-tailored dosing adjustments at monthly visits on the basis of predefined response criteria aiming for remission. If no remission was achieved at 4 weeks after reaching the maximum (tolerable) MTX dosage, the route of MTX administration was switched from oral to subcutaneous and if thereafter still no remission was achieved, adalimumab was added. Prednisone or placebo was given in stable dose for 24 months. Details have been described previously.[9]

Ethics approval

The CAMERA-II trial was approved by the medical ethical committee of the University Medical Center Utrecht (medical ethical committee number: 02/042) and by the institutional review boards of all involved hospitals. The following

hospitals were involved: University Medical Center Utrecht, Diakonessenhuis, Utrecht; St. Antonius Hospital, Nieuwegein; Meander Medical Centre, Amersfoort; Tergooi Hospital, Hilversum; St. Jansdal Hospital, Harderwijk; and Flevohospital, Almere, the Netherlands. Patients gave written informed consent before entering the study.

Statistical analyses

For the present analyses, data were used on treatment strategy, current smoking status (yes/no), current smoking level (0, 1-9, 10-19 and ≥20 cigarettes per day), body mass index (BMI, kg/m^2), gender, rheumatoid factor (RF) status, MTX and biological DMARD (bDMARD) use, disease activity score assessing 28 joints (DAS28) at baseline and monthly up to 24 months. We used linear mixed modelling with a random intercept, DAS28 over 24 months follow-up as dependent variable, current smoking status as independent variable, and DAS28 at baseline, time (trial months), time^2 (non-linear course of DAS28 over time), BMI, gender and RF status as covariates. In model 1, we analysed whether the effect of current smoking status (yes/no) on clinical response was different for MTX+pred when compared to MTX+plac. To this end, the interaction term of group allocation and current smoking status was added to the model. Model 2 was similar to model 1, but here we used the categorical variable of the different levels of current smoking as covariate in the model instead of the binary current smoking status variable, to evaluate the presence of a dose-response effect. Also, unadjusted data are shown in a figure on the course of DAS28 in each strategy arm of the CAMERA-II trial, separately for current smokers and non-smokers. We used a random intercept at patient level (i.e., for the variable patient ID) to account for the dependence of repeated measurements over time and the other variables were added as fixed effects in the model. These were current smoking status, gender, group allocation, RF status, DAS28 ESR at baseline, time, BMI; furthermore, the interaction terms time* time, and current smoking status*group allocation were entered in the model. We used a Log-likelihood test to investigate if the model fitted the data well. analyses were performed with SPSS version 26 (IBM Corp.). All tests were 2-sided and $P \leq 0.05$ was considered statistically significant.

Results

Current smoking data was available for 213 of 236 patients of the CAMERA-II trial. The baseline characteristics of these 213 patients were similar between the MTX+pred and MTX+plac strategy groups (Table 1).

Table 1. Baseline characteristics for each strategy group*

	MTX+plac (N=109)	MTX+pred (N=104)
Female gender, n (%)	66 (61)	60 (58)
Age in years, mean (SD)	54 (13)	54 (14)
DAS28, mean (SD)	5.7 (1.2)	5.8 (1.4)
Positive RF status, n/N (%)	63/89 (71)	45/85 (53)
Smoking, n (%)	31 (28)	38 (37)
BMI, kg/m^2, mean (SD)	26 (4)	26 (4)
VAS GH, mm, mean (SD)	54 (25)	56 (23)
28TJC, median (IQR)	9 (6-13)	10 (5-18)
28SJC, median (IQR)	10 (6-15)	11 (6-15)
ESR, mm/h, median (IQR)	30 (15-49)	30 (15-47)
CRP levels, mg/L, median (IQR)	17 (8-45)	16 (0-44)

Baseline characteristics of 213 of the 236 patients included in CAMERA-II, of whom smoking data were available.
** no statistically significant differences between the 2 groups.*
MTX: methotrexate; plac: placebo; pred: prednisone; SD: standard deviation; DAS28: disease activity score assessing 28 joints; RF: rheumatoid factor (n/N indicates number of patients with positive RF status of those with available RF status); BMI: body mass index; kg/m^2: kilogram per square meter; VAS GH: visual analog scale of global health (VAS range 0 to 100 mm, with 100 mm signifying the worst status); 28TJC: tender joint count assessing 28 joints, range 0-28; IQR: interquartile range; 28SJC: swollen joint count assessing 28 joints, range 0-28; ESR: erythrocyte sedimentation rate; mm/h: millimetre per hour; CRP: C-reactive protein; mg/L: milligram per litre.

Current smoking was significantly associated with a smaller reduction of DAS28 over time compared to non-current smoking: β 0.57 (95% confidence interval, 95%CI) 0.22;0.92), p<0.01, see Table 2. This association was not statistically different between the MTX+pred and MTX+plac strategy groups: p-value for interaction term of treatment strategy and current smoking status=0.73. In line with this, the Figure shows a clear effect of current smoking on the course of DAS28, but of similar magnitude in each of the two strategy arms. The regression coefficient for BMI in the model was 0.03 (95%CI 0.00-0.06), p=0.04, see Table 2.

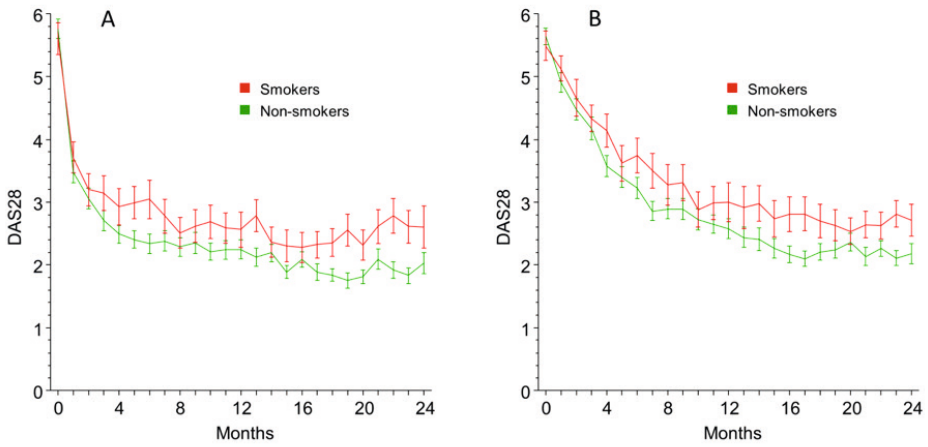

Figure. DAS28 over time for current smokers vs non-smokers in each strategy arm of CAMERA-II.

(A) Strategy initiating methotrexate with prednisone. (B) Strategy initiating methotrexate with placebo-prednisone. DAS28: Disease Activity Score in 28 joints.

The association between current smoking and DAS28 over time was dose dependent (Table 2): β 0.38 (95%CI -0.27;1.02), p=0.25 for patients who smoked 1-9 cigarettes, β 0.59, (95%CI 0.06;1.12), p=0.03 for patients who smoked 10-19 cigarettes, and β 0.66 (95%CI 0.18;1.13), p<0.01 for patients who smoked ≥20 cigarettes per day (Table 2). The interaction terms between treatment strategy and current smoking levels were (again) not statistically different between the MTX+pred and MTX+plac strategy groups. The model we used for the analyses fitted the data well, p<0.00001.

Table 2. Current smoking as predictor and DAS28 over time (24 months) as outcome: 2 models

	Model 1: current smoking yes/no			Model 2: current smoking level	
	β (95%CI)	p		β (95%CI)	p
Current smoking (vs non-smoking)	0.57 (0.22;0.92)	<0.01		-	-
			Current smoking level, cigarettes/day		
			0	=reference category	0.25
			1-9	0.38 (-0.27,1.02)	0.03
			10-19	0.59 (0.06;1.12)	<0.01
			≥ 20	0.66 (0.18;1.13)	<0.01
Male gender (vs female)	-0.45 (-0.69;-0.21)	<0.01	Male gender (vs female)	-0.49 (-0.74;0.24)	
RF positivity (vs negative status)	0.08 (0.34;-0.17)	0.51	RF positivity (vs negative status)	0.05 (0.30;-0.21)	0.71
DAS28 baseline	0.41 (0.32;0.50)	<0.01	DAS28 baseline	0.41 (0.32;0.50)	<0.01
Time	-0.31 (-0.32;-0.29)	<0.01	Time	-0.31 (-0.32;-0.29)	<0.01
BMI	0.03 (0.00;0.06)	0.04	BMI	0.03 (0.00;0.06)	0.06
Time*Time	0.01 (0.01;0.01)	<0.01	Time*Time	0.01 (0.01;0.01)	<0.01
Group allocation: MTX+pred (vs MTX+plac)	-0.65 (-1.06;-0.24)	<0.01	Group allocation: MTX+pred (vs MTX+plac)	-0.74 (-1.03;-0.46)	<0.01

Table 2. Continued.

	Model 1: current smoking yes/no			Model 2: current smoking level	
	β (95%CI)	p		β (95%CI)	p
Current smoking (vs non-smoking)	0.57 (0.22;0.92)	<0.01		-	-
Group allocation*current smoking interaction	0.09 (-0.41;0.58)	0.73	Group allocation*current smoking level interaction, cigarettes/day 0 1-9 10-19 ≥ 20	=reference category -0.17 (-1.05;0.71) -0.25 (-1.04;0.54) 0.12 (-0.56;0.80)	0.71 0.54 0.73

The results of the 2 models, corrected for DAS28 at baseline, time (trial months), time², BMI, gender and RF status. A positive estimate reflects a higher DAS28 over time.
DAS28: disease activity score assessing 28 joints; β: regression coefficient; 95%CI: 95% confidence interval; MTX: methotrexate; pred: prednisone; plac: placebo.

Discussion

To our knowledge, this is the first study investigating the association of current smoking with the clinical effect of concomitant prednisone in RA. In this study in early RA patients, we found a negative effect of current smoking on DAS28 for MTX-based strategies, independent of concomitant use of prednisone or not; this effect was dose dependent. These findings corroborate previously found negative associations between current smoking and response to MTX treatment in early RA patients.[4,12]

BMI had a (small) effect additionally to current smoking. As MTX is often used in daily clinical practice,[7] our results emphasize the importance of not current smoking in early RA patients treated with MTX in clinical practice. Smoking patients also have worse responses to rituximab and anti-TNF treatment [5,6] which many patients require later on in the disease course.[13,14] Not smoking or smoking cessation has other positive effects, such as on cardiovascular risk, which is especially important as this risk is increased in RA.[15-17]

The smaller reduction of disease activity over time induced by current smoking is not statistically significantly different between MTX+pred and MTX+plac; this would indicate that the effect of prednisone is not statistically significantly influenced by current smoking. However, we cannot rule out (in) direct interactions between MTX and prednisone under current smoking and inflammatory conditions. If we may compare our results in RA with those in other chronic diseases: in asthma patients no difference in effect of GCs on relapse rate has been shown for smokers and non-smokers.[18] Also, in a study in 18 healthy male adults, no statistically significantly effect of smoking on systemic availability of prednisolone was observed.[19]

The strength of our analysis is the use of data from a placebo-controlled trial, enabling the comparison of the association of current smoking with clinical response to prednisone and placebo-prednisone. Furthermore, our study examined the relatively long-term effect, i.e. over 24 months, of current smoking on treatment response to MTX as well as concomitant prednisone, while other studies examined the effect of current smoking on treatment effect to MTX only, and only up to a maximum of 6 months of treatment.[3,6]

Our study has limitations. The negative association between current smoking and clinical response might partly be explained by anti-cyclic citrullinated peptide status, but this status had not been assessed in CAMERA-II. However, this does not diminish the clinical relevance of our findings. Data on current smoking of almost 10% of patients were missing. However, as there is no reason

to assume that these missings were not at random, it is unlikely that they have influenced our findings. The number of participating patients was limited, so it cannot be ruled out that current smoking would have a minor negative effect on the benefit of prednisone, but our results show that a clinically relevant effect is absent.

Our finding that current smoking negatively affects the clinical response to MTX-based strategies and that this negative current smoking effect is not different if concomitant prednisone is used, warrants validation, although circumstantial evidence supports this finding.

Conflicts of interest

No conflicts of interest were reported.

Authors' contributors

MSK, JMvL, MJHdH.and JWGJ and PMJW contributed to the study design. MSK and MJdH contributed to the data collection. MSK, MJHdH and PMJW analysed the data. All authors wrote the manuscript.

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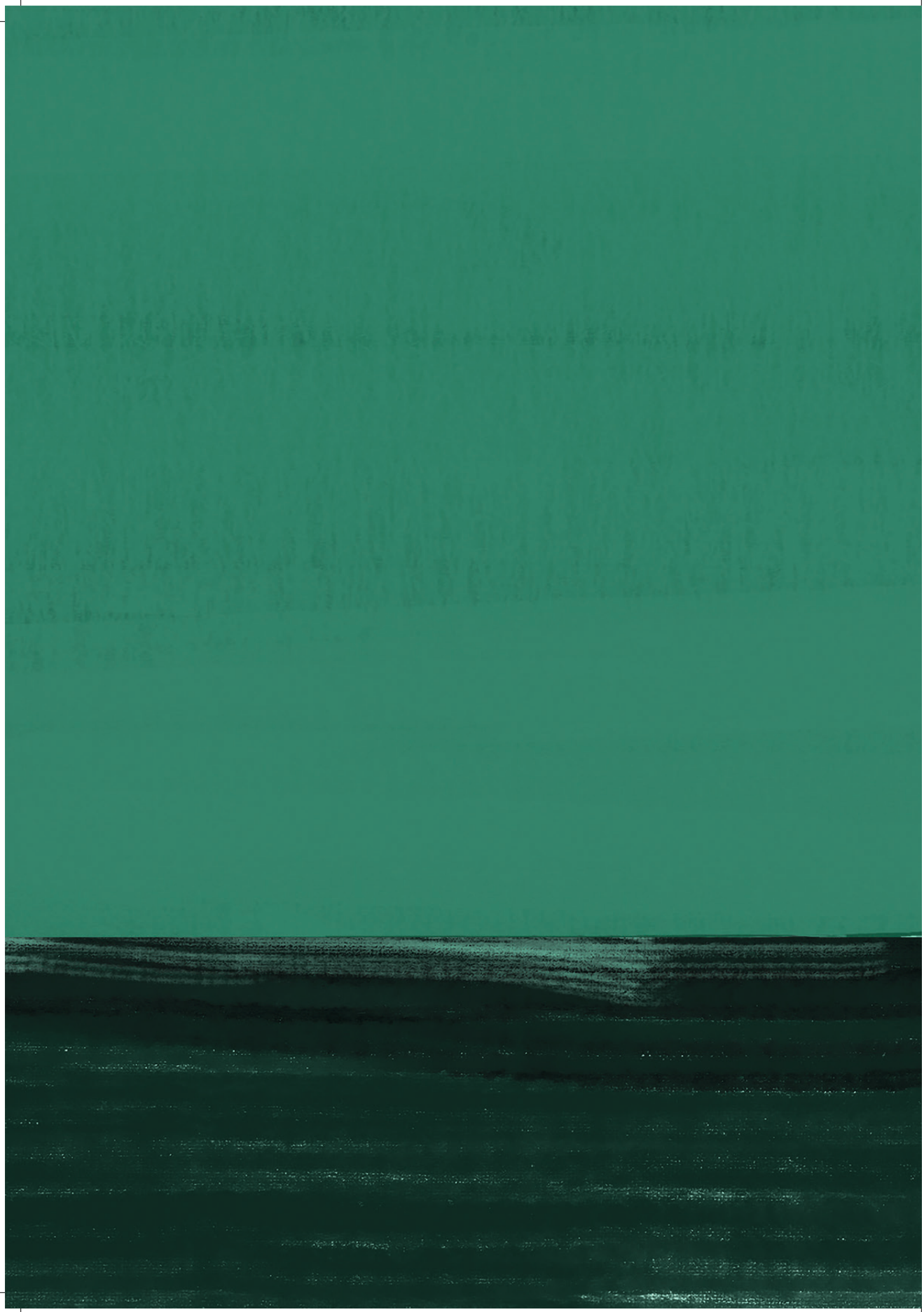

6.

Efficacy and safety of selective glucocorticoid receptor modulators in comparison to glucocorticoids in arthritis, a systematic review

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Abstract

Background. Long-term treatment with glucocorticoids (GCs) plays an important role in the management of arthritis patients, although the efficacy/safety balance is unfavourable. Alternatives with less (severe) adverse effects but with good efficacy are needed. Selective GC receptor modulators (SGRMs) are designed to engage the GC receptor with dissociative characteristics: transactivation of genes, which is mainly responsible for unwanted effects, is less strong while trans-repression of genes, reducing inflammation, is maintained. It is expected that SGRMs thus have a better efficacy/safety balance than GCs. A systematic review providing an overview of the evidence in arthritis is lacking.

Objective. To systematically review the current literature on efficacy and safety of oral SGRMs in comparison to GCs in arthritis.

Methods. A search was performed in Medline, Embase and the Cochrane Library, from inception dates of databases until May 2017. Experimental studies involving animal arthritis models or human material of arthritis patients, as well as clinical studies in arthritis patients were included, provided they reported original data. All types of arthritis were included. Data was extracted on the SGRM studied and on the GC used as reference standard; the design or setting of the study was extracted as well as the efficacy and safety results.

Results. A total of 207 articles was retrieved of which 17 articles were eligible for our analysis. Two studies concerned randomised controlled trials (RCT), five studies were pre-clinical studies using human material, and 10 studies involved pre-clinical animal models (acute and/or chronic arthritis induced in mice or rats). PF-04171327, the only compound investigated in a clinical trial setting, had a better efficacy/safety balance compared to GCs: better clinical anti-inflammatory efficacy and similar safety.

Conclusion. Studies assessing both efficacy and safety of SGRMs are scarce. There is limited evidence for dissociation of anti-inflammatory and metabolic effects of the SGRMs studied. Development of many SGRMs is haltered in a preclinical phase. One SGRM showed a better clinical efficacy/safety balance.

Introduction

Glucocorticoids (GCs) are the most commonly used anti-inflammatory drugs worldwide, applied in arthritic diseases, inflammatory bowel disease, and chronic pulmonary disease, for example [1-3]. In rheumatoid arthritis (RA) between 56% and 68% of the patients are treated with GCs [4–6]. GCs not only exhibit anti-inflammatory effects, but also have proven disease modifying effects as they halter radiological damage and improve physical disability in RA patients in addition to reducing disease activity [7–9]. Despite their proven beneficial effects, GCs potentially cause adverse effects. The most common adverse effects associated with GC use are cardiovascular events, endocrine/metabolic effects (weight gain, dysregulation of glucose metabolism and development of diabetes), infections, gastro-intestinal events and osteoporosis [10-11]. These unwanted effects especially occur when used long-term (>6 months) and in high-dose (>10 mg/daily),and limit the dosing and duration of GC treatment [12]. Hence, the quest for alternatives with a better efficacy/safety balance continues, such as selective GC receptor modulators (SGRMs). SGRMs are specifically designed to engage the GC receptor (GR) with dissociative characteristics: after binding to the GR, GCs may either bind to and activate transcription from gene promoters (transactivation) or interact with other transcription factors to change their function (transrepression). It is assumed that SGRMs promote transrepression over transactivation [13].

Transrepression is most critical for the anti-inflammatory effects of GCs, as it leads to decreased production of pro-inflammatory transcription factors such as nuclear factor-kappa B (NF-κB) and activator protein 1 (AP-1). On the contrary, transactivation is thought to cause detrimental effects of GCs [14]. Upon binding of a GC to GC response elements (GRE) transactivation in various gene promotors occurs, such as glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), fatty acid synthase (FAS) and tyrosine aminotransferase (TAT). The protein products of these genes are involved in carbohydrate, lipid and protein metabolism [15]. As such, activation of these genes could lead to aforementioned (metabolic) side effects.

SGRMs may have an improved efficacy/safety balance compared to conventional GCs, by their potentially disparate effects on transrepression and transactivation. However, till date, no SGRM has entered the market yet, suggesting that development of SGRMs meets challenges. A systematic review providing an in-depth overview of both the efficacy and safety of SGRMs is lacking. Our aim was therefore to systematically investigate whether oral SGRMs have a superior efficacy/safety balance compared to conventional GCs in arthritis in (pre)clinical settings.

Methods

Search and selection

A systematic literature search was performed, to assess efficacy and safety of oral SGRMs in arthritis, compared to GCs. MEDLINE (PubMed), Embase and the Cochrane Library were searched until May 2017. The search (S1 Box) was established after consultation of a librarian at the University Medical Center (UMC) Utrecht with expertise in systematic literature searches (P.H.W.). Duplicates were excluded. Two authors (M.S. and M.J.H.H.) independently screened titles and abstracts for eligibility. Studies were included if fulfilling the following criteria: investigating efficacy and safety of an oral SGRM; studying GC as reference compound; performed in arthritis. Both in vivo and in vitro, were included. Subsequently, the same authors independently screened full texts of eligible articles. Selection was based on mutual agreement. Studies were excluded if not investigating a GC as reference compound; if performed in non-arthritic disease(s); if investigating non-selective GRMs, or if investigating SGRMs with administration route other than oral. Review articles without presentation of original data were also excluded. Of the selected articles, references and citing publications were additionally screened.

Data extraction

Data was extracted using the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE's) guideline ([16]. This guideline is adapted from the Cochrane risk of bias tool [17] and focuses on laboratory animal studies. We extracted data on the SGRM investigated (experimental compound), the GC that was investigated as reference compound, the animal model or setting of the study, and efficacy and safety results. Initial data extraction was performed by one author (M.S.) and extracted data was re-assed by the second author (M.J.H.H.). The efficacy results concerned pro- and anti-inflammatory effects and the safety results concerned any adverse effect reported, including effects on glucose, fat and bone metabolism, as well as mineralocorticoid effects. For clinical studies, also results on adverse effects of GC were extracted, such as cardiovascular events and infections. Results were reported following the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist [18].

Results

Search and selection

The search and selection is presented in Figure 1. A total of 207 reports was retrieved by the initial search. Excluding duplicates and reports other than articles resulted in 81 articles, of which title and abstract were screened. This

resulted in 40 articles of which full text was screened. Finally, 17 articles were eligible for inclusion and analysis. Risk of bias was not assessed, because of very high heterogeneity in study types and in study design and information presented.

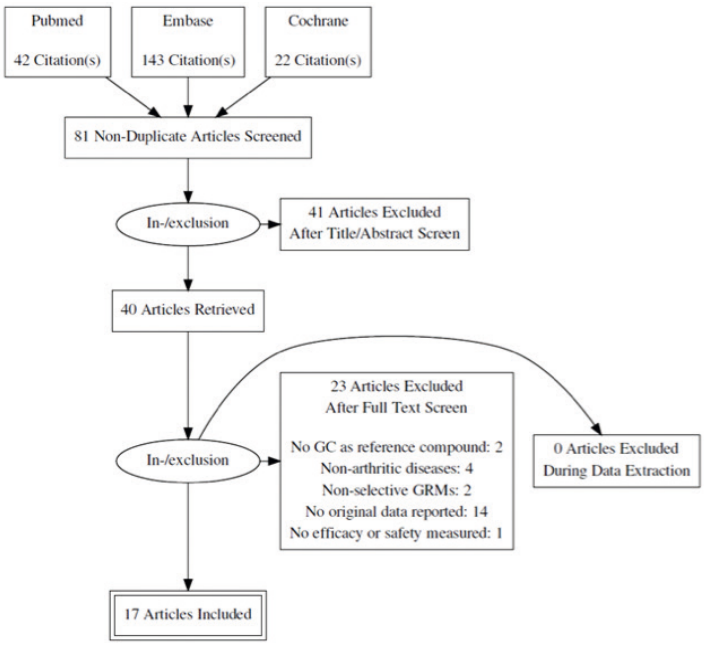

Figure 1. Flow chart of search and selection of studies on efficacy and safety of SGRMs.

SGRMs: selective GRMs; GRMs: glucocorticoid receptor modulators; GCs: glucocorticoids.

Data extraction

Study characteristics are shown in Table 1. Results are reported using the PRISMA checklist (S1 Table). The following SGRMs were investigated: Compound A, PF-04171327, LGD-5552, Compounds 4, 5, and 14, Compounds (R)-16, (R)-18, (R)-21, (R)-35, and (R)-37, Ginsenoside Rg1 and Org 214007-0 [19-35].

Of the 17 studies, 2 studies concerned randomised controlled trials (RCT), 3 were pre-clinical studies using only human material, 10 studies were performed using only a pre-clinical animal model (acute and/or chronic arthritis induced mice or rat model) and 2 pre-clinical studies used both animal as well as human material. Regarding the animal models, an acute arthritis induced model was used to measure pro-inflammatory cytokines, and a chronic induced arthritis model to measure a clinical outcome, such as paw swelling. Dexamethasone was used as reference GC in 7 studies, and prednisone in 11 studies.

Table 1. Overview of studies with efficacy or safety results of a selective glucocorticoid receptor modulator in comparison to a glucocorticoid

Author, year	SGRM tested	Reference GC	Setting	Safety		Conclusion on efficacy and safety in comparison to a glucocorticoid
				Glucose homeostasis/ fat metabolism	Bone markers	
Dewint et al., 2008	Compound A	Dex	B, C	X		Similar efficacy. Better safety of SGRM.
Gossye et al., 2009	Compound A	Dex	B			Similar efficacy. No safety data shown.
Gossye et al., 2010	Compound A	Dex	B, C			Lower efficacy of SGRM. No safety data shown.
Rauch et al., 2011	Compound A	Dex	B		X	Similar efficacy. Better safety of SGRM.
Rauner et al., 2013	Compound A	Dex	C		X	Lower efficacy of SGRM. Better safety of SGRM.
Malaise et al., 2015	Compound A	Pred	B	X		Similar efficacy. Better safety of SGRM.
Yang et al., 2015	Compound 4 and 5	Pred	C			Similar efficacy of compound 4, better efficacy of compound 5. No safety data shown.
Razavi et al., 2014	Compound 14	Pred	C	X		Similar efficacy. Better safety of SGRM.
Riether et al., 2010	Compounds (R)-16 and (R)-37	Pred	C	X		Similar efficacy. Better safety of SGRM.

Harcken et al., 2014	Compounds (R)-18 and (R)-21	Pred	C	X	X	Similar efficacy. Better safety of SGRMs.
Weinstein et al., 2011	Compound 35 and 37	Pred, Dex	C			Better efficacy of SGRMs. No safety data shown.
Miner et al., 2007	LGD-5552	Pred	C			Similar efficacy. No safety data shown.
Lopez et al., 2008	LGD-5552	Pred	C			Similar efficacy. No safety data shown.
Du et al., 2011	Ginsenoside Rg1	Dex	C	X	X	Similar efficacy. Better safety of SGRM.
Van Lierop et al., 2012	Org 214007-0	Pred	C			Similar efficacy. No safety data shown.
Conrado et al., 2015	PF-04171327	Pred	D			Similar efficacy. No safety data shown.
Stock et al., 2017	PF-04171327	Pred	A	X	X	Better efficacy of SGRM. Similar safety.

Studies are sorted on type of SGRM. Efficacy was measured in all 17 studies, safety was measured in nine studies. SGRM: selective glucocorticoid receptor modulator; GC: glucocorticoid; Pred: prednisone; Dex: dexamethasone; A: randomised controlled trial (RCT); B: pre-clinical study with human material; C: pre-clinical study with animal material/model; D: stochastic simulations based on non-published RCT.

Seven studies showed better safety of the studied SGRM compared to dexamethasone or prednisone, with similar efficacy. Four compounds showed similar efficacy of the studied SGRM compared to prednisone, but no safety data was provided. Three compounds showed better efficacy of the studies SGRM than prednisone or dexamethasone, but no safety data was provided for two compounds. In depth results of 6 studies that assessed both efficacy and safety of SGRMs in comparison to GCs are depicted in Table 2 and only studies reporting efficacy and safety results of both SGRMs and GCs were included in this table. Fosdagrocorat (PF-04171327), was the only compound investigated in a clinical setting [35], and of this SGRM both safety and efficacy data was available. In this phase 2 study, 86 RA patients were randomised to receiving either 10 mg or 25 mg fosdagrocorat, or 5 mg prednisone or placebo. A significantly better improvement in DAS28-CRP was observed after two weeks of treatment with 25 mg fosdagrocorat compared to 5 mg prednisone and placebo. Treatment with the 10 mg dose of fosdagrocorat was only compared to placebo, not prednisone. Plasma cortisol levels decreased significantly more in the group of patients treated with 10 mg and 25 mg fosdagrocorat compared to 5 mg prednisone. The number of adverse events was similar between the group of patients receiving 25 mg fosdagrocorat compared to 5 mg prednisone.

Table 2. Details of studies with data on both efficacy and safety of selective glucocorticoid receptor modulator in comparison to a glucocorticoid

Author, year	SGRM tested	GC	Setting/model	Efficacy (SGRM compared to GC)		Safety (SGRM compared to GC)	
Dewint et al., 2008	Compound A	Dex	FLS cells derived from RA patients	Amount of cDNA of TNF = Amount of cDNA of MMP1 = Amount of cDNA of MMP3 =	NA NA NA		
			CIA mice	Arthritis score at day 8 of arthritis ↑ Paw swelling at day 8 of arthritis =	NA NA	Serum levels of insulin ↓ Normal histology of knee joints ↓ mRNA G6P ↓ mRNA PEPCK ↓	NA NA NA NA
Rauner et al., 2013	Compound A	Dex	CIA mice	Arthritis score ↑ Paw swelling ↑ Paw temperature ↓	NA NA NA	Bone loss ↓ Serum P1NP ↓ Serum CTX-1 ↓ Cellular infiltration in paws ↓	NA NA NA NA
			Supernatant from spleen PBMCs from mice, ex vivo stimulated with collagen type II	TNF = IFN-α = NF-kβ =	NA NA NA	Cartilage destruction ↓ Inhibition of number of osteoclasts ↓	NA NA
			mRNA expression in joint tissue from CIA mice	TNF ↑ IL-6 ↓	NA NA		

Table 2. Continued.

Author, year	SGRM tested	GC	Setting/model	Efficacy (SGRM compared to GC)		Safety (SGRM compared to GC)	
Razavi et al., 2014	Compound 14	Pred	Mice inflammation model, LPS stimulated	IL-6 ↓	NA		
				TNF =	NA		
			CIA mice	Arthritis score ↑	NA	Insulin ↑	NA
						Body fat ↑	NA
						Triglycerides ↓	NA
						Free fatty acids ↑	NA
Harcken et al., 2014	Compound R18 and 21	Pred	CIA mice	Arthritis score ↓	NA	Osteocalcin ↑	NA
						Body fat ↑	NA
						Triglycerides ↑	NA
						Free fatty acids ↑	NA
						Insulin ↑	NA
						Femur cortical thickness =	NA S
Du et al., 2011	Ginsenoside Rg1	Dex	Inflamed paw mice model	Paw swelling ↑	NS		
						Body weight ↓	NA
			CIA mice	Arthritis score =	NA	Blood glucose levels ↓	S
						Bone cortical thickness ↑	NA
						Bone content ↑	S
						Trabecular tibial number ↑	S
						Trabecular tibial thickness ↑	NS
						trabecular tibial separation ↑	S

Stock et al., 2017	PF-04171327	Pred	Phase 2 RCT, 86 RA patients, 2 weeks treatment	DAS28-4 (CRP) improvement ↑	S	Fasting glucose = Plasma cortisol ↑ Adverse events = Mean osteocalcin levels = Mean uNTX-1 levels =	NS S NA NA NA

Six studies reporting results on both efficacy and safety of SGRMs compared to GCs are depicted, only studies that reported efficacy and safety of both SGRM and GC are shown in this table. Results have been summarized for each SGRM if multiple dosing schemes were used. SGRM: selective glucocorticoid receptor modulator; GC: glucocorticoid; pred: prednisone, dex: dexamethasone; FLS: fibroblast-like synoviocytes; CIA: collagen induced arthritis; cDNA: copy DNA; TNF: tumour necrosis factor; MMP: matrix metalloproteinase; P1NP: N-terminal propeptide of type 1 collagen; CTX1: collagen type **1** *cross-linked C-telopeptide; PBMCs: peripheral blood mononuclear cells; IFN-α: interferon alpha; NF-kβ: Nuclear Factor kappa-light-chain-enhancer of activated B cells; mRNA: messenger ribonucleic acid; RA: rheumatoid arthritis; DAS28-4 (CRP): disease activity score using 28 joints and c-reactive protein with 4 variables; IL-6: interleukin 6; uNTX-1: N-terminal telopeptide 1 in urine; NA: not statistically analysed; NS: not significant; S: significant; RCT: randomised controlled trial; ↑: results higher for SGRM compared to GC; ↓: results lower for SGRM compared to GC; =: results similar for SGRM compared to GC.*

Discussion

This paper aimed to systematically review the efficacy and safety of SGRMs compared to conventional GCs. We found 17 studies which investigated a SGRM compared to a GC in arthritis, of which seven showed similar efficacy and better safety compared to GCs. However only one SGRM, fosdagrocorat/PF-04171327, was investigated in a clinical setting.

There are several possible explanations as to why most of these SGRMs did not enter the clinical phase of drug development. One of them being the fact that some adverse effects associated with GC treatment are presumed to be caused by transrepression rather than by transactivation. For example, the immunosuppressive effects of GCs, leading to an increased risk of infections, are predominantly caused by transrepression rather than transactivation and therefore this clinically important adverse effect will not be reduced by a dissociative compound [36]. Other side effects, such as osteoporosis, are mediated by both transrepression (osteocalcin transcription) and transactivation (osteoblast apoptosis) [14]. Furthermore, transactivation is not only associated with negative effects, as it has been demonstrated that some genes that are upregulated by transactivation, such as mitogen-activated protein kinase phosphatase-1 (MKP-1, a crucial anti-inflammatory gene), GC-induced leucine zipper (GILZ, a protein which inhibits NFκB and AP-1) and the anti-inflammatory interleukin IL-10, have anti-inflammatory functions [37-39]. Thus, the actual effects of transrepression and transactivation are much more complex than suggested by the hypothesized working mechanism of SGRMs, in which it is claimed that transactivation is solely responsible for the adverse effects and transrepression for the desirable anti-inflammatory effects (full dissociation). Besides the described classical genomic mechanisms of action, which require several hours to take place, SGRMs also act by very rapid non-genomic mechanisms, especially at higher doses [40]. These non-genomic mechanisms of action are thought to be mediated by affecting the physicochemical property of cell membranes, or through binding to intracellular or membrane-bound GC receptor, causing inflammatory signal transduction cascades (mitogen-activated protein kinases (MAPK), neutrophil degranulation and phagocytosis by macrophages) [41-44]. Combined with epigenetic effects of SGRMs, these two mechanisms also contribute to the lack of dissociative effects of SGRMs. Furthermore, in vitro studies use simplified GRE reporter systems compared to the more complex GRE systems present in in vivo gene promoters [45]. Another important predicament in development of SGRMs is to establish equipotent doses of GCs and SGRMs. It has been shown that with increasing SGRM dosage, effects but also the SGRM-induced adverse effects increase [26,30] and vice versa. A case in point is deflazacort, an oxazolone derivative of prednisolone

that was believed to have similar efficacy as prednisone but with fewer adverse effects, but in fact this actually proved to be at a lower than equipotent dosage; deflazacort even showed increased adverse effects compared to prednisolone in really equipotent dosages [46-47]. Furthermore, adverse effects measured in most of the experimental studies, such as increased glucose levels and changes in cortical bone thickness, are in fact surrogate markers for clinical adverse effects in patients, respectively development of diabetes mellitus and osteoporosis. Thus, these parameters in preclinical studies do not fully reflect the clinical GC-related adverse effects.

The only SGRM that did manage to enter a clinical phase in RA patients is PF-04171327 (NCT01393639), of which the first results of 12-week follow-up, were presented at the Annual European League Against Rheumatism (EULAR) Congress in 2015 [48]. In 323 RA patients, 15 mg of PF-04171327 daily showed similar efficacy as prednisone 10 mg daily, assessed by American College of Rheumatology (ACR) 20 response and Disease Activity Score 28 (DAS28), while (unwanted) effects on bone formation and plasma glucose level were similar as 5 mg of prednisone daily. These preliminary results suggest that development of SGRMs with a better efficacy/safety balance compared to GCs (better clinical anti-inflammatory efficacy and similar safety) is feasible.

The strengths of our study include the thorough search across several databases and inclusion of pre-clinical and clinical studies. The present systematic review is the first to investigate the benefit and risks of oral SGRMs compared to GCs in arthritis. A limitation of our review is the heterogeneity of the reported studies which made it difficult to compare these studies. We investigated efficacy (transrepression) by measuring effects of the SGRMs on inflammatory markers, and safety (transactivation) by measuring effects on glucose and bone metabolism. However, measurable effects on bone metabolism are more difficult to detect compared to effects on glucose levels in studies with short duration. This could be an explanation why only three of the 17 studies examined effect on bone markers.

In conclusion, studies assessing both efficacy and safety parameters of SGRMs in arthritis are scarce. There is limited evidence for dissociation of anti-inflammatory and metabolic effects of the SGRMs studied. Development of many SGRMs is haltered in a preclinical phase. One SGRM showed a better clinical efficacy/safety balance, compared to prednisone.

Conflicts of interest

MS received a student grant from AstraZeneca. MK is holder of AstraZeneca stock and received an honorary from AstraZeneca as well. AstraZeneca was not involved in interpretation and analysis of the data. FB reported receiving consultancy fees, honoraria and travel expenses from Pfizer. He served as principal investigator in a Pfizer sponsored trial in rheumatoid arthritis investigating the effects of fosdagrocorate. JMvL received honoraria from MSD, Roche, Pfizer, BMS, Eli Lilly.

Authors' contributors

MS, JWGJ, JMvL contributed to the study design. MS and MHJdH contributed to data collection. All authors contributed to data analysis, data interpretation, writing and review of the manuscript.

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Supplementary files

S1 Box. PubMed search for studies on efficacy and safety of selective glucocorticoid receptor modulators in comparison to glucocorticoids, in arthritis

((selective glucocorticoid[Title/Abstract] OR "dissociated glucocorticoid agonists"[Title/Abstract]) OR "dissociated glucocorticoid receptor"[Title/Abstract]) OR "dissociated glucocorticoids"[Title/Abstract]) OR "selective glucocorticoids"[Title/Abstract]) OR "selective glucocorticosteroid"[Title/Abstract]) OR "sgrm"[Title/Abstract]) OR SGRMs[Title/Abstract]) OR SEGRM[Title/Abstract]) OR SEGRMs[Title/Abstract]) OR segra[Title/Abstract]) OR segras[Title/Abstract]) OR sgra[Title/Abstract]) OR sgras[Title/Abstract]) OR ("Receptors, Glucocorticoid/agonists"[Mesh] OR "Receptors, Glucocorticoid/antagonists and inhibitors"[Mesh])) OR "dagr"[Title/Abstract]) OR "dagrs"[Title/Abstract]) OR "selective gr agonist"[Title/Abstract]) OR "selective gr agonists"[Title/Abstract]) OR "selective gr antagonist"[Title/Abstract]) OR "selective gr antagonists"[Title/Abstract]) OR "selective gr ligand"[Title/Abstract]) OR "selective gr ligands"[Title/Abstract]) OR "selective gr modulator"[Title/Abstract]) OR "selective gr modulators"[Title/Abstract]) OR "non steroidal glucocorticoid receptor"[Title/Abstract]) OR "non steroidal agonist"[Title/Abstract]) OR "non steroidal agonists"[Title/Abstract]) OR "non steroidal gr antagonists"[Title/Abstract]) OR "non steroidal gr ligands"[Title/Abstract]) OR "non steroidal ligand"[Title/Abstract]) OR "glucocorticoid receptor/antagonist"[Title/Abstract]) OR "glucocorticoid receptor agonist"[Title/Abstract]) OR "glucocorticoid receptor ligand"[Title/Abstract]) OR "glucocorticoid receptor ligands"[Title/Abstract]) OR "glucocorticoid receptor modulator"[Title/Abstract]) OR "glucocorticoid receptor modulators"[Title/Abstract]) OR "dagra"[Title/Abstract]) OR "glucocorticoid receptor agonists"[Title/Abstract]) OR (segram[Title/Abstract] OR segrams[Title/Abstract])) AND ("arthritis"[MeSH Terms] OR Arthritis[Title/Abstract])

S1 Table. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Not applicable
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5-6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary Box 1

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Not applicable
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Not applicable
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not applicable
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7, Figure 1

Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Not applicable
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10-11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not applicable
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Not applicable
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not applicable
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11-13
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Not applicable

6

7.

AZD9567 versus prednisolone in patients with active rheumatoid arthritis: a phase IIa, randomised, double blind, efficacy and safety study

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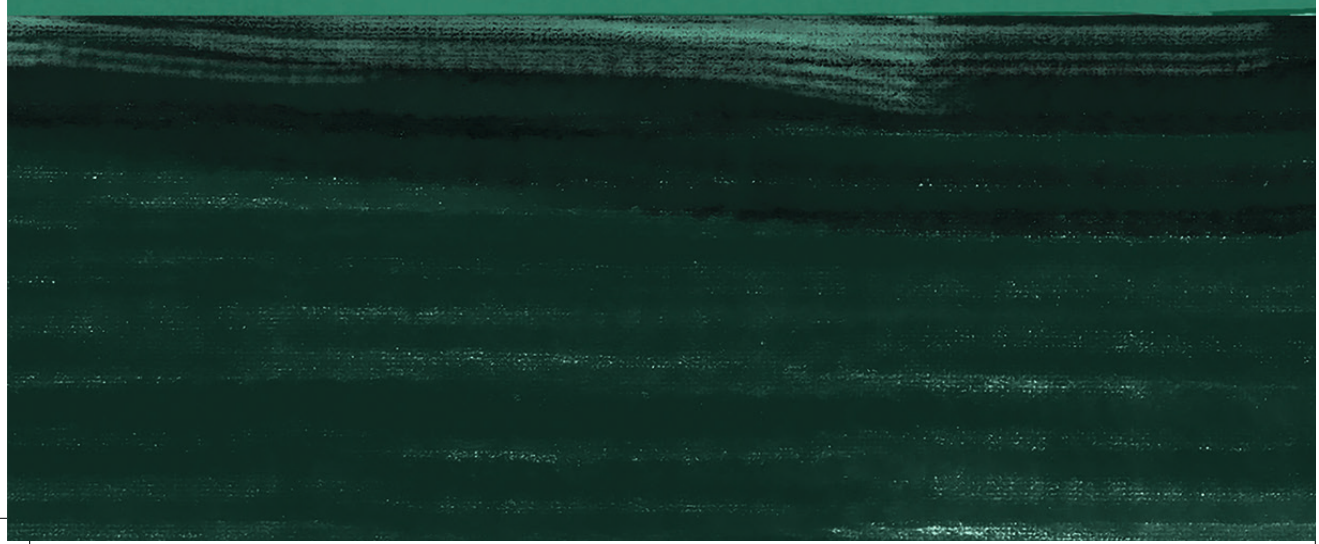

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Abstract

Oral corticosteroid use is limited by side effects, some caused by off-target actions on the mineralocorticoid receptor that disrupt electrolyte balance. AZD9567 is a selective, non-steroidal glucocorticoid receptor modulator. The efficacy, safety, and tolerability of AZD9567 and prednisolone were assessed in a phase IIa study. Anti-inflammatory mechanism of action was also evaluated *in vitro*, in monocytes from healthy donors. In this randomised, double-blind, parallel-group, multicentre study, patients with active rheumatoid arthritis were randomised 1:1 to AZD9567 40 mg or prednisolone 20 mg once daily orally for 14 days. Primary endpoint was change from baseline in DAS28-CRP at day 15. Secondary endpoints included components of DAS28-CRP, ACR response criteria (ACR20, ACR50, and ACR70), and safety endpoints, including serum electrolytes. Overall, 21 patients were randomised to AZD9567 (*n*=11) or prednisolone (*n*=10), and all completed the study. As anticipated, AZD9567 had a similar efficacy profile to prednisolone, with no clinically meaningful (i.e., > 1.0) difference in change from baseline to day 15 in DAS28-CRP between AZD9567 and prednisolone (least-squares mean difference [95% CI], 0.47 [-0.49 to 1.43]). Similar results were observed for the secondary efficacy endpoints. *In vitro* transcriptomic analysis showed that anti-inflammatory responses were similar for AZD9567, prednisolone, and dexamethasone. Unlike prednisolone, AZD9567 had no effect on the serum sodium:potassium ratio, consistent with its higher selectivity for the glucocorticoid receptor over the mineralocorticoid receptor. The safety profile was not different from that of prednisolone. Larger studies of longer duration are required to determine whether AZD9567 40 mg may in the future be an alternative to prednisolone in patients with inflammatory disease.

Introduction

Oral corticosteroids such as prednisolone are potent anti-inflammatory drugs used widely to treat chronic inflammatory diseases, including rheumatoid arthritis (RA).[1-2] However, the duration and dose of oral corticosteroid therapy are limited by serious side effects from unwanted actions on the glucocorticoid receptor, such as hyperglycaemia and reduced bone density, and off-target actions on the mineralocorticoid receptor that disrupt electrolyte balance and increase water retention.[3] In over 60 years of corticosteroid use, the uncoupling of their therapeutic anti-inflammatory effects from their side effects, by identifying novel selective ligands of the glucocorticoid receptor, has not been successfully demonstrated in the clinic.[4] An anti-inflammatory medication with similar efficacy to prednisolone but with a reduced side effect risk would therefore be greatly beneficial to patients requiring long-term oral corticosteroid treatment.

AZD9567 is a first-in-class, oral, selective, non-steroidal glucocorticoid receptor modulator being developed as an alternative to oral corticosteroids for inflammatory disease. *In vitro*, AZD9567 has higher affinity for the glucocorticoid receptor and 10^4-fold lower affinity for the mineralocorticoid receptor than prednisolone,[5] suggesting that it could be less disruptive to electrolyte balance. AZD9567 binds the glucocorticoid receptor differently from steroids.[5] In preclinical experiments, AZD9567 had similar anti-inflammatory effects to prednisolone, both *in vivo* in a rat model of joint inflammation and *ex vivo* by inhibition of lipopolysaccharide-stimulated tumour necrosis factor α (TNFα) release in human whole blood.[5] However, AZD9567 has been shown to have a less deleterious effect than prednisolone on glucose homeostasis *in vitro*: it does not upregulate transcription of gluconeogenic enzymes in human hepatocytes, unlike prednisolone,[5-6] and inhibition of glucose-stimulated insulin secretion in human pancreatic islets is twofold lower with AZD9567[6]. Phase I study data in healthy volunteers support preclinical findings: *ex vivo* inhibition of lipopolysaccharide-stimulated TNFα release in whole blood and results of oral glucose tolerance tests indicate that AZD9567 has an improved anti-inflammatory–dysglycemic side-effect profile versus prednisolone.[6] Furthermore, AZD9567 had no clinically meaningful effects on serum electrolytes in healthy volunteers.[6]

The aim of this proof-of-principle phase IIa study was to assess the efficacy and safety of AZD9567 versus prednisolone in patients with active RA, using a clinical disease activity score to evaluate efficacy. Preclinical evaluation aimed to elucidate underlying mechanisms that drive anti-inflammatory effects for AZD9567, prednisolone and dexamethasone.

Methods

Phase IIa clinical study

Study design, participants, and procedures

This was a phase IIa randomised double-blind parallel-group multicentre study in patients aged 18–80 years with active RA, defined as a disease activity score in 28 joints with serum C-reactive protein (DAS28-CRP) of ≥3.2 despite stable treatment with conventional disease-modifying anti-rheumatic drugs. The study was conducted across five sites: University Medical Center Utrecht, Maastricht University Medical Center, and Medisch Spectrum Twente in the Netherlands, and Sahlgrenska University Hospital and Skåne University Hospital Lund in Sweden. The study was conducted according to the principles of the Declaration of Helsinki and the Good Clinical Practice Guideline of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Independent ethics committees at University Medical Center Utrecht (for sites in the Netherlands) and Regionala etikprövningsnämnden i Göteborg (for sites in Sweden) prospectively approved the study protocol, before it was reviewed at participating sites. All participants provided written informed consent before inclusion. This trial is registered with ClinicalTrials.gov, number NCT03368235. The study protocol is available online via https://clinicaltrials.gov/ct2/show/NCT03368235.

AZD9567 and prednisolone were administered at doses predicted to be equipotent, based on a dose–response analysis of *ex vivo* inhibition of TNFα release in whole blood from phase I studies in healthy volunteers[7]. Within 7 days post-screening, eligible participants were randomised 1:1 to either AZD9567 40 mg or prednisolone 20 mg once daily orally for 14 days. On day 5, study staff checked participants' well-being via telephone. Participants attended clinic visits on days 8 and 15 and a follow-up visit approximately 14 days after the last dose.

Full details of study inclusion criteria, assessments, and analyses are in the supplementary material.

Randomization and masking

Randomization was performed using a computer-generated randomization code supplied by the sponsor. Randomization was done via a centralized interactive voice/web response system. Patients were block randomised 1:1 to either AZD9567 or prednisolone. The study was conducted in a double-blind manner, with patients, study site personnel, and sponsor personnel blinded to treatment assignment.

AZD9567 was provided as a suspension, with a matching placebo. Prednisolone was provided as a capsule, with a matching placebo. Because AZD9567 and prednisolone were not similar in appearance, a double dummy method was used: at each dosing occasion, participants receiving AZD9567 were also given a placebo for prednisolone, and participants receiving prednisolone were also given a placebo for AZD9567.

Endpoints

The primary endpoint was change from baseline to day 15 in DAS28-CRP. Secondary endpoints included proportions of patients achieving American College of Rheumatology (ACR) 20, 50, and 70 response criteria at day 15; change from baseline in 68 tender joint count (TJC68) and 66 swollen joint count (SJC66); change from baseline in scores of the individual components of DAS28-CRP and ACR response; safety and tolerability, including adverse events, clinical chemistry (including, but not limited to, fasting plasma glucose and serum electrolytes), and vital signs; and AZD9567 pharmacokinetics. Exploratory endpoints included prednisolone pharmacokinetics; anti-inflammatory effects on lipopolysaccharide-stimulated cytokine release in whole blood *ex vivo*; and hypothalamic–pituitary–adrenal axis activity using serum cortisol levels. Bone formation/resorption balance was evaluated using the following serum biomarkers: procollagen-1 N-terminal peptide (P1NP) and osteocalcin (bone formation), C-terminal telopeptide of type 1 collagen (CTX-1; bone resorption), and metabolites of collagens type 1, 3, and 4 (C1M, C3M, and C4M; soft tissue turnover).

DAS28-CRP was evaluated using the formula from Wells *et al.* [8] at screening, baseline, on days 8 and 15, and at follow-up. A clinically meaningful change in DAS28-CRP was defined as a reduction of ≥1.0 point.[9] ACR response criteria, including TJC68 and SJC66, were evaluated according to Felson *et al.* [10] at the same time points. Adverse events were monitored throughout the study. Full details of the sampling schedule are in the Supplementary Methods and Table S1.

Statistical analyses

A sample size of 36 participants (18 per arm) was originally planned, based on a Lalonde-type go/no-go decision framework [11] using a reliability threshold for the DAS28 index of 0.6 [12] and an assumed standard deviation of 2.3 for change from baseline in DAS28-CRP. However, blinded data review and monitoring by the study sponsor revealed that data variability was lower than expected, indicating that a smaller sample size of ≥10 participants per arm would be sufficient to address the study's primary objective. Recruitment was therefore stopped when each treatment group reached ≥10 participants.

The primary endpoint, difference in DAS28-CRP units (i.e., difference between the mean change from baseline with AZD9567 40 mg versus prednisolone 20 mg), was used to estimate average difference in DAS28-CRP between the two treatment groups. This difference was calculated using a mixed model with baseline DAS28-CRP as a covariate and categorical fixed effects of treatment, visit, treatment-by-visit interaction, and country. Changes from baseline in secondary efficacy variables were also analysed using mixed models. Each model included the baseline value for the variable of interest as a covariate, with categorical fixed effects of treatment, visit, treatment-by-visit interaction, and country. The study was not powered for inferential hypothesis testing.

Calculated pharmacokinetic parameters for AZD9567 and prednisolone were area under the concentration–time curve from time 0 to 6 hours post dose ($AUC_{(0-6\ h)}$), time to maximum concentration (t_{max}), maximum concentration (C_{max}), and the last plasma concentration measured before the next dose (C_{trough}). Inhibition of *ex vivo* cytokine release by AZD9567 and prednisolone was assessed separately for each cytokine using a sigmoid maximum effect model from which half-maximal inhibitory concentration (IC_{50}) values were estimated. $AUC_{(0-6\ h)}$ was calculated for serum osteocalcin. Bone balance, a measure incorporating bone resorption and formation,[13] was calculated as the ratio of serum concentrations of CTX-1 to PINP or osteocalcin.

The efficacy analysis population was the intention-to-treat population, including all randomised patients who received ≥1dose of study treatment. All participants who received ≥1dose of study drug were included in the safety analyses. The pharmacokinetic analysis set included all participants with ≥1 quantifiable plasma AZD9567 concentration.

Statistical analyses were performed using SAS version 9.4 or higher (SAS Institute, Inc., Cary, NC). Pharmacokinetic parameters were derived using non-compartmental methods with Phoenix WinNonlin version 8.0 (Certara, Princeton, NJ).

Preclinical methods

To compare molecular mechanisms, monocytes isolated from the blood of six healthy donors were split and treated in vitro with four different single doses of AZD9567 (9–949 nM), prednisolone (32–3162 nM), or dexamethasone (3–316 nM), and incubated for 4 h with and without TNFa stimulation. The comparable doses used were based on half-maximal effective concentration values from a dose-setting experiment that assessed transcriptional effects on a set of glucocorticoid receptor regulated genes. Isolated RNA was transcriptionally

characterized by RNA sequencing, using a paired-end sequencing approach on an Illumina NovaSeq 6000 platform (Illumina).

Full details on preclinical methods are provided in Supplementary Methods and Tables S2 and S3.

Results

The phase IIa study took place from January 18, 2018 to November 12, 2019. Of 27 screened patients, 21 were randomised (AZD9567, *n* = 11; prednisolone, *n* = 10). All 21 participants completed the study and were included in the efficacy, pharmacokinetic, and safety analyses (Figure S1). There were slight imbalances between the AZD9567 and prednisolone groups at baseline (Table 1), with higher mean age, more women, and slightly greater disease severity (indicated by higher mean DAS28-CRP, a higher proportion of patients with radiographic erosions, slightly higher functional class, and a higher number of patients treated with anti-TNFa therapies) in the AZD9567 group.

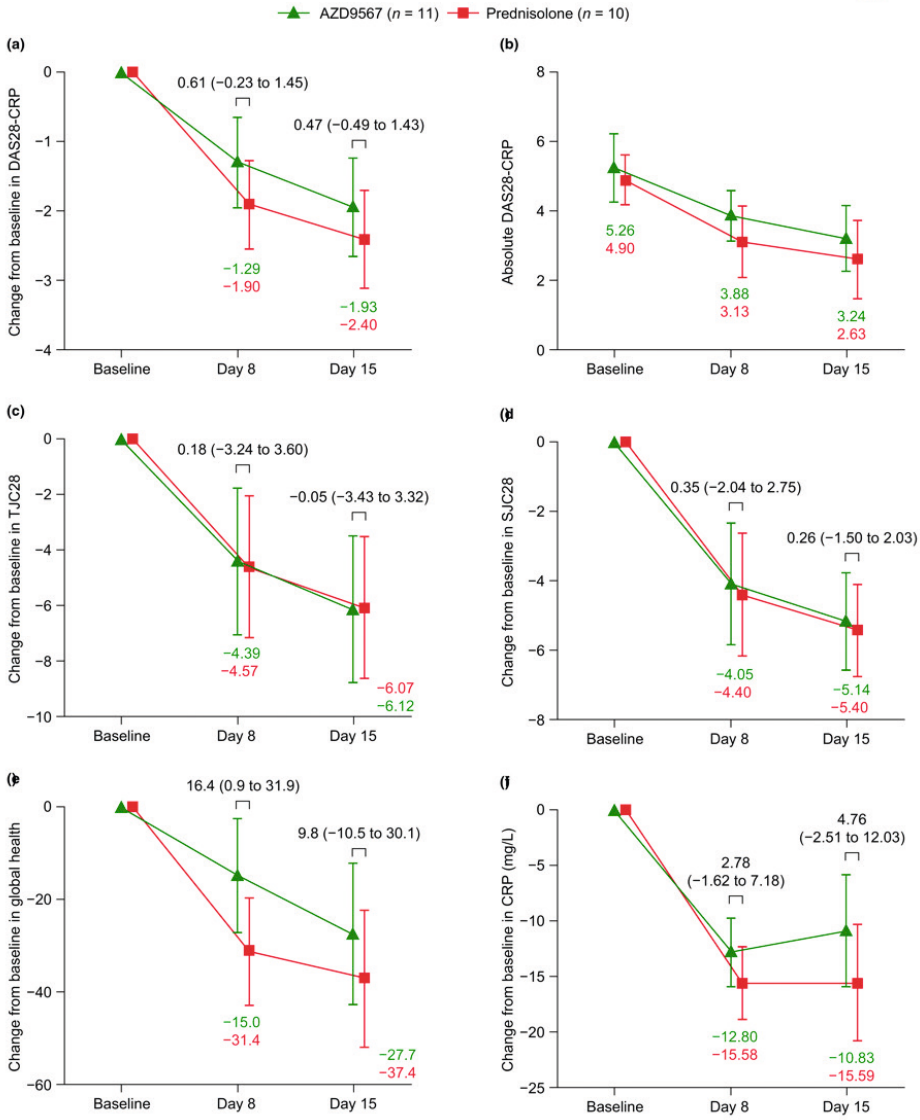

Figure 1. DAS28-CRP and components.

Change from baseline in (a) DAS28-CRP (primary endpoint); (b) absolute DAS28-CRP; and individual components of DAS28-CRP: (c) TJC28, (d) SJC28, (e) global health, and (f) CRP. Data are LS means with 95% CIs. Comparisons are LS mean differences for AZD9567-prednisolone, with 95% CIs. CI, confidence interval; CRP, C-reactive protein; DAS28-CRP, disease activity score in 28 joints with C-reactive protein; LS, least-squares; SJC28, 28 swollen joint count; TJC28, 28 tender joint count.

Table 1. Participant baseline demographics and disease characteristics

	AZD9567 (***n*** = 11)	Prednisolone (***n*** = 10)	Overall (***N*** = 21)
Age (years)	64.5 (8.4)	55.5 (13.6)	60.2 (11.8)
Age group (years)			
18–40	0	2 (20.0%)	2 (9.5%)
41–65	7 (63.6%)	6 (60.0%)	13 (61.9%)
>65	4 (36.4%)	2 (20.0%)	6 (28.6%)
Female	8 (72.7%)	5 (50.0%)	13 (61.9%)
White	11 (100%)	10 (100%)	21 (100%)
Height (cm)	169.3 (9.4)	171.0 (10.9)	170.1 (9.9)
Weight (kg)	78.43 (13.28)	80.67 (23.34)	79.50 (18.29)
Years since onset of RA symptoms	14.73 (14.59)	13.35 (10.91)	14.07 (12.67)
Years since RA diagnosis	13.20 (15.24)	12.79 (11.21)	13.01 (13.14)
Presence of radiographic erosions	7 (63.6%)	5 (50.0%)	12 (57.1%)
Rheumatoid factor positive	9 (81.8%)	9 (90.0%)	18 (85.7%)
Functional capacity class			
Class I	0	2 (22.2%)	2 (10.5%)
Class II	7 (70.0%)	5 (55.6%)	12 (63.2%)
Class III	3 (30.0%)	1 (11.1%)	4 (21.1%)
Class IV	0	1 (11.1%)	1 (5.3%)
Previously treated with TNFα antagonist	6 (54.5%)[a]	3 (30.0%)	9 (42.9%)
Reason for TNFα antagonist discontinuation			
No response	1 (20.0%)	1 (33.3%)	2 (25.0%)
Subsequent loss of response	2 (40.0%)	1 (33.3%)	3 (37.5%)
Adverse effect/intolerance	1 (20.0%)	1 (33.3%)	2 (25.0%)
Other	1 (20.0%)	0	1 (12.5%)
DAS28-CRP	5.26 (0.98)	4.90 (0.74)	5.09 (0.87)
Comorbidities[b]			
Hypertension	3 (27.3%)	4 (40.0%)	7 (33.3%)
Hypothyroidism	3 (27.3%)	1 (10.0%)	4 (19.0%)
Haematuria	0	3 (30.0%)	3 (14.3%)

Table 1. Continued.

	AZD9567 (***n*** = 11)	Prednisolone (***n*** = 10)	Overall (***N*** = 21)
Concomitant medications			
Folic acid and derivatives	8 (72.7%)	7 (70.0%)	15 (71.4%)
Immunosuppressants, including methotrexate	8 (72.7%)	7 (70.0%)	15 (71.4%)
Anilides, including paracetamol/ acetaminophen	4 (36.4%)	3 (30.0%)	7 (33.3%)
Non-steroidal anti-inflammatory and anti-rheumatic agents, including hydroxychloroquine	5 (45.5%)	2 (20.0%)	7 (33.3%)

Data are n (%) or mean (SD).
DAS28-CRP, disease activity score in 28 joints with C-reactive protein; RA, rheumatoid arthritis; SD, standard deviation; TNFa, tumour necrosis factor a.
[a]*One patient who was previously treated with TNFa antagonist continued treatment during the study.*
[b]*Reported by at least three patients overall.*

Efficacy results

In the primary efficacy analysis, the least-squares mean difference in improvement from baseline to day 15 in DAS28-CRP between the AZD9567 and prednisolone groups was 0.47 (95% confidence interval [CI], -0.49 to 1.43), with the numerical difference between the groups being clinically non-meaningful (i.e., < 1.0) (Figure 1a; Table S4). At all-time points, least-squares mean DAS28-CRP overlapped with the 95% CI for the comparator group (Figure 1b; Table S4). Similar results were observed for the change from baseline in the four individual components of DAS28-CRP: TJC28, SJC28, global health, and CRP levels (Figure 1c–f; Table S4).

Similar proportions of patients in both treatment groups achieved the ACR20, ACR50, and ACR70 response criteria, although proportions were numerically lower with AZD9567 (Figure S2a). Improvements in TJC68 and SJC66 from baseline to day 15 were similar in each group (Figure S2b,c; Table S4); however, the reduction was numerically greater with AZD9567 for TJC68 and with prednisolone for SJC66. Similar results were observed for change from baseline in the three other individual components of the ACR response: pain score, disease activity, and physical function (Figure S2d–f; Table S4).

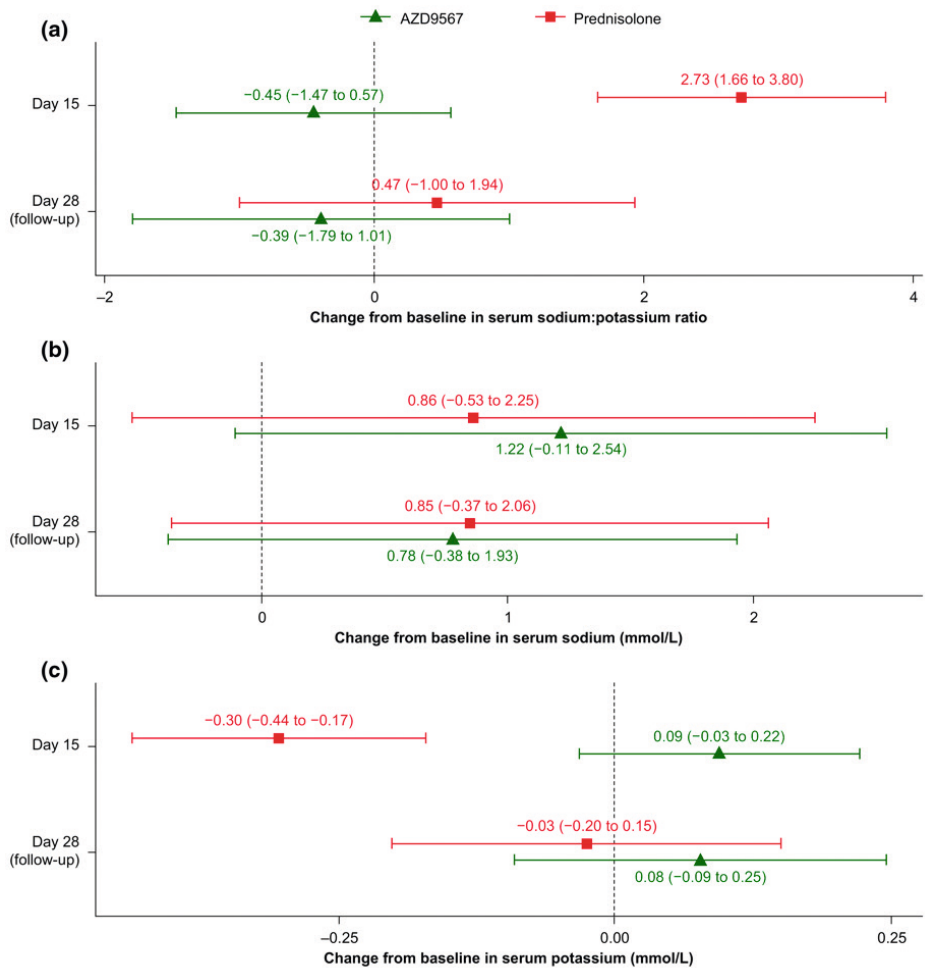

Figure 2. Morning serum sodium and potassium levels.

Change from baseline in (a) sodium: potassium ratio; (b) serum sodium; and (c) serum potassium. Data are least-squares means with 95% confidence intervals.

Serum sodium:potassium ratio

Nuclear hormone receptor binding profiles of AZD9567, prednisolone, and dexamethasone in human monocytes in the preclinical study showed that AZD9567 was more selective for the glucocorticoid receptor than for the mineralocorticoid receptor (Table S5; supplementary data). Significantly greater selectivity for the glucocorticoid receptor was observed with AZD9567 than for prednisolone (P = 0.0001) and dexamethasone (P = 0.0003).

Consistent with this, the serum sodium:potassium ratio was unchanged from baseline to day 15 in the AZD9567 group in the phase IIa study, but increased in the prednisolone group (Figure 2; Figure S3) because decreased potassium in the prednisolone group was not reported in the AZD9567 group. No changes in sodium levels were reported in either group (Figure 2; Figure S3). Both the serum potassium level and sodium:potassium ratio returned to baseline values at follow-up in the prednisolone group.

Clinical chemistry

Fasting plasma glucose levels were similar between the AZD9567 and prednisolone groups throughout the study, with a mean decrease from baseline of approximately 0.5 mmol/L at day 8 and day 15 in both groups. There were no other clinically relevant findings in clinical chemistry, haematology, or urinalysis. There were also no clinically relevant findings in electrocardiographic or physical assessments, including body weight, or in vital signs, including systolic and diastolic blood pressure (although there was a slight increase from baseline in mean systolic blood pressure in the AZD9567 group at day 15; Figure S4).

AZD9567 and prednisolone pharmacokinetics

Pharmacokinetic analyses showed that AZD9567 and prednisolone were rapidly absorbed, with a median t_{max} of 0.7 hours and 1.5 hours, respectively (Table 2). Following C_{max}, elimination of both compounds appeared monophasic and plasma concentrations remained quantifiable until the last sampling time at 6 hours post dose.

Table 2. Pharmacokinetic parameters of AZD9567

Parameter	Summary statistic	AZD9567 (***n*** = 11)	Prednisolone (***n*** = 10)
$AUC_{(0-6\ h)}$ (h·nmol/L)	Geometric mean (CV%)	17,740 (35)	3,591 (22)
t_{max} (h)	Median (min, max)	0.7 (0.3, 1.0)	1.5 (1.0, 1.5)
C_{max} (nmol/L)	Geometric mean (CV%)	4,468 (27)	980 (26)
C_{trough} (nmol/L)	Geometric mean (CV%)	382 (96)	8 (102)

AUC_{0-6}, area under the concentration–time curve from time zero to 6 hours after dose; C_{max}, maximum observed concentration; C_{trough}, observed trough plasma concentration; CV, coefficient of variation; t_{max}, time to maximum observed concentration.

Lipopolysaccharide-stimulated cytokine release

After lipopolysaccharide stimulation of whole blood *ex vivo*, both AZD9567 and prednisolone inhibited the release of all cytokines assessed (TNFα, interferon-γ, interleukins 6 and 8, and macrophage inflammatory protein [MIP]-1α and -1β); the relative inhibitory potency of AZD9567 versus prednisolone was similar for each cytokine (albeit with wide confidence intervals) (Figure S5).

Serum cortisol levels

Morning serum cortisol levels were reduced at day 15 versus baseline in both treatment groups, and the reduction was more pronounced with AZD9567 (Figure S6). Cortisol levels returned to near baseline values at follow-up in both groups, without intervention.

Bone and soft tissue turnover biomarkers

No differences in individual bone and soft tissue biomarker levels in serum were observed between treatment groups other than for P1NP, which was decreased from baseline at day 15 with AZD9567 versus prednisolone, and C1M, which was decreased from baseline at day 15 with prednisolone versus AZD9567 (Figure S7). Bone balance, assessed as change from baseline to day 15 in CTX-1:P1NP and CTX-1:osteocalcin ratios, was similar with AZD9567 and prednisolone.

Safety

Similar numbers of participants in each group reported treatment-emergent adverse events (AZD9567, *n* = 10, prednisolone, *n* = 9) (Table 3). Most adverse events were mild in severity. Six patients in the AZD9567 group and three patients in the prednisolone group reported adverse events assessed by the investigator as related to study treatment. The most common adverse events were cough (AZD9567, 2 patients; prednisolone, 1 patient), fatigue (AZD9567, 3 patients), headache (AZD9567, 2 patients; prednisolone, 1 patient), and hot flash (AZD9567, 3 patients). After completion of treatment, one serious adverse event of severe suicidal depression was reported by the patient's physician as related to AZD9567. The event resolved after approximately 1 month, and the patient was not hospitalized nor given any medical intervention.

Table 3. Summary of participants with adverse events

	AZD9567 (***n*** = 11)	Prednisolone (***n*** = 10)
Any adverse event	10 (90.9%)	9 (90.0%)
Mild	6 (54.5%)	8 (80.0%)
Moderate	3 (27.3%)	1 (10.0%)
Severe	1 (9.1%)	0
Any serious AE	1 (9.1%)[a]	0
Any treatment-related AE	6 (54.5%)	3 (30.0%)
Any AE leading to discontinuation	0	0
AE by preferred term[b]		
Abdominal pain (upper)	2 (18.2%)	0
Cough	2 (18.2%)	1 (10.0%)
Dry mouth	2 (18.2%)	0
Eye pain	2 (18.2%)	0
Fatigue	3 (27.3%)	0
Headache	2 (18.2%)	1 (10.0%)
Hot flash	3 (27.3%)	0
Increased appetite	1 (9.1%)	1 (10.0%)
Insomnia	2 (18.2%)	0
Nasopharyngitis	1 (9.1%)	1 (10.0%)
Treatment-related AE by preferred term[b]		
Abdominal pain (upper)	2 (18.2%)	0
Dry mouth	2 (18.2%)	0
Hot flash	2 (18.2%)	0
Increased appetite	1 (9.1%)	1 (10.0%)

Data are n (%). Table includes AEs that started on or after the date of the first dose, up to and including 14 days after the date of last dose of study treatment (i.e., the follow-up period).
AE, adverse event.
[a]One event of severe suicidal depression was reported in the AZD9567 group, reported by the patient's physician as related to study treatment.
[b]AEs reported by at least two patients overall; patients with multiple events of the same preferred term are counted only once in that preferred term; preferred terms were coded by the Medical Dictionary for Regulatory Activities (MedDRA) version 22.1.

Human monocytes

Transcriptional profiling demonstrated clear dose–response data for AZD9567, prednisolone, and dexamethasone (Figure S8) and revealed that AZD9567 gene regulation exhibited a comprehensive overlap with the two corticosteroids (Figure 3a and Table S6). Predicted upstream regulator analysis and pathway analyses of differentially expressed genes confirmed that AZD9567, prednisolone, and dexamethasone exhibited similar pharmacological profiles (glucocorticoid receptor activation) typical of steroids, and predicted a similar anti-inflammatory response in terms of leukocyte activation (Figure 3b).

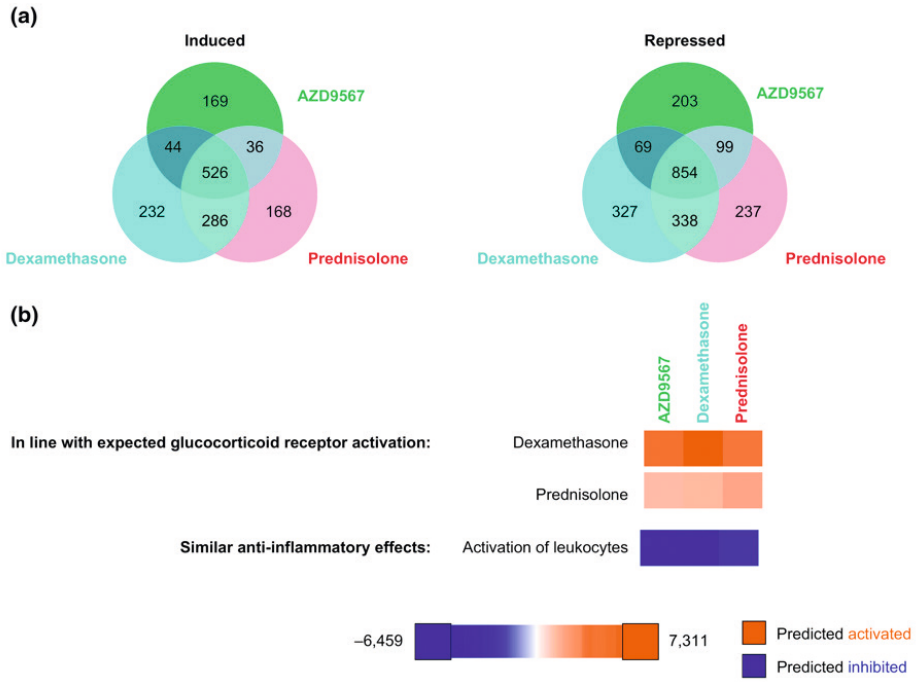

Figure 3. Overall AZD9567 treatment effects on gene transcription in primary monocytes stimulated with TNFα at 4 hours (preclinical study).

(a) Venn diagrams of protein-coding genes induced or repressed by AZD9567, prednisolone and dexamethasone at their highest concentrations (949 nM, 3162 nM, and 316 nM, respectively; FDR < 0.05); (b) predicted upstream regulator analysis and pathway analyses of differentially expressed genes.

A full list of protein-coding genes induced or repressed by AZD9567, prednisolone and dexamethasone at their highest concentrations is included in Table S6 (Supplementary Materials). Colour by z-score: blue for predicted inhibition (negative z-score) and orange for predicted activation (positive z-score).

FDR, false discovery rate; GR, glucocorticoid receptor; TNFα, tumour necrosis factor α.

A global view of all differentially expressed genes induced by both dexamethasone and prednisolone treatment showed that the AZD9567 and prednisolone responses correlated strongly with the dexamethasone response, with R2 values of 0.968 for prednisolone log2 fold-change versus dexamethasone log2 fold-change, and 0.841 for AZD9567 log2 fold-change versus dexamethasone log2 fold-change (Figure 4). For the downregulated genes, R2 values were 0.901 and the slope (b) was 0.87 for prednisolone log2 foldchange versus dexamethasone log2 fold-change, and R2 values were 0.717 and the slope (b) was 0.77 for AZD9567 log2 fold-change versus dexamethasone log2 fold-change. For the upregulated genes, R2 values were 0.931 and the slope (b) was 0.93 for prednisolone log2 fold-change versus dexamethasone log2 fold-change, and R2 values were 0.658 and the slope (b) was 0.54 for AZD9567 log2 fold-change versus dexamethasone log2 fold-change. The data suggest that AZD9567 acts as a partial agonist in gene activation, inducing lower fold-change in those genes while acting as a full agonist for gene repression, indicated by overlapping fold-changes.

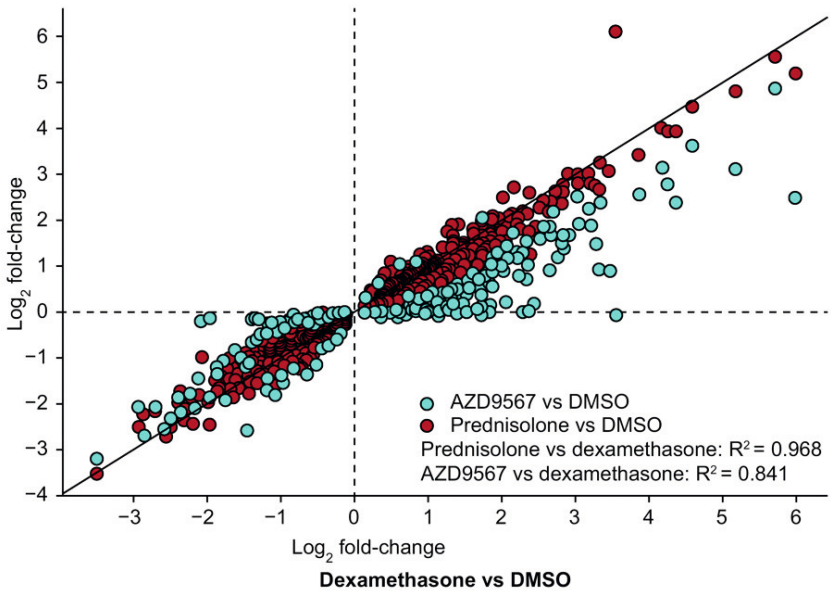

Figure 4. Log2 fold-change of AZD9567 or prednisolone versus dexamethasone for differentially expressed genes common to prednisolone and dexamethasone (preclinical study).

AZD9567 (red) or prednisolone (blue) effects compared with dexamethasone (highest concentration, respectively) for all differentially expressed genes (FDR < 0.05) common to prednisolone and dexamethasone treatments in primary human monocytes stimulated with TNFa, at 4 hours.

DMSO, dimethyl sulfoxide; FDR, false discovery rate; TNFa, tumour necrosis factor a.

Transcript analysis revealed that, although primary monocytes exhibited significant expression of glucocorticoid receptor, the other analysed steroid receptor family members, including mineralocorticoid receptor, were not expressed (Figure S9).

Discussion

In this phase IIa study in patients with active RA, the selective glucocorticoid receptor modulator AZD9567 40 mg had a similar efficacy profile to prednisolone 20 mg based on clinical disease activity measures. AZD9567 40 mg had previously been predicted to be equipotent to prednisolone 20 mg based on pharmacokinetic/pharmacodynamic modelling of anti-inflammatory biomarkers in phase I studies.[7]

Consistent with these data, AZD9567 and prednisolone performed similarly on multiple measures of anti-inflammatory efficacy in patients with active RA, including reductions in the number of tender joints and swollen joints, reductions in serum CRP levels, and improvements in global health and treatment response (ACR20, ACR50, and ACR70). Moreover, AZD9567 and prednisolone inhibited the release of TNFα and other pro-inflammatory cytokines assessed after lipopolysaccharide stimulation of whole blood *ex vivo*, with a similar relative potency against each cytokine, demonstrating that AZD9567 40 mg has a similar broad anti-inflammatory profile to prednisolone 20 mg and further confirming previous biomarker findings in healthy volunteers.[6-7] Although many of the improvements in efficacy measures were numerically smaller in the AZD9567 group than in the prednisolone group, differences were not clinically meaningful, suggesting a similar efficacy profile. Numerical differences may have resulted from the imbalance between the groups in age, sex, and disease severity at baseline, as well as from the small sample size. The differences may also have resulted from the choice of AZD9567 dose. This was based on an estimate of *ex vivo* equipotency of AZD9567 40 mg with prednisolone 20 mg in healthy volunteers,[7] which was accompanied by a 95% CI from 29 to 54 mg.

An *in vitro* transcriptomic analysis (preclinical data) suggests an anti-inflammatory response of AZD9567 consistent with those of prednisolone and dexamethasone. A higher selectivity of AZD9567 for the glucocorticoid receptor over the mineralocorticoid receptor was observed; consistent with this, and unlike prednisolone, AZD9567 had no effect on the serum sodium:potassium ratio. However, it should be noted that in general, very few mineralocorticoid receptor transcriptomic published data exist,[14] and many genes are regulated by both glucocorticoid and mineralocorticoid receptors.[15-16] Indazole ethers are great tools to explore glucocorticoid receptor-exclusive profiles,[17] but

there are currently no equivalent tools for mineralocorticoid receptor-exclusive profiles, and even aldosterone binds to the glucocorticoid receptor, albeit at a lower affinity than to the mineralocorticoid receptor.[18]

In the clinical study, serum cortisol levels were reversibly reduced with both AZD9567 and prednisolone (more so in the AZD9567 group). Both AZD9567 and prednisolone were well tolerated, and there were no new safety findings of concern. Together with the reduced dysglycemia observed with AZD9567 40 mg compared with prednisolone 20 mg in previous phase I studies,[6] these findings suggest that AZD9567 is mechanistically differentiated from prednisolone. Further studies are warranted to support these preliminary data.

Transcriptomic and *in vitro* analysis in monocytes (preclinical data) revealed an anti-inflammatory response to AZD9567 similar to that of prednisolone and dexamethasone. AZD9567 gene regulation exhibited a comprehensive overlap with the two corticosteroids, and AZD9567, prednisolone and dexamethasone exhibited similar pharmacological profiles (glucocorticoid receptor activation), typical of steroids, and predicted a similar anti-inflammatory response with regard to leukocyte activation. This is in stark contrast with the functional profiling in primary hepatocytes, in which AZD9567 showed a clearly differentiated profile, suggestive of a reduced risk for hyperglycaemia as measured by mRNA levels of tyrosine aminotransferase (a key enzyme in gluconeogenesis).[5]

Some of the side effects of corticosteroids, such as oedema, result from off-target actions on the mineralocorticoid receptor that disrupt electrolyte balance and increase water retention.[3,19] The absence of AZD9567 effects on serum potassium levels in the present study and in previous phase I studies [6] is consistent with its demonstrated higher affinity for the glucocorticoid receptor and lower affinity for the mineralocorticoid receptor than prednisolone. [5] Together, these data support a mechanistic differentiation between AZD9567 and prednisolone.

Corticosteroid treatment also disrupts the regulation of endogenous cortisol concentrations via constant activation of the glucocorticoid receptor, suppressing hypothalamic–pituitary–adrenal axis activity and thus reducing levels of cortisol.[19-20] Here, both AZD9567 and prednisolone reduced morning cortisol levels, demonstrating activation of the glucocorticoid receptor. Cortisol suppression appeared more pronounced in the AZD9567 group than in the prednisolone group. These changes were reversible, and levels had spontaneously returned to near baseline values at follow-up, 2 weeks after discontinuation of treatment.

Similar effects on fasting plasma glucose were reported with AZD9567 and prednisolone, although observable differences in this parameter between the drugs were not expected based on previous findings.[6,21] Reduced disruption of glycaemic control with AZD9567 versus prednisolone was evident in a healthy volunteer study: with AZD9567 doses up to 80 mg, plasma glucose after an oral glucose tolerance test was similar to that observed with prednisolone 5 mg.[6] The effects of the study drugs on glycaemic control were not assessed in the present phase IIa study; however, a phase IIa study in adults with type 2 diabetes has been conducted to evaluate these effects (NCT04556760).

Similar changes from baseline in serum CTX-1:P1NP and CTX-1:osteocalcin ratios were observed with AZD9567 and prednisolone. This may indicate that these drugs have similar effects on bone balance, a measure of overall bone metabolism that assesses the equilibrium between bone formation and resorption using ratios of biomarker levels (such as osteocalcin or P1NP for formation and CTX-1 for resorption)[13] Safety and exploratory endpoint findings for AZD9567 in this phase IIa study should be interpreted with caution, owing to the small sample size and the imbalance in demographics and disease severity between treatment groups; however, findings are consistent with previous studies reporting the effects of cortisol on bone biomarkers in healthy volunteers.[3]

Systemic exposure to AZD9567 and prednisolone was 60% and 30% higher, respectively, in patients with RA in the present study versus healthy volunteers from a previous study.[6] Differences in body composition, organ capacity [22], and presence of an inflammatory condition [23] among the participants of the two studies are factors potentially affecting metabolism-dependent drug elimination, and there were also differences in participant body weight and age. Interpretation of this finding is limited by the short pharmacokinetic profile of AZD9567 generated in this study due to participants' limited time in the clinic. Thus, the elimination half-life of AZD9567 could not be evaluated.

The main limitation of the phase IIa study is the small sample size, such that it was not powered to evaluate non-inferiority of AZD9567; therefore, larger studies are needed to assess this. Additionally, the small sample size may have contributed to an imbalance in baseline population and disease characteristics between the randomised groups, although the analyses were performed using models adjusted for baseline values. Nevertheless, the participants' demographics were sufficiently representative of the intended study population to confirm that AZD9567 has similar anti-inflammatory effects to prednisolone in a population with active RA. Although the study design was sufficient to observe an anti-inflammatory effect, another limitation of this study was the short duration of

treatment, which did not permit the assessment of adverse effects that have a low incidence or may take longer to manifest, such as bone remodelling. Despite these limitations, the findings were consistent with a similar efficacy profile and a potentially improved safety profile of AZD9567 versus prednisolone in terms of mineralocorticoid receptor-mediated effects on serum potassium. In addition, findings from the preclinical study support the findings of this phase IIa study.

In conclusion, these results demonstrate that AZD9567 is mechanistically differentiated from prednisolone, showing consistent anti-inflammatory response without any impact on electrolyte balance. AZD9567 40 mg had a similar efficacy profile to prednisolone 20 mg in patients with active RA in this phase IIa study. *In vitro* transcriptomic analysis suggests that the anti-inflammatory response of AZD9567 is consistent with that of the steroid comparators, prednisolone, and dexamethasone. Additionally, AZD9567 showed broad overlap with prednisolone and dexamethasone gene regulation and similar glucocorticoid receptor activation, predicting a comparable anti-inflammatory (leukocyte activation) response. Unlike prednisolone, AZD9567 had no effect on the morning serum sodium:potassium ratio, which is consistent with the higher selectivity of AZD9567 for the glucocorticoid receptor over the mineralocorticoid receptor. Both drugs were well tolerated, with no new safety findings of concern. These results support further clinical trials of AZD9567 in patients with chronic inflammatory disease.

Conflicts of interest

JMvL has received grants from AstraZeneca, MSD, Roche, and Thermo Fisher and honoraria from AbbVie, Arxx Therapeutics, Boehringer Ingelheim, Galapagos, Gesynta, Leadiant, Magenta and Sanofi Genzyme. MSK received a student grant from AstraZeneca. AL, JA, GB, KE, LÖ, BRA, IDi, PB, DE, IDa., CA, MGB, SN, AP, SP, SS, PS and CK are employees of, and may own shares in, AstraZeneca.

Authors' contributors

All authors wrote the manuscript. JMvL, AL, MSK, SN, SP, KE, LÖ and GB designed the research. JMvL, MSK, KE, LÖ, GB, IDi, BRA and PB performed the research. JMvL, AL, MSK, JA, GB, KE, LO, BA, ID, PB, DE, ID, CA, MB, SN, AP, SP, SS, PS, and CK analysed the data.

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Supplementary files

Supplementary Methods

- Full inclusion criteria
  - Men or women aged 18–80 years at screening.
  - Established rheumatoid arthritis (RA) diagnosis according to the 2010 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification or the 1987 American Rheumatism Association criteria.[1-2]
  - Active RA (disease activity score in 28 joints with serum C-reactive protein [DAS28-CRP] ≥ 3.2) with at least three swollen joints and three tender joints using the DAS28 joint count.
  - On stable dosing of conventional disease-modifying antirheumatic drugs and/or subcutaneous/intravenous biologicals for the past 8 weeks before screening.
  - CRP levels > 5 mg/L at screening if seronegative for rheumatoid factor and anti-cyclic citrullinated peptide antibody, or > 2 mg/L if seropositive for either marker.
  - Body mass index from 18 to 35 kg/m^2 (inclusive).
  - Negative pregnancy test (serum) for female patients of childbearing potential.
  - Female patients were required to be 1 year post-menopausal, surgically sterile, or using an acceptable method of contraception (defined as a barrier method in conjunction with a spermicide) for the duration of the study.
  - Male patients were required to be surgically sterile or using an acceptable method of contraception (defined as barrier methods in conjunction with spermicides) for the duration of the study (from the time they signed consent) and for 1 month after the last dose of study drug.
  - Patients who were blood donors could not donate blood during the study and for 3 months after their last dose of study drug.
  - Able and willing to give written informed consent.

- Full exclusion criteria
  - Past or present inflammatory rheumatic disease other than RA (secondary Sjögren's syndrome was excluded).
  - Past or present clinically important disease that may have put the patient at risk when participating in the study, influenced the patient's ability to participate in the study, or influenced the study outcomes.
  - Any clinical contraindications to treatment with steroids.

- Oral or parenteral steroids (beyond study treatment) 8 weeks before study start and during the study. Stable use and dose of topical and inhaled steroids for longer than 4 weeks before randomization was acceptable.
- Use of any prohibited medication during the study, or if the required washout time of such medication was not adhered to.
- History of severe allergy/hypersensitivity or ongoing clinically important allergy/hypersensitivity to drugs of a similar class to the study drugs.
- Any concomitant medications that were known to be associated with torsades de pointes.
- Any clinically significant electrocardiogram, vital signs, or laboratory abnormalities identified at screening or before randomization.
- History of drug or alcohol abuse in the past year before screening.
- Involvement in the planning and/or conduct of the study.
- Previous randomization in the present study.
- Participation in another clinical study of an investigational product during the past 3 months.

- Restrictions during the study

Patients were required to abstain from donating blood and plasma during the study. No change or addition of non-steroidal anti-inflammatory drugs (including topical administration) was permitted less than 24 hours before joint evaluation, or of acetaminophen/paracetamol and other painkillers less than 12 hours before joint evaluation. Owing to the risk of drug–drug interactions, the use of digoxin, statins, inhibitors, or inducers of CYP3A, and QT-prolonging medications, was restricted. Men were required to refrain from fathering a child or donating sperm during the treatment and until 1 month after the last dose. All women of childbearing potential using hormonal contraceptives were required to abstain from use of any medication with CYP3A4 enzyme-inducing properties from 3 months before screening until 1 month after the last dose of study drug.

- Pharmacokinetic analysis of AZD9567 and prednisolone

Blood samples for analysis of AZD9567 and prednisolone in plasma were taken pre dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 hours post dose on day 15.

AZD9567 was quantified in plasma samples using a validated bioanalytical method at Covance Bioanalytical laboratory, Harrogate, UK. The validated method employed protein precipitation followed by liquid chromatography with tandem mass spectrometric detection (LC–MS/MS) in the positive ion mode, with a lower limit of quantification (LLOQ) of 5.0 nM. The intra-batch and inter-batch precision, reported as coefficient of variation, were ≤ 15% at all levels, except ≤ 20% at the LLOQ. Intra-batch and inter-batch bias were within 15% of the nominal concentration at all levels, except ± 20% at the LLOQ.

Prednisolone was quantified in plasma samples using a well-characterized bioanalytical method at AstraZeneca, Gothenburg, Sweden. The method employed protein precipitation followed by ultra-fast LC–MS/MS in negative electrospray ionization mode, with an LLOQ of 1.0 nM. The inter-batch precision reported as coefficient of variation was ≤ 15% at all calibration curve levels.

- Inhibition of lipopolysaccharide-induced cytokine release in human whole blood

Assessments of *ex vivo* cellular function were performed using blood samples from patients receiving AZD9567 or prednisolone taken at baseline and on day 15, pre dose, and 1, 2, 3, 4, and 6 hours post dose. TruCulture tubes (Myriad RBM, Austin, TX) were prepared in-batch with lipopolysaccharide (*Escherichia coli* serotype O55:B5) resuspended in a volume of 2 mL buffered media and maintained at -20°C until time of use. Blood was obtained from the antecubital vein using a 60 mL syringe containing sodium heparin (50 IU/mL final concentration). Within 15 minutes after collection, 1 mL of whole blood was distributed into each of the prewarmed TruCulture tubes, inserted into a dry block incubator, and maintained at 37°C room air for 24 hours. At the end of the incubation period, the tubes were opened, and a valve was inserted to separate the sedimented cells from the supernatant and to stop the stimulation reaction. Liquid supernatants were aliquoted and immediately frozen at -80°C until time of use. Plasma supernatants from whole blood stimulation systems were analyzed at Myriad RBM using Luminex xMAP technology (Bio-Rad Laboratories, Inc.,Hercules, CA).

To compare the relative potency of AZD9567 and prednisolone on inhibition of cytokine release, concentration–response models were developed for selected cytokines with roles in the mechanisms of action of anti-inflammatory drugs: tumour necrosis factor α (TNFα), interferon-γ (IFNγ), interleukin-6 (IL-6) and IL-8, and macrophage inflammatory protein (MIP)-1α and -1β. The concentration–response models were defined as:

$$E = E_0 \times \left(1 - \frac{I_{\mathrm{MAX}} \times C^{\gamma}}{(\mathrm{IC}_{50})^{\gamma} + C^{\gamma}}\right),$$

where E is the observed cytokine concentration, E_0 the baseline cytokine concentration after stimulation with lipopolysaccharide in the absence of any drug, I_{MAX} the maximum inhibition relative to baseline, C the total drug concentration in plasma, IC_{50} the concentration of drug producing a half-maximal inhibition, and γ the sigmoidicity parameter. Parameters of the concentration–response models were estimated from plasma concentrations of AZD9567 and prednisolone time-matched with cytokine data, and from lipopolysaccharide-stimulated cytokine release data before any drug

administration (i.e., with zero drug concentration). For each cytokine, separate values of the model parameters were estimated for AZD9567 and prednisolone, except for the baseline cytokine concentration, which was a shared parameter between the two compounds. Log-normal interindividual variability was assumed for the baseline cytokine concentration. The residual error was modeled as log-normally distributed (additive normal error on log-transformed data). A log-normal prior distribution for the sigmoidicity parameter, with 95% of the probability density in the interval 0.69–4.35, was used to stabilize the model. The prior was determined by fitting a log-normal distribution to the 12 different estimates (one for each biomarker and each compound) of the sigmoidicity parameter from the corresponding phase I biomarker analysis, then increasing the standard deviation by 100%. Model estimation was performed using the non-linear mixed effects modeling software NONMEM version 7.3.0 (Icon Development Solutions, Hanover, MD). Visual predictive checks and goodness-of-fit plots were used for model evaluation.

Serum cortisol analysis

Blood samples for analysis of serum cortisol were taken pre dose at baseline, day 15, and at follow-up. Baseline and day 15 samples were taken at approximately 08:00 under fasting conditions. Follow-up samples were taken at an unspecified time and not under fasting conditions. Cortisol was quantified in serum samples using a well-characterized bioanalytical method at AstraZeneca, Gothenburg, Sweden. The method employed protein precipitation followed by ultra-fast LC–MS/MS in negative electrospray ionization mode, with an LLOQ of 1.0 nM. The inter-batch precision reported as coefficient of variation was ≤ 15% at all calibration curve levels.

- ### Bone and tissue biomarker analysis

Procollagen-1 N-terminal peptide (P1NP), osteocalcin, C-terminal telopeptide of type 1 collagen (CTX-1), and metabolites of collagens type 1, 3, and 4 (C1M, C3M, and C4M) were assessed in serum samples taken at baseline (day 1), on day 15, and at follow-up. Osteocalcin was also assessed in samples taken 1, 2, 3, 4, and 6 hours post dose at baseline (day 1) and on day 15. Baseline and day 15 samples were taken at approximately 08:00 under fasting conditions. Follow-up samples were taken at an unspecified time and not under fasting conditions. Osteocalcin and CTX-1 were quantified using the cobas system (Roche Diagnostics, Rotkreuz, Switzerland), as described previously[3], and P1NP, C1M, C3M, and C4M were quantified using manual enzyme-linked immunosorbent assay (ELISA), as described previously [4-7].Osteocalcin was analyzed at AstraZeneca (Gothenburg, Sweden) and the other bone and tissue biomarkers were analyzed at Nordic Bioscience (Herlev, Denmark). Bone balance [8] at baseline (day 1), day 15, and follow-up was assessed as the ratio of CTX-1 to P1NP or osteocalcin.

Sampling schedule

Blood samples for clinical chemistry and hematology assessments were taken at approximately 08:00 under fasting conditions, at screening, baseline, on day 8, day 15, and at follow-up. Other safety assessments included urinalysis, vital signs (including blood pressure), electrocardiography, and physical assessments. Pharmacokinetic analysis of AZD9567 and prednisolone in plasma was performed using blood samples taken pre dose and at intervals post dose on day 15. The *ex vivo* anti-inflammatory effects of AZD9567 and prednisolone (exploratory endpoint) were assessed using blood samples taken pre dose and at intervals post dose at baseline and on day 15. After lipopolysaccharide stimulation of leukocytes in whole blood *ex vivo*, the inhibition of release of TNFα, IFNγ, IL-6, IL-8, MIP-1α, and MIP-1β were measured. Serum cortisol and bone and soft tissue biomarkers were assessed in blood samples taken pre dose at approximately 08:00 under fasting conditions, at baseline (day 1), day 15, and follow-up. Serum osteocalcin was also assessed at intervals up to 6 hours post dose on days 1 and 15.

- **Monocyte dose-finding study: isolation of monocytes, cell culturing, and treatments**

Peripheral blood mononuclear cells (PBMCs) from three healthy human donors were prepared from fresh blood samples (100 mL) from an internal AZ resource of healthy donors (approval number from the Ethics Committee in Gothenburg, Sweden, Dnr T705-14 Ad 033-10) using Ficoll density gradient centrifugation (Ficoll-Paque Plus, GE Healthcare, cat# 17-1440-03, Piscataway, NJ), with a yield of 140–238 million PBMCs. Monocytes were then isolated from PBMCs by positive selection using human CD14 MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany cat# 130-050-201), according to the manufacturer's protocol, with a yield of 19.6–44.7 million monocytes of 95.3–98.3% purity.

Cells were seeded at 350,000 to 450,000 cells/well in a 48-well plate and left overnight at 37°C, 5% CO_2. On day 2, TNFα (Peprotech, cat# 300-01A, Rocky Hill, NJ; 10 ng/mL final assay concentration) or medium were added, together with 7-point concentration–response curves (**Table S2**) for the compounds AZD9567, prednisolone, and dexamethasone or dimethyl sulfoxide (DMSO), and cells were incubated for 4 hours at 37°C, 5% CO_2. Final assay volume was 500 µL and the DMSO concentration in assay was 0.1%. Medium was carefully removed, and lysis buffer was added. Cell lysates were transferred to gDNA eliminator spin columns, spun down, and stored frozen at –80°C until RNA purification.

- Monocyte dose-finding study: RNA extraction, cDNA synthesis, and qPCR

RNA was purified according to the manufacturer's protocol using the RNeasy® Plus Mini Kit (Qiagen, cat# 74136, Hilden, Germany) and eluted in 30 µL RNAse-free water. Total RNA concentration for each sample was measured on the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 260 nm.

Complementary DNA (cDNA) was synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat# 4368813, Foster City, CA) according to the manufacturer on the Veriti 96-Well Thermal Cycler (Applied Biosystems Foster City, CA). Plates used were MicroAmp Optical 96-Well Reaction Plate (Applied Biosystems, cat# N8010560). After synthesis, cDNA plates were stored frozen at –20°C. Duplicate cDNA samples were pooled. Quantitative PCR (qPCR) was performed on TaqMan Custom Array Cards (Format 24, Life Technologies, cat# 4342249, lot# B3598, Pleasanton, CA) on a panel of 20 known glucocorticoid receptor (GR)-response genes (*TSC22D3, FKBP5, ZBTB16, SGK1, IL1B, CCL2, ICAM1, IL4I1, CXCL8, SERPINB2, PER1, KLF9, TNFAIP8L3, NFKBIA, TNFAIP3, IL6, TNFRSF11B, HBEGF, RGS2, IL23A*; including up- and down-regulated genes) plus three control genes (GAPDH, HMBS, B2M). Median threshold cycles (Ct) for control genes showed gene-specific dose-response behavior, following stimulation with GR modulators. This indicates that these control genes cannot be used for normalization. Instead, all Ct values were normalized to the sample's RNA concentration relative to the mean RNA concentration across all samples. EC_{50} values were estimated by fitting a four-parameter log-logistic function; any combination of gene and compound for which the fit did not converge were excluded from further analysis. The low variability in estimated EC_{50}/IC_{50} values across the genes supported that an average EC_{50} value could be estimated for each compound.

- Monocyte RNA-seq study: isolation of monocytes

Monocyte isolation was performed exactly as described for the dose-finding study, except PBMCs from six healthy human donors were prepared from fresh blood samples (100 mL) from an internal AZ resource of healthy donors (approval number from the Ethics Committee in Gothenburg, Sweden, Dnr T705-14 Ad 033-10).

Monocyte RNA-seq study: cell culturing and treatments

Cell culturing and treatments were performed as described for the dose-finding study; however, the EC_{50} values from that study were used to set the concentrations in the RNA sequencing study. Assay concentrations were set to -0.5 log to EC_{50}, EC_{50}, +0.5 log to EC_{50}, and +1.5 log to EC_{50} for each compound

tested at 4 hours, and only EC_{50} concentrations were used for each compound at 24 hours (**Table S3**), all with and without TNFα (10 ng/mL).

- **Monocyte RNA-seq study: RNA isolation, library preparation, and sequencing**

Total RNA was isolated with Qiagen RNeasy plus 96 according to manufacturer´s protocol, followed by an RNA concentration using RNAClean XP beads, Beckman Coulter Inc., Indianapolis, IN in a 1:1.8 (sample:beads) ratio to achieve the required concentration for library preparation. RNA integrity and concentrations were assessed on a Fragment Analyzer (Agilent) instrument using the High Sensitivity RNA kit (Agilent, DNF-472-0500). RNA was used as input to create libraries using TruSeq Stranded TotalRNA kit (Illumina Inc., San Diego, CA) with dual indexing (Illumina) following standard protocols. Libraries were validated on the Fragment Analyzer platform (Advanced Analytical Technologies Inc. [AATI], Ames, IA) using standard sensitivity NGS fragment analysis kit (Agilent, cat# DNF-473-0500, Santa Clara, CA). Sample libraries were pooled in four batches at equimolar concentrations and bead washed with AMPure XP beads (Beckman Coulter, Inc., Indianapolis, IN) in a 1:0.9 (sample:bead) ratio to remove primer-dimers. The individual pools were quality checked on the Fragment Analyzer system using an NGS Standard Sensitivity kit (Agilent, cat# DNF-473-0500, Santa Clara, CA), and the concentration was determined using Qubit dsDNA Broad Range and High Sensitivity assay kit on the Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA). Sample libraries were pooled in four batches at equimolar concentrations, and sequenced on an NovaSeq 6000 S4 flow cell paired-end 2 × 150 bp, Illumina, Inc.,San Diego, CA.

- **Monocyte RNA-seq study: RNA-seq processing and analysis**

RNA-seq fastq files were processed using bcbio-nextgen (v. 1.0.9)[9], with which reads were mapped to the human genome build hg38 using hisat2 (v. 2.1.0) [10], yielding from 31 million to 197 million mapped reads (average: 79 million) and from 69% to 92% mapping frequency (average: 86%) per sample. Gene and transcript level quantifications, including transcript per million (TPM), were generated with salmon (v. 0.9.1)[11], all within bcbio. R (v. 3.5.1) was used for further data analysis[12]. Differential gene expression was assessed with DESeq2[13], using tximport (v. 1.8.0)[14] to generate estimated counts from salmon quantification and apeglm (v. 1.2.1)[15] for foldchange shrinkage. Genes were considered significantly differentially expressed if Benjamini–Hochberg adjusted *P*-value (false discovery rate [FDR]) < 0.05. Functional analyses were conducted through the use of Qiagen Ingenuity Pathway Analysis.

- Nuclear hormone receptor binding analysis

Binding potency at various nuclear hormone receptors, expressed as pKi, was determined using a radioligand binding assay (**Table S5**).

Nuclear receptor lysate preparation

SF9 insect cells were split to 1.5 × 10^6/mL and infected with 10 µL, 100 µL, or 1 mL of nuclear receptor, according to nuclear receptor, incubated on a shaking platform at 27°C/110 rpm for 72 or 96 hours, then harvested at 3,000 rpm for 10 minutes. Cell pellets were resuspended in 4 mL/g of lysis buffer (20 mM trisaminomethane [pH 7.5], 0.5 mM ethylenediaminetetraacetic acid [EDTA], 2 mM dithiothreitol [DTT], 20% glycerol, 0.4 M potassium chloride, 20 mM sodium molybdate), and protease inhibitor on ice. Lysate was flash-frozen in liquid nitrogen, and then thawed at 30°C. The freeze/thaw step was repeated three times. After freeze thaw, lysate was spun at 18,000 rpm for 2 hours. Supernatant was recovered, aliquoted, and snap frozen, ready for use in the assay.

Assays

Assay protocol

Radioligand was mixed with nuclear receptor lysate to a total volume of 50 µL. Bound and free radioligand were then separated by the addition of a charcoal suspension. Plates were centrifuged and 20 µL of the supernatant added to 100 µL of MicroScint™ (Perkin-Elmer, Inc., Waltham, MA), then read on Packard Topcount plate reader (Packard Instrument Company, Meriden, CT).

The following assay buffers and radioligands were used:

- Human androgen receptor assay: assay buffer: 50 mM trisaminomethane (tris), 800 mM sodium chloride, 10% glycerol (pH 7.5); charcoal buffer: 2% charcoal, 0.5% dextran, 10% glycerol, 50 mM tris, 800 mM sodium chloride, 2 mM dithiothreitol (DTT) (pH 8); radioligand: [^{3}H]-dihydrotestosterone (5 nM final concentration)
- Estrogen α and β receptor assay: assay buffer: 10 mM tris, 1.5 mM EDTA, 10% glycerol (pH 7.4); charcoal buffer: 2% charcoal, 0.5% dextran in 10 mM tris, 1 mM EDTA (pH 7.5); radioligand: [^{3}H]-estradiol (1 nM final concentration)
- Glucocorticoid receptor assay: assay buffer: 10 mM tris, 1.5 mM EDTA, 10% glycerol (pH 7.4); charcoal buffer: 10 mM tris, 1 mM EDTA, 1.5% charcoal, 0.5% dextran (pH 7.4); radioligand: [^{3}H]-dexamethasone (20 nM final concentration)
- Mineralocorticoid receptor assay: assay buffer: 10 mM tris, 1.5 mM EDTA, 10% glycerol (pH 7.4); charcoal buffer: 10 mM tris, 1 mM EDTA, 1.5% charcoal, 0.5% dextran (pH 7.4); radioligand: [^{3}H]-aldosterone (5nM final concentration) or [^{3}H]-progesterone (5 nM final concentration)

- Progesterone receptor assay: assay buffer: 10 mM tris, 1.5 mM EDTA, 10% glycerol (pH 7.4); charcoal buffer: 1.5% charcoal, 0.5% dextran in 10 mM tris, 1 mM EDTA (pH 7.4); radioligand: [^{3}H]-progesterone (20 nM final concentration)

Compound testing

All compounds were tested against human androgen, estrogen α and β, and glucocorticoid, mineralocorticoid and progesterone receptors at 10 concentrations in triplicate, in 4–6 independent experiments. The inhibition of binding of the respective radioligand was used to measure the binding potency of the compound at each nuclear hormone receptor. For compounds with clearly defined inhibition concentration effect curves, the potency was expressed as the pKi (pIC_{50} with adjustment for concentration of radioligand used).

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Supplementary Figures

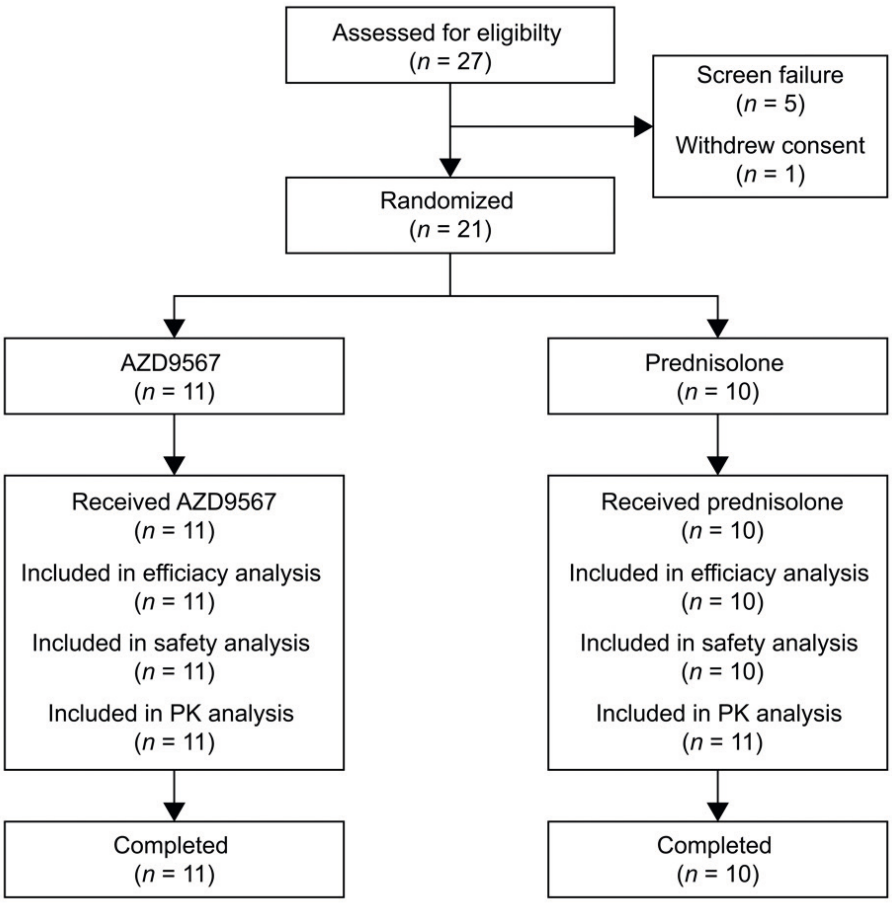

Figure S1. Participant flow through the study. PK, pharmacokinetic.

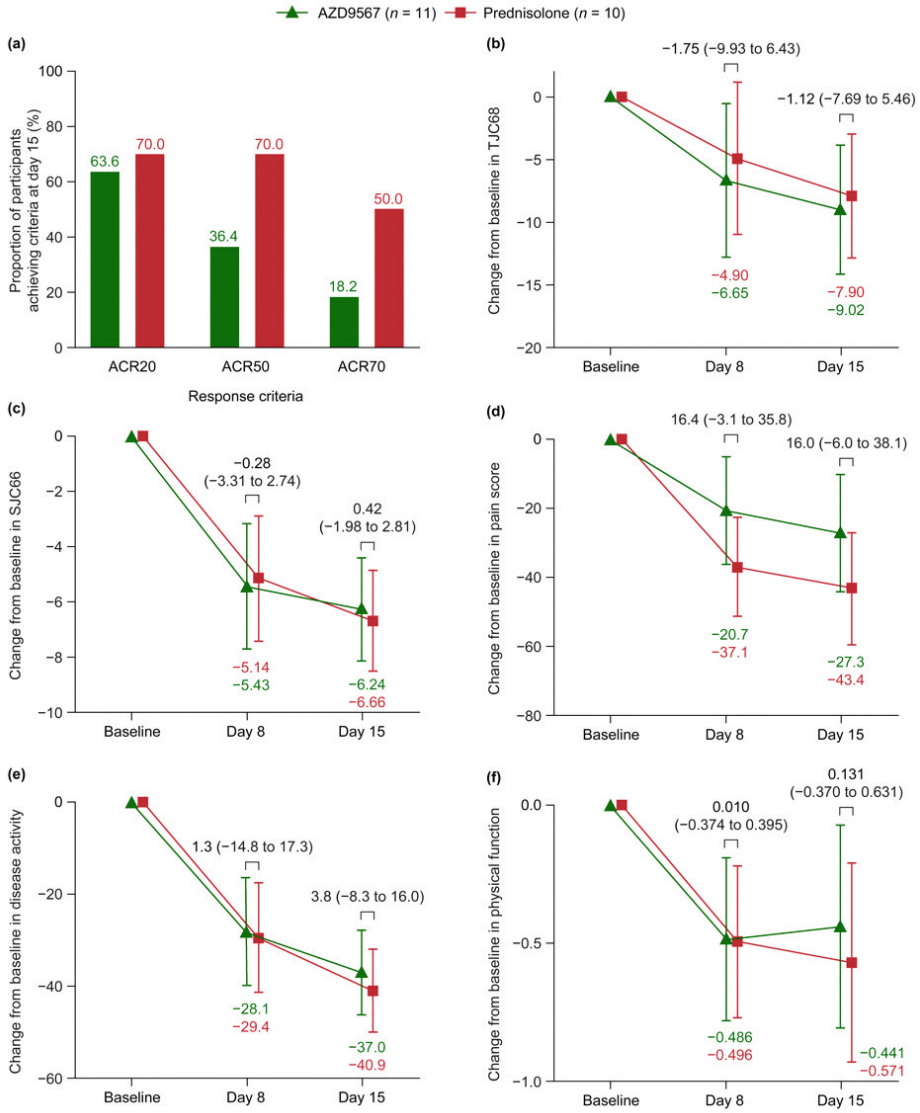

Figure S2. ACR score and components.

(a) Proportions of patients achieving ACR20, ACR50, and ACR70 criteria at day 15; change from baseline in individual components of ACR: (b) TJC68, (c) SJC66, (d) pain score, (e) disease activity, and (f) physical function. Data in b–f are LS means with 95% confidence intervals (CIs). Comparisons for b–f are LS mean differences for AZD9567–prednisolone, with 95% CIs. Supporting data are shown in Table S2. ACR, American College of Rheumatology; LS, least-squares; SJC66, 66 swollen joint count; TJC68, 68 tender joint count.

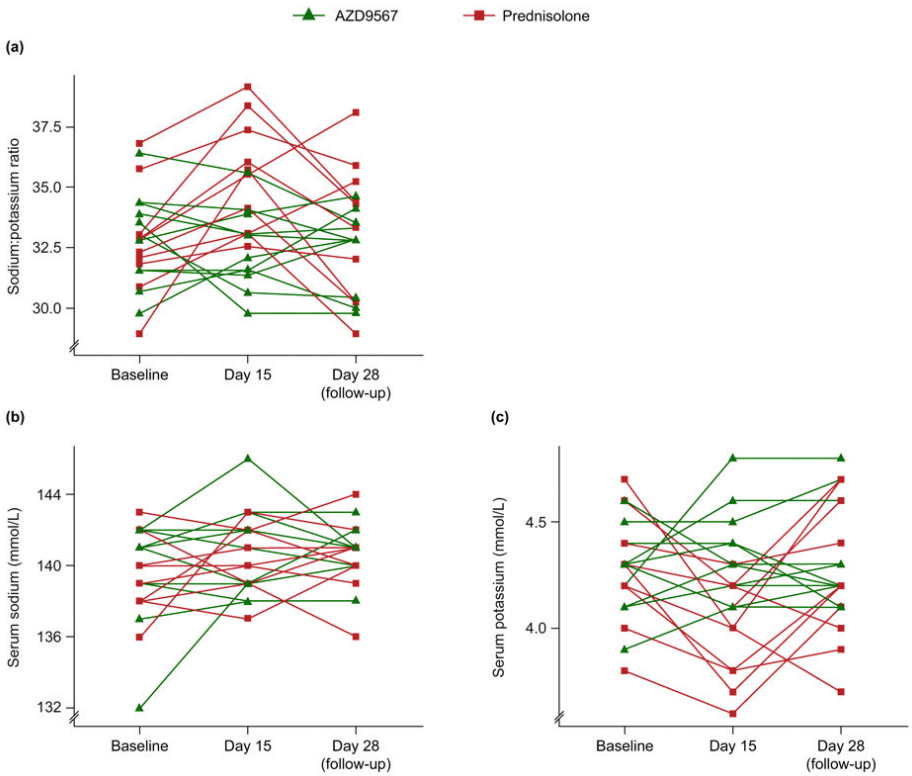

Figure S3. Morning serum sodium and potassium levels.

(a) Sodium:potassium ratios; (b) serum sodium; and (c) serum potassium, shown as individual-level data.

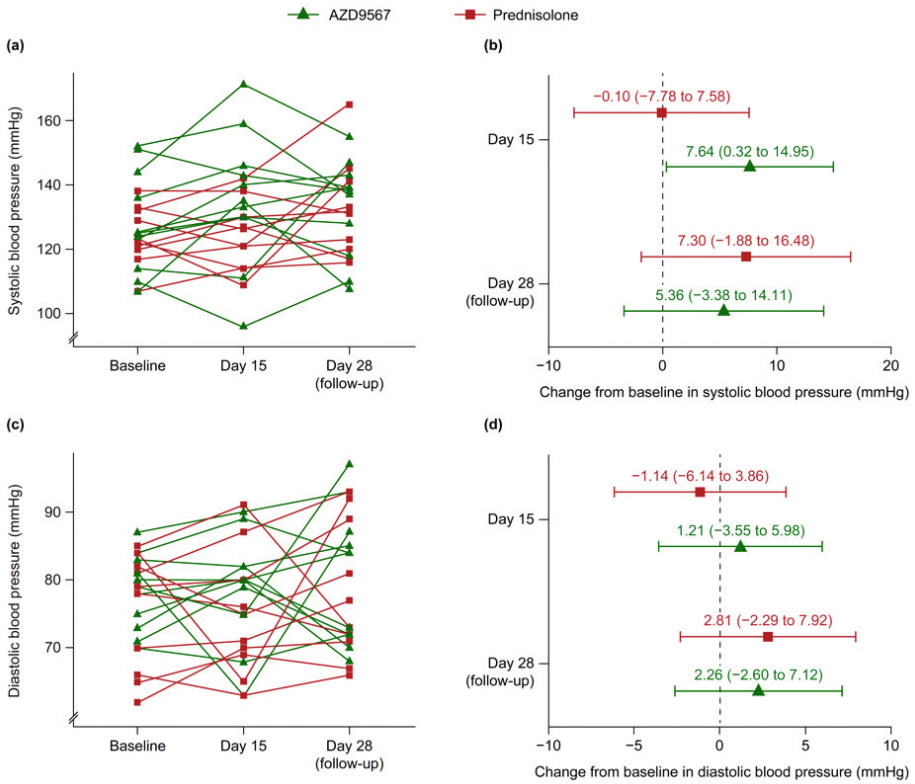

Figure S4. Blood pressure.

(a,b) Systolic and (c,d) diastolic blood pressure, shown as individual-level data in (a) and (c), and change from baseline in least-squares means, with 95% confidence intervals, in (b) and (d).

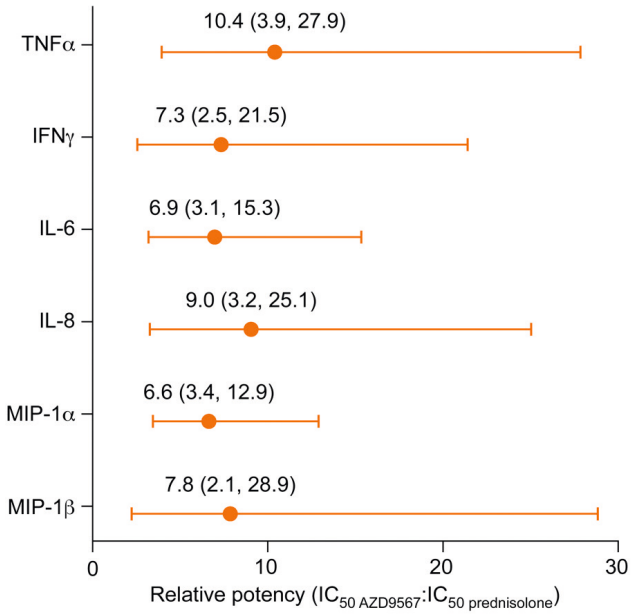

Figure S5. Relative potency of AZD9567 versus prednisolone to inhibit cytokine release from whole blood *ex vivo*.

Relative potency defined as the ratio of the IC_{50} value of AZD9567 to the IC_{50} value of prednisolone. Error bars show 95% confidence intervals. IC_{50} calculations based on total drug concentrations in plasma (N.B.: AZD9567 is more protein-bound than prednisolone. [1] IFNγ, interferon-γ; IL, interleukin; MIP, macrophage inflammatory protein; TNFα, tumour necrosis factor.

Supplementary Reference

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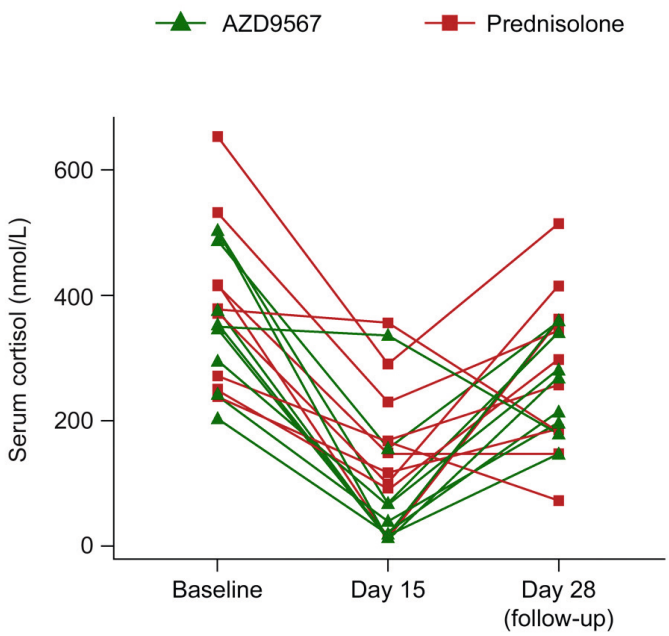


Figure S6. Morning serum cortisol levels, shown as individual-level data.

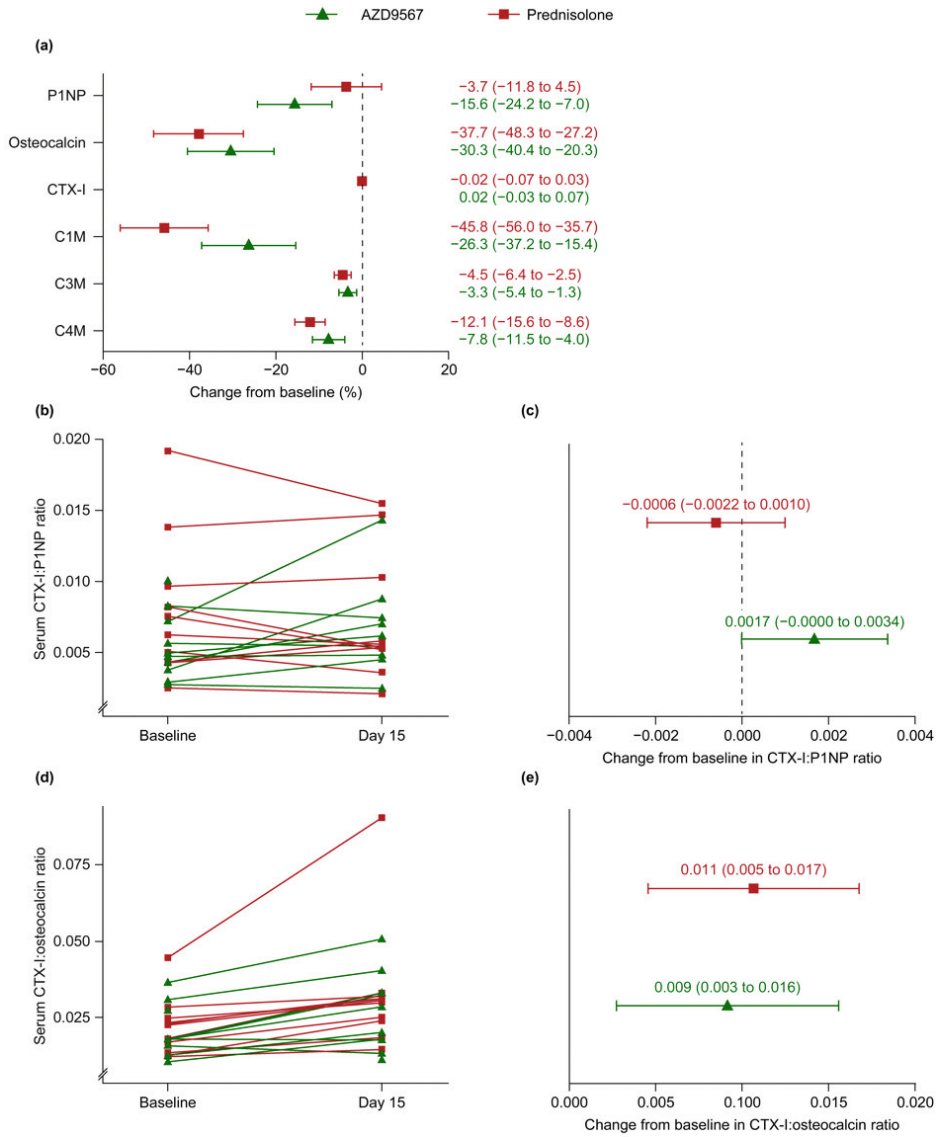


Figure S7. Bone and tissue biomarkers in serum.

(a) Change from baseline to day 15 in LS mean P1NP, osteocalcin, CTX-1, C1M, C3M, and C4M, with 95% confidence intervals (CIs). (b) Individual-level data for CTX-1:P1NP ratio. (c) Change from baseline to day 15 in LS mean CTX-1:P1NP ratio with 95% CIs. (d) Individual-level data for CTX-1:osteocalcin ratio. (e) Change from baseline to day 15 in LS mean CTX-1:osteocalcin ratio with 95% CIs. Osteocalcin data (including in CTX-1:osteocalcin ratios) are $AUC_{(0-6\ h)}$ from samples taken pre dose and 1, 2, 3, 4, and 6 hours post dose; other biomarker data are pre-dose concentrations. C1M, C3M, C4M, metabolites of collagens type 1, 3, 4; CTX-1, C-terminal telopeptide of type 1 collagen; LS, least-squares; P1NP, procollagen-1 N-terminal peptide.

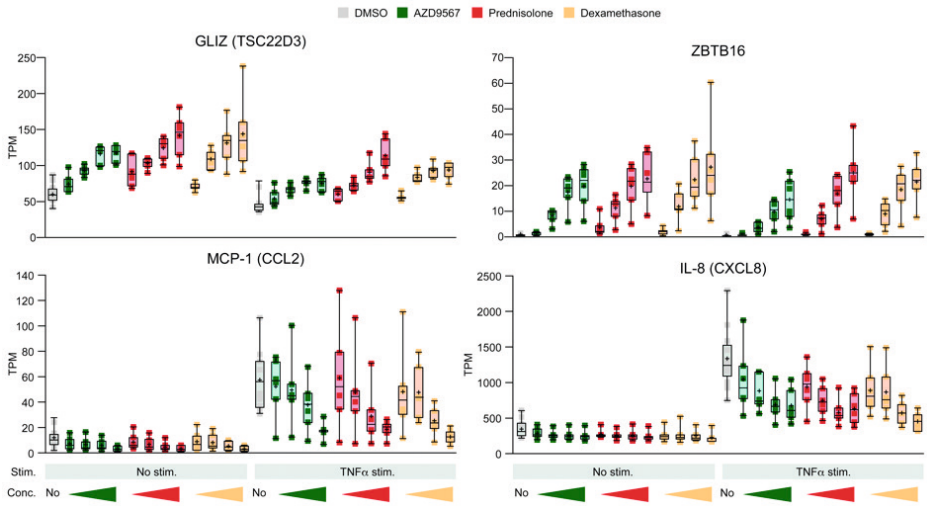


Figure S8. Transcriptional response to AZD9567, prednisolone, and dexamethasone in human monocytes with and without stimulation with TNFα at 4 hours (preclinical study).

Dose-response for AZD9567 (red), prednisolone (blue), and dexamethasone (green) in unstimulated and TNFα-stimulated conditions at 4 hours for two induced genes (TSC22D3, ZBTB16) and two repressed genes (CCL2, CXCL8). The concentrations are 0.5 log below EC_{50}, EC_{50}, 0.5 log above EC_{50}, and 1.5 log above EC_{50}, respectively (Table S3). DMSO used as control (gray). conc., concentration; DMSO, dimethyl sulfoxide; EC_{50}, half maximal effective concentration; stim., stimulation;TNFα, tumour necrosis factor α; TPM, transcript per million. Lines within the box plots represent the median, crosses represent the mean, and upper and lower lines (outside each box plot) represent maximum and minimum values, respectively.

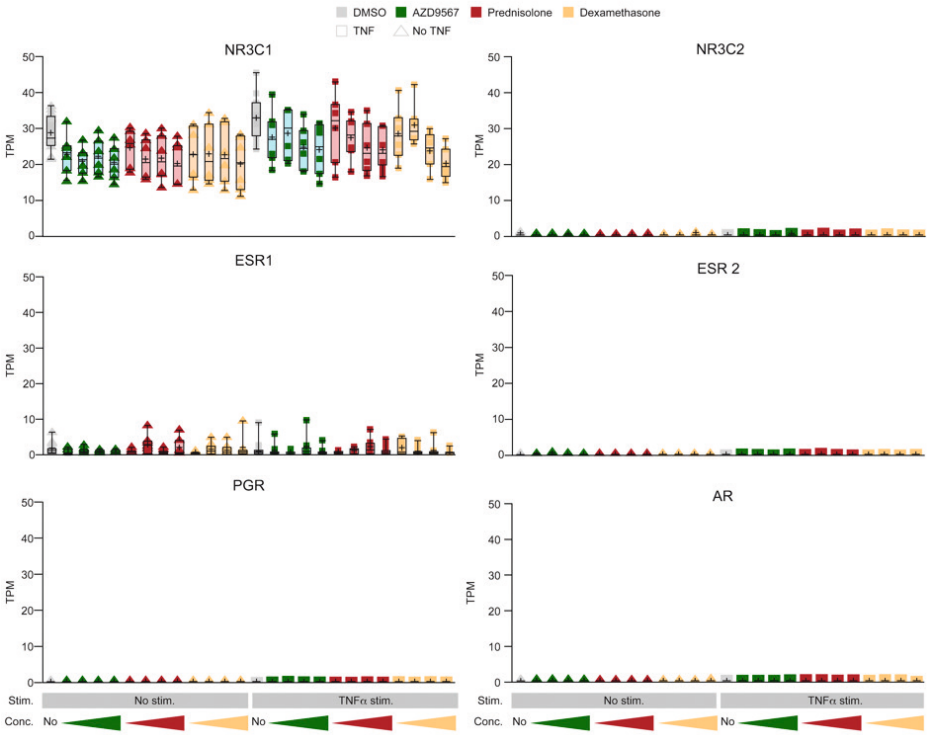


Figure S9 Nuclear receptor expression in response to AZD9567, prednisolone, and dexamethasone in human monocytes stimulated with TNFα at 4 hours (preclinical study).

Dose-response for AZD9567, prednisolone, and dexamethasone in unstimulated and TNFα stimulated conditions at 4 hours for six nuclear receptors: glucocorticoid receptor (NR3C1), mineralocorticoid receptor (NR3C2), estrogen receptor 1 (ESR1) and 2 (ESR2), progesterone receptor (PGR), and androgen receptor (AR). The concentrations are 0.5 log below EC_{50}, EC_{50}, 0.5 log above EC_{50}, and 1.5 log above EC_{50}, respectively. conc., concentration; DMSO, dimethyl sulfoxide; stim., stimulation; TPM, transcript per million. Lines within the box plots represent the median, crosses represent the mean, and upper and lower lines (outside each box plot) represent maximum and minimum values, respectively.

Supplementary Tables

Table S1. Sampling schedule

	Screening: day -7 to -1	Baseline: day 1	Day 8 ± 1	Day 15 ± 1	Follow-up: day 28 ± 2
Clinical chemistry and hematology	08:00[a]	Pre dose at 08:00[a]	Pre dose at 08:00[a]	Pre dose at 08:00[a]	Single time point
AZD9567 pharmacokinetics	–	–	–	Pre dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 hours post dose	–
Prednisolone pharmacokinetics	–	–	–	Pre dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 hours post dose	–
Lipopolysaccharide-stimulated cytokines	–	Pre dose and 1, 2, 3, 4, and 6 hours post dose	–	Pre dose and 1, 2,3, 4, and 6 hours post dose	–
Serum cortisol	–	Pre-dose at 08:00[a]	–	Pre dose at 08:00[a]	Single time point
Serum bone and tissue biomarkers (except osteocalcin)	–	Pre-dose at 08:00[a]	–	Pre dose at 08:00[a]	Single time point
Serum osteocalcin	–	Pre dose at 08:00 and 1, 2, 3, 4, and 6 hours post dose[a]	–	Pre dose at 08:00 and 1, 2, 3, 4, and 6 hours post dose[a]	Single time point

[a]*Fasting conditions.*

Table S2. Concentrations used in the monocyte dose-finding experiment (preclinical study)

Dose (nM)	1	2	3	4	5	6	7	8
AZD9567	0	5	25	75	125	250	750	2,000
Dexamethasone	0	0.1	1	5	10	50	200	1,000
Prednisolone	0	1	10	50	100	250	750	2,000

Table S3. Concentrations used in the monocyte transcriptomics profiling experiment (preclinical study)

Dose	-0.5 log from EC_{50} [nM]	EC_{50} [nM][a]	+0.5 log from EC_{50} [nM]	+1.5 log from EC_{50} [nM]
AZD9567	9	30	95	949
Dexamethasone	3	10	32	316
Prednisolone	32	100	316	3,162

[a]*EC_{50} estimated from dose-setting experiment with 20 genes.*

Table S4. Change from baseline in clinical disease activity measures

	AZD9567 (***n*** = 11)		Prednisolone (***n*** = 10)		Comparison (AZD9567 vs. prednisolone)	
	LSM CFB (SE)	95% CI	LSM CFB (SE)	95% CI	LSMD (SE)	95% CI
DAS28-CRP						
Day 8	-1.29 (0.31)	-1.95 to -0.64	-1.90 (0.30)	-2.53 to -1.28	0.61 (0.40)	-0.23 to 1.45
Day 15	-T1.93 (0.35)	-2.66 to -1.21	-2.40 (0.34)	-3.11 to -1.70	0.47 (0.46)	-0.49 to 1.43
TJC28 score						
Day 8	-4.39 (1.27)	-7.05 to -1.73	-4.57 (1.22)	-7.15 to -1.99	0.18 (1.62)	-3.24 to 3.60
Day 15	-6.12 (1.25)	-8.76 to -3.49	-6.07 (1.21)	-8.61 to -3.52	-0.05 (1.60)	-3.43 to 3.32
SJC28 score						
Day 8	-4.05 (0.85)	-5.81 to -2.28	-4.40 (0.85)	-6.17 to -2.63	0.35 (1.14)	-2.04 to 2.75
Day 15	-5.14 (0.65)	-6.51 to -3.76	-5.40 (0.63)	-6.73 to -4.08	0.26 (0.84)	-1.50 to 2.03
Global health score						
Day 8	-15.0 (5.8)	-27.3 to -2.7	-31.4 (5.5)	-43.0 to -19.9	16.4 (7.4)	0.9 to 31.9
Day 15	-27.7 (7.3)	-42.8 to -12.5	-37.4 (7.1)	-52.3 to -22.6	9.8 (9.7)	-10.5 to 30.1
CRP, mg/L						
Day 8	-12.80 (1.49)	-15.91 to -9.69	-15.58 (1.54)	-18.80 to -12.36	2.78 (2.10)	-1.62 to 7.18
Day 15	-10.83 (2.42)	-15.89 to -5.78	-15.59 (2.52)	-20.87 to -10.31	4.76 (3.47)	-2.51 to 12.03
TJC68 score						
Day 8	-6.65 (2.91)	-12.79 to -0.51	-4.90 (2.87)	-10.98 to 1.19	-1.75 (3.84)	-9.93 to 6.43

Day 15	-9.02 (2.46)	-14.21 to -3.82	-7.90 (2.36)	-12.88 to -2.91	-1.12 (3.12)	-7.69 to 5.46
SJC66 score						
Day 8	-5.43 (1.08)	-7.69 to -3.16	-5.14 (1.07)	-7.39 to -2.89	-0.28 (1.44)	-3.31 to 2.74
Day 15	-6.24 (0.89)	-8.13 to -4.36	-6.66 (0.86)	-8.48 to -4.85	0.42 (1.14)	-1.98 to 2.81
Pain						
Day 8	-20.7 (7.4)	-36.3 to -5.1	-37.1 (6.8)	-51.4 to -22.7	16.4 (9.2)	-3.1 to 35.8
Day 15	-27.3 (8.2)	-44.4 to -10.2	-43.4 (7.7)	-59.6 to -27.2	16.0 (10.5)	-6.0 to 38.1
Disease activity						
Day 8	-28.1 (5.6)	-39.8 to -16.4	-29.4 (5.6)	-41.2 to -17.5	1.3 (7.6)	-14.8 to 17.3
Day 15	-37.0 (4.4)	-46.2 to -27.8	-40.9 (4.3)	-49.9 to -31.8	3.8 (5.7)	-8.3 to 16.0
Physical function						
Day 8	-0.486 (0.141)	-0.782 to -0.190	-0.496 (0.130)	-0.772 to -0.221	0.010 (0.182)	-0.374 to 0.395
Day 15	-0.441 (0.176)	-0.808 to -0.073	-0.571 (0.171)	-0.931 to -0.211	0.131 (0.238)	-0.370 to 0.631

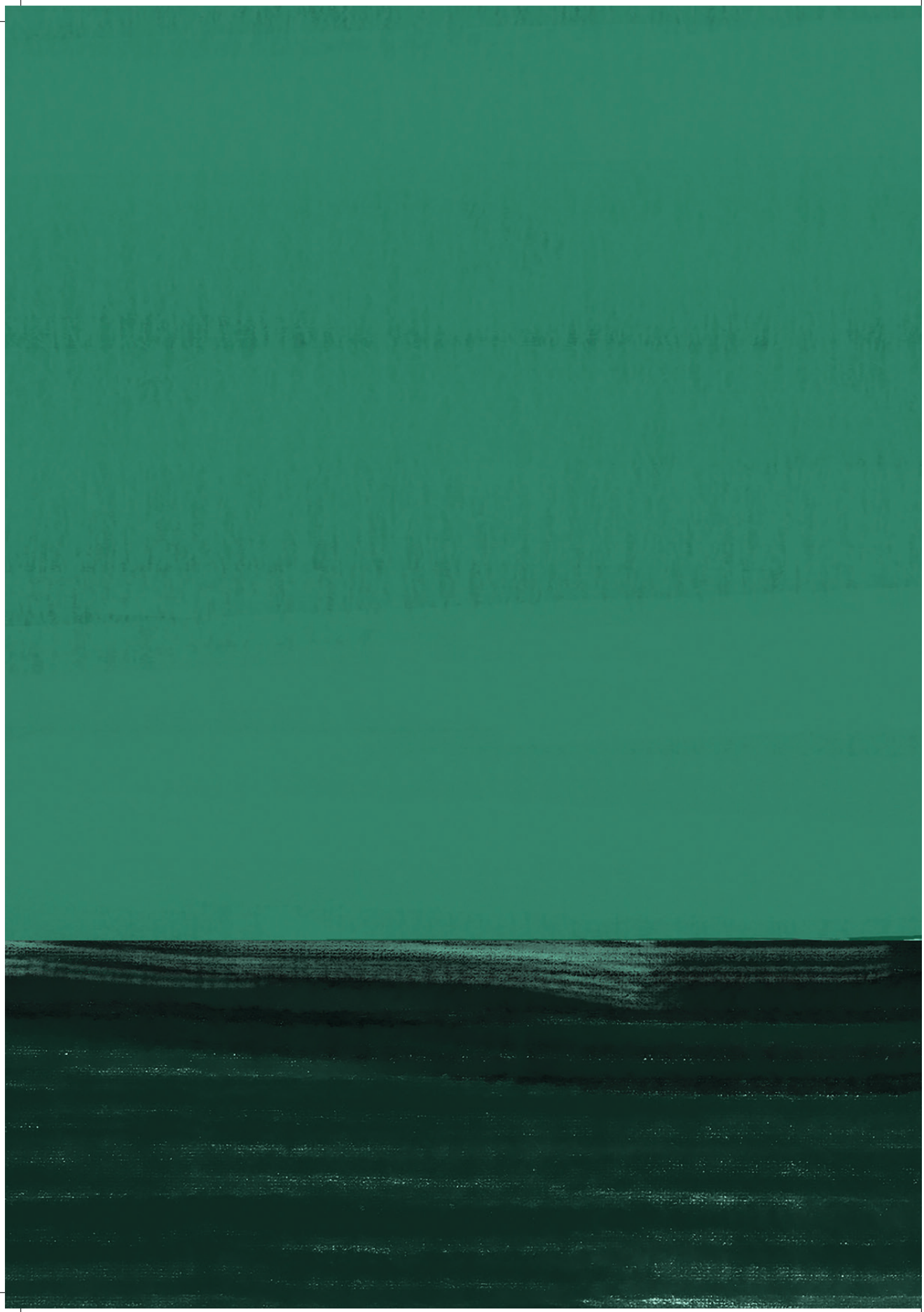
CFB, change from baseline; DAS28-CRP, disease activity score in 28 joints with serum C-reactive protein; LSM, least-squares mean; LSMD, least-squares mean difference; SE, standard error; SJC, swollen joint count; TJC, tender joint count.

Table S5. Nuclear hormone receptor binding profile for AZD9567, prednisolone, and dexamethasone, with binding potencies for each ligand at each receptor defined as pKi (preclinical study)

Mean pKi	GR	MR using [3H] Aldosterone	MR using [3H] Progesterone	PR	AR	ERa	ERb
AZD9567	8.8	--	--	5.6	--	--	--
Prednisolone	8.0[a]	8.2	7.9	5.4	--	--	--
Dexamethasone	8.0[b]	8.1	7.7	5.4	4.8	--	--

[a]P = 0.0001 (versus AZD9567) (unpaired t test).
[b]P = 0.0003 (versus AZD9567) (unpaired t test).
-- indicates no definable inhibition curve; pKi < 4.5 .
AR, androgen receptor; ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor.

7



8.

Summary and general discussion

Summary

The aims of this thesis were to: 1) resolve various underexplored clinical issues relating to glucocorticoid (GC) therapy for rheumatoid arthritis (RA), and 2) evaluate the effectiveness and safety of selective glucocorticoid receptor modulators (SGRMs).

1) Underexplored clinical issues relating to GC therapy

The second Computer-Assisted Management in Early Rheumatoid Arthritis (CAMERA-II) trial had shown that the addition of medium-dose prednisone resulted in a significantly faster reduction of disease activity, less erosive joint damage after two years, and less frequent initiation of tumour necrosis factor (TNF)-inhibitor treatment.[1] Our 2-year post-trial follow-up evaluating the longer-term effectiveness and safety of low-dose GCs revealed that in the former methotrexate (MTX) plus prednisone (pred) strategy group, fewer patients during the follow-up initiated a first biological disease modifying antirheumatic drug (bDMARD), compared to in the former MTX plus placebo (plac) group **(Chapter 2)**. Additionally, after the 2-year follow-up, the former MTX+pred group had less radiographic joint damage than the former MTX+plac group. There were no significant differences in the onset of GC-related comorbidities between the former strategy groups.

Our observation that less frequently bDMARDs were initiated, not only during the 2-year CAMERA-II trial, but also during the 2-year follow up in the former MTX+pred strategy group raised our interest in the potential impact of background GC use in randomised clinical trials (RCTs) evaluating the effectiveness of bDMARDs. Our research showed that in these trials, comparing RA patients on stable background oral GC versus those not on GCs, no statistically significant differences were found in efficacy outcome measures, except for less radiographic progression associated with GC usage in one MTX arm. Serious adverse event rates did not show any significant differences either **(Chapter 3)**.

The multiple-biomarker disease activity (MBDA) test, which reportedly objectively measures RA activity, might also be used for monitoring GC response in patients with RA. Our study in a subgroup of patients from the CAMERA-II trial showed that MBDA and disease activity score assessing 28 joints (DAS28) had similar response profiles, i.e., MBDA was able to track treatment response in CAMERA-II, similarly to DAS28 **(Chapter 4).**

Previous studies had indicated that current smoking reduces the clinical response to DMARDs in RA. We evaluated whether smoking would predict a lesser clinical response to an MTX-based treatment strategy with or without

pred, using data from the CAMERA-II trial **(Chapter 5).** Current smoking was associated with higher DAS28 over time, and this negative effect was dose-dependent. Additionally, smoking significantly reduced the clinical effect of MTX-based strategy in patients with early RA, regardless of whether they also received pred or not.

2) Effectiveness and safety of SGRMs.

For our systematic review of studies investigating the efficacy and safety of SGRMs compared to GCs in arthritis **(Chapter 6),** out of the 207 articles retrieved, only 17 were found to be eligible. Two of these articles involved randomised controlled trials of which both investigated PF-04171327, five were pre-clinical studies that used human samples, and the remaining 10 studies involved pre-clinical animal models of induced acute and/or chronic arthritis in mice or rats. The only compound that was investigated in a clinical trial setting, known as PF-04171327, showed better efficacy/safety balance in comparison to GCs. This was due to its superior clinical anti-inflammatory efficacy and similar safety.

In our phase 2a proof-of-principle controlled trial investigating the anti-inflammatory effects of the SGRM "AZD9567" compared to those of pred **(Chapter 7)**, patients with active RA were randomised to either AZD9567 or pred orally for 14 days. The primary goal of the study was to assess the change in DAS28-CRP. Secondary goals included safety endpoints, such as serum electrolytes. At day 15 from baseline, AZD9567 showed a similar efficacy and safety profile, compared to pred. However, unlike pred, AZD9567 did not affect the serum sodium: potassium ratio, suggesting it is more selective than pred.

Discussion

Our 2-year follow-up study after the 2-year CAMERA-II study shows favourable longer-term results of 10 mg pred daily adjunctive therapy during the first 2 years of early RA. Better outcome, less need of initiation of expensive bDMARDs, and, in contrast to expectations, not more adverse effects, such as diabetes mellitus and bone fractures. It should be noted, however, that all patients received a bisphosphonate and a calcium carbonate preparation with vitamin D during the 2-year CAMERA-II study.[1] Furthermore, one should appreciate the difficult to unravel- interplay of GC therapy, inflammatory disease, and negative effects, which might be adverse effects of GC therapy, but also negative manifestations of the disease itself.[2] Inflammatory diseases have been proven to exert negative effects, which also are attributed to (especially medium and high-dose) glucocorticoids. Glucocorticoids suppress the inflammatory disease and thus also these negative disease-related effects, see Figure.

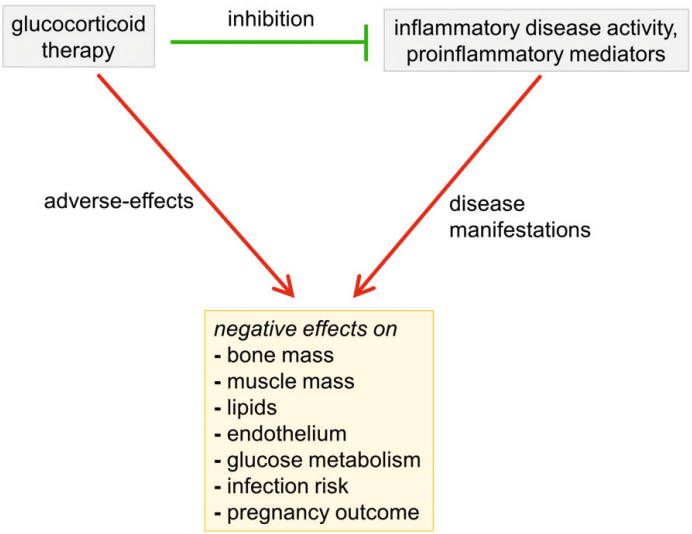


Figure. Effects of glucocorticoids.
With permission from BMJ Publishing Group.[3]

In all, GC therapy in RA, whether as 10 mg pred daily during the first two years of the disease (CAMERA-II trial), or as 60 mg pred daily tapered in six weeks to 7.5 mg/daily (COBRA trial) or as COBRA-light trial, starting with 30 mg pred daily, or in established RA as 5 mg daily (GLORIA trial), seems underappreciated as strategy, especially given their lower price and wider global availability.[4-6] And if adding a bDMARD to the therapeutic strategy is needed, this is no problem, as can be inferred from the result described in this thesis, that inclusion also of RA patients on stable background oral GC in bDMARD RCTs seems not a major issue.

Another underrated, preventive, strategy in RA is smoking cessation. Smoking not only reduces the effectiveness of several drug therapies for RA,[7-8] also of MTX+pred as described in this thesis, but also increases the prevalence of anti-CCP antibodies, increasing the risk of acquiring RA and in RA patients the risk of less well treatable RA (partly via reduced effectiveness of drug therapies).[9-11] In addition, smoking increases the risk of cancer and cardiovascular disease.[12] Without doubt, the risk of cardiovascular disease is already increased by inflammatory diseases such as RA.[13] So, in our opinion, there are several good arguments that an active and, if needed, ongoing additional strategy in smoking RA patients should be helping them stop smoking.

Clinical research into the efficacy and safety of SGRMs is not easy, possibly explaining the lack of clinical studies. First, there is the issue of finding an

equivalent dose, when comparing the effects of a SGRM or investigational synthetic GC like deflazacort with those of conventional GC.[14] A SGRM probably has one major mode of action, i.e., GC receptor modulation, triggering transrepression, but not, or less so, transactivation, to exert a GC-like effect, while the class of GCs is known to have many modes of action, via genomic mechanisms by binding to the glucocorticoid receptor, causing transrepression and transactivation, as well as by manifold nongenomic mechanisms.[15-16] To arrive at an equivalent therapeutic clinical effect, the impact of the SGRM on the GC receptor probably has to be greater than that of a GC, which also has other mechanisms to yield a therapeutic effect.[17] Then, if this hypothesis holds, even if the SGRM relatively has a percentage-wise much smaller transactivation effect than a GC, the absolute transactivation effect might be not so different from that of the equivalent dosed GC, seemingly diluting clinically the selective character of the SGRM. Furthermore, the idea that the anti-inflammatory effects of GCs are largely due to transrepression, while transactivation is accountable for the greater part of GC treatment-associated side effects has turned out to be too simplistic.[18-19] Apart from that, the wanted anti-inflammatory effect has as a downside the increased infection risk as adverse-effect.[20] Since GCs have multiple modes of action and a SGRM probably just one, the DMARD effect of GCs, i.e., inhibiting (progression of) radiographic joint damage, still has to be proven for a SGRM. To gain more insight into the spectrum of effects of a SGRM versus those of a GC, biomarker tests need be developed, since in our research specific biomarkers showed differentiated response profiles, e.g., some markers responding to MTX+pred, but not to MTX+plac.

In our phase IIa study, although the SGRM AZD9567 did not have an effect on the serum sodium-potassium ratio in contrast to pred, clear clinical superiority of this SGRM over pred could not be demonstrated. This may (also) be due to limitations in the study design, including a small sample size of only 21 patients and the short treatment duration of only 2 weeks. Additionally, the two randomised, double-blind treatment groups differed in disease severity, age, and duration, all to the disadvantage of AZD9567. This imbalance in randomised groups is due to chance, the risk of imbalance being higher in small groups, but it nevertheless may have influenced the study results. Further studies are necessary to compare effectiveness and anti-inflammatory and metabolic effects of AZD9567 with those of pred, also on the longer term.

The research described in this thesis has made a small step towards resolving clinical issues of GC and SGRMs in RA. While it is generally a characteristic of scientific research, that scientific studies often yield more questions than answers, it is confronting that even after several decades of research, still more research in the field of GC is needed.

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Nederlandse samenvatting

Inleiding

Reumatoïde artritis (RA) is een chronische auto-immuun ontstekingsziekte van vooral gewrichten (artritis=gewrichtsontsteking), vaak leidend tot gewrichtsschade en minder goed lichamelijk functioneren.[1] Ook andere organen, zoals ogen, speekselklieren en bloedvaten, kunnen aangetast raken.[2] Het belangrijkste doel van medicamenteuze behandeling van RA is de ziekteactiviteit van RA (volledig) te onderdrukken. Daarbij moeten eventuele bijwerkingen en patiënt-gerelateerde factoren, waaronder risicofactoren, verwachtingen en wensen, in het oog worden gehouden. De eerste stap in de medicamenteuze behandeling van beginnende RA is methotrexaat (MTX), een van de zogeheten 'disease modifying anti-rheumatic drugs' (DMARDs). DMARDs zijn medicijnen die het ziekteproces van RA in positieve zin veranderen: ze onderdrukken niet alleen de ontstekingsactiviteit van RA, maar kunnen ook het ontstaan van schade aan gewrichten voorkomen of afremmen. Als MTX, een chemische DMARD, onvoldoende effect heeft, kan er als tweede stap in de medicamenteuze behandeling een biologische DMARD (bDMARD), aan de behandeling met MTX toegevoegd worden, en/of een glucocorticoïd. Een bDMARD is een DMARD bestaand uit een eiwit, vandaar de term biologisch.[3]

Glucocorticoïden voor reumatoïde artritis: hoe worden ze toegepast?

Glucocorticoïden (GCs), zoals prednison, zijn hormonale geneesmiddelen, afgeleid van het bijnierschorshormoon cortisol. GCs hebben veel verschillende werkingsmechanismen en effecten.[4] Ze kunnen in verschillende doseringsschema's en via verscheidene toedieningswegen worden gebruikt. Zo kunnen GCs als tablet of capsule worden ingenomen, of toegediend worden als injectie in een spier of ontstoken gewricht, of via een infuus.[5] GCs kunnen ook het ontstaan van schade aan gewrichten voorkomen of afremmen; ze worden daarom tot de DMARDs gerekend.[6-11] Bij langdurig GC-gebruik, vooral in hogere doseringen, ontstaan vaak bijwerkingen. Maar slechts 35% van de RA-patiënten die GCs gebruiken kan er (helemaal) mee stoppen, doordat dan de klachten door RA weer toenemen.[12-13] Roken vermindert het therapeutisch effect van veel DMARDs, maar het is niet bekend of dat ook geldt voor GCs.[14]

GCs dempen het afweersysteem en remmen zo (reumatoïde) ontsteking, onder andere door zich te binden aan DNA in celkernen.[15] Dit leidt tot stimulering van aanmaak in de cel van bepaalde eiwitten (zogeheten transactivatie) en remming van aanmaak van andere eiwitten (transrepressie).[16-18] Van deze eiwitten hebben meerdere een functie bij ontsteking en daarmee de activiteit van RA. Vooral transrepressie door GCs is verantwoordelijk voor onderdrukken van RA, en vooral transactivatie door GCs veroorzaakt de bijwerkingen van GCs.

[19] Maar dit onderscheid is helemaal niet zo scherp. Een voorbeeld: remming van de afweer vermindert de activiteit van RA, een positief effect, maar vergroot tegelijk ook de infectiegevoeligheid, een negatieve bijwerking.[20]

Zo remmen GCs de aanmaak van ontstekingseiwitten (cytokines).[21] De zogeheten MBDA-test meet het niveau van verschillende cytokines in bloed.[22] Het was nog niet bekend of met de MBDA-test, of met concentraties van individuele cytokines van de MBDA-test, het effect van behandeling van RA-patiënten goed vastgesteld kan worden. En of de test en de individuele cytokines dan verschillen zouden laten zien bij hen die een GC gebruiken, in vergelijking bij hen die geen GC gebruiken.

Zoals gezegd kan langdurig gebruik van GCs, vooral in hoge doseringen, leiden tot bijwerkingen, bijvoorbeeld infecties, botfracturen (osteoporose), hart- en vaatziekte, diabetes en verhoogd risico op overlijden.[23] Nieuwe medicamenten die vooral de gunstige werking van GCs zouden hebben met minder risico op deze bijwerkingen zouden zeer van pas komen.

Alternatieven voor gewone GCs
In het verleden zijn GCs waar een bepaald vetlaagje eromheen is aangebracht (liposomale GCs), GCs gebonden aan stikstofmonoxide (nitro-steroïden) en zogeheten selectieve glucocorticoïd receptor modulatoren (SGRMs) onderzocht.[24-25] Liposomale GCs komen na toediening vooral op plekken in het lichaam terecht waar bloedvaten verhoogd doorlaatbaar zijn. Dat zijn ontstoken weefsels, bijvoorbeeld door RA ontstoken gewichten. Daar treedt dan een hoge concentratie op van GCs met veel gunstige, ontstekingsremmende werking. Tegelijk is de concentratie in andere weefsels veel lager; dientengevolge zouden liposomale GCs minder bijwerkingen hebben. Tot zover de theorie.[26] Onderzoek bij RA patiënten laat tot op heden niet zodanig overtuigende voordelen van liposomale GCs zien, dat ze in de klinische praktijk mogen worden voorgeschreven .[27]

Er is geen onderzoek bij mensen verricht naar de effectiviteit van nitro-steroïden, die minder afbraak van bot (osteoporose) zouden veroorzaken.[20]

SGRMS zijn geneesmiddelen die vooral transrepressie veroorzaken en minder transactivatie, en evenmin andere werkingsmechanismen hebben, die GCs wel hebben. Dit zou kunnen betekenen dat ze een therapeutisch effect hebben als GCs, maar minder bijwerkingen.[28] Er was bij de start van het onderzoek leidend tot dit proefschrift nog maar één onderzoek bij RA patiënten gedaan met een SGRM, fosdagrocorat geheten.[29] Tien mg fosdagrocorat daags bleek even effectief als 10 mg prednison (=standaard GC). Hoewel verwacht werd dat

fosdagrocorat vanwege zijn specifieke werking minder bijwerkingen zou hebben, was dat niet zo. De bijwerkingen van beide medicijnen kwamen overeen.

Resultaten van het onderzoek beschreven in dit proefschrift

Geïnspireerd door bovenstaande vragen en feiten had het onderzoek beschreven in dit proefschrift twee overkoepelende doelen. Ten eerste verschillende onderbelichte klinische aspecten met betrekking tot glucocorticoïdtherapie voor RA te verhelderen. Ten tweede de effectiviteit en veiligheid van SGRMs te evalueren.

Het in het verleden verrichte, tweede 'Computer Assisted Management in Early Rheumatoid Arthritis' (CAMERA-II) onderzoek toonde aan dat starten bij beginnende RA met MTX gecombineerd met 10 mg prednison daags (MTX+Pred) de ziekteactiviteit sneller en beter vermindert dan starten met MTX en placebo-prednison (MTX+Plac), dus eigenlijk MTX alleen.[30] Dit ging gelukkig niet gepaard met meer bijwerkingen in de MTX+Pred groep. Verder bleek er in CAMERA-II minder gewrichtsschade na twee jaar behandeling te zijn in de groep gestart met MTX+Pred dan in de groep gestart met MTX+Plac. Ook was het in de MTX+Pred groep minder vaak nodig nog een bDMARD aan het therapeutische regime toe te voegen, omdat de RA te actief bleek.

In het kader van het proefschrift werd nog een meerjarige observatie (follow-up) na het 2 jaar durende CAMERA-II onderzoek verricht. Tijdens deze follow up periode, waarin de prednison zo veel mogelijk werd verminderd en gestopt, bleek dat in de groep die tijdens het CAMERA-II onderzoek gestart was met MTX+Pred, nog steeds minder patiënten bDMARD therapie nodig hadden gehad, vergeleken met patiënten in de groep die gestart was met MTX+Plac. Na 2 jaar follow-up, dus 4 jaar na de start van CAMERA-II onderzoek, werden weer röntgenfoto's van gewrichten gemaakt. De voormalige MTX+Pred groep had ook toen minder gewrichtsschade dan de voormalige MTX+Plac groep. Er waren ook tijdens follow-up geen duidelijke verschillen tussen deze voormalige strategiegroepen wat betreft bijwerkingen. Een verklaring is dat tijdens het CAMERA-II onderzoek de patiënten ook medicijnen tegen botontkalking, dat een bijwerking van GCs is, gebruikten. Een bijkomende, mogelijke verklaring is de volgende. Het vaker vóórkomen van andere bijwerkingen door GCs in de voormalige MTX+Pred groep werd mogelijk gecompenseerd door het minder vaak voorkomen van bijwerkingen door ontstekingsremmende pijnstillers, zoals naproxen en diclofenac, in die groep. Want tijdens de follow-up werd een lager gebruik van ontstekingsremmende pijnstillers vastgesteld in de voormalige MTX+Pred groep, vergeleken met de voormalige MTX+Plac groep. De resultaten van de follow-up analyses staan meer gedetailleerd beschreven in **hoofdstuk 2**.

Dat starten met de eerste bDMARDs minder vaak nodig was gedurende het CAMERA-II onderzoek én gedurende de follow-up periode in de (voormalige) MTX+Pred groep wekte onze belangstelling naar de mogelijke invloed van al dan niet GC-gebruik door patiënten in onderzoeken naar het effect van bDMARDs. Gebruikmakend van de gegevens van vier verrichte onderzoeken vergeleken wij het effect van de bDMARD bij patiënten met en zonder GC-therapie bij de start van elk onderzoek. Wij vonden daarbij geen verschil tussen patiënten met en zonder GC-therapie wat betreft effectiviteit en bijwerkingen van de bDMARD, met uitzondering van minder gewrichtsschade in een van de vier onderzoeken onder de patiënten die een GC gebruikten, zie **hoofdstuk 3**.

Bij RA is de aanmaak van cytokines (ontstekingseiwitten) verhoogd; GCs en andere DMARDs remmen deze aanmaak.[3] Wij vroegen ons af of met de MBDA-test, of door meting van concentraties van de individuele cytokines van de MBDA-test, het effect van behandeling van RA-patiënten goed vastgesteld kan worden, bij hen die ook GCs gebruiken en bij hen die geen GC gebruiken. We analyseerden gegevens van (een deel van) patiënten uit de CAMERA-II trial. We gebruikten als gouden standaard de alom toegepaste ziekte-activiteitscore (disease activity score) DAS28, waarbij 28 verwijst naar het scoren op ontstekingsverschijnselen van 28 gewrichten per patiënt. Met de MBDA kon het effect op behandeling vergelijkbaar met DAS28 vastgesteld worden, **hoofdstuk 4**. Combinaties van individuele cytokines van de MBDA-test leverden verschillende profielen op in de MTX+Pred en de MTX+Plac groep.

Uitgaande van het gegeven dat roken het therapeutische effect van veel DMARDs vermindert, onderzochten we, gebruik makend van gegevens uit CAMERA-II, of roken een andere invloed had op de effectiviteit van de MTX+Pred behandelstrategie dan op die van de MTX+Plac strategie, **hoofdstuk 5**. We stelden vast dat roken gepaard gaat met een hogere DAS28, dus meer ziekteactiviteit van RA, dan niet roken. Dit wijst op een nadelige invloed van roken op de effectiviteit van de medicatie. Meer roken had nog een slechter effect dan minder roken. Het negatieve effect van roken was niet verschillend voor de MTX+Pred strategie en de MTX+Plac strategie.

Hoe effectief en veilig zijn SGRMs?
Om deze vraag te beantwoorden zochten we naar artikelen voor het doen van een systematisch overzichtsonderzoek naar de effectiviteit en veiligheid van SGRMs bij artritis (**hoofdstuk 6**). Er bleken slechts 17 van de 207 gevonden artikelen in de eerste fase voor ons doel in aanmerking te komen. Vijf betroffen laboratoriumonderzoek met menselijk materiaal en 10 waren poefdieronderzoeken (muizen of ratten met acute of langdurige experimenteel opgewekte artritis). Twee van de 17 artikelen beschreven patiëntgebonden

onderzoek, waarvan één, met analyse van gegevens van een reeds verricht onderzoek, keek naar de relatie tussen dosis en alleen therapeutisch effect. Over bleef het andere onderzoek, een oorspronkelijk vergelijkend onderzoek waarin patiënten met RA op MTX therapie lootten voor toevoeging aan de medicatie van dagelijks de SGRM met de codenaam 'PF-04171327' =fosdagrocorat 10 mg, of fosdagrocorat 25 mg, of prednison 5 mg of placebo. Fosdagrocorat 25 mg had het beste effect, zonder dat dit ten koste ging van meer bijwerkingen.

Wij verrichtten zelf ook een vergelijkend onderzoek bij RA patiënten met een SGRM met de codenaam 'AZD9567', **zie hoofdstuk 7**. Hierbij werden effectiviteit (veranderingen van herhaalde metingen met de DAS28) en veiligheid (kijkend naar bijwerkingen, onder andere ongewenste verschuivingen van elektrolyten in het bloed, die kunnen voorkomen bij behandeling met bepaalde GCs) van AZD9567 vergeleken met die van prednison. AZD9567 had een vrijwel overeenkomend werkzaamheids- en veiligheidsprofiel als prednison op dag 15 na start van het onderzoek. Maar in tegenstelling tot prednison liet AZD9567 echter geen verschuiving van elektrolyten in het bloed zien. Dit suggereert dat AZD9567 een selectiever werkend middel is dan prednison, m.a.w. dat AZD9567 mogelijk minder van de bijwerkingen, die pas optreden bij langdurig gebruik, veroorzaakt, bij gelijkblijvende effectiviteit.

Discussie

GCs zijn echt wonderlijke medicijnen. Ze worden al sedert de vijftiger jaren van vorige eeuw gebruikt, zijn goedkoop maar zeer effectief bij allerlei ziekten en zijn daar vaak onmisbaar, maar ze hebben ook bekende bijwerkingen[4,31]. Sedert hun ontdekking zijn we steeds meer over de vele werkingsmechanismen van GCs te weten gekomen, maar toch weten we, na vele decennia onderzoek, nog niet alles daarover. Zo wisten we bijvoorbeeld niet of het al dan niet gebruiken van GCs door patiënten die werden ingesloten in onderzoek met een bDMARD, het behandelingseffect zou kunnen beïnvloeden. Dat was, blijkens ons onderzoek, niet het geval.

GCs hebben vooral bij patiënten een slechte naam vanwege gevreesde bijwerkingen, maar ons onderzoek van de follow-up van CAMERA-II laat zien dat dat niet altijd helemaal terecht is, wanneer GCs in lagere dosering gebruikt worden. Dat onderzoek liet zien dat er geen belangrijke verschillen waren in bijwerkingen tussen patiënten die GCs gebruikten en die geen GCs gebruikten. Misschien speelt mee dat bepaalde ziekteverschijnselen van RA, zoals osteoporose, ook bijwerkingen van GCs zijn, maar door patiënten alleen toegeschreven worden aan het gebruik van een GC. Maar, mede gebaseerd op inzichten in werkingsmechanismen, wordt ook al vele jaren gezocht naar nieuwe

medicijnen met minstens dezelfde effectiviteit, maar minder bijwerkingen. Opvallend is dat dit tot nu toe niet geresulteerd heeft in een middel dat goedgekeurd is, en vrij te gebruiken. Momenteel wordt veel onderzoek gedaan naar SGRMs Deze middelen hebben, in tegenstelling tot GCs, maar een zeer nauw omschreven werkingsmechanisme, vooral transrepressie, dat voornamelijk onderdrukking van ontstekingsactiviteit veroorzaakt.[32-33] Hoe kan het dan zijn dat van deze middelen tenminste even goede effectiviteit met minder bijwerkingen zo moeilijk aan te tonen is? Een verklaring is dat de tweedeling transrepressie-effectiviteit en transactivatie-bijwerkingen zeker lang niet zo duidelijk is als voorheen werd gedacht. Een andere mogelijke verklaring is het smalle werkingsmechanisme van SGRMs. Doordat GCs op veel meer manieren ontstekingsactiviteit remmen, kunnen zij relatief lager gedoseerd worden, en moeten SGRMs, om dezelfde effectiviteit te verkrijgen, mogelijk relatief hoger gedoseerd worden, met daardoor weer wat meer bijwerkingen in absolute zin. [34-35]

Dus voorlopig blijft het bij pogingen om de behandeling met GCs van patiënten met RA te optimaliseren. Met de MBDA-test kan de effectiviteit van de behandelingsstrategie, die ook een GC kan bevatten, nagegaan worden bij patiënten met RA.[22] Maar door de prijs van de test en het niet onmiddellijk voorhanden zijn van de uitslag en niet beter vaststellen van ontstekingsactiviteit is het uitermate onwaarschijnlijk dat de MBDA-test de DAS28, die binnen luttele minuten af te nemen is, maar verder niets kost.

De nadelen van roken, vooral bij patiënten met RA, vormen een belangrijke boodschap van ons onderzoek voor behandelend artsen en patiënten. Roken gaat de effectiviteit van veel DMARDs tegen, zoals ook ons onderzoek beschreven in het proefschrift laat zien. Maar er zijn veel meer nadelen van roken voor RA-patiënten. RA verhoogt de kans op bepaalde vormen van kanker enigszins; van roken zijn allerlei vormen van kanker als niet zeldzame complicatie goed bekend. Mogelijk is er dan een extra verhoogde kans op kanker bij RA-patiënten die roken. [36-39] Actieve RA en andere auto-immuun ontstekingsziekten verhogen de kans op hartvaatziekten; roken doet dat ook en draagt dus verder bij aan die kans. [40] Dus om al deze redenen vinden wij dat roken door RA-patiënten zeer actief tegengegaan dient te worden in de spreekkamer, te beginnen met voorlichting aan rokende patiënten met RA.

Wij hopen met ons onderzoek, beschreven in dit proefschrift, weer een steentje te hebben bijgedragen aan het optimaliseren van de behandeling van patiënten met RA.

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Curriculum Vitae

Mary Safy-Khan was born on April 20, 1987, in Kabul, Afghanistan. When she was five years old, Mary, her parents and brother fled Afghanistan because of the war. The family spent six months in an asylum seekers' centre before being granted residency permits. Mary finished high school (atheneum) with honours at "Sg. St. Canisius" in Almelo in 1999. Thereafter, she started her medical training at Groningen University to become a physician. During the first four years of her medical study, Mary daily travelled from Almelo to Groningen by train since she also helped her father in his mobile phone shop and because of her parents' cultural preference that she should not move out. However, she rarely missed a class and obtained her medical Bachelor's degree cum laude. A highlight of her studies was a two-month extracurricular internship at the gastro-intestinal surgery department in a public hospital, Maiwand Hospital, in Kabul, Afghanistan. In May 2013, she earned her medical degree from the University of Groningen.

Mary, having a particularly interest in Africa, had studied medicine because she wanted to work for Doctors Without Borders in developing countries. However, her mother felt that this kind of work would be too dangerous for her only daughter. So, Mary changed her plan and opted to work as a researcher in a developing country. She moved to Amsterdam in 2013 with the aim of working at the Global Health Institute, but due to funding issues of projects in developing countries, she started working as a research physician at the department of Clinical Immunology and Rheumatology of AMC in Amsterdam. There she met dr. MJH de Hair and was she responsible for running several clinical trials in patients with rheumatoid arthritis.

In 2013, Mary several times met with prof. Joep Lange of the Global Health Institute, a renowned AIDS-researcher, who inspired her with his research projects in Africa. She dreamed of joining one of his PhD programmes in Africa. Dramatically, Joep perished in the MH17 disaster, and with him those plans died. Mary continued working at AMC until she got the opportunity in 2016 to start as a PhD student at the department Clinical Immunology & Rheumatology of the UMCU, under the supervision of prof. van Laar, dr. JWG Jacobs, and dr. MJH de Hair, who had been appointed to that same department. This research was funded by AstraZeneca, which gave her a unique insight as a medical doctor into the drug development process of a pharmaceutical company, enabling her to guide a first-in-patient trial with one of their compounds. Prof. JWJ Bijlsma gave her the opportunity to also work as a subinvestigator of the Esperence trial, in which for the first time liposomal glucocorticoid was investigated in patients with rheumatoid arthritis.

After finishing the research for her thesis, Mary started a family with her husband and therefore decided not pursue a career in Africa but rather stay in the Netherlands. After having trodden some medical side paths, in 2021 she decided to become a general practitioner, entering the general practitioner programme of the AMC/UvA. During the first year of her training, she worked in a general practice. After maternity leave, she started the second year of her training with several internships at a Psychiatry Department, a nursing home and an Emergency Department.

Mary lives in Wilnis, the Netherlands with her husband, Kashif Khan, and their daughters Nyla (2021) and Nora (2022), and their cat Billy.

List of publications

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