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This report

Abstract

Rituximab versus tocilizumab and B-cell status in TNF-alpha inadequate-responder rheumatoid arthritis patients: the R4-RA RCT

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Background: Although biological therapies have transformed the outlook for those with rheumatoid arthritis, there is a lack of any meaningful response in approximately 40% of patients. The role of B cells in rheumatoid arthritis pathogenesis is well recognised and is supported by the clinical efficacy of the B-cell-depleting agent rituximab (MabThera, F. Hoffman La-Roche Ltd, Basel, Switzerland). Rituximab is licensed for use in rheumatoid arthritis following failure of conventional synthetic disease-modifying antirheumatic drugs and tumour necrosis factor inhibitor therapy. However, over 50% of patients show low/absent synovial B-cell infiltration, suggesting that, in these patients, inflammation is driven by alternative cell types. This prompted us to test the hypothesis that, in synovial biopsy B-cell-poor patients, tocilizumab (RoActemra, F. Hoffman La-Roche Ltd, Basel, Switzerland) (targeting interleukin 6) is superior to rituximab (targeting $CD20^+$/B cells).

Design: The R4–RA (A Randomised, open-labelled study in anti-TNFalpha inadequate responders to investigate the mechanisms for Response, Resistance to Rituximab versus Tocilizumab in Rheumatoid Arthritis patients) trial is a 48-week Phase IV, open-label, randomised controlled trial conducted in 19 European centres that recruited patients failing on or intolerant to conventional synthetic disease-modifying antirheumatic drug therapy and at least one tumour necrosis factor inhibitor.

Participants: Synovial tissue was obtained at trial entry and classified histologically as B-cell rich or B-cell poor to inform balanced stratification. Patients were randomised on a 1 : 1 basis to receive standard therapy with rituximab or tocilizumab. B-cell-poor/-rich molecular classification was also carried out. The study was powered to test the superiority of tocilizumab over rituximab at 16 weeks in the B-cell-poor population.

ABSTRACT

Main outcome measures: The primary end point was defined as an improvement in the Clinical Disease Activity Index (CDAI) score of ≥ 50% from baseline. In addition, patients were considered to be non-responders if they did not reach an improvement in CDAI score of ≥ 50% and a CDAI score of < 10.1, defined for simplicity as CDAI major treatment response (CDAI-MTR). Secondary outcomes included the assessment of CDAI response in the B-cell-rich cohort, in which the non-inferiority of rituximab compared with tocilizumab was evaluated. Safety data up to week 48 are reported.

Results: In total, 164 patients were randomised: 83 patients received rituximab and 81 received tocilizumab. Eighty-one out of 83 rituximab patients and 73 out of 81 tocilizumab patients completed treatment up to week 16 (primary end point). Baseline characteristics were comparable between the treatment groups. In the histologically classified B-cell-poor population ($n = 79$), no significant difference was observed in the primary outcome, an improvement in CDAI score of ≥ 50% from baseline (risk ratio 1.25, 95% confidence interval 0.80 to 1.96). A supplementary analysis of the CDAI-MTR, however, did reach statistical significance (risk ratio 1.96, 95% confidence interval 1.01 to 3.78). In addition, when B-cell-poor classification was determined molecularly, both the primary end point and the CDAI-MTR were statistically significant (risk ratio 1.72, 95% confidence interval 1.02 to 2.91, and risk ratio 4.12, 95% confidence interval 1.55 to 11.01, respectively). Moreover, a larger number of secondary end points achieved significance when classified molecularly than when classified histologically. In the B-cell-rich population, there was no significant difference between treatments in the majority of both primary and secondary end points. There were more adverse events and serious adverse events, such as infections, in the tocilizumab group than in the rituximab group.

Conclusion: To our knowledge, this is the first biopsy-based, multicentre, randomised controlled trial of rheumatoid arthritis. We were unable to demonstrate that tocilizumab was more effective than rituximab in patients with a B-cell-poor pathotype in our primary analysis. However, superiority was shown in most of the supplementary and secondary analyses using a molecular classification. These analyses overcame possible unavoidable weaknesses in our original study plan, in which the histological method of determining B-cell status may have misclassified some participants and our chosen primary outcome was insufficiently sensitive. Given the significant results observed using the molecular classification, future research will focus on refining this stratification method and evaluating its clinical utility.

Trial registration: Current Controlled Trials ISRCTN97443826.

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ACPA	anti-citrullinated peptide antibodies		ITT	intention to treat
ACR	American College of Rheumatology		MAR	missing at random
ANCOVA	analysis of covariance		MATURA	Maximising Therapeutic Utility for Rheumatoid Arthritis
AUC	area under the curve		MCS	Mental Component Summary
CDAI	Clinical Disease Activity Index		MPAG	Maximising Therapeutic Utility for Rheumatoid Arthritis (MATURA) Patient Advisory Group
CRP	C-reactive protein			
csDMARD	conventional synthetic disease-modifying antirheumatic drug		MTR	major treatment response
DAS28	Disease Activity Score in 28 joints		NICE	National Institute for Health and Care Excellence
DIA	digital image analysis		NIHR	National Institute for Health and Care Research
ESR	erythrocyte sedimentation rate		PCS	Physical Component Summary
EULAR	European League Against Rheumatism		PD	power Doppler
			PI	principal investigator
FACIT	Functional Assessment of Chronic Illness Therapy		PP	per protocol
			PPI	patient and public involvement
FANTOM5	Functional ANnoTation Of the Mammalian genome		QMTAG	Queen Mary Trials Advisory Group
FC	fold change		QMUL	Queen Mary University of London
FDC	follicular dendritic cell		QoL	quality of life
GC	germinal centre		R4–RA	A Randomised, open-labelled study in anti-TNFalpha inadequate responders to investigate the mechanisms for Response, Resistance to Rituximab versus Tocilizumab in Rheumatoid Arthritis patients
GLMM	generalised linear mixed model			
H&E	haematoxylin and eosin			
HAQ	Health Assessment Questionnaire			
HIV	human immunodeficiency virus			
IL-6	interleukin 6		RA	rheumatoid arthritis
IMP	investigational medicinal product		RCT	randomised controlled trial
IQR	interquartile range		RF	rheumatoid factor
ISRCTN	International Standard Randomised Controlled Trial Number		RNA	ribonucleic acid
			RNA-seq	ribonucleic acid sequencing

RNase	ribonuclease	TNFi	tumour necrosis factor inhibitor
RR	risk ratio	TSC	Trial Steering Committee
SD	standard deviation	VAS	visual analogue scale
SF-36	Short Form-36 items	VST	variance-stabilising transformation
ST	synovial thickness		

Plain English summary

Rheumatoid arthritis is a common disease in which the immune system attacks the joints, causing pain, stiffness and swelling.

In 40% of patients, conventional chemical drugs do not work and expensive 'biological' therapies are prescribed; however, these therapies can have serious side effects. The first biological drug to be prescribed is usually a type called a tumour necrosis factor inhibitor drug. If that fails, a second biological drug, rituximab (MabThera, F. Hoffman La-Roche Ltd, Basel, Switzerland), is recommended.

Rituximab reduces inflammation by attacking a specific type of immune cell (B cells). However, in about half of patients it has been found that, when the joint lining is examined under a microscope, B cells are present in only small numbers or are completely absent.

We reasoned that these patients, said to be B-cell poor, would respond better to another drug, tociluzimab, which works by a different mechanism. If this is the case, it might be possible, by examining a small piece of tissue from the lining of the joint, to predict which patients will respond better to each drug.

We examined the joint lining from 164 rheumatoid arthritis patients from hospitals in the UK, Belgium, Italy, Portugal and Spain who had not responded to tumour necrosis factor inhibitors. Of these patients, 79 were found to have few or no B cells and are described as B-cell poor. Approximately equal numbers of patients were treated with rituximab or tocilizumab (RoActemra, F. Hoffman La-Roche Ltd, Basel, Switzerland). After 16 weeks of treatment, we evaluated the number of tender and/or swollen joints.

We found that disease improvement in B-cell-poor patients was slightly better with tocilizumab than with rituximab, but that the difference was not enough to be sure that it did not arise by chance. However, when we assessed the joint lining tissue using newer, molecular methods and when we measured different outcomes, we found some promising results.

This means that we cannot yet be sure which drugs should be offered to which patients, but the results of this study could lead to further research to improve how we test and treat patients to get the best improvement in their arthritis.

Scientific summary

Background

Although biological therapies have transformed the outlook for those with rheumatoid arthritis, the lack of any meaningful response in approximately 40% of patients, the potential side effects and the high cost of these drugs have highlighted the need to define predictive biomarkers of response and to stratify patients according to therapeutic outcome. The importance of B cells in rheumatoid arthritis pathogenesis is supported by the efficacy of the B-cell-depleting agent rituximab (MabThera, F. Hoffman La-Roche Ltd, Basel, Switzerland). Rituximab is licensed for use with rheumatoid arthritis following the failure of conventional synthetic disease-modifying antirheumatic drugs and tumour necrosis factor inhibitor biologicals. In this increasing therapeutically resistant cohort, only 30% of patients achieve a major treatment response at 6 months. However, over 50% of patients show low/absent synovial B-cell infiltration, suggesting that synovial inflammation is driven by alternative cell types. This prompted us to test the hypothesis that, in synovial-biopsy B-cell-poor patients, tocilizumab (RoActemra, F. Hoffman La-Roche Ltd, Basel, Switzerland) (targeting interleukin 6) is superior to rituximab (targeting CD20$^+$/B cells).

Objectives

The main aim of this study was to test the hypothesis that the presence or absence of specific synovial cellular and molecular signatures (B cells and B-cell-associated signatures), assessed following a synovial tissue biopsy, will enrich for response/non-response to the B-cell-depleting anti-CD20 monoclonal antibody, rituximab.

Methods

Design
We conducted a Phase IV, open-label, multicentre, randomised controlled trial. Patients were randomised to receive rituximab or tocilizumab and were stratified according to histological classification of baseline synovial biopsy (B-cell poor, B-cell rich, germinal centre positive or unknown) and by site (Queen Mary University of London vs. all other sites).

Patients were followed up at 4-weekly intervals throughout the 48-week trial treatment period, at which times the rheumatoid arthritis disease activity measurements and safety data were collected. An optional repeat synovial biopsy of the same joint sampled at baseline was performed at 16 weeks.

Setting
Rheumatology outpatient clinics in 19 European centres.

Participants
Patients aged ≥ 18 years fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis, and who were eligible for treatment with rituximab therapy in accordance with UK National Institute for Health and Care Excellence guidelines [i.e. failing on or intolerant to conventional synthetic disease-modifying antirheumatic drug therapy and at least one biological therapy (excluding trial investigational medicinal products)], were eligible for recruitment to the study and identified through rheumatology outpatient clinics at each study site.

Sample size

A sample size of 82 B-cell-poor patients was planned to provide 90% power to detect a 35% difference (assuming 55% response in tocilizumab and 20% response in rituximab) in the proportion of patients who had a response. The assumed proportions of B-cell-poor, B-cell-rich and germinal centre-positive recruited patients were 60%, 35% and 5%, respectively. After accounting for 10% ungradable biopsy samples and a 5% dropout rate, we estimated that a total of 160 patients was required to achieve 90% power for the study. No power calculation was conducted for the B-cell-rich population.

Interventions

Patients underwent a synovial biopsy of a clinically active joint at trial entry. Synovial tissue was retained for both histological analysis and ribonucleic acid extraction. Histological classification of synovial tissue was performed following immunohistochemical staining for $CD20^+$ B cells according to a predefined algorithm to stratify patients into B-cell-rich and B-cell-poor categories. Following synovial biopsy and subsequent randomisation, rituximab was administered at baseline as two 1000-mg infusions 2 weeks apart, or tocilizumab was administered at baseline as an 8-mg/kg infusion at 4-weekly intervals.

Outcome measures

The study was powered to test in the B-cell-poor population superiority of tocilizumab over rituximab at 16 weeks. The primary end point was defined as an improvement in the Clinical Disease Activity Index (CDAI) score of $\geq 50\%$ from baseline. In addition, patients were considered to be non-responders if they did not reach an improvement in CDAI score of $\geq 50\%$ and a CDAI score of < 10.1, defined for simplicity as CDAI major treatment response (CDAI-MTR).

Patients who were deemed to be responders at 16 weeks continued on their allocated treatment, with rituximab infusions being repeated at 24 weeks. Non-responders were switched to the alternative biological therapy (switch patients) and treatment response was determined at 16 weeks post switch.

Secondary outcomes included assessment of CDAI response (as defined for primary outcome analysis) at 16 weeks in the B-cell-rich cohort. Additional secondary efficacy analyses were performed in both the B-cell-rich and the B-cell-poor populations with and without week 16 switch patients based on the following parameters at week 16: mean improvement in Disease Activity Score in 28 joints (DAS28) C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR); number of patients in remission, defined as DAS28 of ≤ 2.6, or with low disease activity, defined as DAS28 of ≤ 3.2; and percentage of patients with low disease activity, defined as a CDAI score of < 10.1. Additional key secondary end points included the rates of low disease activity and remission as measured by DAS28(ESR) and DAS28(CRP) and patient-reported outcomes such as fatigue up to week 48. The incidence and severity of all adverse events were recorded.

Exploratory end points included the evaluation of change in synovial molecular signatures and therapeutic response to tocilizumab or rituximab.

Analysis

The primary end point and other binary end points were analysed using a two-sided alpha of 0.05 significance level by chi-squared test or Fisher's exact test, as appropriate. For continuous secondary outcomes, an analysis of covariance was performed with treatment as the factor and baseline score as the covariate. Changes from baseline within groups were analysed using paired Wilcoxon signed-rank tests. All statistical analyses were carried out using R version 3.5.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients and demographics

Of the 212 patients screened, 190 consented and 164 were randomised. The trial ended because the recruitment targets were reached. Eighty-three patients were randomised to receive rituximab and 81 patients were randomised to receive tocilizumab. A total of 161 patients received the investigational medicinal product. Baseline characteristics, disease activity and histological groups were balanced across the treatment groups. Most patients were female (80%) and the majority were seropositive for rheumatoid factor (67%) or anticitrullinated peptide antibodies (77%). The median disease duration was 9 years (interquartile range 4–19 years). Disease activity was high [mean DAS28(ESR) of 5.8 (standard deviation 1.2)] and a total of 49% of patients were classified as B-cell poor compared with 40% of patients being classified as B-cell rich.

Treatment response in the B-cell-poor population

Seventy-nine (49%) patients who received the investigational medicinal product were classified as B-cell poor histologically, 38 (48%) of whom were randomised to rituximab and 41 (52%) to tocilizumab. At 16 weeks, there was no significant difference between groups in the primary outcome, an improvement in CDAI score of $\geq$ 50% response rate (risk ratio 1.25, 95% confidence interval 0.8 to 1.96). However, a supplementary analysis of CDAI-MTR did reach statistical significance (risk ratio 1.96, 95% confidence interval 1.01 to 3.78). When B-cell-poor classification was determined by ribonucleic acid sequencing, 67 (50%) patients were classified as B-cell poor, of whom 33 (49%) were randomised to rituximab and 34 (51%) to tocilizumab. In this case, the primary end point and CDAI-MTR were both met (risk ratio 1.72, 95% confidence interval 1.02 to 2.91, and risk ratio 4.12, 95% confidence interval 1.55 to 11.01, respectively). Similar results were obtained for other secondary end points, with significantly more patients in the tocilizumab group than in the rituximab group achieving DAS28(ESR) remission and DAS28 (ESR) moderate/good European League Against Rheumatism response and tocilizumab-treated patients achieving significantly greater decreases in DAS28(ESR), DAS28(CRP) and Functional Assessment of Chronic Illness Therapy (FACIT) fatigue score between baseline and week 16 than those in the rituximab group. The area under the curve of mean change in DAS28(ESR/CRP) between baseline and 16 weeks was also significantly greater in patients treated with tocilizumab than in those treated with rituximab. We performed further analyses including patients who responded to first-line investigational medicinal product and those who were switched to alternative investigational medicinal product after failing to achieve the primary end point (improvement in CDAI score of $\geq$ 50%) or CDAI-MTR (improvement in CDAI score of $\geq$ 50% and CDAI score of $<$ 10.1) at 16 weeks. Treatment responses at 16 weeks following treatment initiation again demonstrated significantly higher response rates to tocilizumab through the majority of outcome measures evaluated. Per-protocol analyses showed results consistent with the intention-to-treat analysis.

Treatment response in the B-cell-rich population

Although the study was not powered for the analysis of the B-cell-rich group, 64 (40%) patients who received investigational medicinal product were classified as B-cell rich. Of these, 33 (52%) were randomised to the rituximab group and 31 (48%) to the tocilizumab group. At week 16, there were no significant differences in the number or proportion of patients achieving the primary end point (improvement in CDAI score of $\geq$ 50%: risk ratio 1.31, 95% confidence interval 0.76 to 2.26) or the CDAI-MTR (an improvement in CDAI score of $\geq$ 50% and CDAI score of $<$ 10.1: risk ratio 2.34, 95% confidence interval 0.92 to 5.97) when patients were classified histologically or molecularly. Likewise, there were no significant differences between treatment groups in secondary end points, except that the number of patients achieving DAS28(ESR) remission was significantly larger in the tocilizumab group than in the rituximab group, and that the mean decrease in DAS28(ESR) was significantly greater in the tocilizumab group. Analyses included patients who switched to the alternative investigational medicinal product at week 16 (following treatment failure to the primary drug) and per-protocol analyses, which showed consistent results.

Interaction between the treatment response and the B-cell status

The likelihood ratio test that was performed through logistic regression showed no evidence of an interaction between the investigational medicinal product and the histologically defined B-cell subgroups for the primary end point ($p = 0.95$) or CDAI-MTR ($p = 0.82$). When testing the interaction between the ribonucleic acid sequencing-defined B-cell subgroup and the investigational medicinal product, no significant interaction was observed when using the primary end point ($p = 0.096$), but a significant interaction was observed when using the CDAI-MTR ($p = 0.049$). The study was not powered for this analysis because this would require a larger number of patients.

Treatment response and immune histological parameters

There were no differences in the baseline histological parameters that were evaluated between the rituximab-treated and tocilizumab-treated groups. A paired week 16 synovial biopsy was available in 41 patients who were treated with rituximab and 24 patients who were treated with tocilizumab. In the rituximab group, we saw a significant decrease in CD20+ and CD79a+ B-cell scores, synovitis scores, CD138+ plasma cell scores and CD68+ sublining macrophage scores at 16 weeks. In the rituximab-treated group, when patients were stratified into week 16 responder and non-responder (improvement in CDAI score of ≥ 50%) groups, CD20+ and CD79a+ B-cells decreased significantly between baseline and 16 weeks in both groups, although the percentage decrease was larger in the responder group. For rituximab-treated patients, the CD138+ plasma cell score was the only histological parameter that demonstrated a significant decrease in the responder group only. In patients treated with tocilizumab in whom a paired biopsy was available, the only significant change at week 16 was a decrease in CD68+ sublining macrophages. Changes in histological parameters when tocilizumab-treated patients were stratified into responder and non-responder groups were less notable than in the rituximab-treated group, with only CD68+ sublining macrophages showing a significant change from baseline.

Ribonucleic acid sequencing analysis

Ribonucleic acid sequencing analysis was carried out in patients for whom high-quality data could be obtained following quality control of library preparation and principal components analysis evaluation. We analysed patients who were treated with rituximab ($n = 101$) and tocilizumab ($n = 82$) separately to assess the change in gene expression between the baseline and the primary end point in responders and non-responders to treatment (European League Against Rheumatism good/moderate vs. none). As well as analysing the change in responders and non-responders individually, the difference in the change between these two groups was also investigated.

In the gene-level analysis, the expression of *MS4A1* (the gene encoding CD20) decreased significantly ($p < 0.05$) in the rituximab-treated group in both responders and non-responders, but the reduction in *MS4A1* expression over time was more significant in responders, which may suggest that individuals responded better because of improved B-cell depletion in synovial tissue. The expression of the interleukin-6 gene in the tocilizumab-treated group decreased in responders ($p < 0.05$), but there was no significant change in non-responders.

Next, we examined the change in module or pathway expression between groups and found a significant decrease in B-cell module expression in the responders ($p < 0.05$), but not in the non-responders in the rituximab-treated group. There were no significant changes in the degree of interleukin-6 pathway expression in the tocilizumab-treated group in either the responders or the non-responders.

Safety and adverse events

There were more adverse events in patients treated with tocilizumab than in those treated with rituximab (327 vs. 284), and also more serious adverse events (18 vs. 8, respectively). One patient in the rituximab-treated group and three patients in the tocilizumab-treated group discontinued the investigational medicinal product because of serious adverse events. The serious adverse events included six infections, three in each group, and five ischaemic cardiac events, four in the tocilizumab group and one in the rituximab group. One death because of suicide was reported in the rituximab group. No malignancies were reported. Importantly, no serious adverse events related to synovial biopsy were reported.

Conclusions

To our knowledge, this is the first biopsy-based, multicentre, randomised controlled trial for rheumatoid arthritis, and we were unable to demonstrate that tocilizumab is more effective than rituximab in patients with a B-cell-poor pathotype in our primary analysis. However, superiority was shown in some supplementary and secondary analyses. The supplementary and secondary analyses overcame possible unavoidable weaknesses in our original study plan, in which the histological method of determining B-cell status may have misclassified some participants, and our chosen primary outcome that was insufficiently sensitive. In more detail, when synovial B-cell-poor status was defined histologically, no significant difference was observed in the primary outcome. However, superiority was found in the supplementary analyses that examined the proportion of patients treated with tocilizumab or rituximab who achieved CDAI-MTR. In addition, when B-cell-poor classification was determined molecularly, both the primary end point and the CDAI-MTR were met. Other secondary end points showed similar findings, with statistically more tocilizumab-treated patients than rituximab-treated patients achieving favourable outcomes.

Although the study was not powered to detect differences in a B-cell-rich rheumatoid arthritis population, the results of the analyses, whether patients were classified histologically or molecularly, suggested that clinical outcomes were similar when patients were treated with rituximab or tocilizumab. Importantly, our results were consistent in both the intention-to-treat and the per-protocol cohort analyses, and similar outcome results were demonstrated through to week 24.

Analysis of the synovial histological response to rituximab is in line with previously published data from observational cohorts, which report significant decreases in synovial B cells following treatment with rituximab, although no significant associations between the degree of synovial B-cell depletion and the clinical response were observed. This result may have been influenced by a skewed population of participants who agreed to have a second biopsy and which included a larger number of non-responder patients. Importantly, in line with previous reports, our data identified CD138+ plasma cell depletion as a significant marker of response to rituximab. In addition, the significant decrease in expression of genes associated with B cells, at both module and single-gene level (*MS4A1*, the gene encoding CD20, which is the target of rituximab) in responders but not in non-responders, suggests that modulation of specific target expression levels measured by ribonucleic acid sequencing may be a more sensitive method than analysing the number of B cells histologically to determine the mechanisms of treatment response.

The safety analysis showed a larger number of serious adverse events and adverse events in patients treated with tocilizumab than in those treated with rituximab; these adverse events were largely unrelated to the study drug but may suggest, in this first head-to-head trial of rituximab and tocilizumab, that tocilizumab is less well tolerated than rituximab. Importantly, there were no serious adverse events related to synovial biopsy.

The study does have limitations, including uncertainty about the optimal B-cell-poor/-rich classification (cellular vs. molecular) and the inclusion of an active comparator (tocilizumab), which, similar to rituximab, modulates B-cell function and survival. The selection of tocilizumab was a pragmatic choice based largely on the accessibility of NHS trusts participating in the trial to this biological treatment. An additional limitation is the lack of double blinding for the investigational medicinal product. Finally, the adoption of the CDAI as a primary outcome measure rather than the DAS28/European League Against Rheumatism response has significantly influenced the clinical response rates to investigational medicinal products.

In conclusion, the trial failed to demonstrate superiority of tocilizumab over rituximab when the biopsy was analysed histologically, suggesting that this method cannot be used for drug selection. However, the molecular classification showed stronger correlations with clinical responses indicating that in B-cell-poor rheumatoid arthritis patients tocilizumab is significantly more likely to induce a clinical response than

rituximab. Future studies will be required to establish whether or not molecular pathology analysis of synovial tissue has clinical utility in accurately identifying patients with low B-cell infiltrate and in guiding biological choice (e.g. rituximab) in rheumatoid arthritis.

Trial registration

This trial is registered as ISRCTN97443826.

Funding

Chapter 1 Introduction

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by synovitis and joint damage that results in considerable morbidity and an increased mortality.[2] Although biological therapies have transformed the outlook for RA patients, the lack of any meaningful response to treatment in approximately 40% of patients, the potential side effects and the high cost of these drugs means that there is a need to define predictive markers of response and stratify patients according to therapeutic outcome.[3]

B cells are pivotal to RA pathogenesis, driving synovial inflammation through the production of local disease-specific autoantibodies,[4] the secretion of pro-inflammatory and osteoclastogenic cytokines,[5] and acting as antigen-presenting cells. The pivotal role of B cells is also confirmed by the efficacy of the B-cell-depleting agent, rituximab (MabThera, F. Hoffman La-Roche Ltd, Basel, Switzerland).[6] Rituximab is licensed for use in RA following failure of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and tumour necrosis factor inhibitor (TNFi) therapy. However, in this more therapy-resistant patient cohort, clinical response to rituximab is heterogeneous, with only 30% of patients achieving a 50% improvement in the American College of Rheumatology (ACR) response criteria at 6 months.[7]

Given the mechanism of action of rituximab, it could be hypothesised that the analysis of pre- or post-treatment circulating B-cell numbers could be used to predict treatment response. However, post hoc analyses of several randomised controlled trials (RCTs) have shown that the numbers of pretreatment peripheral B cells or the depth of their depletion, as measured by conventional flow cytometry, are not associated with clinical outcome.[6–14] In contrast to the complete depletion of peripheral blood B cells, variable depletion of B cells and other immune cells in synovia following treatment with rituximab has been described.[15–23] In particular, the number of pretreatment synovial CD79a+ B cells,[15,19] the pretreatment synovial molecular signatures[22] and a reduction in synovial plasma cells[17] have been identified as factors associated with response to rituximab. However, the small number of patients analysed and the use of different time points make it difficult to draw conclusions about the association of synovial B-cell signatures with treatment response to rituximab.

We have recently demonstrated synovial heterogeneity in patients with early RA,[24] with ≥ 50% of patients showing low/absent synovial B-cell infiltration and defined synovial cellular and molecular markers predictive of prognosis and therapeutic response to csDMARDs. Given that these patients presented with high levels of disease activity, it can be plausibly reasoned that synovial inflammation in their joints is driven by alternative cell types. This prompted us to test the hypothesis that, in patients lacking significant synovial B-cell infiltration, an alternative biological agent to rituximab that targets different biological pathways [e.g. interleukin 6 (IL-6)], inhibited by a specific biological agent [tocilizumab (RoActemra, F. Hoffman La-Roche Ltd, Basel, Switzerland)], may be more effective. We report results from the first pathobiology-driven, multicentre randomised controlled trial (RCT) in RA [R4-RA (A Randomised, open-labelled study in anti-TNFalpha inadequate responders to investigate the mechanisms for Response, Resistance to Rituximab versus Tocilizumab in Rheumatoid Arthritis patients)] that evaluated whether or not patient stratification according to synovial B-cell-rich/poor status enriches for response/non-response to rituximab.

Chapter 2 Methods

Trial design

We conducted a Phase IV, open-label RCT in 19 European centres. Given that ultrasound-guided biopsy is not part of routine care and that patients are not familiar with the biopsy procedure, multiple centres were required because of the relatively small number of RA patients presenting with TNFi-resistant RA[25] at each centre. The study was conducted in compliance with the Declaration of Helsinki,[26] International Conference on Harmonisation Guidelines for Good Clinical Practice,[27] and local country regulations. The final protocol, amendments and documentation of consent were approved by the institutional review board of each study centre and relevant independent ethics committees. The trial was supported by an unrestricted grant from the National Institute for Health and Care Research (NIHR). The study protocol is available at www.r4ra-nihr.whri.qmul.ac.uk (accessed 12 October 2020).

Participants

Patients aged $\geq$ 18 years fulfilling the 2010 ACR/European League Against Rheumatism (EULAR) classification criteria for RA[28] who were eligible for treatment with rituximab therapy in accordance with UK National Institute for Health and Care Excellence (NICE) guidelines [i.e. failing or intolerant to csDMARD therapy and at least one biological therapy, excluding trial investigational medicinal products (IMPs)][29] were eligible for recruitment to the study and identified through rheumatology outpatient clinics at each study site. A complete list of the inclusion and exclusion criteria is provided in *Appendix 1, Table 5*. All patients provided written informed consent.

Several updates to the eligibility criteria were made during the trial. This was to provide further clarification to investigators rather than to amend the patient population to be included in the trial. Substantial amendment no. 3 involved the addition of the exclusion criterion 'Prior exposure to rituximab or tocilizumab for the treatment of RA and oral prednisolone more than 10 mg per day or equivalent $\leq$ 4 weeks prior to biopsy visit' (previously specified elsewhere in the protocol, but not in the exclusion criteria), and minor amendments to several other exclusion criteria already listed. Substantial amendment no. 6 specified that a hepatitis B screening test must be performed at or in the 3 months preceding the screening visit. Substantial amendment no. 9 specified that participants must be $\geq$ 18 years of age (previously stated 'over 18 years of age'). The exclusion criteria were also updated to provide further clarification (changes shown in italics): 'History of or current primary inflammatory joint disease, or primary *rheumatological* autoimmune disease other than RA *(if secondary to RA, then the patient is still eligible)*'; 'Known HIV [human immunodeficiency virus] or *active* hepatitis B/C infection. Hepatitis B screening test must be performed at or in the preceding 3 months of screening visit'; 'Patients unable to tolerate synovial biopsy or in whom this is contraindicated *including* patients on anti-coagulants *(oral anti-platelet agents are permitted)*'.

Interventions

Synovial biopsy, histological and molecular classification/analyses
Patients underwent a synovial biopsy of a clinically active joint at entry to the trial, which was performed according to the expertise of the local centre either as an ultrasound-guided or as an arthroscopic procedure, as previously described.[30]

Histological classification/analysis

For the histological analysis, a minimum of six synovial biopsies were paraffin-embedded en masse by the Core Pathology Department at Queen Mary University of London (QMUL) . Tissue sections that were 3–5 μm thick were stained with haematoxylin and eosin (H&E) for immunohistochemical markers CD20 (B cells), CD3 (T cells), CD138 (plasma cells), CD21 [follicular dendritic cells (FDCs)] and CD68 (macrophages) in an automated Ventana Autostainer machine (F. Hoffman La-Roche Ltd, Basel, Switzerland). CD79a (B cells) and CD3 (T cell) staining was performed on deparaffinised tissue after antigen retrieval (30 minutes at 95°C) followed by peroxidase and protein blocking steps. The primary antibodies CD79a clone JCB117 (Dako, Agilent, Santa Clara, CA, USA) or CD3 clone (F7.238, Dako) were used at a dilution factor of 1 : 50 or 1 : 80 for 60 minutes at room temperature. Visualisation of antibody binding was achieved by a 30-minute incubation with Dako EnVision™+ before completion by the addition of 3,3′-diaminobenzidine (DAB+) for 10 seconds. Slides were counterstained with haematoxylin.

Following immunohistochemical staining, sections underwent semiquantitative scoring (0–4) to determine the levels of CD20+ and CD79a+ B cells, CD3+ T cells, CD138+ plasma cells and CD68+ lining (L) and sublining (SL) macrophages (see *Appendix 1, Figure 6*) adapted from a previously described score.[15,31-33] The CD20+ B-cell score was validated against digital image analysis (DIA) and the transcript levels were determined using the gene set derived from the Functional ANnoTation Of the Mammalian genome (FANTOM5) project.[34,35] Haematoxylin- and eosin-stained slides also underwent evaluation to determine the level of synovitis.[36] If CD20+ cells were identified, staining for CD21 was also performed.[4] Patients were then classified as B-cell rich or B-cell poor if definite synovial tissue could be identified (see *Appendix 1, Figure 7*) in the NHS pathology laboratory of Barts Health NHS Trust by a consultant pathologist (HR). In addition, synovial tissue underwent independent histological classification in the research laboratories of QMUL by a second expert in synovial pathology (GT), and any discrepancies in classification were resolved through mutual agreement. The B-cell status of patients in whom definite synovial tissue could not be identified was classified as 'unknown'. Patients were classified as B-cell rich or B-cell poor according to a semiquantitative score: patients with a CD20 score of ≥ 2 and with CD20+ B-cell aggregates were classified as B-cell rich whereas those with a CD20 score of < 2 were classified as B-cell poor.[19] The score was validated against DIA and the transcript levels were determined using the gene set derived from FANTOM5.[34,35] B-cell-rich samples were further classified as germinal centre (GC) positive if CD21+ FDC networks were subsequently identified (see *Appendix1, Figure 8*). Only patients classified as B-cell rich or B-cell poor were included in the primary analysis of the trial presented here, that is GC-positive patients or patients unclassifiable histologically were excluded from analysis, as predefined in the statistical analysis plan.

Molecular classification/analysis

A minimum of six synovial samples per patient were immediately immersed in RNA*later* (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and ribonucleic acid (RNA) was extracted from synovial tissue using one of two protocols: either using phenol–chloroform isolation or via a Zymo Direct-zol™ RNA MicroPrep – total RNA/miRNA extraction kit (Zymo Research, Irvine, CA, USA). In both methods, tissue was lysed in Trizol solution (Thermo Fisher Scientific) using a LabGen125 homogeniser (Cole Parmer Instrument Co. Ltd, Saint Neots, UK). Briefly, for the phenol–chloroform extraction method, 1–10 mg of tissue was lysed and sheared using a 21-gauge needle. The tissue lysate was then mixed vigorously with chloroform before centrifugation. The aqueous phase was removed and mixed with ice-cold isopropanol for 30 minutes. After further centrifugation, the RNA pellet was washed in 70% ethanol before air drying and resuspending in ribonuclease (RNase)-free water. Extraction of samples using Zymo Direct-zol RNA MicroPrep – total RNA/miRNA extraction kits was carried out in accordance with the manufacturer's instructions. Briefly, 1–10 mg of tissue lysate was run through the Zymo-Spin™ ion chromatography column. Columns were then washed using the appropriate kit wash buffers before RNA was eluted and resuspended in RNase-free water. Quality control was

carried out by quantifying samples by spectrophotometer readings on a NanoDrop ND2000C (Thermo Fisher). RNA integrity was measured using Pico chip (Agilent, Santa Clara, CA, USA) technology on a 2100 Bioanalyzer (Agilent) to determine a RNA integrity number (RIN). All RNA samples were sent to Genewiz UK Ltd (Takeley, UK) for RNA sequencing (RNA-seq).

RNA sequence data processing

A total of 184 paired-end RNA-seq samples of 150 base pairs were trimmed to remove the Illumina (San Diego, CA, USA) adaptors using BBDuk from the BBMap package version 37.93 (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbmap-guide/; accessed 12 October 2020) using the default parameters. Transcripts were then quantified using Salmon version 0.13.1[37] (https://combine-lab.github.io/salmon; accessed 12 October 2020) and an index generated from the GENCODE release 29 transcriptome following the standard operating procedure. Tximport version 1.13.10 (https://bioconductor.org/packages/release/bioc/html/tximport.html; accessed 12 October 2020) was used to aggregate the transcript-level expression data to genes, and counts were then subject to variance-stabilising transformation (VST) using the DESeq2 version 1.25.9 package (https://bioconductor.org/packages/release/bioc/html/DESeq2.html; accessed 12 October 2020).[38] One sample was removed as an outlier with the assistance of principal component analysis.

Bioinformatic analysis

Starting with the 'length scaled tpm counts' from tximport, the limma voom method[39] was followed to normalise the data and calculate the weights for linear modelling. For the linear mixed modelling, limma was used, using the 'duplicate Correlation' function method, in which patient ID is fitted as a random effect to take into account added correlation between the repeated measurements on an individual patient. For gene-set-level analysis using the B-cell module or *IL-6* pathway, we used the limma 'fry' function.

Synovial tissue from 162 patients was available for RNA extraction and was subsequently sent for RNA-seq analysis. Patients classified histologically as GC+ (*n* = 9) were excluded from subsequent analyses. Of the remaining 153 patients, one patient was withdrawn before the IMP was administered and 28 were excluded following RNA-seq quality control or because of poor mapping. Therefore, 124 patients had RNA-seq data available for subsequent analysis.

Patients were classified as B-cell poor/rich according to a previously developed B-cell-specific gene module derived from the analysis of FANTOM5 gene expression data,[34] which was validated using RNA-seq of drug-naive early RA synovial biopsies.[40] In addition, this method was used to validate the histopathology score (see *Histological classification/analysis*),[35] applied to a RNA-seq data set derived from the synovial biopsies. Given that no predetermined cut-off points for B-cell transcript classification were found in the literature, and to avoid potential bias, patients were classified as B-cell poor/rich according to the median transcript module value (see *Appendix 1, Figure 9*).

Randomisation and masking

Patients were randomised to receive rituximab or tocilizumab and were stratified into four blocks according to histological classification of their baseline synovial biopsy (i.e. B-cell poor, B-cell rich, GC+ or unknown) and by site (QMUL vs. all other sites). Patients were randomised within blocks (1 : 1), with random block size of six and four. The randomisation list was prepared by the trial statistician and securely embedded with the application code so that it was not accessible to end users. The randomisation result was sent electronically by the R4–RA trial office to all of the clinical trial staff at each site, except the named joint assessor (research nurse/assistant) who remained blinded to study drug allocation. Thus, determination of the primary outcome [improvement in the Clinical Disease Activity Index (CDAI) of ≥ 50% from baseline] and secondary outcomes, including joint counts, was performed by a joint assessor blinded to study drug allocation. Clinical trial staff remained blinded to histological subtypes throughout the study.

Trial procedures

Following synovial biopsy and subsequent randomisation, either rituximab as two 1000-mg infusions at an interval of 2 weeks or tocilizumab infused at a dose of 8 mg/kg at 4-weekly intervals was administered at baseline. Throughout the 48-week trial treatment period, patients were followed up every 4 weeks, at which point RA disease activity measurements and safety data were collected (see *Appendix 1, Figure 10*). An optional repeat synovial biopsy of the same joint that was sampled at baseline was carried out at 16 weeks, with the histological analyses described above repeated.

Outcomes

The study was powered to test the superiority of tocilizumab over rituximab at 16 weeks in the B-cell-poor population. The primary end point was defined as an improvement in the CDAI score[41] of ≥ 50% from baseline.

Secondary outcomes included the B-cell-rich assessment of CDAI response (as defined for primary outcome analysis) at 16 weeks, where the efficacy of rituximab compared to tocilizumab was evaluated.

Patients deemed to be responders at 16 weeks continued with their allocated treatment, and rituximab infusions were repeated at 24 weeks. Non-responders were defined, as per the primary end point, as not reaching an improvement in the CDAI score of ≥ 50% from baseline. In addition, as prespecified in the protocol, patients were considered to be non-responders if they did not achieve an improvement in the CDAI score of ≥ 50% and a CDAI score of < 10.1, which is defined from now on for simplicity as CDAI-major treatment response (CDAI-MTR). Non-responders were switched to the alternative biological therapy (switch patients) and treatment response to the switched therapy was determined at 16 weeks post switch.

The primary efficacy analysis evaluated the number of patients achieving the primary end point (improvement in CDAI score of ≥ 50%). Supplementary efficacy analysis included evaluating the number of B-cell-rich and B-cell-poor patients (classified according to the molecular methodology; see *Histological and molecular classification/analyses* and *Synovial biopsy*) achieving CDAI score of ≥ 50% improvement and CDAI-MTR. Additional secondary efficacy analyses of the following parameters were performed at week 16 in the B-cell-rich and B-cell-poor populations separately, with and without week 16 switch patients: mean improvement in Disease Activity Score in 28 joints (DAS28); C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR); number of patients in remission (DAS28 of < 2.6) and with low disease activity (DAS28 of < 3.2); and percentage of patients with low disease activity (CDAI score of < 10.1). Other key secondary end points, such as rate of DAS28 CRP ESR, low disease activity and remission (at time points other than week 16), and patient-reported outcomes, such as fatigue, are defined in *Appendix 1, Table 6*. Additional exploratory analyses included evaluation of B-cell molecular signatures of response to rituximab and tocilizumab.

The incidence and severity of all adverse events were recorded. The incidence and severity of treatment and procedure emergent adverse events were monitored throughout the study; adverse event coding was carried out in accordance with Medical Dictionary for Regulatory Activities (MedDRA) version 22 (www.meddra.org; accessed 12 October 2020).

Sample size

A sample size of 82 B-cell-poor patients was planned to provide 90% power to detect a 35% difference in the proportion of patients who had a response (assuming 55% response in the tocilizumab group and 20% response in the rituximab group). The assumed proportions of B-cell-poor, B-cell-rich and GC+ recruited patients were 60%, 35% and 5%, respectively. After accounting for 10% ungradable biopsy samples and a 5% dropout rate, we estimated that a total of 160 patients were required to achieve 90% power for the study. No power calculation was conducted on the B-cell-rich population.

Statistical analyses

The primary end point and other binary end points were analysed using a two-sided chi-squared test or, where appropriate, a Fisher's exact test with an alpha of 0.05. For each continuous secondary outcome, an analysis of covariance (ANCOVA) was performed on the score change from baseline, with treatment as factor and baseline score as covariate, to analyse the difference between treatment groups. If the residuals of the model were not normally distributed (normality was tested using the Shapiro–Wilk test), the data were log-transformed before the analysis and the estimates were back transformed or analysed through non-parametric ANCOVA. Changes from baseline in the groups were analysed by a paired Wilcoxon signed-rank test. As an additional secondary measure, the area under the curve (AUC) of mean change in DAS28 (ESR/CRP) was calculated for each individual as a function of time. A generalised linear mixed model (GLMM) with treatment as a fixed effect and baseline DAS28 as a covariate was run to test the treatment effect. Although we did not power the study on the B-cell-rich group, we tested non-inferiority of rituximab to tocilizumab in such patients with a 0.2 margin on the relative risk of response as a supplementary analysis.

The analysis of the interaction between treatments and pathotypes (B-cell poor and B-cell rich, excluding GC) was conducted using the likelihood ratio test between two nested logistic regression models: one with pathotype and treatment as covariates and the other with pathotype, treatment and their interaction as covariates. For all of the binary and continuous outcomes, separate analyses were computed for B-cell-poor and B-cell-rich subpopulations.

All efficacy analyses were carried out in the intention-to-treat (ITT) population and then on the per-protocol (PP) population to assess the robustness of the results. The PP population included all subjects from the ITT population who did not have any major protocol violations. The list of deviations that exclude a subject from the PP population was reviewed at a classification meeting prior to data lock and is documented in the trial master file. Safety analyses were carried out on the safety analysis set (by ITT, including only participants who received at least one dose of the trial medication), in which patients were analysed according to their actual treatment in case this differed from the scheduled treatment (i.e. randomised or switched). A separate analysis was conducted on patients who switched treatment at week 7 or 8 (either from rituximab to tocilizumab or from tocilizumab to rituximab) to test the treatment effect through a chi-squared/Fisher's exact test. All statistical analyses were carried using R, version 3.5.1 (The R Foundation for Statistical Computing, Vienna, Austria). The trial was registered on the International Standard Randomised Controlled Trial Number (ISRCTN) database (as ISRCTN97443826). An independent Data Monitoring and Ethics Committee met on a 6-monthly basis during the trial to review the accruing trial data and assess whether or not there were any safety issues, and to make recommendations to the Trial Steering Committee (TSC).

Handling of missing data

Missing values for the primary outcome were considered separately from other variables. The CDAI response at visit 8 was used as the primary outcome for five patients who did not attend visit 7, and the CDAI response at visit 6 was used as the primary outcome for one patient who withdrew from the study at visit 6. Primary end-point data that were missing under other circumstances (for three patients) were imputed assuming that data were missing at random (MAR). The same assumption was used for missing data on the components for all secondary end points: ESR, CRP, swollen joint count, tender joint count, visual analogue scale (VAS) score, patient global assessment and physician global assessment. When applicable, the estimates were log-transformed or transformed to the squared root for the imputation and for use in secondary measures and then back-transformed for reporting. Missing values assumed to be MAR were imputed using multiple imputation by chained equations (MICE) and implemented using R package 'Amelia' 1.7.5. The imputation model included treatment, histological pathotype, delta CDAI, CDAI at visit 7, sex, ethnicity, age, body mass index (BMI) and blood pressure. Such imputation was run five times and the pooled results were used for the analysis. The performance of the imputation was examined through the convergence and marginal distributions, and no substantial issue was detected.

Chapter 3 Results

Patients and demographics

Patient disposition in a Consolidated Standards of Reporting Trials (CONSORT) format is shown in *Figure 1*: 212 patients were screened, 190 provided consent and 164 were randomised. The first patient visit was on 28 February 2013 and the last patient visit was on 17 January 2019. The trial ended because recruitment targets were reached. A total of 83 patients were randomised to receive treatment with rituximab and 81 with tocilizumab. In total, 161 patients received the IMP (rituximab, *n* = 82; tocilizumab, *n* = 79), of whom 99% (81/82) and 92% (73/79) of patients, respectively, completed treatment to the primary end point at week 16 (see *Figure 1*). The largest proportion of patients (38%, 62/164) was recruited at Barts Health NHS Trust (see *Appendix 1, Table 7*). Patient disposition from week 16 to week 48 is summarised in *Appendix 1, Figure 11* (B-cell-poor group), and *Appendix 1, Figure 12* (B-cell-rich group).
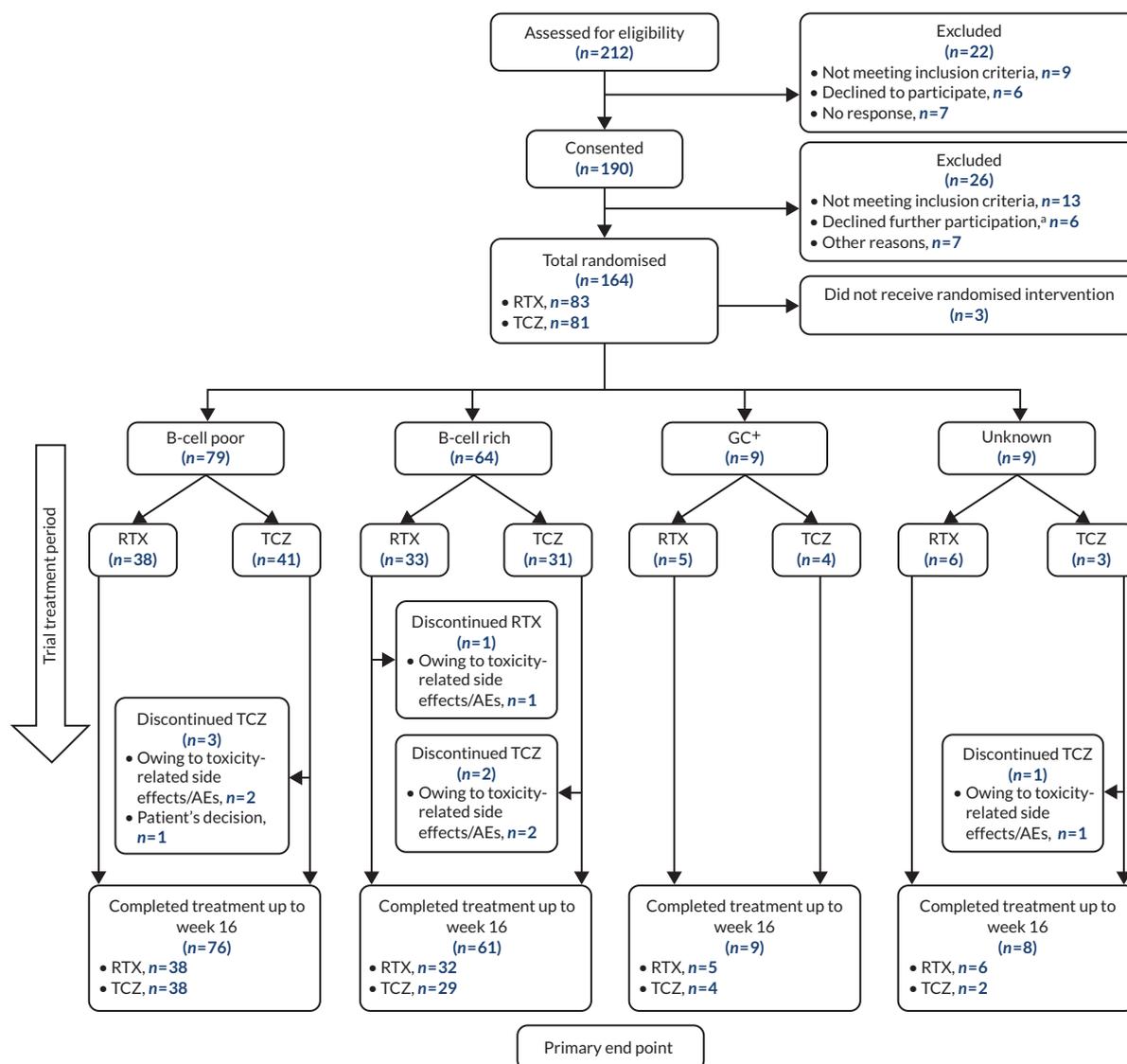
FIGURE 1 Patient disposition to week 16 (primary end point). a, Owing to biopsy. AE, adverse event; RTX, rituximab; TCZ, tocilizumab.

Baseline characteristics, disease activity and histological groups were balanced across the treatment groups (*Table 1* and see *Appendix 1*, *Table 8*). Most patients were female (80%) and the majority were seropositive for rheumatoid factor (RF) (74%) or anti-citrullinated peptide antibodies (ACPAs) (80%). The median disease duration was 9 years [interquartile range (IQR) 4–19 years]. Disease activity was high, with a mean DAS28(ESR) of 5.8 [standard deviation (SD) 1.2]. Almost half of the patients (49%, 79/161) were classified as B-cell poor, 40% (64/161) as B-cell rich, 6% (9/161) as GC+ and 6% (9/161) as unknown status.

TABLE 1 Baseline characteristics of patients

Patient characteristic	Total (*N* = 161)	Treatment group	
		Rituximab (*N* = 82)	Tocilizumab (*N* = 79)
Pathotype, *n* (%)			
B-cell poor	79 (49)	38 (46)	41 (52)
B-cell rich	64 (40)	33 (40)	31 (39)
GC+	9 (6)	5 (6)	4 (5)
Unknown	9 (6)	6 (7)	3 (4)
Sex (female), *n* (%)	128 (80)	62 (76)	66 (84)
Age (years), median (IQR)	55.5 (47.4–65.3)	55.7 (47.7–65.5)	55.5 (47.3–65.1)
Disease duration (years), median (IQR)	9 (4.0–19.0)	10 (4.0–20.7)	9 (4.0–18.0)
CDAI score, median (IQR)	29.8 (21.7–40.6)	30.6 (22.8–40.6)	29.4 (21.5–40.3)
ESR (mm/hour), median (IQR)	31.0 (17.0–48.0)	34.5 (17.0–48.0)	28.0 (18.5–46.5)
CRP (mg/l), median (IQR)	11.0 (5.0–27.0)	10.0 (5.0–23.0)	15.0 (5.0–32.0)
RF/ACPA positive, *n* (%)	140 (87)	73 (89)	67 (85)
RF positive, *n* (%)	119 (74)	64 (78)	55 (70)
ACPA positive, *n* (%)	128 (80)	67 (82)	61 (77)
Haemoglobin (g/l), median (IQR)	123.0 (110.5–131.5)	121.0 (109.0–131.0)	123.0 (111.5–131.7)
Tender joint count (28), median (IQR)	11.0 (6.0–18.0)	10.5 (6.2–18.7)	11.0 (6.0–16.0)
Swollen joint count (28), median (IQR)	6.0 (3.0–10.0)	6.00 (4.0–9.0)	6.0 (3.0–10.5)
DAS28(ESR), mean (SD)	5.81 (1.25)	5.84 (1.19)	5.78 (1.31)
DAS28(CRP), mean (SD)	5.31 (1.20)	5.30 (1.15)	5.33 (1.26)
Ultrasound 12max[a] score (PD), median (IQR)	4.0 (1.0–9.0)	4.0 (0.2–8.0)	6.0 (1.5–10.0)
Ultrasound 12max[a] score (ST), median (IQR)	15.0 (11.5–22.0)	16.0 (13.0–22.0)	15.0 (10.0–20.2)
SHSS: total score, mean (IQR)	8.50 (2.50–39.62)	8.50 (2.50–39.62)	9.25 (2.50–36.88)
SHSS: JSN score, mean (IQR)	6.75 (1.12–29.62)	6.25 (1.25–29.62)	7.00 (1.38–29.00)
SHSS: ER score, median (IQR)	1.50 (0.00–8.88)	3.25 (0.12–8.88)	0.75 (0.00–8.38)
SHSS: ER progressive (score of ≥ 1), *n* (%)	45 (55)	24 (63)	21 (48)
Methotrexate, *n* (%)	161 (100)	82 (100)	79 (100)
Prednisolone, *n* (%)	90 (56)	44 (54)	46 (58)
Previous biologicals, *n* (%) [anti-TNF/other][b]			
1	116 (72) [116/0]	62 (76) [62/0]	54 (68) [54/0]
2	36 (22) [32/4]	14 (17) [11/3]	22 (28) [21/1]
3+	9 (6) [5/4]	6 (7) [3/3]	3 (4) [2/1]

ACPA, anticyclic citrullinated peptide; ER, erosion; JSN, joint space narrowing; PD, power Doppler; RF, rheumatoid factor; SHSS, Sharp van der Heijde Scale Score; ST, synovial thickness.
a 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.
b A total of eight patients used non-TNFi biologicals [seven abatacept (Orencia, Bristol-Myers Squibb, New York, NY, USA) and one 'vaccine RA TNF-K-006' for a clinical study].
There were no significant differences among treatment groups at baseline.

Treatment response in the B-cell-poor population: 16 week outcomes

Histological classification

Almost half (49%, 79/161) of the patients who received the IMP were classified as B-cell poor histologically. Of these patients, 48% (38/79) were randomised to the rituximab group and 52% (41/79) were randomised to the tocilizumab group (see *Figure 1*). At 16 weeks, no significant difference between groups was observed in the primary outcome, the proportion of patients achieving an improvement in CDAI score of $\geq$ 50% [risk ratio (RR) 1.25, 95% confidence interval (CI) 0.8 to 1.96]. A supplementary analysis of CDAI-MTR, however, did reach statistical significance (RR 1.96, 95% CI 1.01 to 3.78). In addition, response rates were higher in the tocilizumab-treated patients on a number of secondary end points, including disease remission (CDAI score of < 10.1) (RR 1.6, 95% CI 0.88 to 2.91), DAS28(ESR) moderate/good EULAR response (RR 1.33, 95% CI 1.03 to 1.72), DAS28(ESR) low disease activity (RR 1.67, 95% CI 0.88 to 3.15), DAS28(ESR) remission (RR 2.32, 95% CI 1 to 5.35), DAS28(CRP) moderate/good EULAR response (RR 1.35, 95% CI 0.98 to 1.85), DAS28(CRP) low disease activity (RR 1.47, 95% CI 0.83 to 2.6) and DAS28(CRP) remission (RR 1.72, 95% CI 0.77 to 3.85) (*Figure 2*).

Other week 16 secondary end points also favoured tocilizumab, including greater decreases in DAS28 (ESR/CRP) and CDAI score (see *Appendix 1, Table 9*). Quality-of-life (QoL) outcome measures [Functional Assessment of Chronic Illness Therapy (FACIT) and the Short Form questionnaire-36 items (SF-36) scores] also had higher levels of improvement between baseline and 16 weeks in tocilizumab-treated patients (see *Appendix 1, Table 9*). We observed little difference in ultrasound synovial thickness (ST) or power Doppler (PD) or Health Assessment Questionnaire (HAQ) scores between the IMP groups (see *Appendix 1, Table 9*). In addition, the AUC of mean change in DAS28(ESR/CRP) between baseline and 16 weeks was significantly greater in patients treated with tocilizumab than in those treated with rituximab (see *Appendix 1, Figure 13*).

Molecular classification

Using the median B-cell module value, 65 out of 124 patients were classified as B-cell poor. When clinical outcomes were compared between treatment groups in the group of patients categorised as B-cell poor according to RNA-seq, we observed a significantly higher response rate in the tocilizumab group than in the rituximab group, as determined by the number of patients achieving an improvement in CDAI score of $\geq$ 50%, the primary outcome measure (RR 1.72, 95% CI 1.02 to 2.91), and CDAI-MTR (RR 4.12, 95% CI 1.55 to 11.01). A number of secondary outcomes, including EULAR DAS28(ESR/CRP) good/moderate response, DAS28(ESR/CRP) low disease activity (DSA28 $\leq$ 3.2) and DAS28(ESR)

Variables	Rituximab (N = 38), n (%)	Tocilizumab (N = 41), n (%)	RR (95% CI)	
CDAI 50%	17 (44.7)	23 (56.1)	1.25 (0.8 to 1.96)	
CDAI 50% and CDAI < 10.1	9 (23.7)	19 (46.3)	1.96 (1.01 to 3.78)	
CDAI < 10.1	11 (28.9)	19 (46.3)	1.60 (0.88 to 2.91)	
EULAR DAS28(ESR)[a]	25 (65.8)	36 (87.8)	1.33 (1.03 to 1.72)	
DAS28(ESR) ≤ 3.2	10 (26.3)	18 (43.9)	1.67 (0.88 to 3.15)	
DAS28(ESR) ≤ 2.6	6 (15.8)	15 (36.6)	2.32 (1.00 to 5.35)	
EULAR DAS28(CRP)[a]	22 (57.9)	32 (78.0)	1.35 (0.98 to 1.85)	
DAS28(CRP) ≤ 3.2	12 (31.6)	19 (46.3)	1.47 (0.83 to 2.60)	
DAS28(CRP) ≤ 2.6	7 (18.4)	13 (31.7)	1.72 (0.77 to 3.85)	

0.50 1.0 2.0 4.0   16.0

← Risk ratio →

Favours RTX   Favours TCZ

FIGURE 2 Primary and secondary efficacy end-point analyses: B-cell-poor population (histological classification). a, Moderate/good EULAR response. RTX, rituximab; TCZ, tocilizumab.

remission (DSAR $\leq$ 2.6) (*Figure 3*), also favoured tocilizumab. In addition, the decrease in CDAI and DAS28(ESR/CRP) between baseline and 16 weeks was greater in the tocilizumab group than in the rituximab group (see *Appendix 1, Table 10*), and there was a trend towards greater improvements in QoL measures [FACIT and SF-36 Mental Component Summary (MCS) and Physical Component Summary (PCS)] in tocilizumab-treated patients (see *Appendix 1, Table 10*).

Using the molecular classification, 28 patients were excluded following RNA-seq quality control, as explained in *Chapter 2, Histological classification/analysis, Bioinformatic analysis*. To understand whether the molecular classification identifies a subset of the histological B-cell-poor population or a different group, we also compared the two classification methods.

Of the 124 patients for whom RNA-seq data were available, four were ungraded by histology. Of the remaining 120 patients, the majority (96 patients, 80%) were classified in the same category by both histology and RNA-seq, whereas only 24 patients (20%) were mismatched. Out of these 24 mismatched patients, 14 histological B-cell-poor patients, those with a semiquantitative B-cell score of 0 or 1, were classified as B-cell rich by molecular analyses. On the other hand, 10 histological B-cell-rich patients, all with a semiquantitative B-cell score of 2, were classified as B-cell poor by molecular analyses. Importantly, all patients with a histological semiquantitative B-cell score of 3 or 4 were confirmed to be B-cell rich by molecular analysis, which clearly indicates the presence of a grey area in the histological classification, particularly around the cut-off point.

Treatment response in the B-cell-rich population: 16-week outcomes

Histological classification
In total, 40% (64/161) of the patients who received an IMP were classified as B-cell rich, 52% (33/64) of whom were randomised to the rituximab group and 48% (31/64) to the tocilizumab group (see *Figure 1*). Although the study was not powered for the analysis of the B-cell-rich group week 16 response rates, we observed similar response rates for the majority of end points analysed, including the proportion of patients achieving an improvement in CDAI score of $\geq$ 50% (RR 1.31, 95% CI 0.76 to 2.26) and CDAI-MTR (RR 2.34, 95% CI 0.92 to 5.97) (*Figure 4*). A number of additional secondary end points showed similar results, including larger decreases in DAS28(ESR/CRP) between baseline and 16 weeks in tocilizumab-treated patients than in rituximab-treated patients (see *Appendix 1, Table 11*). In comparison with the analysis in the B-cell-poor group, we saw minimal differences in QoL measures

Variables	Rituximab (N = 33), n (%)	Tocilizumab (N = 32), n (%)	RR (95% CI)
CDAI 50%	12 (36.4)	20 (62.5)	1.72 (1.02 to 2.91)
CDAI 50% and CDAI < 10.1	4 (12.1)	16 (50.0)	4.12 (1.55 to 11.01)
CDAI < 10.1	5 (15.2)	16 (50.0)	3.30 (1.37 to 7.94)
EULAR DAS28(ESR)[a]	21 (63.6)	30 (93.8)	1.47 (1.12 to 1.94)
DAS28(ESR) ≤ 3.2	6 (18.2)	17 (53.1)	2.92 (1.32 to 6.46)
DAS28(ESR) ≤ 2.6	3 (9.1)	13 (40.6)	4.47 (1.40 to 14.22)
EULAR DAS28(CRP)[a]	18 (54.5)	27 (84.4)	1.55 (1.10 to 2.18)
DAS28(CRP) ≤ 3.2	7 (21.2)	16 (50.0)	2.36 (1.12 to 4.96)
DAS28(CRP) ≤ 2.6	4 (12.1)	10 (31.2)	2.58 (0.90 to 7.39)

Risk ratio: 0.50 1.0 2.0 4.0 16.0

← Favours RTX   Favours TCZ →

FIGURE 3 Primary and secondary efficacy end-point analyses: B-cell-poor population (molecular classification).
a, Moderate/good EULAR response. RTX, rituximab; TCZ, tocilizumab.

Variables	Rituximab (N = 33), n (%)	Tocilizumab (N = 31), n (%)	RR (95% CI)
CDAI 50%	13 (39.4)	16 (51.6)	1.31 (0.76 to 2.26)
CDAI 50% and CDAI < 10.1	5 (15.2)	11 (35.5)	2.34 (0.92 to 5.97)
CDAI < 10.1	7 (21.2)	12 (38.7)	1.82 (0.83 to 4.03)
EULAR DAS28(ESR)[a]	25 (75.8)	27 (87.1)	1.15 (0.91 to 1.46)
DAS28(ESR) ≤ 3.2	8 (24.2)	13 (41.9)	1.73 (0.83 to 3.60)
DAS28(ESR) ≤ 2.6	2 (6.1)	11 (35.5)	5.85 (1.41 to 24.34)
EULAR DAS28(CRP)[a]	23 (69.7)	25 (80.6)	1.16 (0.87 to 1.54)
DAS28(CRP) ≤ 3.2	12 (36.4)	13 (41.9)	1.15 (0.62 to 2.13)
DAS28(CRP) ≤ 2.6	4 (12.1)	9 (29.0)	2.40 (0.82 to 6.99)

Risk ratio — Favours RTX ← → Favours TCZ (0.50 1.0 2.0 4.0 25.0)
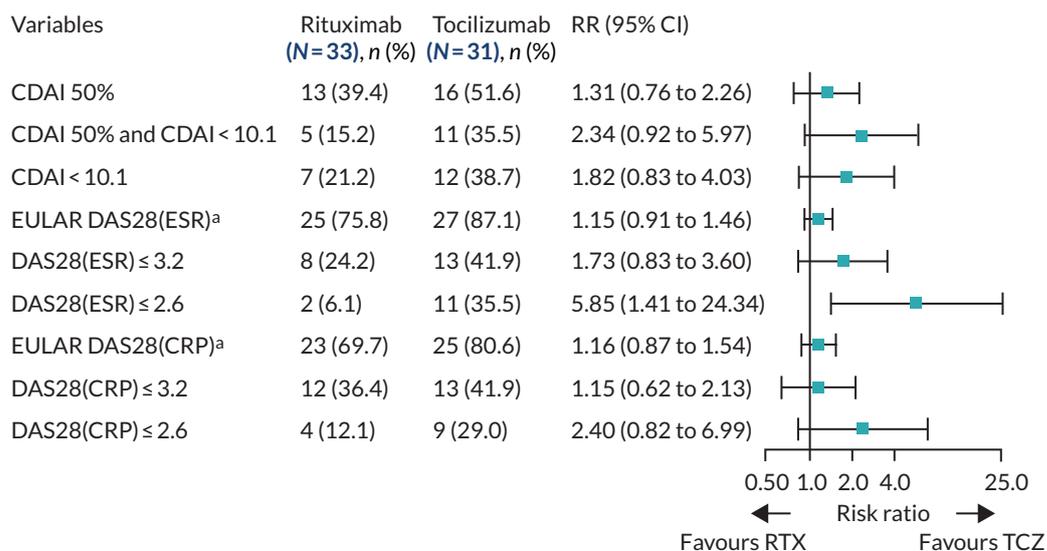
FIGURE 4 Primary and secondary efficacy end-point analyses: B-cell-rich population (histological classification). a, Moderate/good EULAR response. RTX, rituximab; TCZ, tocilizumab.

(FACIT and SF-36) between the rituximab- and tocilizumab-treated groups (see *Appendix 1, Table 11*). We also observed larger decreases in ultrasound ST and PD scores between baseline and 16 weeks in the tocilizumab-treated patients. As observed in the B-cell-poor subgroup, the AUC of mean change in DAS28(ESR/CRP) between baseline and 16 weeks was significantly greater in B-cell-rich patients treated with tocilizumab than in those treated with rituximab (see *Appendix 1, Figure 14*).

Molecular classification

The 16-week outcomes were then evaluated between treatment groups in patients classified as B-cell rich according to RNA-seq classification criteria (*n* = 59). No significant difference between rituximab- and tocilizumab-treated patients was observed in the primary end point (improvement in CDAI score of ≥ 50%), and outcomes for the majority of the primary and secondary efficacy end points that were evaluated were generally similar in the rituximab- and tocilizumab-treated groups (*Figure 5*; see also *Appendix 1, Table 12*).

Variables	Rituximab (N = 30), n (%)	Tocilizumab (N = 29), n (%)	RR (95% CI)
CDAI 50%	15 (50.0)	14 (48.3)	0.97 (0.57 to 1.62)
CDAI 50% and CDAI < 10.1	7 (23.3)	9 (31.0)	1.33 (0.57 to 3.10)
CDAI < 10.1	10 (33.3)	10 (34.5)	1.03 (0.51 to 2.11)
EULAR DAS28(ESR)[a]	24 (80.0)	24 (82.8)	1.03 (0.81 to 1.32)
DAS28(ESR) ≤ 3.2	9 (30.0)	10 (34.5)	1.15 (0.55 to 2.41)
DAS28(ESR) ≤ 2.6	3 (10.0)	10 (34.5)	3.45 (1.05 to 11.28)
EULAR DAS28(CRP)[a]	23 (76.7)	23 (79.3)	1.03 (0.79 to 1.36)
DAS28(CRP) ≤ 3.2	14 (46.7)	11 (37.9)	0.81 (0.44 to 1.48)
DAS28(CRP) ≤ 2.6	4 (13.3)	8 (27.6)	2.07 (0.70 to 6.13)

Risk ratio — Favours RTX ← → Favours TCZ (0.50 1.0 2.0 4.0 25.0)

FIGURE 5 Primary and secondary efficacy end-point analyses: B-cell-rich population (molecular classification). a, Moderate/good EULAR response. RTX, rituximab; TCZ, tocilizumab.

Interaction between treatment response and B-cell status
The likelihood ratio test was performed through logistic regression. No evidence of an interaction between the IMP and the histologically defined B-cell subgroups was observed for the primary end point ($p = 0.95$) or CDAI-MTR ($p = 0.82$). When testing the interaction between RNA-seq-defined B-cell subgroup and IMP, no significant interaction was observed for the primary end point ($p = 0.096$) but a significant interaction was observed for CDAI-MTR ($p = 0.049$). The study was not powered for this analysis, as it would require a larger number of patients.

Treatment responses beyond 16 weeks: B-cell-poor population

Patients not reaching predetermined CDAI targets at 16 weeks and/or developing side effects to treatment at 16 weeks and beyond were switched to the alternative IMP (from rituximab to tocilizumab or from tocilizumab to rituximab) (see *Appendix 1, Figure 10*), as PP. Therefore, the number of patients continuing on the initial IMP decreased beyond 16 weeks (see *Appendix 1, Figure 10*): not because of patients dropping out but because of the PP treatment switch. At 24 weeks, 55% (21/38) of the B-cell-poor patients continued with rituximab and 65% (27/41) continued with tocilizumab. At 36 weeks, 53% (20/38) of patients continued with rituximab and 54% (22/41) continued with tocilizumab. At 48 weeks, 45% (17/38) of patients continued with rituximab and 51% (21/41) continued with tocilizumab (see *Appendix 1, Figure 11* and *Table 13*). The analysis of secondary end points at 24, 36 and 48 weeks in the B-cell-poor population was consistent with the 16-week analysis, with response rates favouring tocilizumab for the majority of outcome measures, including an improvement in CDAI score of $\geq$ 50% and CDAI-MTR responses and changes in CDAI score and DAS28(ESR/CRP) between baseline and 16 weeks (see *Appendix 1, Table 13*).

We performed further analyses comparing patients who responded to the first-line IMP ($n = 38$) with those who switched to the alternative IMP after failing to achieve primary end point at 16 weeks (switch patients, $n = 18$). We evaluated treatment responses at 16, 24 and 32 weeks following treatment initiation. The results again demonstrated higher response rates in the tocilizumab-treated groups than in the rituximab-treated groups for the majority of outcome measures evaluated (see *Appendix 1, Table 14*). Per-protocol analyses showed results consistent with the ITT analysis (see *Appendix 1, Table 15*).

Treatment responses beyond 16 weeks: B-cell-rich population

At the 24-week follow-up in the group of patients classified as B-cell rich, 18 out of 33 patients continued treatment with rituximab and 20 out of 31 patients continued treatment with tocilizumab (see *Appendix 1, Figure 12* and *Table 16*). At 36 weeks, 17 out of 33 patients continued with rituximab and 16 out of 31 patients continued with tocilizumab. At 48 weeks, 17 out of 33 patients continued with rituximab and 16 out of 31 patients continued with tocilizumab. Overall, the results at 24, 36 and 48 weeks were consistent with the primary analysis at week 16, with similar response rates in the tocilizumab- and rituximab-treated groups (see *Appendix 1, Table 16*). Analyses including patients who switched to the alternative IMP at week 16 (following treatment failure to the primary drug) (see *Appendix 1, Table 17*) and the PP analysis (see *Appendix 1, Table 18*) were consistent with the primary analyses.

Treatment responses in the total trial population independent of pathotype stratification

To establish whether or not tocilizumab was more effective than rituximab, regardless of the B-cell-poor/-rich classification, clinical outcomes were analysed in the total patient population at 16 weeks. Although we observed a trend for higher response rates in the group treated with

tocilizumab than in the group treated with rituximab, there was no significant difference in response rate between the treatment groups for the primary outcome (improvement in CDAI score of $\geq 50\%$: RR 1.23, 95% CI 0.91 to 1.68); however, a significant difference was observed for CDAI-MTR (improvement in CDAI score of $\geq 50\%$ and CDAI score of < 10.1: RR 2.14, 95% CI 1.31 to 3.49) (see *Appendix 1, Table 19*). We continued to observe larger improvements in patient QoL measures (FACIT and SF-36) in the tocilizumab-treated groups (see *Appendix 1, Table 19*).

Treatment response to rituximab

We next evaluated differences in response rates to rituximab treatment, defined as an improvement in CDAI score of $\geq 50\%$, between patients classified histologically as B-cell rich or B-cell poor and found no significant differences in outcome (Fisher's exact test $p = 0.81$).

Modulation of synovial histopathobiology following treatment

Immunohistochemical parameters

No differences were found between the rituximab- and tocilizumab-treated groups in the baseline histological parameters evaluated, which included histological synovitis, CD20+ and CD79+ B cells, CD138+ plasma cells, CD3+ T cells, and CD68+ L and SL layer macrophages (*Table 2*). A paired week 16 synovial biopsy was available for 41 patients treated with rituximab and 24 patients treated with tocilizumab. In the rituximab group, we saw a significant decrease in CD20+ and CD79a+ B cells, synovitis score ($p < 0.001$), CD138+ plasma cells and CD68+ SL macrophages ($p < 0.05$) at 16 weeks (see *Table 2*). The decreases in CD20+ and CD79a B cells were also significantly greater in the rituximab group than in the tocilizumab group (-81% vs. -20% and -49% vs. -5%, respectively; $p < 0.05$).

When patients in the rituximab-treated group were stratified into week 16 responders ($n = 15$) and non-responders ($n = 26$) (response being defined as an improvement in CDAI score of $\geq 50\%$), significant decreases in CD20+ and CD79a+ B cells were observed between baseline and 16 weeks in both groups, although the percentage decrease was larger in the responder group (CD20+ -96% vs. -72%; CD79a -56% vs. -43%; $p < 0.001$) (*Table 3*). Similar effects were seen for the synovitis score (-34% vs. -28%). Changes in CD68+ SL macrophages were identical between responder and non-responder groups (-31%); however, this finding was significant in the non-responder group only ($p < 0.05$), probably because of the different numbers of patients in each group. CD138+ plasma cells was the only histological parameter that decreased significantly only in those who responded to rituximab treatment (-1.07 vs. -0.58; $p < 0.05$) (see *Table 3*). In patients treated with tocilizumab for whom a paired biopsy was available ($n = 24$), the only significant change at week 16 was a decrease in CD68+ SL macrophages (absolute change -1.04, percentage change -54%; $p < 0.05$), and this decrease was also significantly greater than that observed in rituximab-treated patients (-54% vs. -31%; $p < 0.05$), with decreases in CD20+ or CD79a+ B cells, CD138+ plasma cells and CD3+ T cells being minimal or only modest (see *Table 2*). When tocilizumab-treated patients were stratified into responder ($n = 10$) and non-responder ($n = 14$) groups as above, the changes in histological parameters were less notable than in the rituximab-treated group on the B-cell lineage, with only CD68+ SL macrophages showing a significant change from baseline and a significant difference in change between responders and non-responders (absolute change -1.7, percentage change 77% vs. absolute change -0.57, percentage change -33%; $p < 0.05$). Notably, when all patients ($n = 161$) were stratified into those undergoing a 16-week repeat biopsy ($n = 65$) or not ($n = 96$), although there were no significant differences in baseline clinical parameters between the groups, the number of patients who failed to achieve an improvement in CDAI score of $\geq 50\%$ or CDAI-MTR was significantly larger in the repeat biopsy group than in the no biopsy group (see *Appendix 1, Table 20*). These results indicated that the repeat biopsy group was skewed by the inclusion of more patients failing to respond to first-line IMP. This is in line with expectations because patients responding well to treatment would be less keen to have a second biopsy.

TABLE 2 Synovial histological analysis stratified according to IMP at baseline and 16 weeks

| | Unpaired analysis (all patients) | | Paired analysis | | | | | | | Treatment effect |
| | Baseline biopsy[a] | | Rituximab group (n = 41) | | | Tocilizumab group (n = 24) | | | | |
Variable	Rituximab (n = 82), mean (SD)	Tocilizumab (n = 79), mean (SD)	Baseline, mean (SD)	Week 16, mean (SD)	Absolute change (percentage change)	Baseline, mean (SD)	Week 16, mean (SD)	Absolute change (percentage change)		Least squares mean difference (95% CI)
CD20+	1.62 (1.3)	1.5 (1.4)	1.88 (1.4)	0.35 (0.8)	−1.53[b] (−81)	1.67 (1.3)	1.33 (1.3)	−0.34 (−20)		1.02 (0.52 to 1.52)[d]
CD79a+	1.54 (1.3)	1.6 (1.4)	1.77 (1.4)	0.9 (1.1)	−0.87[b] (−49)	1.54 (1.3)	1.47 (1.2)	−0.07 (−5)		0.55 (0.04 to 1.06)[d]
CD138+	1.43 (1.3)	1.42 (1.4)	1.68 (1.3)	0.92 (1.1)	−0.76[c] (−45)	1.58 (1.4)	1.25 (1.1)	−0.33 (−21)		0.36 (−0.16 to 0.88)
CD3+	1.43 (1.1)	1.47 (1.2)	1.63 (1.1)	1.52 (1.2)	−0.11 (−7)	1.58 (1.1)	1.42 (1.2)	−0.16 (−10)		−0.08 (−0.64 to 0.49)
CD68+ L	1.11 (1)	1.2 (1.1)	1.2 (1)	1.07 (0.9)	−0.13 (−11)	1.46 (1.1)	1.38 (1.1)	−0.08 (−5)		0.2 (−0.27 to 0.66)
CD68+ SL	1.67 (1)	1.75 (1.1)	1.88 (0.8)	1.3 (0.6)	−0.58[c] (−31)	1.92 (1)	0.88 (0.7)	−1.04[c] (−54)		−0.43 (−0.78 to −0.08)[d]
Synovial score	3.99 (2.6)	3.88 (2.9)	4.63 (2.5)	3.23 (2)	−1.4[b] (−30)	4.38 (2.8)	3.46 (2.4)	−0.92 (−21)		0.32 (−0.69 to 1.32)

a No significant difference between treatments was observed for the presented values (tested by Mann–Whitney U-test).
b $p < 0.001$ for the within-group change from baseline (paired Wilcoxon signed-rank test comparing baseline values with values at 16 weeks for the same patients).
c $p < 0.05$ for the within-group change from baseline (paired Wilcoxon signed-rank test comparing baseline values with values at 16 weeks for the same patients).
d $p < 0.05$ for the comparison with non-responders of the change from baseline. The difference in the changes from baseline between treatments was tested by a non-parametric ANCOVA model with treatment as factor and baseline score as covariate.

Note
No adjustments were made for multiple comparisons.

TABLE 3 Change in synovial immune cell infiltrate between baseline and 16 weeks stratified according to response (improvement in CDAI score of ≥ 50%) and IMP

	Treatment group												
	Rituximab						Tocilizumab						
	Non-responders (n = 26)			Responders (n = 15)			Treatment effect	Non-responders (n = 14)			Responders (n = 10)			Treatment effect
Variable	Baseline, mean (SD)	Week 16, mean (SD)	Absolute change (percentage change)	Baseline, mean (SD)	Week 16, mean (SD)	Absolute change (percentage change)	Least squares mean difference (95% CI)	Baseline, mean (SD)	Week 16, mean (SD)	Absolute change (percentage change)	Baseline, mean (SD)	Week 16, mean (SD)	Absolute change (percentage change)	Least squares mean difference (95% CI)
CD20^{+}	1.88 (1.3)	0.52 (1)	−1.36 (−72)[a]	1.87 (1.5)	0.07 (0.3)	−1.8 (−96)[a]	−0.46 (−0.97 to 0.06)	1.71 (1.3)	1.21 (1.3)	−0.5 (−29)	1.6 (1.3)	1.5 (1.3)	−0.1 (−6)	0.34 (−0.64 to 1.33)
CD79a^{+}	1.54 (1.4)	0.88 (1.1)	−0.66 (−43)[a]	2.13 (1.4)	0.93 (1.1)	−1.2 (−56)[a]	−0.25 (−0.88 to 0.38)	1.64 (1.4)	1.31 (1.2)	−0.33 (−20)	1.4 (1.3)	1.83 (1.2)	0.43 (31)	0.4 (−0.57 to 1.36)
CD138^{+}	1.58 (1.4)	1.0 (1.3)	−0.58 (−37)	1.87 (1.2)	0.8 (0.9)	−1.07 (−57)[a]	−0.31 (−1.01 to 0.38)	1.5 (1.4)	1.21 (1.1)	−0.29 (−19)	1.7 (1.4)	1.3 (1.2)	−0.4 (−24)	0.01 (−0.84 to 0.86)
CD3^{+}	1.58 (1.1)	1.4 (1.2)	−0.18 (−11)	1.73 (1.1)	1.73 (1.3)	0 (0)	0.27 (−0.45 to 0.98)	1.57 (1.2)	1.57 (1.1)	0 (0)	1.6 (1.1)	1.2 (1.4)	−0.4 (−25)	−0.38 (−1.38 to 0.61)
CD68^{+} L	1.08 (0.9)	1.08 (0.9)	0 (0)	1.4 (1.2)	1.07 (1)	−0.33 (−24)	−0.11 (−0.69 to 0.48)	1.5 (1.3)	1.71 (1.2)	0.21 (14)	1.4 (0.8)	0.9 (0.9)	−0.5 (−36)	−0.76 (−1.51 to 0)
CD68^{+} SL	1.85 (0.8)	1.28 (0.6)	−0.57 (−31)[a]	1.93 (1)	1.33 (0.7)	−0.6 (−31)	0.04 (−0.39 to 0.47)	1.71 (1.1)	1.14 (0.8)	−0.57 (−33)	2.2 (0.9)	0.5 (0.5)	−1.7 (−77)[a]	−0.7 (−1.31 to −0.09)[b]
Synovitis score	4.46 (2.2)	3.2 (2.1)	−1.26 (−28)[a]	4.93 (3)	3.27 (1.8)	−1.66 (−34)[a]	−0.16 (−1.29 to 0.98)	4.43 (3)	4.0 (2.5)	−0.43 (−10)	4.3 (2.6)	2.7 (2.3)	−1.6 (−37)	−1.26 (−3.26 to 0.74)

a p < 0.05 for the within group change from baseline. This comes from a paired Wilcoxon signed-rank test comparing baseline values with values at 16 weeks for the same patients.
b p < 0.05 for the comparison with non-responders of the change from baseline. The difference in the changes from baseline between treatments was tested through a non-parametric ANCOVA model with treatment as factor and baseline score as covariate.

Note
No adjustments were made for multiple comparisons. A p-value of ≤ 0.05 was considered to indicate statistical significance.

Change in gene expression levels between baseline and 16 weeks

The rituximab (baseline, $n = 68$; week 16, $n = 33$) and tocilizumab (baseline, $n = 65$; week 16, $n = 17$) patient samples were analysed separately to assess the differences in the change in gene expression (from baseline to week 16) between responders and non-responders without the confoundment of different biological therapy. Linear mixed models were used because the data had quite a few unmatched samples between visits.

In the gene-level analysis, we examined the change in *MS4A1* in the rituximab-treated group in the responders and non-responders separately, and the response interaction (see *Appendix 1, Figure 15*). The decrease in *MS4A1* expression was greater in responders [$\log_2$ fold change (FC) = −0.51] than in non-responders ($\log_2 FC = −0.45$). The p-value was smaller in responders ($p = 1.07 \times 10^{-7}$) than in non-responders ($p = 0.0031$), which may suggest that individuals responded better because of a more profound B-cell depletion in synovial tissue. However, the responders group was larger ($n = 65$) than the non-responders group ($n = 37$), and the interaction p-value was not significant. Next, we examined the change in *IL-6* gene expression in the tocilizumab-treated group for responders and non-responders, and performed a statistical test for interaction (see *Appendix 1, Figure 16*). In responders, there was a significant reduction in *IL-6* gene expression over time ($p = 0.038$; $n = 65$), but there was no significant difference in non-responders ($n = 17$). The interaction p-value was not significant.

Next, we examined the change in module or pathway expression using the same statistical comparisons that were made at the gene level for the B-cell module (see *Appendix 1, Figure 17*) and *IL-6* module genes (see *Appendix 1, Figure 18*). Over time, the B-cell module decreased significantly in responders in the rituximab-treated group ($\log_2 FC = −0.13$; $p = 0.0013$), but not in rituximab non-responders ($\log_2 FC = −0.07$), with a significant p-value for interaction ($p < 0.05$). There were no significant changes in *IL-6* module expression in the tocilizumab-treated group in this preliminary analysis.

Safety and adverse events

The safety and adverse events data are summarised in *Table 4* and *Appendix 1, Table 21*. It can be seen that more adverse events (327 vs. 284) and serious adverse events (18 vs. 8; $p < 0.05$) were observed

TABLE 4 Safety data from weeks 0 to 48 (+ 30 days)

Variable	Treatment group, *n* (%)		Total,[a] *n*
	Rituximab	Tocilizumab	
Patients exposed to IMP	108	116	224
Patients with adverse events	60 (55.6)	70 (60.3)	135
Patients with serious adverse events	8 (7.4)	12 (10.3)	20
Patients with serious infections	3 (2.8)	3 (2.6)	6
Patients who discontinued the study drug because of serious adverse event	2 (1.9)	2 (1.7)	4
Patients with confirmed cancer[b]	0 (0)	0 (0)	0
Deaths	1 (0.9)	0 (0)	1
Total events			
Adverse events	280	331	659
Serious adverse events	8	18[c]	26
Infections	3	3	6

a The total number of events/patients with events up to week 48 is reported here. This includes some adverse events occurring before the first prescription of the IMP.
b No cancer was observed during the treatment period. However, there was one kidney carcinoma after week 48.
c $p < 0.05$ for the comparison with rituximab, tested by Fisher's exact test.

in patients treated with tocilizumab than in those treated with rituximab. One patient in the rituximab-treated group who reported a serious unexpected serious adverse reaction (corneal melt) and three patients in the tocilizumab group who reported serious adverse events (pleural effusion, chest pain and cytokine release syndrome) discontinued the IMP because of these events. The serious adverse events comprised three infections in each group, four ischaemic cardiac events in the tocilizumab group and one ischaemic cardiac event in the rituximab group (see *Appendix 1, Table 21*). One death because of suicide was reported in the rituximab group. No malignancies were reported during the 48-week trial period. Three patients underwent randomisation but did not receive a study drug and no serious adverse events were reported in these patients. Importantly, no serious adverse events related to synovial biopsy were reported.

Chapter 4 Discussion

Rituximab remains a pivotal therapeutic option for RA patients; however, response to therapy remains heterogeneous, with only 20% of patients achieving clinically meaningful American College of Rheumatology 70% (ACR70) response rates[6] when rituximab is used as first-line biological therapy. On the other hand, rituximab therapy can be very effective, transforming the life of many patients. Thus, understanding the mechanism of response/non-response is critical to avoid the unnecessary use of an expensive and potentially toxic drug. Although B cells are considered key players in RA pathogenesis, particularly in the development of systemic autoimmunity in RF/ACPA-positive patients, which may precede clinical manifestations by years, their contribution in seronegative RA is less clear.[42] In addition, at the disease tissue level (synovium), synovial B-cell infiltration is highly heterogeneous, being low or absent in a significant proportion of patients (approximately 50%) despite high disease activity.[24] This suggests that in these patients synovial inflammation is sustained by alternative cell types and that, if CD20+ B cells are absent from the disease tissue or present at only a very low level, the therapeutic response to rituximab may be poor and an alternative therapy with a mode of action not dependent on B-cell depletion may be more effective. The R4–RA study was designed and supported by the NIHR Efficacy and Mechanism Evaluation programme and aimed to determine whether or not specific cellular (CD20+ B cells) and molecular (B-cell-associated) signatures in the synovial biopsy can mechanistically explain specific disease outcomes. To our knowledge, this is the first biopsy-based randomised controlled trial in RA and, although the histological classification of patients as B-cell poor did not appear to stratify patients for clinical response to rituximab or tocilizumab when response was defined as an improvement in CDAI score of ≥ 50%, we observed no significant difference in the primary end point (improvement in CDAI score of ≥ 50% from baseline) between the IMP groups. However, a supplementary analysis evaluating a prespecified definition of non-response as failure to achieve an improvement in CDAI score of ≥ 50% and a CDAI score of < 10.1 (defined as CDAI-MTR) did reach statistical significance. Moreover, when patients were classified as B-cell poor/rich according to the FANTOM5-derived[34] B-cell molecular module (73 genes), which became available at the end of the trial, based on RNA sequencing of the synovial biopsy, both primary end points (improvement in CDAI score of ≥ 50%) and CDAI-MTR (improvement in CDAI score of ≥ 50% and CDAI score of < 10.1) reached statistical significance. Furthermore, although logistic regression analysis showed no evidence of an interaction between the IMP and the histologically defined B-cell subgroups for the primary end point, a statistically significant interaction between RNA-seq-defined B-cell subgroup and IMP was observed when using CDAI-MTR ($p = 0.049$), providing evidence that the treatment effect was different between RNA-seq-defined B-cell-rich and B-cell-poor groups.

The reasons for these differences are likely to relate to the sensitivity of the classification technique. CD20+ staining was evaluated at three cutting levels using a semiquantitative score on a minimum of six biopsies, as recommended for use in clinical trials and reported to be representative of the whole joint tissue.[43] However, although the semiquantitative score used for balanced stratification of patients prior to randomisation had been validated against both DIA and the transcript levels determined using the FANTOM5-derived gene set,[34,35] the cut-off point was set arbitrarily as 0–1 for B-cell-poor patients and 2–4 for B-cell-rich patients. Given that a binary classification has never been attempted before and no published 'gold standard' is available, it is possible that the histological cut-off point may not have been set at an optimal level.

Turning to the molecular B-cell-poor/-rich classification, this was determined by applying the same FANTOM5-derived module used to validate the semiquantitative score and included 73 genes associated with B cells[34,35] scored against the RNA-seq of six pooled homogenised biopsy samples. This provides richer granularity of the pathobiological processes (expression of 30,000 genes) of the entire active joint and, arguably, a more precise estimate of the number not only of mature CD20+ B cells but also of B cells at different stages of differentiation, with the gradual loss of *CD20* gene expression that in itself is likely to influence the response to rituximab, which specifically targets *CD20*. Thus, the application of the molecular classification, an objective method using the transcript-level median value of a B-cell

module gene set derived from FANTOM5, enables a number of limitations of histological classification to be overcome, including the relatively subjective assessment of synovial B-cell infiltration by histopathology.

We recognise that a proportion of patients (28 out of 152, 18.4%) could not be classified by molecular analysis, as their samples failed RNA-seq quality control. The most likely reason for this is the decision to use deep genotyping (150-bp-long reads, 50 million reads per sample), which allows, for instance, the analysis of split variants, but requires larger amounts of RNA. Despite this limitation, the use of RNA-seq has allowed the identification of specific signatures, such as the FANTOM5-derived B-cell module that we used to classify patients into B-cell-rich and B-cell-poor groups. In the future, it will be possible to overcome such limitation by analysing these modules with alternative molecular techniques, such as NanoString (NanoString Technologies, Seattle, WA, USA), which is based on a selection of specific gene probes, thus requiring less material and most likely resulting in a smaller number of unclassified patients.

Importantly, we also evaluated clinical outcomes in the overall study population (irrespective of pathotype). Although we observed a trend for higher response rates in the tocilizumab-treated patients, there was no significant difference in the numbers achieving the primary outcome (CDAI score improvement $\geq$ 50%) at 16 weeks, and so, in this first head-to-head trial of rituximab versus tocilizumab, the data do not justify modification of current biological prescribing algorithms to favour the use of tocilizumab over rituximab in patients failing to respond to TNFi therapy.

Data from the longitudinal analysis of the synovial tissue following treatment with rituximab are in line with previously published data from observational cohorts, which report significant decreases in synovial B cells post treatment with rituximab, but no significant associations between the degree of synovial B-cell depletion and the clinical response.[15,17,18] However, our molecular analysis demonstrated a greater decrease in both *MS4A1* and B-cell module gene expression in patients who responded to rituximab treatment than in non-responders, suggesting an increased sensitivity of molecular versus histological techniques to detect change. To confirm that the number of CD20^{+} B cells was not underestimated owing to internalisation of the *CD20* molecule, we also evaluated the expression of *CD79a*, with the results being consistent for both markers.[44] Importantly, our data identified CD138^{+} plasma cell depletion as a significant marker of response to rituximab, in line with a previous study that found that poor depletion of CD138^{+} plasma cells was associated with non-response to rituximab treatment.[17] The concept that synovial plasma cells are an important therapeutic target is supported by data from an independent cohort, in which decreases in synovial immunoglobulin synthesis were associated with response to rituximab.[18] The effects of tocilizumab on synovial immune cell infiltrate were less pronounced, with only CD68^{+} SL macrophage numbers decreasing significantly in both the total and the responder cohorts. The molecular findings were in line with the histological results, with insignificant decreases in *IL6* and *IL6* pathway gene expression in either responder or non-responder groups in the tocilizumab-treated patients. Although others have also reported a decrease in CD68^{+} macrophage numbers post tocilizumab treatment,[45] it was associated with decreases in other immune cell types, something that we did not observe. However, when interpreting data from our analysis, the significantly larger numbers of non-responders to the IMP in the population of patients undergoing a second biopsy may be attributed to patients who responded well to treatment being less keen to have a second biopsy and/or having no suitable joints to biopsy. This resulted in a skewed population that is likely to underestimate the immunomodulatory effects of IMP on synovial immune cell infiltrate and may also explain the lack of consistent significant decreases in CD68^{+} SL macrophage numbers in responder groups that may have been predicted in the light of previous studies identifying this marker as a validated outcome measure.[46,47] However, notably, other studies evaluating synovial response to rituximab have not identified significant decreases in CD68 SL in responders to therapy.[17,18]

Although we report a larger number of serious adverse events and adverse events in patients treated with tocilizumab than in those treated with rituximab, these were largely unrelated to the study drug; nevertheless, this finding in this first head-to-head trial of rituximab and tocilizumab may suggest

that tocilizumab is less well tolerated. Importantly, there were no serious adverse events related to synovial biopsy, supporting previously published data relating to the safety of minimally invasive synovial biopsy techniques performed by rheumatologists.[30,48]

Study limitations

To our knowledge, this is the first randomised controlled biopsy-based trial in RA and, inevitably, the study had limitations:

1. The important issue of the binary B-cell-poor/-rich classification discussed will require further analysis of the trial to determine a more accurate cut-off point or may lead in the future to a different quantification method using, for example, continuous variable data (e.g. transcript levels) as a sensitive tool to predict clinical response as reported for other therapeutic targets, for example programmed cell death-1 (PD1).[49]
2. The choice of tocilizumab as an active comparator to rituximab might not have been optimal because tocilizumab itself modulates B-cell function and survival.[50] Thus, if a biological lacking direct B-cell modulatory effects had been selected (e.g. an alternative TNFi or abatacept), a more pronounced effect on treatment differences within pathotype groups may have been observed.
3. Of critical relevance, tocilizumab is known to act faster than rituximab and the study design might have favoured the fast-acting drug, despite the deliberate choice of a relatively late primary time point (16 weeks). In the same vein, the lack of double blinding for the IMP may have amplified the clinical response to tocilizumab, which is given as a monthly infusion rather than, as with rituximab, at 24 weeks. Although the clinical staff remained blind at all times to the pathotype status of patients, the frequency of the infusions may have created an unconscious bias, potentially in both patients and clinical staff. The selection of tocilizumab was a pragmatic choice based largely on the accessibility of NHS trusts participating in the trial to this biological treatment beyond second-line therapy, and it was considered unethical to inconvenience those patients randomised to the rituximab group with a monthly placebo infusion.
4. It is conceivable that, despite a washout period for previous biological therapies, as well as standardisation of steroid and csDMARD therapy prior to trial entry, these therapies and/or other concomitant therapies may have modulated baseline synovial pathobiology. Future studies evaluating synovial pathobiological markers and clinical response to biological treatment in patients naive to therapy are certainly warranted to address this issue.
5. Finally, the choice of an improvement in CDAI score of $\geq$ 50% as a primary binary outcome rather than, for example, EULAR/DAS28(ESR) response rates, illustrates the lack of precision of current assessment methods, as the choice of the latter would have led to a positive trial based on the results of even by the histopathological classification. Thus, to achieve precision, future rheumatology studies should aim to refine both the current assessment tools (originally designed mainly for regulatory purposes) and the pathobiological classification so that a truly integrated clinical and molecular pathology algorithm can be developed for the optimal management of RA.

Conclusion

We report the results from what is, to our knowledge, the first pathobiology-driven, multicentre, randomised controlled trial in RA. Although we were unable in our primary analysis to demonstrate that tocilizumab is more effective than rituximab in patients with a B-cell-poor pathotype, superiority was suggested in some supplementary and secondary analyses. These analyses overcame possible unavoidable weaknesses in our original study plan, in which the histological method of determining B-cell status may have misclassified some participants and our chosen primary outcome was insufficiently sensitive. In particular, using the molecular classification, the results suggest that in B-cell-poor RA patients tocilizumab is significantly more likely than rituximab to induce a clinical response, whereas in

patients presenting with a B-cell-rich synovium rituximab is as effective as tocilizumab. However, owing to the limitations of the study discussed above, these finding are insufficiently robust to justify change in clinical practice. Future research will focus on the refinement of the molecular pathology classification and replication of the R4–RA findings in independent studies to determine whether or not stratification of patients according to cellular and molecular signatures may have clinical utility for treatment allocation of specific targeted biological therapies depending on the level of expression of their cognate target in the disease tissue.

The ability to target biological therapies to the right patients, rather than continue the current practice of trial and error, may enrich for clinical response with the potential to have a significant impact on the health economics of RA with reduced exposure of patients to expensive and potentially toxic drugs. This would also align clinical practice in rheumatology with specialties such as oncology in which stratification of patients according to the tissue expression of drug targets has been adopted in routine clinical practice.[51]

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Principal investigators and sites

As part of our biopsy-driven research programme on stratified medicine for the treatment of RA, we worked closely with the Medical Research Council-funded Maximising Therapeutic Utility for Rheumatoid Arthritis (MATURA) Consortium, which has a dedicated patient and public involvement (PPI) stream. The MATURA Patient Advisory Group (MPAG) forged links with local patient support groups to raise the profile of research studies and identify barriers to recruitment. The group also assisted with activities such as advising on participant information materials, with a focus on the acceptability of the biopsy and understanding of our stratified medicine concept. The group met regularly to receive updates on our research portfolio and this included R4–RA.

Support from PPI groups for R4-RA included:

- Reading and critiquing the patient documentation (e.g. patient information leaflet, consent forms and end of trial letter).
- Representing the wider patient community by participating in meetings.
- Organising educational events for RA patients on the potential of stratified medicines, particularly at hospitals recruiting patients to trials coordinated by the Centre for Experimental Medicine and Rheumatology.
- A patient who had undergone synovial biopsy discussed their experience of the biopsy procedure in a video. This was provided for patients who were considering taking part in the Centre for Experimental Medicine and Rheumatology biopsy-driven studies. This video was approved by the Ethics Committee on 30 August 2017 for use in the R4–RA trial.
- A recruitment poster was created for R4–RA, which was reviewed by a PPI group prior to being submitted to the Ethics Committee.

The MPAG members were invited to the MATURA symposium (RCP CPD 127665) on 30 September 2019, where the results of R4–RA were presented; they will have another opportunity to discuss the results at the next MPAG meeting, where we will also consult on the dissemination of the results.

A patient representative (Di Skingle, National Rheumatoid Arthritis Society Trustee) joined the R4-RA TSC in 2016, and attended the TSC meetings thereafter to provide oversight of the conduct of the trial from a patient's perspective.

Contributions of authors

Frances Humby (https://orcid.org/0000-0003-1280-9133) (Co-Investigator, Consultant Rheumatologist) contributed to the design of the study, data collection, data analysis and interpretation, and was the lead in drafting the report.

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Data-sharing statement

The anonymised raw data will be stored in a non-publicly available repository called TranSMART (once the final study report has been published). More information can be found here: www.matura.whri. qmul.ac.uk/TranSMART.php (accessed 26 October 2020). All data requests should be submitted to the corresponding author for consideration. Access to anonymised data may be granted following review.

Patient data

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation.

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Appendix 1 Additional figures and tables
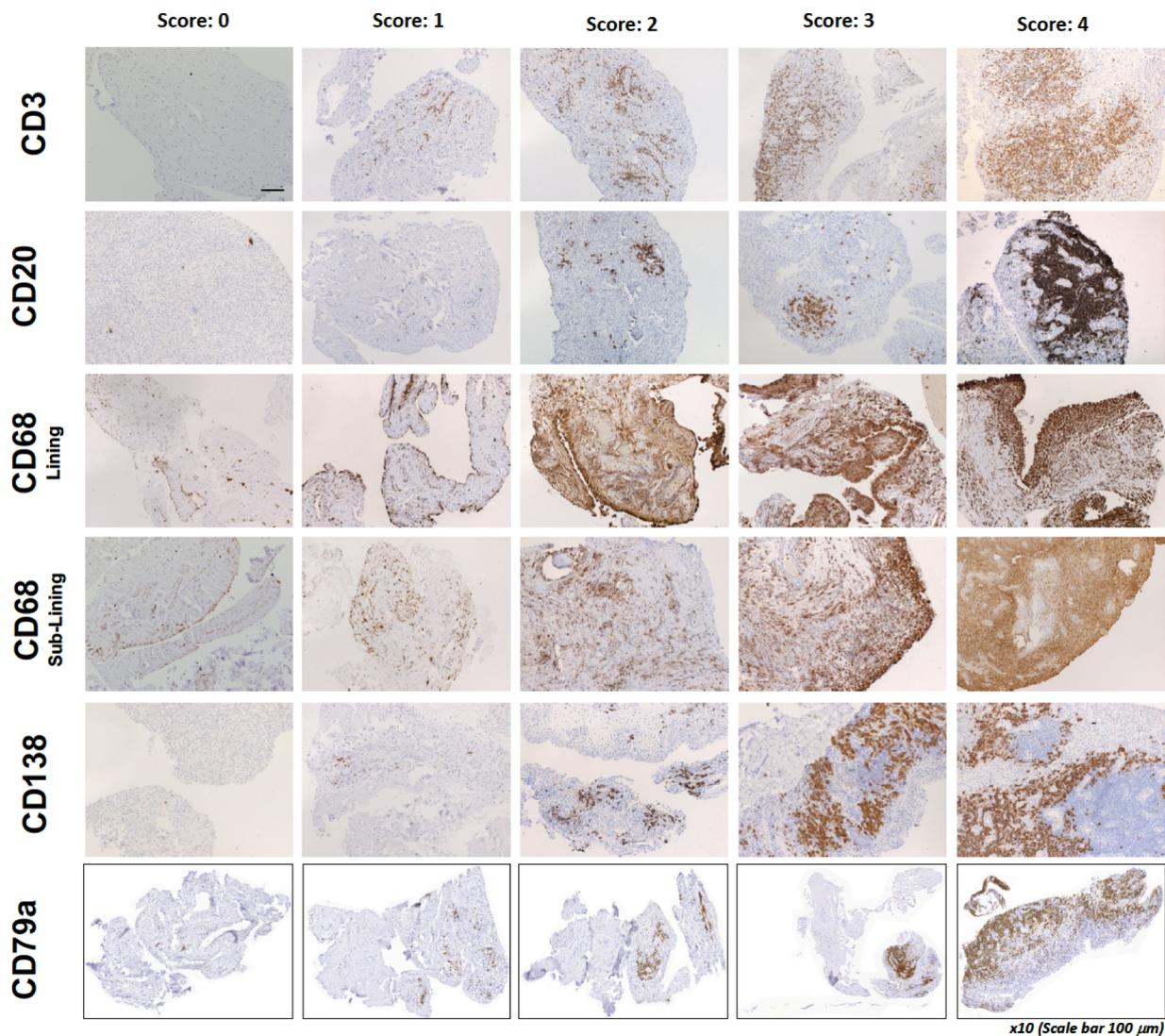
FIGURE 6 Reference atlas for immunohistochemical scores for synovial tissue (0–4).
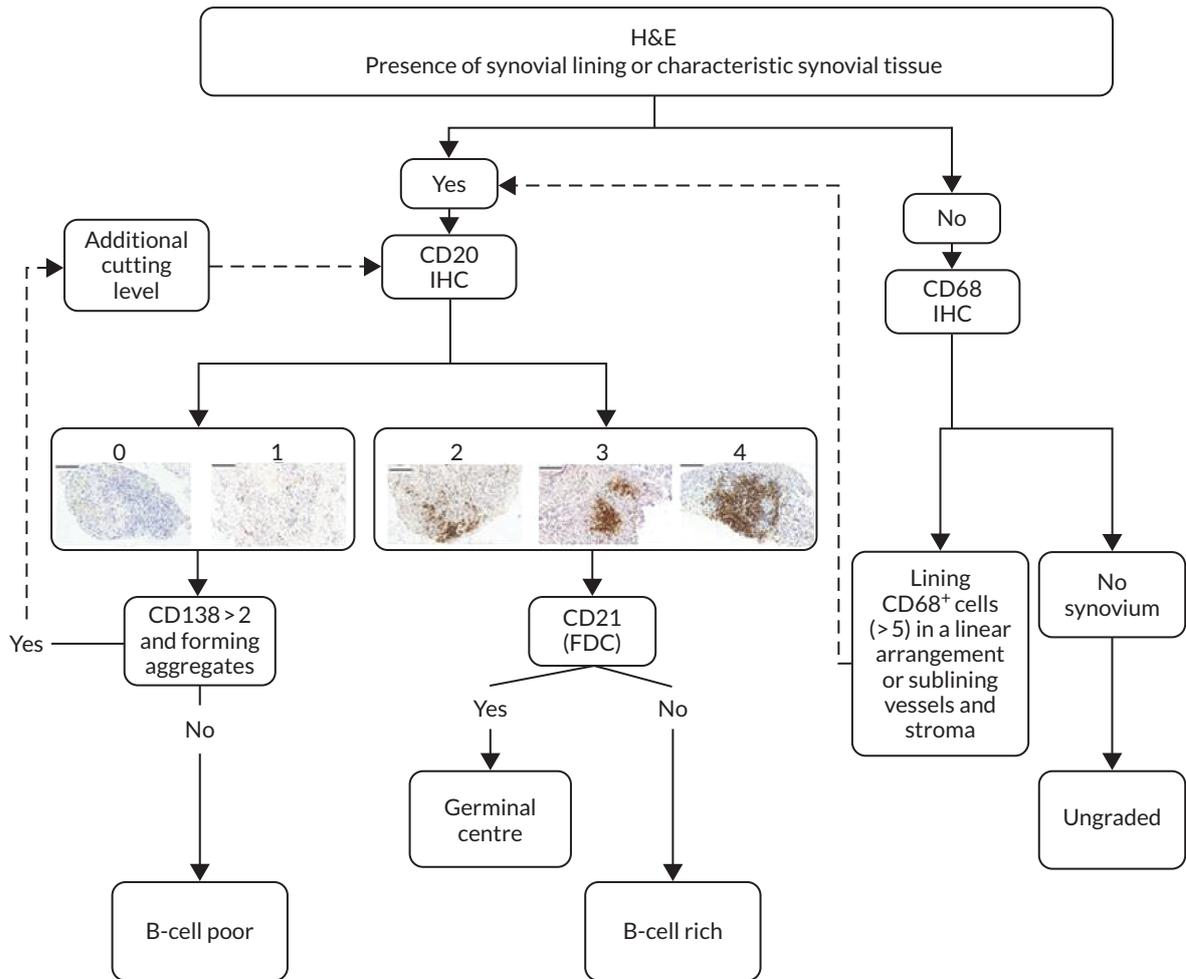
FIGURE 7 Histological classification of samples. IHC, immunohistochemistry. Line: 100 μm.
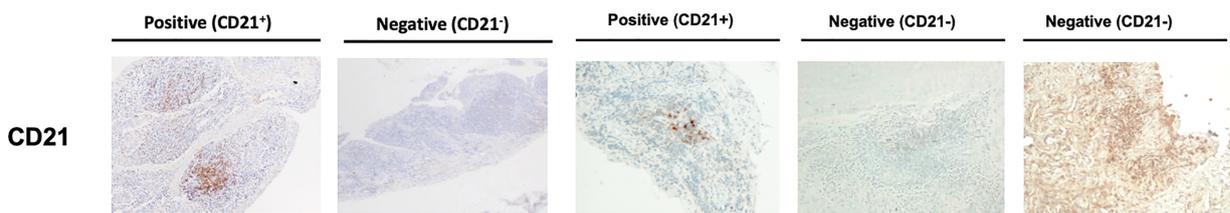
FIGURE 8 Reference atlas for immunohistochemical staining for CD21^{+} FDCs.
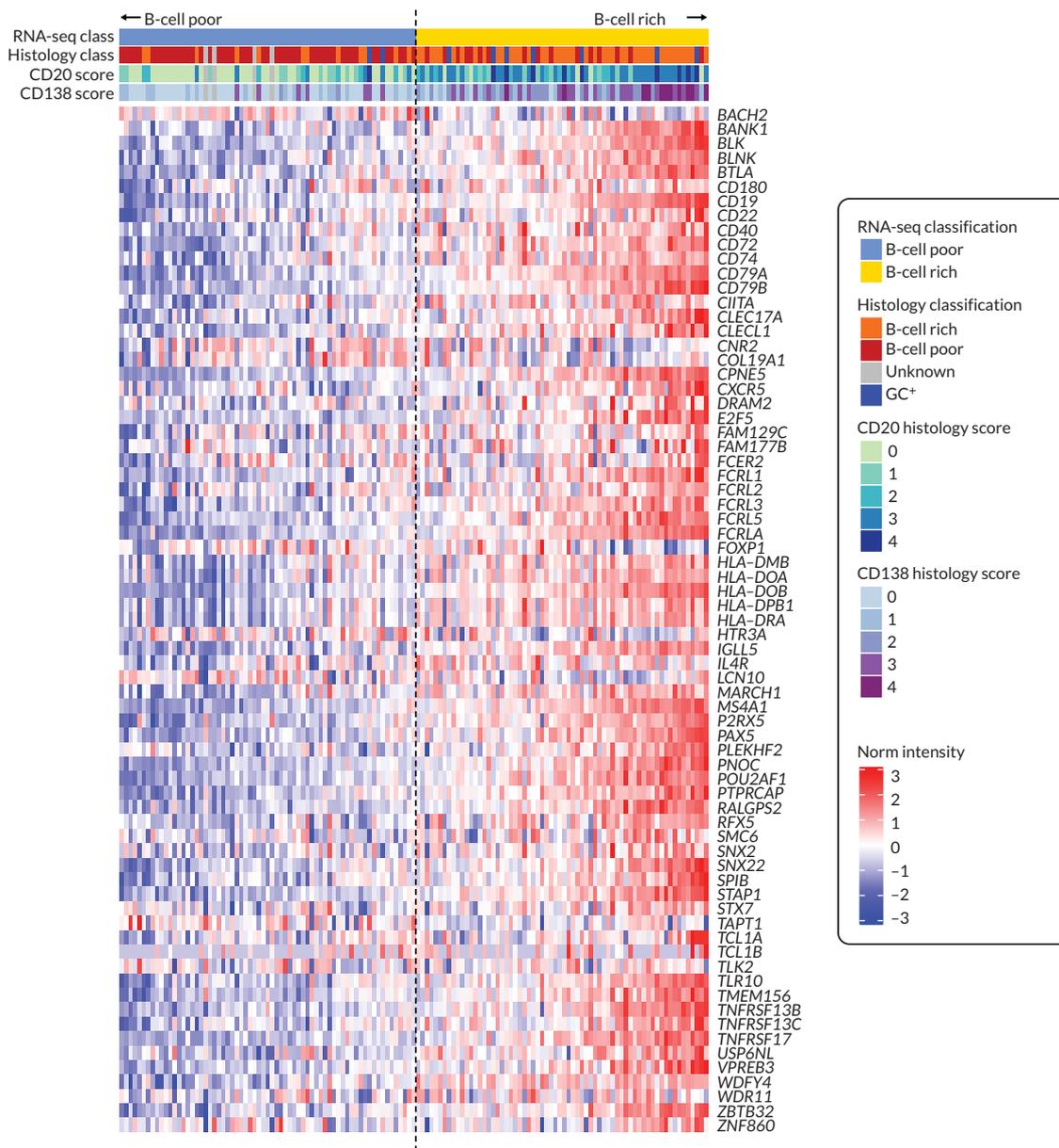
FIGURE 9 Heat map of RNA-seq B-cell module gene expression across the whole cohort. Samples are ranked by RNA-seq B-cell module score from lowest to highest demonstrating the reclassification of patients into RNA-seq B-cell poor and rich categories. The top tracks show the original histology class, CD20 and CD138 histology scores. GC, germinal centre as classified by histology.

Visit 1: −6 to −1 weeks	Screening
Visit 2: −3 to −1 weeks	Synovial biopsy Patient stratification according to histopathology
Visit 3: baseline	Disease activity assessment at baseline

Randomisation
1 : 1 within each stratum

Rituximab   Tocilizumab

| Visit 7: week 16 | Primary end point: disease activity assessment at 16 weeks |

Responder   Non-responder   Responder   Non-responder

Continue rituximab   Switch to tocilizumab   Continue tocilizumab   Switch to rituximab

| Visit 15: week 48 | Disease activity assessment at 48 weeks: end of treatment period |
| Visit 17: week 96 | Long-term follow-up |

FIGURE 10 Trial design.

FIGURE 11 Patient disposition in weeks 16 to 48 (B-cell-poor population).
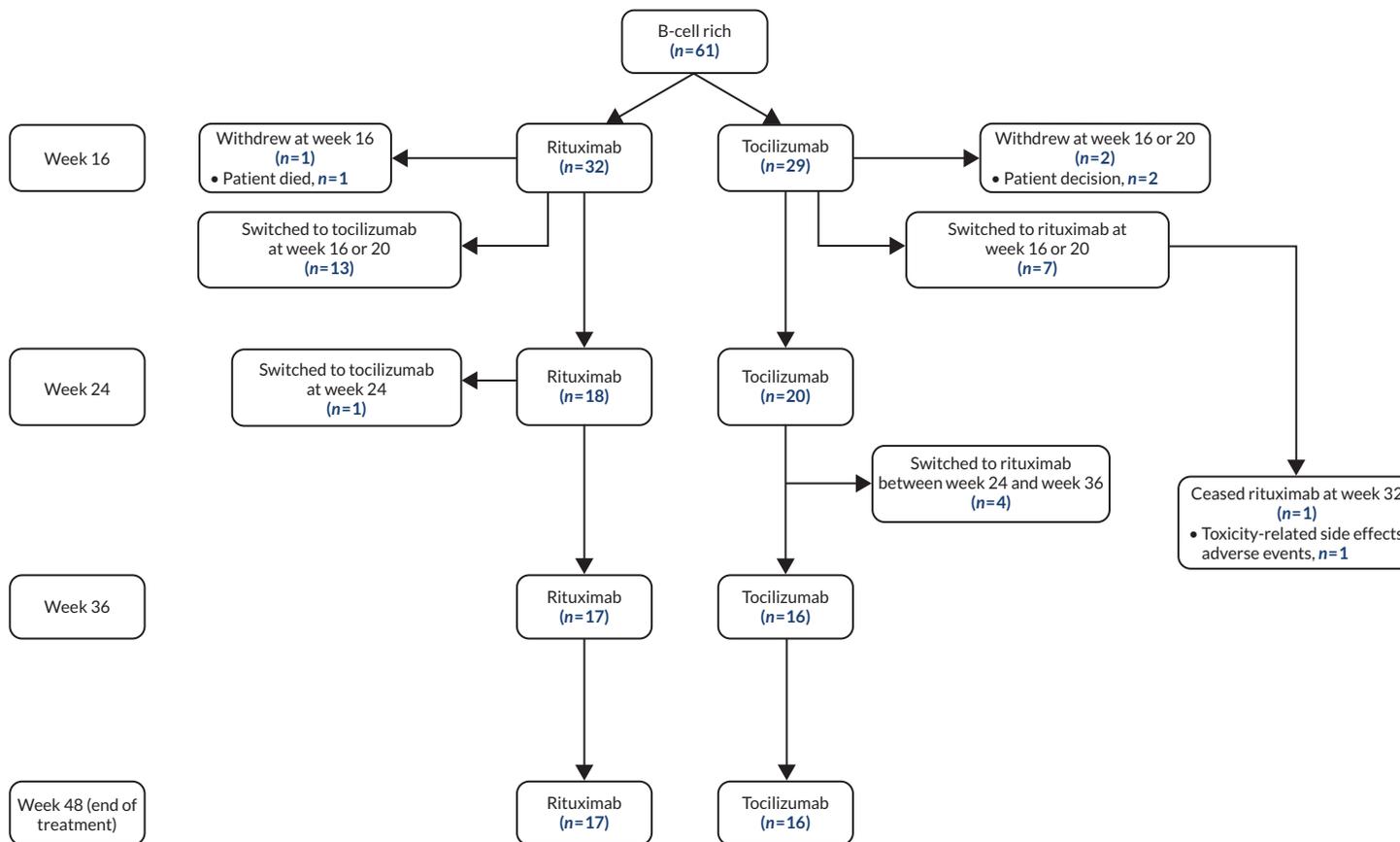
FIGURE 12 Patient disposition in weeks 16 to 48 (B-cell-rich population).
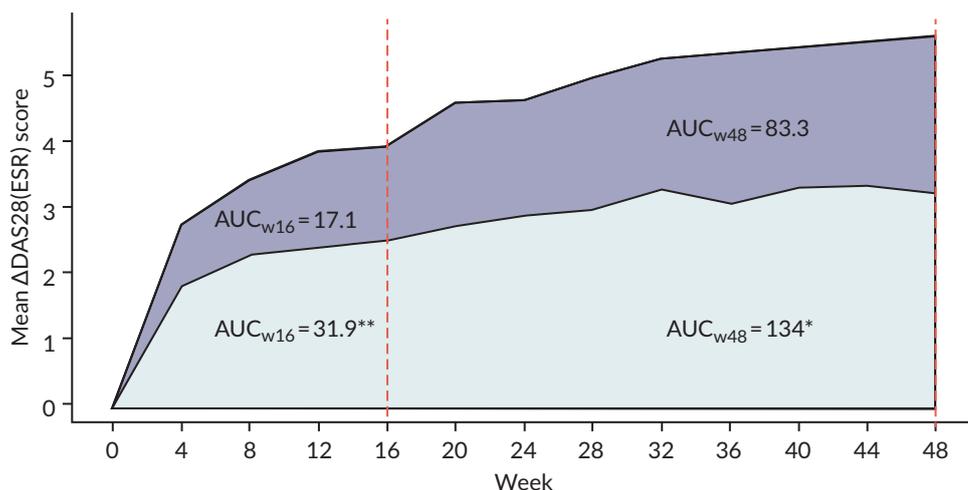
FIGURE 13 Area under the curve of the mean change in DAS28(ESR) score from baseline to 48 weeks (B-cell-poor population). The purple area represents rituximab and the light-blue area represents tocilizumab. Areas are here presented as stacked. *$p < 0.05$ for the comparison of tocilizumab vs. rituximab by GLMM; **$p < 0.01$ for the comparison of tocilizumab vs. rituximab by GLMM.
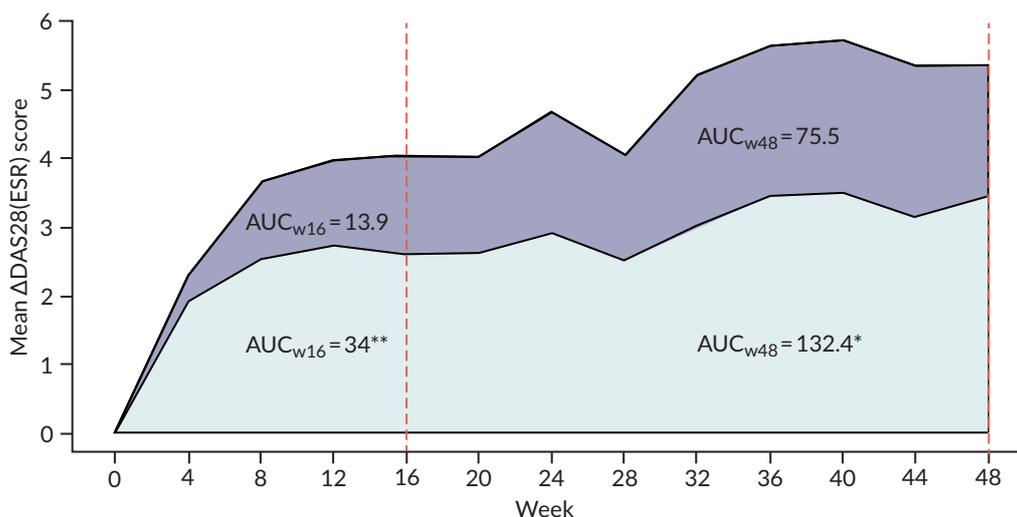
FIGURE 14 Area under the curve of mean change in the DAS28(ESR) score from baseline to 48 weeks (B-cell-rich population). The purple area represents rituximab and the light-blue area represents tocilizumab. Areas are here presented as stacked. *$p < 0.05$ for the comparison of tocilizumab vs. rituximab by GLMM; **$p < 0.01$ for the comparison of tocilizumab vs. rituximab by GLMM.
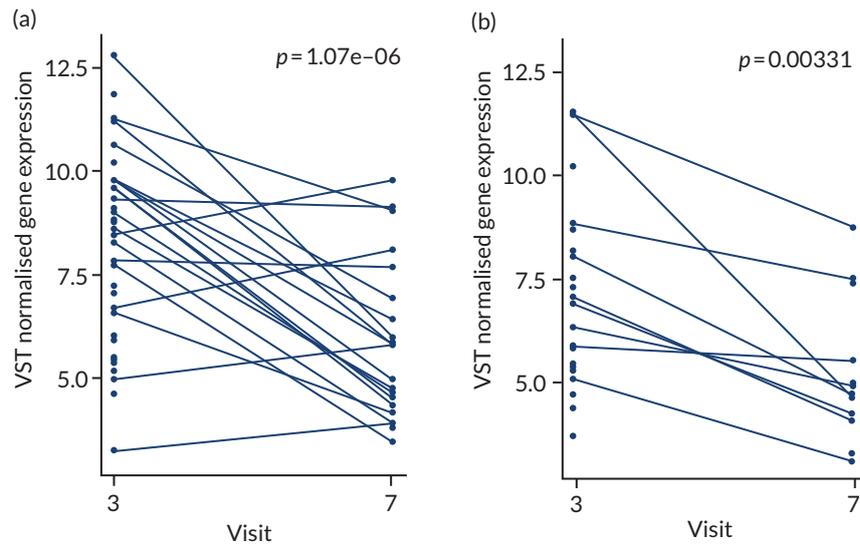
FIGURE 15 Investigating the differences in the change in *MS4A1* gene expression between responders and non-responders in rituximab-treated patients (*n* = 101) with respect to EULAR response. (a) Good or moderate; and (b) none. In this analysis, linear mixed models were used in limma5 with patient ID added as a random effect. Although *p*-values for the change in gene expression within responders or non-responders were significant in some comparisons, the interaction *p*-values were not. Each line represents the change in a patient's gene expression, with the dots representing individual samples.
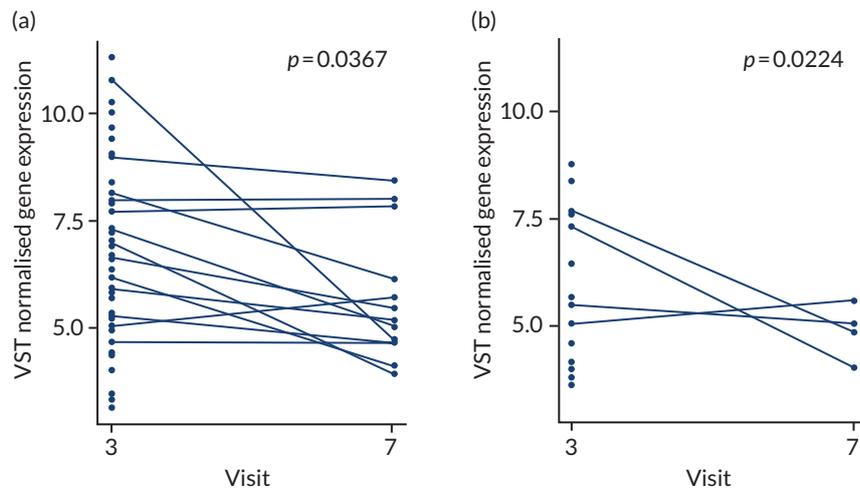
FIGURE 16 Investigating the differences in the change in *IL6* gene expression between responders and non-responders in tocilizumab-treated patients (*n* = 82) with respect to EULAR response. (a) Good or moderate; and (b) none. Each line represents the change in a patient's gene expression, with the dots representing individual samples.
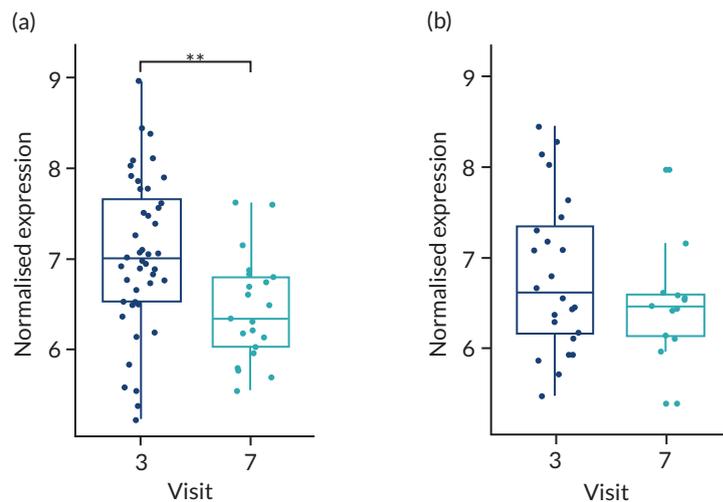
FIGURE 17 Investigating the differences in the change in B-cell module gene expression between responders and non-responders in rituximab-treated patients. (a) Good or moderate; and (b) none. Blue shading represents visit 3 and green shading represents visit 7. The same type of analysis was performed as in *Figure 15* but at the gene-set level with the limma fry function. The change in expression of genes in the B-cell module pathway was examined. Significance below the $p < 0.01$ threshold is indicated by two stars (**). The y-axis corresponds to the mean VST normalised gene expression data for all of the genes in the set.
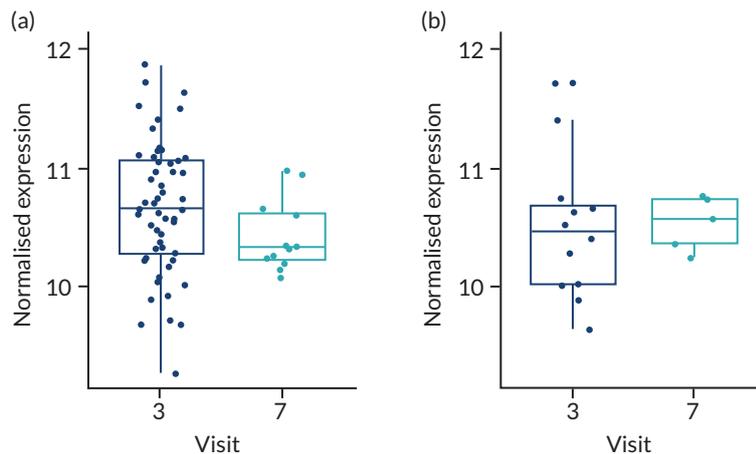
FIGURE 18 Investigating the differences in the change in *IL6*-pathway gene expression between responders and non-responders in tocilizumab-treated patients. (a) Good or moderate; and (b) none. Blue shading represents visit 3 and green shading represents visit 7. The same type of analysis was performed as in *Figure 16* but at the gene set level with the limma fry function. The change in expression of genes in the *IL6* pathway was examined. The y-axis corresponds to the mean VST normalised gene expression data for all of the genes in the set.

TABLE 5 Inclusion and exclusion criteria

Inclusion criteria	
1	Patients who have failed anti-TNF therapy (inadequate responders). Note that this includes patients who have failed anti-TNF therapy because of reactions
2	Patients who are eligible for rituximab therapy in accordance with the UK NICE guidelines[29]
3	Patients should be receiving a stable dose of methotrexate for at least 4 weeks prior to biopsy visit
4	Patients fulfil 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis for a diagnosis of RA
5	Patients who are aged ≥ 18 years
6	Patients are capable of giving informed consent
7	Patients willing and able to comply with scheduled visits, treatment plans and laboratory tests and other study procedures
Exclusion criteria	
1	Women who are pregnant or breastfeeding
2	Women of child-bearing potential, or males whose partners are women of child-bearing potential, who are unwilling to use effective contraception during the study and for at least 12 months after stopping study treatment
3	Patients with a history of or current primary inflammatory joint disease or primary rheumatological autoimmune disease other than RA (if secondary to RA, then the patient is still eligible)
4	Patients with prior exposure to rituximab or tocilizumab for the treatment of RA
5	Patients with treatment with any IMP ≤ 4 weeks prior to baseline (or < 5 half-lives of the investigational drug, whichever is the longer)
6	Patients with intra-articular or parenteral corticosteroids ≤ 4 weeks prior to biopsy visit
7	Patients with active infection
8	Patients with septic arthritis in a native joint within the last 12 months
9	Patients with sepsis of a prosthetic joint within 12 months or indefinitely if the joint remains in situ
10	Patients with known HIV or active hepatitis B/C infection. Hepatitis B screening test must be performed at or in the preceding 3 months of the screening visit
11	Patients with latent tuberculosis infection unless they have completed adequate antibiotic prophylaxis
12	Patients with malignancy (other than basal cell carcinoma) within the last 10 years
13	Patients with NYHA grade 3 or 4 congestive cardiac failure
14	Patients with demyelinating disease
15	Patients with latex allergy or allergy to any excipients of rituximab or tocilizumab
16	Patients with any other contraindication to the study medications as detailed in their SmPC, including low (IgG) levels at the clinician's discretion
16	Patients in receipt of live vaccine < 4 weeks prior to first infusion
18	Patients with major surgery in the 3 months prior to first infusion
19	Patients with a transplanted organ (with the exception of a corneal transplant > 3 months prior to screening)
20	Patients with known recent substance abuse (drug or alcohol)
21	Patients with poor tolerability of venepuncture or lack of adequate venous access for required blood sampling during the study period
21	Patients unable to tolerate synovial biopsy or in whom this is contraindicated, including patients on anticoagulants (oral antiplatelet agents are permitted)

TABLE 5 Inclusion and exclusion criteria (*continued*)

Exclusion criteria
23	Patients currently recruited to other clinical trial(s) involving an IMP (except any observational follow-up periods not involving an IMP)
24	Patients with other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that would impart, in the judgement of the investigator, excess risk associated with study participation or study drug administration, or which, in the judgement of the investigator, would make the patient inappropriate for entry into this study

IgG, immunoglobulin G; NYHA, New York Heart Association; SmPC, summary of product characteristics.

TABLE 6 Additional secondary end points

Additional secondary end points
1	Patients deemed treatment failures at 16 weeks will be switched to the other therapeutic option. Such patients will be considered a new patient starting at week 0 with treatment response assessed again at 16 weeks for primary response. Treatment difference before and after the switch will be compared in B-cell poor and B-cell rich
2	For the B-cell-rich synovial pathotypes, we aim to show non-inferiority of rituximab compared with tocilizumab. The same analysis as for the primary end point will be repeated

For the following end points, the treatment difference will be assessed separately in B-cell-poor, B-cell-rich and in the switched patients

3	AUC of mean improvement in DAS28 between 0 and 16 weeks, and between 0 and 48 weeks
4	Percentage of patients with low disease activity (DAS28 of < 3.2) at 16, 24, 36, 48 and 96 weeks
5	Percentage of patients in remission (DAS28 of < 2.6) at 16, 48 and 96 weeks
6	Percentage of patients with a low CDAI score at 16, 48 and 96 weeks
7	Mean percentage change in DAS28 between baseline and 16, 48 and 96 weeks
8	Mean percentage change in SF-36 score between baseline and 16, 48 and 96 weeks
9	Mean percentage change in CDAI score between baseline and 16, 48 and 96 weeks
10	Mean change in HAQ score between baseline and 16, 48 and 96 weeks
11	Change in fatigue score between baseline and 16, 48 and 96 weeks
12	Serious adverse events over 12 months; the rate of serious adverse events in the 16 week period following a switch from one technology to the other will be compared
13	Mean change in erosive score by the van der Heijde/Sharp scoring system[52] at baseline and week 24
14	Reduction in ultrasound 2D grey scale and power Doppler signal at baseline, 16 and 48 weeks
15	Mean change in synovial immune cell infiltrate determined immunohistologically (C20, CD68, CD138 and CD3) between baseline, 16 and 48 weeks
16	Mean change in synovial gene expression between baseline, 16 weeks and 48 weeks
17	EULAR response based on DAS28 (good and moderate responder/non-responders)

TABLE 7 Participating sites and recruitment numbers

Site name	Principal investigator	Randomised patients, n
Barts Health NHS Trust, London, UK	Dr Fran Humby	62
Cliniques Universitaires Saint Luc, Louvain, Belgium	Professor Patrick Durez	25
Santa Maria Hospital, Lisbon, Portugal	Professor João Eurico Fonseca	9
Azienda ospedaliera Maggiore della Carità, Novara, Italy	Dr Pier Paolo Sainaghi	8
University Hospital of Wales, Cardiff, UK	Professor Ernest Choy	6
Royal Victoria Infirmary, Newcastle, UK	Professor John Isaacs	6
Southampton General Hospital, Southampton, UK	Professor Christopher Edwards	6
Basildon University Hospital, Basildon, UK	Dr Nagui Gendi	6
Hospital Clínic de Barcelona, Barcelona, Spain	Dr Juan D Cañete	6
Southend University Hospital, Southend-on-Sea, UK	Professor Bhaskar Dasgupta	4
Chapel Allerton Hospital, Leeds, UK	Professor Maya Buch	4
Azienda Ospedaliero Universitaria di Cagliari, Cagliari, Italy	Professor Alberto Cauli	4
Homerton University Hospital, London, UK	Dr Piero Reynolds	3
Nuffield Orthopaedic Hospital, Oxford, UK	Professor Peter Taylor	3
Aintree University Hospital, Liverpool, UK	Professor Robert Moots	3
Manchester Royal Infirmary, Manchester, UK	Dr Pauline Ho	3
Guy's Hospital, London, UK	Dr Nora Ng	3
Fondazione IRCCS Policlinico San Matteo, Pavia, Italy	Professor Carlomaurizio Montecucco	2
Universitair Ziekenhuis Leuven, Leuven, Belgium	Professor Patrick Verschueren	1

TABLE 8 Additional demographics of patients

Patient characteristic	Total (n = 161)	Treatment group	
		Rituximab (n = 82)	Tocilizumab (n = 79)
Creatinine (µmol/l), median (IQR)	61 (53–71)	63 (53–73)	60 (54–68)
ALT (IU/l), median (IQR)	16 (12–22)	16 (12–21)	16 (12–23)
AST (IU/l), median (IQR)	19 (15–22)	19 (15–22)	18 (16–22)
Haemoglobin (g/l), median (IQR)	123 (111–132)	121 (109–131)	123 (112–132)
White blood cells (10^9/l), median (IQR)	8.1 (6.8–10.5)	8.0 (6.6–10.2)	8.5 (7.0–10.5)
Platelets (10^9/l), median (IQR)	303 (254–385)	302 (256–344)	304 (252–395)
Neutrophils (10^9/l), median (IQR)	5.70 (4.43–7.21)	5.70 (4.20–7.30)	5.60 (4.62–7.11)
Lymphocytes (10^9/l), median (IQR)	1.70 (1.33–2.30)	1.70 (1.20–2.30)	1.80 (1.40–2.40)
VAS: patient's GA, median (IQR)	72 (51–85)	71 (50–82)	74 (52–88)
VAS: physician's GA, median (IQR)	61 (48–78)	60 (49–80)	64 (46–77)
VAS: early-morning stiffness, median (IQR)	45 (20–100)	35 (20–100)	60 (20–100)
VAS: tiredness, median (IQR)	68 (50–83)	67 (45–79)	70 (50–87)
VAS: pain, median (IQR)	70 (45–86)	67 (48–84)	72 (43–87)
HAQ score, median (IQR)	1.75 (1.25–2.13)	1.75 (1.25–2.13)	1.75 (1.25–2.13)
FACIT score, median (IQR)	22 (15–32)	23 (15–32)	21 (13–34)

TABLE 8 Additional demographics of patients (*continued*)

Patient characteristic	Total (*n* = 161)	Treatment group	
		Rituximab (*n* = 82)	Tocilizumab (*n* = 79)
SF-36: physical functioning, median (IQR)	30 (15–45)	30 (10–49)	30 (15–45)
SF-36: role limitations (PH), median (IQR)	0 (0–25)	0 (0–25)	0 (0–0)
SF-36: role limitations (EP), median (IQR)	0 (0–67)	33 (0–92)	0 (0–67)
SF-36: energy fatigue, median (IQR)	35 (20–45)	35 (20–50)	30 (20–45)
SF-36: emotional well-being, mean (SD)	60 (20)	61 (19)	59 (21)
SF-36: social functioning, median (IQR)	38 (25–63)	38 (25–63)	50 (25–75)
SF-36: pain, median (IQR)	23 (13–45)	23 (23–45)	23 (10–45)
SF-36: general health, median (IQR)	35 (25–50)	35 (25–45)	35 (25–50)
Methotrexate, *n* (%)	161 (100)	82 (100)	79 (100)
Prednisolone, *n* (%)	90 (56)	44 (54)	46 (58)
Additional DMARDs, *n* (%)			
0	85 (53)	42 (51)	43 (54)
1	31 (19)	14 (17)	17 (22)
2	32 (20)	20 (24)	12 (15)
3 +	13 (8)	6 (7)	7 (9)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; EP, emotional problems; ER, erosions; GA, global assessment; JSN, joint space narrowing; PH, physical health.
a There were no significant differences among treatment groups at baseline.
b A total of eight patients used non-anti-TNF biologicals: seven patients used abatacept and one patient used 'vaccine RA TNF-K-006' for a clinical study.

TABLE 9 Additional secondary efficacy end-point analyses at 16 weeks: B-cell-poor (histological classification) pathotype subgroup

Variable	Least squares mean		Effect size (least squares mean difference) (95% CI)
	Change from baseline, mean (SD)		
	Rituximab group (*n* = 38)	Tocilizumab group (*n* = 41)	
CDAI	−12.11 (1.93)	−15.71 (1.86)	3.6 (−1.74 to 8.94)
DAS28(ESR)	−1.46 (0.22)	−2.56 (0.21)	1.1 (0.49 to 1.72)
DAS28(CRP)	−1.31 (0.22)	−1.96 (0.21)	0.66 (0.06 to 1.26)
HAQ	−0.26 (0.08)	−0.36 (0.08)	0.1 (−0.14 to 0.33)
FACIT	1.57 (1.13)	5.55 (1.12)	−3.98 (−7.16 to −0.81)
SF-36 PCS	4.05 (1.45)	7.29 (1.47)	−3.24 (−7.38 to 0.89)
SF-36 MCS	−0.67 (1.57)	2.13 (1.6)	−2.79 (−7.27 to 1.69)
Ultrasound ST 12max[a]	−0.17 (1.54)	0.35 (1.6)	−0.53 (−5.14 to 4.08)
Ultrasound PD 12max[a]	0.32 (1.11)	−1.07 (1.11)	1.39 (−2.05 to 4.83)

a 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 10 Additional secondary efficacy end-point analyses at 16 weeks: B-cell-poor (molecular analysis) pathotype subgroup

| Variable | Least squares mean | | Effect size (least squares mean difference) (95% CI) |
| | Change from baseline, mean (SD) | | |
	Rituximab group (n = 33)	Tocilizumab group (n = 32)	
CDAI	−10.67 (1.96)	−17.21 (1.93)	6.54 (1.03 to 12.04)
DAS28(ESR)	−1.24 (0.22)	−2.77 (0.22)	1.54 (0.91 to 2.16)
DAS28(CRP)	−1.12 (0.22)	−2.15 (0.21)	1.03 (0.42 to 1.64)
HAQ	−0.21 (0.08)	−0.26 (0.08)	0.05 (−0.18 to 0.28)
FACIT	2.04 (1.39)	5.14 (1.37)	−3.1 (−7.02 to 0.82)
SF-36 PCS	3.51 (1.54)	5.38 (1.54)	−1.87 (−6.21 to 2.47)
SF-36 MCS	0.67 (1.81)	4.9 (1.81)	−4.23 (−9.37 to 0.91)
Ultrasound ST 12max[a]	−1.25 (1.69)	2.23 (1.61)	−3.48 (−8.35 to 1.39)
Ultrasound PD 12max[a]	0.12 (1.11)	−1.09 (0.99)	1.21 (−1.96 to 4.38)

a 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 11 Additional secondary efficacy end-point analyses at 16 weeks: B-cell-rich (histological classification) pathotype subgroup

| Variable | Least squares mean | | Effect size (least squares mean difference) (95% CI) |
| | Change from baseline, mean (SD) | | |
	Rituximab group (n = 33)	Tocilizumab group (n = 31)	
CDAI	−13.21 (2.05)	−14.21 (2.11)	1.01 (−4.87 to 6.88)
DAS28(ESR)	−1.46 (0.22)	−2.57 (0.23)	1.1 (0.47 to 1.74)
DAS28(CRP)	−1.47 (0.2)	−2.03 (0.21)	0.56 (−0.02 to 1.14)
HAQ	−0.31 (0.1)	−0.42 (0.1)	0.11 (−0.16 to 0.39)
FACIT	8.48 (1.9)	7.75 (2)	0.73 (−4.81 to 6.27)
SF-36 PCS	7.01 (1.95)	8.54 (2.05)	−1.53 (−7.22 to 4.15)
SF-36 MCS	5.37 (2.23)	3.32 (2.35)	2.05 (−4.45 to 8.55)
Ultrasound ST 12max[a]	−0.84 (1.13)	−2.46 (1.17)	1.62 (−1.74 to 4.99)
Ultrasound PD 12max[a]	−1.43 (1.04)	−2.81 (1.12)	1.38 (−1.81 to 4.57)

a 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 12 Additional secondary efficacy end-point analyses at 16 weeks: B-cell-rich (molecular analysis) pathotype subgroup

| | Least squares mean | | |
| | Change from baseline, mean (SD) | | |
Variable	**Rituximab group (n = 30)**	**Tocilizumab group (n = 29)**	**Effect size (least squares mean difference) (95% CI)**
CDAI	−14.61 (2)	−13.43 (2.13)	−1.18 (−7.02 to 4.66)
DAS28(ESR)	−1.65 (0.21)	−2.41 (0.22)	0.76 (0.16 to 1.37)
DAS28(CRP)	−1.58 (0.2)	−1.91 (0.21)	0.33 (−0.25 to 0.91)
HAQ	−0.28 (0.08)	−0.54 (0.09)	0.26 (0.02 to 0.51)
FACIT	6.18 (1.59)	8.23 (1.79)	−2.05 (−6.86 to 2.75)
SF-36 PCS	6.27 (1.75)	10.74 (1.97)	−4.47 (−9.75 to 0.81)
SF-36 MCS	3.03 (2.1)	4.06 (2.37)	−1.03 (−7.39 to 5.34)
Ultrasound ST 12max[a]	−0.4 (1.11)	−4.05 (1.34)	3.64 (0.03 to 7.25)
Ultrasound PD 12max[a]	−0.11 (1.22)	−2.63 (1.41)	2.52 (−1.45 to 6.48)

a 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 13 Additional secondary efficacy end-point analyses at 24, 36 and 48 weeks: B-cell-poor (histological classification) pathotype subgroup

Variable	Week 24			Week 36			Week 48		
	Rituximab group (N = 21), n (%)/least squares mean change (SD)	Tocilizumab group (N = 27), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 20), n (%)/least squares mean change (SD)	Tocilizumab group (N = 22), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 17), n (%)/least squares mean change (SD)	Tocilizumab group (N = 21), n (%)/least squares mean change (SD)	Effect size (95% CI)
Binary response: effect size expressed as RR									
CDAI improvement of ≥ 50%	11 (52.4%)	21 (80.8%)	1.54 (0.98 to 2.42)	14 (70%)	16 (72.7%)	1.04 (0.71 to 1.53)	15 (88.2%)	16 (80%)	0.91 (0.69 to 1.2)
CDAI-MTR	5 (23.8%)	15 (55.6%)	2.33 (1.01 to 5.38)	9 (45%)	12 (54.5%)	1.21 (0.65 to 2.25)	11 (64.7%)	14 (66.7%)	1.03 (0.65 to 1.64)
CDAI < 10.1	6 (28.6%)	16 (59.3%)	2.07 (0.98 to 4.37)	9 (45%)	13 (59.1%)	1.31 (0.72 to 2.38)	11 (64.7%)	14 (66.7%)	1.03 (0.65 to 1.64)
EULAR DAS28(ESR)[a]	17 (81%)	23 (85.2%)	1.05 (0.81 to 1.37)	17 (85%)	22 (100%)	1.18 (0.98 to 1.41)	16 (94.1%)	21 (100%)	1.06 (0.94 to 1.2)
DAS28(ESR) of ≤ 3.2	7 (33.3%)	17 (63%)	1.89 (0.97 to 3.69)	8 (40%)	14 (63.6%)	1.59 (0.85 to 2.97)	8 (47.1%)	16 (76.2%)	1.62 (0.93 to 2.83)
DAS28(ESR) of ≤ 2.6	3 (14.3%)	14 (51.9%)	3.63 (1.2 to 11)	5 (25%)	10 (45.5%)	1.82 (0.75 to 4.41)	3 (17.6%)	13 (61.9%)	3.51 (1.19 to 10.33)
EULAR DAS28(CRP)[a]	16 (76.2%)	22 (81.5%)	1.07 (0.79 to 1.44)	18 (90%)	22 (100%)	1.11 (0.96 to 1.29)	15 (88.2%)	20 (95.2%)	1.08 (0.89 to 1.32)
DAS28(CRP) of ≤ 3.2	9 (42.9%)	17 (63%)	1.47 (0.83 to 2.6)	11 (55%)	14 (63.6%)	1.16 (0.7 to 1.92)	9 (52.9%)	13 (61.9%)	1.17 (0.67 to 2.05)
DAS28(CRP) of ≤ 2.6	3 (14.3%)	9 (33.3%)	2.33 (0.72 to 7.56)	3 (15%)	9 (40.9%)	2.73 (0.86 to 8.68)	5 (29.4%)	11 (52.4%)	1.78 (0.77 to 4.13)
Change from baseline: effect size expressed as least squares mean difference									
CDAI	−16.39 (1.93)	−21.03 (1.7)	4.64 (−0.54 to 9.83)	−17.89 (1.59)	−22.05 (1.52)	4.16 (−0.31 to 8.62)	−20.03 (1.55)	−22.79 (1.4)	2.76 (−1.48 to 7.01)
DAS28(ESR)	−1.79 (0.27)	−2.92 (0.24)	1.14 (0.42 to 1.86)	−2.29 (0.24)	−3.13 (0.22)	0.85 (0.19 to 1.5)	−2.43 (0.23)	−3.26 (0.21)	0.83 (0.2 to 1.45)
DAS28(CRP)	−1.6 (0.26)	−2.33 (0.23)	0.72 (0.03 to 1.41)	−1.98 (0.19)	−2.6 (0.19)	0.62 (0.07 to 1.16)	−2.03 (0.23)	−2.64 (0.21)	0.62 (−0.02 to 1.25)
HAQ	−0.25 (0.14)	−0.45 (0.12)	0.2 (−0.17 to 0.57)	−0.35 (0.13)	−0.65 (0.12)	0.3 (−0.07 to 0.66)	−0.41 (0.14)	−0.65 (0.13)	0.24 (−0.14 to 0.63)
FACIT	1.95 (1.8)	6.6 (1.65)	−4.65 (−9.59 to 0.3)	5.08 (1.85)	7.84 (1.76)	−2.76 (−7.96 to 2.43)	5.58 (2.19)	7.11 (2.02)	−1.52 (−7.59 to 4.55)
SF-36 PCS	7.7 (2.08)	8.73 (1.94)	−1.03 (−6.81 to 4.74)	7.96 (2.36)	13.29 (2.3)	−5.33 (−12.07 to 1.4)	8.6 (2.53)	16.18 (2.39)	−7.58 (−14.68 to −0.47)
SF-36 MCS	−2.66 (2.86)	3.91 (2.67)	−6.57 (−14.48 to 1.33)	−0.98 (2.2)	3.82 (2.15)	−4.79 (−11.02 to 1.43)	1.54 (2.69)	4.7 (2.54)	−3.15 (−10.68 to 4.37)
SHSS: total score	0.09 (0.58)	1.2 (0.43)	−1.12 (−2.61 to 0.38)				0.02 (0.69)	1.15 (0.56)	−1.14 (−3.02 to 0.75)
SHSS: ER score	0.13 (0.24)	0.39 (0.18)	−0.26 (−0.89 to 0.37)				0.08 (0.29)	0.32 (0.24)	−0.25 (−1.04 to 0.55)
SHSS: JSN score	−0.08 (0.37)	0.61 (0.28)	−0.69 (−1.65 to 0.28)				−0.12 (0.44)	0.58 (0.35)	−0.7 (−1.89 to 0.5)
Ultrasound ST 12max[b]							−2.16 (2.43)	2.62 (2.88)	−4.78 (−13.33 to 3.77)
Ultrasound PD 12max[b]							−4.9 (1.54)	−6.46 (1.79)	1.56 (−5.16 to 8.28)

a Moderate/good EULAR response.
b 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 14 Secondary efficacy end-point analyses including patients switching to alternative IMP at week 16 in the B-cell-poor pathotype subgroup

Variable	Week 16			Week 24			Week 36		
	Rituximab group (N = 45), n (%)/least squares mean change (SD)	Tocilizumab group (N = 55), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 26), n (%)/least squares mean change (SD)	Tocilizumab group (N = 40), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 25), n (%)/least squares mean change (SD)	Tocilizumab group (N = 35), n (%)/least squares mean change (SD)	Effect size (95% CI)
Binary response: effect size expressed as RR									
CDAI improvement of ≥ 50%	18 (40%)	28 (50.9%)	1.27 (0.82 to 1.98)	12 (46.2%)	28 (70%)	1.52 (0.96 to 2.41)	16 (64%)	24 (68.6%)	1.07 (0.74 to 1.55)
CDAI-MTR	9 (19.6%)	24 (43.6%)	2.23 (1.15 to 4.31)	5 (18.5%)	19 (46.3%)	2.5 (1.06 to 5.9)	9 (34.6%)	17 (48.6%)	1.4 (0.75 to 2.63)
CDAI < 10.1	11 (23.9%)	24 (43.6%)	1.82 (1 to 3.31)	6 (22.2%)	20 (48.8%)	2.2 (1.01 to 4.75)	9 (34.6%)	20 (57.1%)	1.65 (0.9 to 3.01)
EULAR DAS28(ESR)[a]	28 (60.9%)	48 (87.3%)	1.43 (1.11 to 1.85)	20 (74.1%)	37 (90.2%)	1.22 (0.95 to 1.56)	19 (73.1%)	33 (94.3%)	1.29 (1.01 to 1.65)
DAS28(ESR) of ≤ 3.2	10 (21.7%)	23 (41.8%)	1.92 (1.02 to 3.61)	7 (25.9%)	28 (68.3%)	2.63 (1.35 to 5.15)	8 (30.8%)	22 (62.9%)	2.04 (1.09 to 3.84)
DAS28(ESR) of ≤ 2.6	6 (13%)	20 (36.4%)	2.79 (1.22 to 6.36)	3 (11.1%)	21 (51.2%)	4.61 (1.52 to 13.96)	5 (19.2%)	16 (45.7%)	2.38 (1 to 5.65)
EULAR DAS28(CRP)[a]	27 (58.7%)	42 (76.4%)	1.3 (0.98 to 1.73)	19 (70.4%)	33 (80.5%)	1.14 (0.86 to 1.52)	21 (80.8%)	30 (85.7%)	1.06 (0.84 to 1.34)
DAS28(CRP) of ≤ 3.2	12 (26.1%)	24 (43.6%)	1.67 (0.94 to 2.96)	9 (33.3%)	25 (61%)	1.83 (1.02 to 3.29)	11 (42.3%)	22 (62.9%)	1.49 (0.89 to 2.49)
DAS28(CRP) of ≤ 2.6	7 (15.2%)	17 (30.9%)	2.03 (0.92 to 4.47)	3 (11.1%)	13 (31.7%)	2.85 (0.9 to 9.08)	3 (11.5%)	14 (40%)	3.47 (1.11 to 10.83)
Change from baseline: effect size expressed as least squares mean difference									
CDAI	−9.86 (1.7)	−12.83 (1.49)	2.97 (−1.51 to 7.45)	−11.84 (2.07)	−18.28 (1.65)	6.44 (1.16 to 11.72)	−16.76 (2.02)	−20.41 (1.74)	3.65 (−1.73 to 9.03)
DAS28(ESR)	−1.26 (0.2)	−2.14 (0.17)	0.88 (0.37 to 1.4)	−1.27 (0.23)	−2.88 (0.18)	1.61 (1.03 to 2.18)	−1.95 (0.26)	−3.1 (0.23)	1.15 (0.45 to 1.85)
DAS28(CRP)	−1.08 (0.18)	−1.61 (0.16)	0.53 (0.05 to 1)	−1.17 (0.21)	−2.14 (0.17)	0.97 (0.43 to 1.51)	−1.7 (0.22)	−2.45 (0.19)	0.75 (0.16 to 1.34)
HAQ	−0.21 (0.08)	−0.36 (0.07)	0.14 (−0.06 to 0.35)	−0.2 (0.11)	−0.45 (0.1)	0.25 (−0.05 to 0.55)	−0.3 (0.12)	−0.6 (0.1)	0.3 (−0.01 to 0.61)
FACIT	1.37 (1.05)	5.19 (0.97)	−3.82 (−6.67 to −0.98)	1.36 (1.5)	6.01 (1.32)	−4.65 (−8.7 to −0.61)	5.18 (1.64)	7.7 (1.38)	−2.53 (−6.9 to 1.84)
SF-36 PCS	2.81 (1.43)	7.73 (1.34)	−4.92 (−8.84 to −1.01)	6.79 (1.98)	9.3 (1.75)	−2.52 (−7.82 to 2.78)	6.65 (2.17)	12.81 (1.85)	−6.16 (−11.92 to −0.41)
SF-36 MCS	−0.96 (1.54)	2.28 (1.44)	−3.24 (−7.43 to 0.95)	−3.36 (2.35)	4.26 (2.08)	−7.61 (−13.89 to −1.34)	−1.74 (1.99)	2.16 (1.69)	−3.9 (−9.16 to 1.36)
Total SHSS				0.08 (0.26)	0.79 (0.21)	−0.7 (−1.38 to −0.03)			
SHSS: ER score				0.1 (0.12)	0.3 (0.09)	−0.2 (−0.5 to 0.1)			
SHSS: JSN score				−0.03 (0.17)	0.52 (0.14)	−0.54 (−0.99 to −0.09)			
Ultrasound ST 12max[b]	0.1 (1.4)	−0.34 (1.4)	0.44 (−3.6 to 4.48)						
Ultrasound PD 12max[b]	−0.22 (0.95)	−0.4 (1)	0.18 (−2.72 to 3.09)						

a Moderate/good EULAR response.
b 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 15 Per-protocol analyses of secondary efficacy end points at 16, 24, 36 and 48 weeks in the B-cell-poor pathotype

Variable	Week 16			Week 24			Week 36			Week 48		
	Rituximab group (N = 37), n (%)/least squares mean change (SD)	Tocilizumab group (N = 30), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 20), n (%)/least squares mean change (SD)	Tocilizumab group (N = 23), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 19), n (%)/least squares mean change (SD)	Tocilizumab group (N = 21), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 16), n (%)/least squares mean change (SD)	Tocilizumab group (N = 20), n (%)/least squares mean change (SD)	Effect size (95% CI)
Binary response: effect size expressed as RR												
CDAI improvement ≥ 50%	16 (43.2%)	21 (70%)	1.62 (1.05 to 2.51)	10 (50%)	20 (87%)	1.74 (1.09 to 2.77)	13 (68.4%)	16 (76.2%)	1.11 (0.76 to 1.64)	14 (87.5%)	16 (84.2%)	0.96 (0.74 to 1.26)
CDAI-MTR	9 (24.3%)	17 (56.7%)	2.33 (1.22 to 4.46)	5 (25%)	13 (56.5%)	2.26 (0.98 to 5.23)	9 (47.4%)	12 (57.1%)	1.21 (0.66 to 2.2)	11 (68.8%)	14 (70%)	1.02 (0.66 to 1.58)
CDAI < 10.1	11 (29.7%)	17 (56.7%)	1.91 (1.06 to 3.42)	6 (30%)	13 (56.5%)	1.88 (0.88 to 4.03)	9 (47.4%)	12 (57.1%)	1.21 (0.66 to 2.2)	11 (68.8%)	14 (70%)	1.02 (0.66 to 1.58)
EULAR DAS28 (ESR)[a]	31 (83.8%)	24 (80%)	0.95 (0.76 to 1.2)	14 (70%)	19 (82.6%)	1.18 (0.84 to 1.66)	16 (84.2%)	19 (90.5%)	1.07 (0.85 to 1.36)	14 (87.5%)	18 (90%)	1.03 (0.81 to 1.3)
DAS28(ESR) ≤ 3.2	10 (27%)	14 (46.7%)	1.73 (0.9 to 3.32)	7 (35%)	14 (60.9%)	1.74 (0.88 to 3.44)	7 (36.8%)	13 (61.9%)	1.68 (0.85 to 3.31)	8 (50%)	15 (75%)	1.5 (0.86 to 2.6)
DAS28(ESR) ≤ 2.6	16 (43.2%)	8 (26.7%)	0.62 (0.31 to 1.24)	5 (25%)	7 (30.4%)	1.22 (0.46 to 3.24)	6 (31.6%)	6 (28.6%)	0.9 (0.35 to 2.33)	4 (25%)	4 (20%)	0.8 (0.24 to 2.71)
EULAR DAS28 (CRP)[a]	29 (78.4%)	24 (80%)	1.02 (0.8 to 1.31)	17 (85%)	20 (87%)	1.02 (0.8 to 1.3)	15 (78.9%)	17 (81%)	1.03 (0.75 to 1.4)	11 (68.8%)	16 (80%)	1.16 (0.78 to 1.73)
DAS28(CRP) ≤ 3.2	12 (32.4%)	16 (53.3%)	1.64 (0.93 to 2.92)	9 (45%)	14 (60.9%)	1.35 (0.75 to 2.43)	10 (52.6%)	13 (61.9%)	1.18 (0.68 to 2.02)	9 (56.2%)	13 (65%)	1.16 (0.67 to 1.98)
DAS28(CRP) ≤ 2.6	10 (27%)	9 (30%)	1.11 (0.52 to 2.38)	3 (15%)	8 (34.8%)	2.32 (0.71 to 7.58)	2 (10.5%)	7 (33.3%)	3.17 (0.75 to 13.42)	2 (12.5%)	7 (35%)	2.8 (0.67 to 11.67)

Variable	Week 16			Week 24			Week 36			Week 48		
	Rituximab group (N = 37), n (%)/least squares mean change (SD)	Tocilizumab group (N = 30), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 20), n (%)/least squares mean change (SD)	Tocilizumab group (N = 23), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 19), n (%)/least squares mean change (SD)	Tocilizumab group (N = 21), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 16), n (%)/least squares mean change (SD)	Tocilizumab group (N = 20), n (%)/least squares mean change (SD)	Effect size (95% CI)
Change from baseline: effect size expressed as least squares mean difference												
CDAI	−11.93 (1.92)	−17.88 (2.13)	5.95 (0.21 to 11.7)	−16.29 (1.94)	−21.46 (1.81)	5.17 (−0.22 to 10.55)	−17.28 (1.65)	−21.94 (1.57)	4.67 (0.05 to 9.28)	−20.27 (1.56)	−22.87 (1.39)	2.61 (−1.64 to 6.86)
DAS28(ESR)	−1.47 (0.21)	−2.85 (0.24)	1.38 (0.74 to 2.02)	−1.79 (0.28)	−3.04 (0.26)	1.24 (0.46 to 2.03)	−2.19 (0.24)	−3.2 (0.22)	1.01 (0.34 to 1.67)	−2.49 (0.24)	−3.28 (0.21)	0.79 (0.14 to 1.45)
DAS28(CRP)	−1.3 (0.21)	−2.2 (0.23)	0.9 (0.27 to 1.52)	−1.59 (0.26)	−2.43 (0.24)	0.84 (0.12 to 1.57)	−1.9 (0.2)	−2.63 (0.19)	0.72 (0.18 to 1.27)	−2.08 (0.24)	−2.71 (0.21)	0.62 (−0.02 to 1.27)
HAQ	−0.25 (0.08)	−0.4 (0.09)	0.14 (−0.11 to 0.39)	−0.27 (0.14)	−0.46 (0.13)	0.19 (−0.2 to 0.58)	−0.37 (0.13)	−0.66 (0.13)	0.29 (−0.08 to 0.66)	−0.45 (0.14)	−0.66 (0.13)	0.21 (−0.18 to 0.6)
FACIT	1.75 (1.1)	5.56 (1.18)	−3.81 (−7.04 to −0.58)	2.3 (1.78)	7.4 (1.66)	−5.1 (−10.04 to −0.16)	5.39 (1.91)	8.03 (1.82)	−2.65 (−8.01 to 2.71)	6.28 (2.26)	7.03 (2.07)	−0.75 (−7 to 5.49)
SF-36 PCS	4.25 (1.5)	8.25 (1.67)	−4 (−8.49 to 0.49)	8.17 (2.15)	8.74 (2.09)	−0.57 (−6.67 to 5.53)	8.58 (2.43)	13.34 (2.37)	−4.76 (−11.7 to 2.18)	9.41 (2.49)	17.1 (2.34)	−7.68 (−14.66 to −0.7)
SF-36 MCS	−0.69 (1.57)	2.65 (1.75)	−3.35 (−8.07 to 1.37)	−3.01 (2.84)	2.74 (2.77)	−5.75 (−13.8 to 2.3)	−1.21 (2.31)	3.23 (2.25)	−4.44 (−10.99 to 2.11)	1.1 (2.86)	4.35 (2.7)	−3.25 (−11.3 to 4.8)
Total SHSS				0.17 (0.61)	1.42 (0.51)	−1.25 (−2.93 to 0.44)				0.02 (0.7)	1.26 (0.6)	−1.24 (−3.21 to 0.73)
SHSS: ER score				0.18 (0.26)	0.45 (0.22)	−0.27 (−0.99 to 0.45)				0.09 (0.3)	0.35 (0.26)	−0.26 (−1.09 to 0.58)
SHSS: JSN score				−0.05 (0.4)	0.73 (0.33)	−0.78 (−1.87 to 0.31)				−0.12 (0.44)	0.63 (0.38)	−0.76 (−2 to 0.49)
Ultrasound ST 12max[b]	−0.42 (1.54)	−1.28 (1.85)	0.87 (−4.17 to 5.9)							−2.85 (2.36)	3.73 (3.13)	−6.58 (−15.66 to 2.49)
Ultrasound PD 12max[b]	0.12 (1.11)	−2.39 (1.41)	2.51 (−1.49 to 6.51)							−5.18 (1.44)	−10.14 (2.24)	4.96 (−4.5 to 14.41)

a Moderate/good EULAR response.
b 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 16 Secondary efficacy end-point analyses in the B-cell-rich pathotype subgroup at 24, 36 and 48 weeks

Variable	Week 24			Week 36			Week 48		
	Rituximab group (N = 18), n (%)/least squares mean change (SD)	Tocilizumab group (N = 20), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 17), n (%)/least squares mean change (SD)	Tocilizumab group (N = 16), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 17), n (%)/least squares mean change (SD)	Tocilizumab group (N = 16), n (%)/least squares mean change (SD)	Effect size (95% CI)
Binary response: effect size expressed as RR									
CDAI improvement ≥ 50%	10 (55.6%)	14 (73.7%)	1.33 (0.81 to 2.17)	10 (66.7%)	14 (93.3%)	1.4 (0.95 to 2.05)	9 (52.9%)	13 (81.2%)	1.53 (0.93 to 2.55)
CDAI-MTR	5 (27.8%)	10 (50%)	1.8 (0.76 to 4.27)	7 (41.2%)	11 (68.8%)	1.67 (0.87 to 3.22)	6 (35.3%)	12 (75%)	2.12 (1.05 to 4.29)
CDAI < 10.1	5 (27.8%)	10 (50%)	1.8 (0.76 to 4.27)	8 (47.1%)	11 (68.8%)	1.46 (0.8 to 2.67)	7 (41.2%)	12 (75%)	1.82 (0.97 to 3.44)
EULAR DAS28(ESR)[a]	14 (77.8%)	18 (90%)	1.16 (0.87 to 1.54)	15 (88.2%)	16 (100%)	1.13 (0.95 to 1.35)	15 (88.2%)	14 (87.5%)	0.99 (0.77 to 1.28)
DAS28(ESR) ≤ 3.2	6 (33.3%)	13 (65%)	1.95 (0.94 to 4.04)	9 (52.9%)	11 (68.8%)	1.3 (0.74 to 2.27)	9 (52.9%)	12 (75%)	1.42 (0.83 to 2.41)
DAS28(ESR) ≤ 2.6	2 (11.1%)	9 (45%)	4.05 (1.01 to 16.32)	3 (17.6%)	10 (62.5%)	3.54 (1.19 to 10.58)	4 (23.5%)	12 (75%)	3.19 (1.29 to 7.86)
EULAR DAS28(CRP)[a]	14 (77.8%)	16 (80%)	1.03 (0.74 to 1.43)	13 (76.5%)	15 (93.8%)	1.23 (0.92 to 1.64)	12 (70.6%)	14 (87.5%)	1.24 (0.87 to 1.77)
DAS28(CRP) ≤ 3.2	10 (55.6%)	13 (65%)	1.17 (0.69 to 1.98)	7 (41.2%)	12 (75%)	1.82 (0.97 to 3.44)	7 (41.2%)	12 (75%)	1.82 (0.97 to 3.44)
DAS28(CRP) ≤ 2.6	2 (11.1%)	9 (45%)	4.05 (1.01 to 16.32)	4 (23.5%)	11 (68.8%)	2.92 (1.17 to 7.32)	5 (29.4%)	9 (56.2%)	1.91 (0.81 to 4.49)
Change from baseline: effect size expressed as least squares mean difference									
CDAI	−13.65 (2.27)	−17.23 (2.15)	3.59 (−2.76 to 9.93)	−16.8 (1.78)	−21.81 (1.83)	5.02 (−0.2 to 10.23)	−15.61 (2.06)	−22.51 (2.12)	6.9 (0.87 to 12.93)
DAS28(ESR)	−1.87 (0.29)	−2.82 (0.28)	0.94 (0.12 to 1.76)	−2.26 (0.25)	−3.39 (0.26)	1.12 (0.38 to 1.87)	−1.98 (0.34)	−3.39 (0.35)	1.41 (0.41 to 2.41)
DAS28(CRP)	−1.64 (0.29)	−2.15 (0.27)	0.52 (−0.28 to 1.32)	−1.88 (0.22)	−2.72 (0.22)	0.84 (0.2 to 1.48)	−1.76 (0.26)	−2.73 (0.27)	0.97 (0.22 to 1.73)
HAQ	−0.47 (0.11)	−0.38 (0.11)	−0.08 (−0.4 to 0.23)	−0.59 (0.14)	−0.55 (0.14)	−0.04 (−0.45 to 0.37)	−0.58 (0.16)	−0.64 (0.16)	0.06 (−0.4 to 0.52)
FACIT	10.31 (2.1)	6.18 (2.04)	4.12 (−1.98 to 10.22)	13.98 (2.11)	8.09 (2.11)	5.89 (−0.29 to 12.08)	11.41 (2.77)	8.44 (2.86)	2.97 (−5.26 to 11.2)
SF-36 PCS	11.71 (2.41)	10.05 (2.34)	1.66 (−5.22 to 8.53)	11.2 (2.95)	10.16 (2.95)	1.05 (−7.52 to 9.62)	10.22 (2.98)	13.27 (3.07)	−3.04 (−11.78 to 5.69)
SF-36 MCS	2.53 (2.69)	5.7 (2.61)	−3.18 (−10.89 to 4.54)	7.08 (2.36)	3.25 (2.36)	3.83 (−3.08 to 10.74)	7.75 (2.68)	3.37 (2.77)	4.39 (−3.55 to 12.32)
Total SHSS	0.36 (0.21)	0.31 (0.19)	0.06 (−0.52 to 0.64)				0.36 (0.2)	0.25 (0.21)	0.11 (−0.51 to 0.73)
SHSS: ER score	0.36 (0.16)	0.04 (0.15)	0.33 (−0.14 to 0.79)				0.36 (0.18)	0.05 (0.19)	0.31 (−0.23 to 0.85)
SHSS: JSN score	0.23 (0.19)	0.23 (0.17)	0 (−0.54 to 0.54)				0.22 (0.2)	0.2 (0.21)	0.02 (−0.58 to 0.62)
Ultrasound ST 12max[b]							−2.32 (1.63)	−3.35 (1.63)	1.03 (−3.9 to 5.96)
Ultrasound PD 12max[b]							−5.42 (0.98)	−5.8 (0.98)	0.37 (−2.62 to 3.36)

a Moderate/good EULAR response.
b 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 17 Secondary efficacy end-point analyses including patients switching to alternative IMP at week 16. Outcomes were assessed at 16, 24 and 32 weeks post IMP initiation: B-cell-rich pathotype subgroup

Variable	Week 16			Week 24			Week 36		
	Rituximab group (N = 40), n (%)/least squares mean change (SD)	Tocilizumab group (N = 44), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 24), n (%)/least squares mean change (SD)	Tocilizumab group (N = 33), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 23), n (%)/least squares mean change (SD)	Tocilizumab group (N = 29), n (%)/least squares mean change (SD)	Effect size (95% CI)
Binary response: effect size expressed as RR									
CDAI improvement ≥ 50%	16 (40%)	22 (50%)	1.25 (0.77 to 2.02)	14 (58.3%)	22 (68.8%)	1.18 (0.78 to 1.78)	14 (66.7%)	21 (75%)	1.12 (0.78 to 1.63)
CDAI-MTR	7 (17.5%)	14 (31.8%)	1.82 (0.82 to 4.05)	7 (29.2%)	15 (45.5%)	1.56 (0.75 to 3.22)	8 (34.8%)	15 (51.7%)	1.49 (0.77 to 2.88)
CDAI < 10.1	9 (22.5%)	15 (34.1%)	1.52 (0.75 to 3.07)	7 (29.2%)	15 (45.5%)	1.56 (0.75 to 3.22)	9 (39.1%)	15 (51.7%)	1.32 (0.71 to 2.46)
EULAR DAS28(ESR)[a]	28 (70%)	39 (88.6%)	1.27 (1.01 to 1.59)	20 (83.3%)	29 (87.9%)	1.05 (0.85 to 1.31)	20 (87%)	28 (96.6%)	1.11 (0.93 to 1.32)
DAS28(ESR)≤ 3.2	10 (25%)	17 (38.6%)	1.55 (0.8 to 2.97)	8 (33.3%)	17 (51.5%)	1.55 (0.8 to 2.98)	10 (43.5%)	17 (58.6%)	1.35 (0.77 to 2.35)
DAS28(ESR)≤ 2.6	4 (10%)	14 (31.8%)	3.18 (1.14 to 8.87)	2 (8.3%)	13 (39.4%)	4.73 (1.17 to 19.03)	3 (13%)	13 (44.8%)	3.44 (1.11 to 10.64)
EULAR DAS28(CRP)[a]	28 (70%)	37 (84.1%)	1.2 (0.94 to 1.53)	19 (79.2%)	27 (81.8%)	1.03 (0.8 to 1.34)	19 (82.6%)	25 (86.2%)	1.04 (0.82 to 1.32)
DAS28(CRP)≤ 3.2	14 (35%)	16 (36.4%)	1.04 (0.58 to 1.85)	12 (50%)	17 (51.5%)	1.03 (0.61 to 1.73)	8 (34.8%)	17 (58.6%)	1.69 (0.89 to 3.19)
DAS28(CRP)≤ 2.6	6 (15%)	12 (27.3%)	1.82 (0.75 to 4.39)	4 (16.7%)	12 (36.4%)	2.18 (0.8 to 5.94)	5 (21.7%)	14 (48.3%)	2.22 (0.94 to 5.26)
Change from baseline: effect size expressed as least squares mean difference									
CDAI	−12.24 (1.81)	−13.08 (1.69)	0.84 (−4.07 to 5.75)	−17.38 (2.01)	−17.35 (1.65)	−0.03 (−5.22 to 5.15)	−18.79 (2.24)	−18.74 (2)	−0.06 (−6.1 to 5.99)
DAS28(ESR)	−1.5 (0.21)	−2.26 (0.19)	0.76 (0.19 to 1.32)	−1.95 (0.24)	−2.76 (0.2)	0.81 (0.18 to 1.44)	−2.2 (0.27)	−3 (0.24)	0.8 (0.08 to 1.52)
DAS28(CRP)	−1.49 (0.19)	−1.76 (0.17)	0.27 (−0.23 to 0.78)	−1.96 (0.23)	−2.12 (0.19)	0.16 (−0.44 to 0.76)	−2.03 (0.25)	−2.37 (0.22)	0.34 (−0.32 to 1)
HAQ	−0.33 (0.09)	−0.33 (0.08)	0 (−0.24 to 0.24)	−0.38 (0.1)	−0.3 (0.09)	−0.08 (−0.35 to 0.19)	−0.49 (0.12)	−0.34 (0.11)	−0.15 (−0.48 to 0.18)
FACIT	8.29 (1.74)	8.02 (1.63)	0.27 (−4.49 to 5.03)	8.93 (1.9)	7.25 (1.7)	1.68 (−3.48 to 6.85)	10.99 (2.14)	7.11 (1.85)	3.88 (−1.85 to 9.62)
SF-36 PCS	6.58 (1.85)	5.72 (1.7)	0.86 (−4.15 to 5.87)	10.59 (2.06)	6.74 (1.85)	3.84 (−1.72 to 9.4)	8.51 (2.44)	7.76 (2.11)	0.75 (−5.75 to 7.25)
SF-36 MCS	5.38 (2.19)	4.85 (2.02)	0.53 (−5.43 to 6.49)	2.5 (2.15)	7.93 (1.93)	−5.43 (−11.25 to 0.39)	6.03 (2.41)	5.48 (2.08)	0.55 (−5.9 to 7.01)
Total SHSS				0.53 (0.23)	0.46 (0.21)	0.07 (−0.57 to 0.7)			
SHSS: ER score				0.23 (0.11)	0.1 (0.1)	0.12 (−0.18 to 0.43)			
SHSS: JSN score				0.32 (0.16)	0.3 (0.14)	0.02 (−0.41 to 0.46)			
Ultrasound ST 12max[b]	−1.88 (1.04)	−2.16 (1.02)	0.28 (−2.68 to 3.24)						
Ultrasound PD 12max[b]	−0.96 (0.95)	−1.88 (0.95)	0.92 (−1.81 to 3.65)						

a Moderate/good EULAR response.
b 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 18 Per-protocol analyses of secondary efficacy end points in the B-cell-rich pathotype subgroup at 16, 24, 36 and 48 weeks

	Week 16			Week 24			Week 36			Week 48		
Variable	Rituximab group (N = 31), n (%)/least squares mean change (SD)	Tocilizumab group (N = 23), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 17), n (%)/least squares mean change (SD)	Tocilizumab group (N = 16), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 16), n (%)/least squares mean change (SD)	Tocilizumab group (N = 13), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 16), n (%)/least squares mean change (SD)	Tocilizumab group (N = 13), n (%)/least squares mean change (SD)	Effect size (95% CI)
Binary response: effect size expressed as RR												
CDAI improvement ≥ 50%	12 (38.7%)	12 (52.2%)	1.35 (0.75 to 2.43)	9 (52.9%)	12 (75%)	1.42 (0.83 to 2.41)	9 (64.3%)	12 (92.3%)	1.44 (0.94 to 2.19)	8 (50%)	10 (76.9%)	1.54 (0.87 to 2.73)
CDAI-MTR	5 (16.1%)	7 (30.4%)	1.89 (0.69 to 5.2)	5 (29.4%)	7 (43.8%)	1.49 (0.59 to 3.74)	6 (37.5%)	9 (69.2%)	1.85 (0.89 to 3.83)	6 (37.5%)	9 (69.2%)	1.85 (0.89 to 3.83)
CDAI < 10.1	7 (22.6%)	8 (34.8%)	1.54 (0.65 to 3.64)	5 (29.4%)	7 (43.8%)	1.49 (0.59 to 3.74)	7 (43.8%)	9 (69.2%)	1.58 (0.82 to 3.07)	7 (43.8%)	9 (69.2%)	1.58 (0.82 to 3.07)
EULAR DAS28(ESR)[a]	24 (77.4%)	22 (95.7%)	1.24 (1 to 1.52)	15 (88.2%)	13 (81.2%)	0.92 (0.69 to 1.23)	15 (93.8%)	12 (92.3%)	0.98 (0.8 to 1.2)	15 (93.8%)	11 (84.6%)	0.9 (0.69 to 1.18)
DAS28(ESR) ≤ 3.2	8 (25.8%)	11 (47.8%)	1.85 (0.89 to 3.86)	6 (35.3%)	10 (62.5%)	1.77 (0.84 to 3.74)	9 (56.2%)	9 (69.2%)	1.23 (0.7 to 2.16)	9 (56.2%)	9 (69.2%)	1.23 (0.7 to 2.16)
DAS28(ESR) ≤ 2.6	6 (19.4%)	10 (43.5%)	2.25 (0.95 to 5.29)	3 (17.6%)	6 (37.5%)	2.12 (0.64 to 7.1)	4 (25%)	4 (30.8%)	1.23 (0.38 to 3.99)	6 (37.5%)	7 (53.8%)	1.44 (0.64 to 3.22)
EULAR DAS28(CRP)[a]	24 (77.4%)	18 (78.3%)	1.01 (0.76 to 1.35)	12 (70.6%)	14 (87.5%)	1.24 (0.87 to 1.77)	10 (62.5%)	9 (69.2%)	1.11 (0.66 to 1.87)	12 (75%)	12 (92.3%)	1.23 (0.89 to 1.7)
DAS28(CRP) ≤ 3.2	10 (32.3%)	10 (43.5%)	1.35 (0.68 to 2.69)	10 (58.8%)	10 (62.5%)	1.06 (0.61 to 1.84)	6 (37.5%)	10 (76.9%)	2.05 (1.02 to 4.13)	7 (43.8%)	9 (69.2%)	1.58 (0.82 to 3.07)
DAS28(CRP) ≤ 2.6	6 (19.4%)	9 (39.1%)	2.02 (0.84 to 4.88)	4 (23.5%)	4 (25%)	1.06 (0.32 to 3.55)	4 (25%)	3 (23.1%)	0.92 (0.25 to 3.41)	5 (31.2%)	5 (38.5%)	1.23 (0.45 to 3.35)

Variable	Week 16			Week 24			Week 36			Week 48		
	Rituximab group (N = 31), n (%)/least squares mean change (SD)	Tocilizumab group (N = 23), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 17), n (%)/least squares mean change (SD)	Tocilizumab group (N = 16), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 16), n (%)/least squares mean change (SD)	Tocilizumab group (N = 13), n (%)/least squares mean change (SD)	Effect size (95% CI)	Rituximab group (N = 16), n (%)/least squares mean change (SD)	Tocilizumab group (N = 13), n (%)/least squares mean change (SD)	Effect size (95% CI)
Change from baseline: effect size expressed as least squares mean difference												
CDAI	−12.84 (2.1)	−12.58 (2.43)	−0.27 (−6.72 to 6.19)	−13.16 (2.04)	−16.03 (2.1)	2.87 (−3.1 to 8.84)	−15.86 (1.93)	−20.54 (2.14)	4.67 (−1.25 to 10.6)	−14.94 (2.16)	−20.37 (2.39)	5.43 (−1.2 to 12.07)
DAS28(ESR)	−1.42 (0.22)	−2.62 (0.26)	1.2 (0.51 to 1.89)	−1.84 (0.25)	−2.76 (0.26)	0.92 (0.17 to 1.67)	−2.2 (0.27)	−3.28 (0.3)	1.08 (0.25 to 1.9)	−1.92 (0.36)	−3.07 (0.39)	1.15 (0.05 to 2.24)
DAS28(CRP)	−1.39 (0.21)	−1.97 (0.24)	0.58 (−0.06 to 1.22)	−1.59 (0.24)	−2.03 (0.25)	0.44 (−0.27 to 1.15)	−1.8 (0.23)	−2.65 (0.26)	0.85 (0.13 to 1.56)	−1.7 (0.27)	−2.46 (0.3)	0.76 (−0.08 to 1.59)
HAQ	−0.28 (0.09)	−0.3 (0.1)	0.02 (−0.24 to 0.28)	−0.42 (0.11)	−0.28 (0.12)	−0.14 (−0.48 to 0.2)	−0.58 (0.13)	−0.36 (0.13)	−0.22 (−0.59 to 0.16)	−0.51 (0.14)	−0.35 (0.15)	−0.16 (−0.58 to 0.26)
FACIT	7.76 (1.91)	7.45 (2.19)	0.31 (−5.55 to 6.18)	10.64 (1.88)	4.13 (1.94)	6.5 (0.85 to 12.16)	13.16 (2.1)	6.44 (2.18)	6.72 (0.36 to 13.08)	10.66 (2.72)	4.72 (3.02)	5.94 (−2.56 to 14.44)
SF-36 PCS	6.35 (1.83)	6.41 (2.09)	−0.07 (−5.66 to 5.52)	11.57 (2.39)	9.13 (2.47)	2.43 (−4.63 to 9.49)	10.99 (2.95)	7.96 (3.06)	3.04 (−5.73 to 11.8)	10.27 (2.75)	9.06 (3.05)	1.21 (−7.24 to 9.66)
SF-36 MCS	4.37 (2.25)	4.18 (2.58)	0.19 (−6.7 to 7.08)	1.67 (2.57)	3.29 (2.66)	−1.61 (−9.25 to 6.02)	6.81 (2.43)	1.05 (2.53)	5.76 (−1.6 to 13.12)	5.98 (2.65)	0.59 (2.94)	5.39 (−2.8 to 13.59)
Total SHSS				0.36 (0.21)	0.37 (0.21)	0 (−0.64 to 0.63)				0.36 (0.21)	0.28 (0.23)	0.08 (−0.58 to 0.73)
SHSS: ER score				0.36 (0.17)	0.05 (0.17)	0.32 (−0.2 to 0.83)				0.36 (0.18)	0.06 (0.2)	0.3 (−0.27 to 0.88)
SHSS: JSN score				0.22 (0.2)	0.28 (0.2)	−0.05 (−0.64 to 0.54)				0.22 (0.2)	0.23 (0.22)	−0.01 (−0.65 to 0.62)
Ultrasound ST 12max[b]	−0.88 (1.18)	−2.57 (1.32)	1.69 (−2.01 to 5.38)							−2.55 (1.55)	−4.87 (1.76)	2.32 (−2.76 to 7.4)
Ultrasound PD 12max[b]	−1.47 (1.07)	−2.91 (1.26)	1.44 (−2.04 to 4.92)							−5.52 (0.81)	−7.05 (0.92)	1.53 (−1.17 to 4.23)

a   Moderate/good EULAR response.
b   12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 19 Treatment response to IMP in the total patient population, independent of pathotype

| Variable | Treatment group | | Effect size (95% CI) |
	Rituximab (N = 82), n (%)/least squares mean change (SD)	Tocilizumab (N = 79), n (%)/least squares mean change (SD)	
Binary response: effect size expressed as RR			
CDAI improvement ≥ 50%	37 (45.1%)	44 (55.7%)	1.23 (0.91 to 1.68)
CDAI-MTR	17 (20.7%)	35 (44.3%)	2.14 (1.31 to 3.49)
CDAI < 10.1	21 (25.6%)	36 (45.6%)	1.78 (1.15 to 2.76)
EULAR DAS28(ESR)[a]	60 (73.2%)	69 (87.3%)	1.19 (1.02 to 1.39)
DAS28(ESR) ≤ 3.2	21 (25.6%)	36 (45.6%)	1.78 (1.15 to 2.76)
DAS28(ESR) ≤ 2.6	10 (12.2%)	31 (39.2%)	3.22 (1.69 to 6.12)
EULAR DAS28(CRP)[a]	54 (65.9%)	62 (78.5%)	1.19 (0.98 to 1.45)
DAS28(CRP) ≤ 3.2	26 (31.7%)	37 (46.8%)	1.48 (1 to 2.19)
DAS28(CRP) ≤ 2.6	12 (14.6%)	27 (34.2%)	2.34 (1.27 to 4.28)
Change from baseline: effect size expressed as least squares mean difference			
CDAI	−13.23 (1.28)	−14.82 (1.31)	1.59 (−2.03 to 5.2)
DAS28(ESR)	−1.5 (0.14)	−2.52 (0.15)	1.02 (0.62 to 1.43)
DAS28(CRP)	−1.38 (0.14)	−1.98 (0.14)	0.61 (0.22 to 0.99)
HAQ	−0.26 (0.06)	−0.4 (0.06)	0.13 (−0.03 to 0.29)
FACIT	4.58 (1.01)	6.67 (1.05)	−2.09 (−4.96 to 0.78)
SF-36 PCS	4.86 (1.1)	8.34 (1.16)	−3.48 (−6.64 to −0.33)
SF-36 MCS	1.67 (1.23)	3.23 (1.3)	−1.56 (−5.11 to 1.99)
Ultrasound ST 12max[b]	−0.52 (0.94)	−1.01 (0.98)	0.49 (−2.23 to 3.21)
Ultrasound PD 12max[b]	−0.47 (0.72)	−2.06 (0.77)	1.58 (−0.54 to 3.71)

a EULAR good/moderate response.
b 12max, ultrasound summary score calculated as the mean of synovial thickening (ST) and power Doppler (PD) scores (0–3) for 12 joints, including five metacarpophalangeal joints, plus the maximum score in the wrist joint, for both hands.

TABLE 20 Demographics and disease activity of patients undergoing paired week 16 biopsy

| Baseline characteristics | Total cohort (N = 161) | Did the patient consent to a second biopsy? | | p-value |
		No (N = 96)	Yes (N = 65)	
Sex (male), n (%)	33 (20.5)	23 (24.0)	10 (15.4)	0.261
Age (years), mean (SD)	55.7 (12.9)	56.4 (12.4)	54.6 (13.7)	0.381
Disease duration (years), median [IQR]	9.0 [4.0–19.0]	9.0 [5.0–21.0]	10.0 [4.0–6.0]	0.357
CDAI, median [IQR]	29.8 [21.7–0.6]	29.4 [21.2–41.8]	30.4 [24.5–39.7]	0.707
ESR (mm/h), median [IQR]	31.5 [17.0–48.0]	28.0 [17.0–48.0]	35.0 [19.0–50.0]	0.202
CRP (mg/l), median [IQR]	11.0 [5.0–27.5]	14.4 [5.0–32.2]	10.0 [5.0–25.0]	0.462
RF positive, n (%)	105 (67.3)	60 (65.2)	45 (70.3)	0.621
ACPA positive, n (%)	119 (76.8)	70 (76.9)	49 (76.6)	1.000
Tender joint count (28), median [IQR]	11.0 [6.0–18.0]	10.5 [5.8–17.0]	11.0 [7.0–18.0]	0.248
Swollen joint count (28), median [IQR]	6.0 [3.0–10.0]	6.0 [3.0–11.2]	6.0 [4.0–9.0]	0.919
DAS28(ESR), mean (SD)	5.8 (1.2)	5.7 (1.4)	5.9 (1.0)	0.311
DAS28(CRP), mean (SD)	5.3 (1.2)	5.3 (1.3)	5.3 (1.0)	0.829

TABLE 20 Demographics and disease activity of patients undergoing paired week 16 biopsy (*continued*)

Baseline characteristics	Total cohort (N = 161)	Did the patient consent to a second biopsy?		p-value
		No (N = 96)	Yes (N = 65)	
Week 16 treatment response, n (%)				
CDAI ≥ 50% response	81 (50.3)	56 (58.3)	25 (38.5)	0.016[a]
CDAI-MTR	52 (32.3)	40 (41.7)	12 (18.5)	0.002[a]
Baseline histological score, mean (SD)				
CD20$^+$ B cells	1.56 (1.3)	1.38 (1.3)	1.8 (1.3)	0.052
CD138$^+$ plasma cells	1.43 (1.4)	1.26 (1.4)	1.65 (1.4)	0.077
CD68$^+$ L macrophages	1.15 (1)	1.05 (1)	1.29 (1.1)	0.141
CD68$^+$ SL macrophages	1.71 (1)	1.57 (1.1)	1.89 (0.9)	0.04[a]
CD3$^+$ T cells	1.45 (1.2)	1.32 (1.2)	1.62 (1.1)	0.081
Synovitis score	3.93 (2.8)	3.48 (2.8)	4.54 (2.6)	0.019[a]

a Significant *p*-value.

TABLE 21 Additional safety data

Serious adverse event	Treatment group	
	Rituximab (N = 82), n (%)	Tocilizumab (N = 79), n (%)
Discontinuation of study drug because of serious adverse event[a]	1	3
Infection[b]	3 (37.5)	3 (16.6)
Cardiac-related disorder[c]	1 (12.5)	4 (22.2)
Gastrointestinal disorder[d]	2 (25)	1 (33.3)
Surgical or medical procedure[e]	0 (0)	3 (16.6)
Pleural effusion[f]	0 (0)	2 (11.1)
Unspecified leg pain[g]	0 (0)	1 (5.5)
Parathyroid adenoma	0 (0)	1 (5.5)
Seizure	0 (0)	1 (5.5)
Urinoma	0 (0)	1 (5.5)
Cytokine release syndrome	1 (12.5)	0 (0)
Corneal melt[h]	0 (0)	1 (100)
Malignancy	0 (0)	0 (0)
Death[i]	1 (12.5)	0 (0)
Total	8	18

a SAEs that resulted in patients withdrawing from trial treatment were corneal melt; right pleural effusion; sudden onset chest pain; and cytokine release syndrome.
b Pneumonia (*n* = 1), lower respiratory tract infection (*n* = 1), pilonidal sinus/abscess (*n* = 2), urinary tract infection (*n* = 1), infective exacerbation of COPD (*n* = 1).
c Ischaemic coronary artery disorders (*n* = 4), unspecified chest pain (*n* = 1). One event, 'sudden onset chest pain', was reported as a SUSAR.
d Diarrhoea (*n* = 1), abdominal pain (*n* = 1), dental cyst (*n* = 1).
e Toe amputation (*n* = 1), total knee replacement (*n* = 1), osteotomy (*n* = 1).
f Two pleural effusion events reported for one patient.
g Scan confirmed no deep-vein thrombosis.
h Reported as SUSAR.
i Death due to suicide.

EME
HSDR
HTA
PGfAR
PHR

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