



Stratification of biological therapies by pathobiology in biologic-naive patients with rheumatoid arthritis (STRAP and STRAP-EU): two parallel, open-label, biopsy-driven, randomised trials



Felice Ravellese*, Alessandra Nerviani*, Giovanni Giorli, Louise Warren, Edyta Jaworska, Michele Bombardieri, Myles J Lewis, Frances Humby, Arthur G Pratt, Andrew Filer, Nagui Gendi, Alberto Cauli, Ernest Choy, Iain McInnes, Patrick Durez, Christopher J Edwards, Maya H Buch, Elisa Gremese, Peter C Taylor, Nora Ng, Juan D Cañete, Sabrina Raizada, Neil D McKay, Deepak Jadon, Pier Paolo Sainaghi, Richard Stratton, Michael R Ehrenstein, Pauline Ho, Joaquim P Pereira, Bhaskar Dasgupta, Claire Gorman, James Galloway, Hector Chinoy, Désirée van der Heijden, Peter Sasieni, Anne Barton, Costantino Pitzalis, on behalf of the STRAP collaborative group†

Summary

Lancet Rheumatol 2023;
5: e648–59

See [Comment](#) page e640

*Joint first authors

†Members are listed in the
appendix (p 21)

Centre for Experimental
Medicine and Rheumatology,
William Harvey Research
Institute, Queen Mary
University of London, London,
UK (F Ravellese MD,
A Nerviani PhD, G Giorli MSc,
L Warren BAgSc, E Jaworska MSc,
Prof M Bombardieri PhD,
M J Lewis PhD,
Prof C Pitzalis MD); Barts Health
NHS Trust and Barts Biomedical
Research Centre, National
Institute for Health and Care
Research (NIHR), London, UK
(F Ravellese, A Nerviani,
Prof M Bombardieri, M J Lewis,
F Humby MBBS, Prof C Pitzalis);
Rheumatology Department,
Guy's and St Thomas' NHS
Foundation Trust, London, UK
(F Humby, N Ng FRCP);
Translational and Clinical
Research Institute,
Newcastle University,
Newcastle upon Tyne, UK
(A G Pratt PhD); Directorate of
Musculoskeletal Services,
Newcastle upon Tyne Hospitals
NHS Foundation Trust,
Newcastle upon Tyne, UK
(A G Pratt); Rheumatology
Research Group, Institute for
Inflammation and Ageing,
NIHR Birmingham Biomedical
Research Centre and Clinical
Research Facility, University of
Birmingham, Queen Elizabeth
Hospital, Birmingham, UK
(Prof A Filer PhD); Basildon
University Hospital, Basildon
and Thurrock University NHS
Hospitals Foundation Trust,
Basildon, UK (N Gendi FRCP);
Rheumatology Unit, AOU and

Background Despite highly effective targeted therapies for rheumatoid arthritis, about 40% of patients respond poorly, and predictive biomarkers for treatment choices are lacking. We did a biopsy-driven trial to compare the response to rituximab, etanercept, and tocilizumab in biologic-naive patients with rheumatoid arthritis stratified for synovial B cell status.

Methods STRAP and STRAP-EU were two parallel, open-label, biopsy-driven, stratified, randomised, phase 3 trials done across 26 university centres in the UK and Europe. Biologic-naive patients aged 18 years or older with rheumatoid arthritis based on American College of Rheumatology (ACR)–European League Against Rheumatism classification criteria and an inadequate response to conventional synthetic disease-modifying antirheumatic drugs (DMARDs) were included. Following ultrasound-guided synovial biopsy, patients were classified as B cell poor or B cell rich according to synovial B cell signatures and randomly assigned (1:1:1) to intravenous rituximab (1000 mg at week 0 and week 2), subcutaneous tocilizumab (162 mg per week), or subcutaneous etanercept (50 mg per week). The primary outcome was the 16-week ACR20 response in the B cell-poor, intention-to-treat population (defined as all randomly assigned patients), with data pooled from the two trials, comparing etanercept and tocilizumab (grouped) versus rituximab. Safety was assessed in all patients who received at least one dose of study drug. These trials are registered with the EU Clinical Trials Register, 2014-003529-16 (STRAP) and 2017-004079-30 (STRAP-EU).

Findings Between June 8, 2015, and July 4, 2019, 226 patients were randomly assigned to etanercept (n=73), tocilizumab (n=74), and rituximab (n=79). Three patients (one in each group) were excluded after randomisation because they received parenteral steroids in the 4 weeks before recruitment. 168 (75%) of 223 patients in the intention-to-treat population were women and 170 (76%) were White. In the B cell-poor population, ACR20 response at 16 weeks (primary endpoint) showed no significant differences between etanercept and tocilizumab grouped together and rituximab (46 [60%] of 77 patients vs 26 [59%] of 44; odds ratio 1.02 [95% CI 0.47–2.17], p=0.97). No differences were observed for adverse events, including serious adverse events, which occurred in six (6%) of 102 patients in the rituximab group, nine (6%) of 108 patients in the etanercept group, and three (4%) of 73 patients in the tocilizumab group (p=0.53).

Interpretation In this biologic-naive population of patients with rheumatoid arthritis, the dichotomic classification into synovial B cell poor versus rich did not predict treatment response to B cell depletion with rituximab compared with alternative treatment strategies. However, the lack of response to rituximab in patients with a pauci-immune phenotype and the higher risk of structural damage progression in B cell-rich patients treated with rituximab warrant further investigations into the ability of synovial tissue analyses to inform disease pathogenesis and treatment response.

Funding UK Medical Research Council and Versus Arthritis.

Copyright © 2023 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

Introduction

Biological disease-modifying antirheumatic drugs (DMARDs) have transformed the outlook for patients with rheumatoid arthritis. However, the lack of a

meaningful response to treatment in about 40% of patients, particularly when using stringent response criteria such as low disease activity or remission, and the potential side-effects and exposure to potentially ineffective high-cost

Research in context

Evidence before this study

We searched PubMed for clinical trials, observational studies, and review articles published in English between Jan 1, 2013, and Jan 1, 2023, with the search terms “rheumatoid arthritis” AND (“biologic drugs” or “biologic DMARDs” or “biologics”) AND (“biomarkers” or “RNA” or “RNAseq” or “gene expression” or “transcriptomics”), including medical subject heading terms and variations of the above search terms. Several studies described blood and synovial biomarkers associated with response to biological disease-modifying antirheumatic drugs (DMARDs), including genes (DNA, RNA, and miRNA) and proteins. However, most of the evidence came from small observational studies or post-hoc analyses of clinical trials with little predictive value and limited clinical applicability for patient stratification in clinical practice. Only one randomised controlled trial (R4RA, published in 2021) identified predictive signatures of treatment response to rituximab and tocilizumab.

Added value of this study

The stratification of biological therapies for rheumatoid arthritis by pathobiology (STRAP) and STRAP-EU trials are the largest biopsy-driven, multicentre, randomised trials comparing etanercept, tocilizumab, and rituximab in biological DMARD-naïve patients with rheumatoid arthritis. Although the absence of synovial B cell signatures according to a prespecified and arbitrary dichotomic B cell-rich versus B cell-poor classification

was not associated with a significantly different response to rituximab compared with etanercept or tocilizumab (primary outcome), synovial inflammatory patterns (pathotypes) were informative of treatment response, and the presence of a B cell-rich synovitis was associated with structural damage progression in patients treated with rituximab.

Implications of all the available evidence

The R4RA and STRAP trials represent key steps in the journey towards precision medicine in rheumatology. As we learned from other fields—eg, in oncology with PD1 and PDL1 inhibitors—multiple attempts are often needed to identify optimal biomarker cutoffs for patient stratification. In rheumatoid arthritis, the available evidence indicates that synovial tissue analysis is informative of disease evolution and progression; however, the association of low or absent synovial B cell signatures with lack of response to B cell depletion with rituximab observed in the R4RA trial was not observed in the biologic-naïve population of the STRAP trials. Given the heterogeneity of rheumatoid arthritis, future precision medicine studies should move from binary patient stratification towards more comprehensive biomarker-driven approaches, in which molecular and histological signatures are combined into predictive algorithms of treatment responses to individual medication.

drugs, have underlined the need to identify predictive markers of response to ensure the best therapeutic outcome to individual targeted therapeutics and facilitate patient stratification before treatment.¹

Consistent with the well known heterogeneity of rheumatoid arthritis, variable response to biological DMARDs can be linked to the variable expression and pathogenetic relevance of the therapeutic targets in individual patients. Several studies have attempted to define blood and synovial biomarkers associated with response to biological DMARDs, including both genes (DNA, RNA, and miRNA) and proteins. However, most of the evidence comes from small observational studies or post-hoc analyses of clinical trials with little predictive value and limited clinical applicability for patient stratification in clinical practice. One randomised controlled trial (R4RA) from 2021 reported that a low or absent synovial B cell molecular signature in patients who did not respond to at least one tumour necrosis factor (TNF) inhibitor was associated with a lower response to B cell depletion (rituximab) compared with interleukin-6 receptor inhibition (tocilizumab).² Further analyses of the same trial identified molecular predictors of treatment response to the individual therapies, together with a signature associated with multidrug resistance.³

Here, we report results from the stratification of biological therapies for rheumatoid arthritis by pathobiology (STRAP) trials, the main aim of which was to test the utility of

analysing synovial B cell infiltrates as a potential biomarker to guide therapeutic decisions in patients who do not respond to conventional synthetic DMARDs. The primary hypothesis was that patients with a low B cell infiltrate, assessed by combining histomorphology and a B cell-specific molecular score, would have a worse response to B cell depletion with rituximab, as compared with etanercept and tocilizumab grouped together.

Methods

Study design and participants

We did two parallel, open-label, biopsy-driven, stratified, randomised, phase 3 trials at 26 centres across the UK and the EU. The STRAP trial began recruitment in the UK on June 8, 2015. Owing to drug supply issues, a separate trial (STRAP-EU) was opened in 2018 in four EU countries (Belgium, Italy, Portugal, and Spain). The data from both trials were combined, as prespecified in the statistical analysis plan, because the two studies were identical in design up to the 16-week primary endpoint.

Patients aged 18 years or older fulfilling the 2010 American College of Rheumatology (ACR)–European League Against Rheumatism (EULAR) classification criteria for rheumatoid arthritis⁴ with an inadequate response to two conventional synthetic DMARDs and eligible for biological DMARD therapy according to UK National Institute for Health and Care Excellence (NICE)

University of Cagliari, Monserrato, Italy (Prof A Cauli MD); UOC of Radiology, Ospedale SS Trinità, ATS Cagliari, Italy (Prof A Cauli); CREATE Centre, Cardiff University, Cardiff, UK (Prof E Choy MD); Department of Rheumatology, University Hospital of Wales, Cardiff, UK (Prof E Choy); Glasgow Clinical Research Facility, Glasgow Royal Infirmary, Glasgow, UK (Prof I McInnes PhD); Institute of Experimental and Clinical Research, Université Catholique de Louvain, Brussels, Belgium (Prof P Durez MD); Department of Rheumatology, Cliniques Universitaires Saint-Luc, Brussels, Belgium (Prof P Durez); NIHR Southampton Clinical Research Facility, University Hospital Southampton, Southampton, UK (Prof C J Edwards MD); Faculty of Medicine, University of Southampton, Southampton, UK (Prof C J Edwards); Centre for Musculoskeletal Research, Division of Musculoskeletal and Dermatological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK (Prof M H Buch PhD, Prof H Chinoy PhD, Prof A Barton PhD); NIHR Manchester Biomedical Research Centre, Manchester, UK (Prof M H Buch); Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy (E Gremese MD); Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Research Centre, University of Oxford, Oxford, UK (Prof P C Taylor PhD); Rheumatology Department, Hospital Clínic de Barcelona, Barcelona, Spain (J D Cañete MD); Institut d'Investigacions Biomèdiques August Pi I Sunyer, Barcelona, Spain (J D Cañete); New Cross Hospital and Cannock Chase Hospital, Royal Wolverhampton NHS Trust, Wolverhampton, UK (S Raizada PhD); Edinburgh Rheumatology Research Group and Rheumatic Diseases Unit, NHS Lothian, Edinburgh, UK (N D McKay MBBs); Department of Medicine, University of Cambridge, Cambridge, UK (D Jadon MRCP); Department of Rheumatology, University

Eastern Piedmont and Maggiore della Carita Hospital, Novara, Italy (Prof P P Sainaghi PhD); Royal Free Hospital, Royal Free London NHS Foundation Trust, London, UK (Prof R Stratton PhD); Department of Rheumatology, University College London, London, UK (Prof M R Ehrenstein PhD); The Kellgren Centre for Rheumatology, Manchester Royal Infirmary, Manchester University NHS Foundation Trust, Manchester, UK (P Ho PhD); Rheumatology Department, Hospital De Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal (J P Pereira PhD); Rheumatology Research Unit, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal (J P Pereira); Rheumatology Department, Mid and South Essex University Hospitals NHS Foundation Trust, Southend University Hospital, Westcliff-on-Sea, UK (Prof B Dasgupta FRCP); Department of Rheumatology, Homerton University Hospital, Homerton Healthcare NHS Foundation Trust, London, UK (C Gorman PhD); King's College Hospital, King's College Hospital NHS Foundation Trust, London, UK (J Galloway PhD); Department of Rheumatology, Salford Royal Hospital, Northern Care Alliance NHS Foundation Trust, Manchester Academic Health Science Centre, Salford, UK (Prof H Chinoy); Department of Rheumatology, Leiden University Medical Center, Leiden, Netherlands (Prof D van der Heijde MD); King's Clinical Trials Unit, Kings College London, London, UK (Prof P Sasieni PhD); IRCCS Humanitas Research Hospital, Milan, Italy (Prof C Pitzalis)

Correspondence to: Prof Costantino Pitzalis, Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, UK
c.pitzalis@qmul.ac.uk

See Online for appendix

For the protocols see <https://matura-mrc.whri.qmul.ac.uk/documents.php>

guidelines (ie, disease activity score in 28 joints [DAS28] ≥ 5.1) were included. Patients were identified through rheumatology outpatient clinics at participating sites. The appendix contains a complete list of the inclusion and exclusion criteria (p 1) and a list of protocol deviations leading to exclusion from the per-protocol set (p 2). Participants' sex was collected from hospital records. The study was conducted in compliance with the Declaration of Helsinki, International Conference on Harmonisation Guidelines for Good Clinical Practice, and local country regulations. The final protocols for both trials, amendments, and documentation of consent were approved by the regulatory authorities and relevant ethics committees in each country.

Randomisation and masking

Before randomisation, participants were stratified according to synovial histopathology (B cell rich, B cell poor, or unknown B cell status) and methotrexate use, and subsequently randomly assigned (1:1:1) to rituximab, etanercept, or tocilizumab using hierarchical dynamic randomisation. Randomisation was performed by the STRAP Trial Office within the Barts Clinical Trials Unit (London, UK). The randomisation list was prepared by the trial statistician, and the application codes of hierarchical dynamic randomisation were securely embedded with the application code so that it was not accessible to end users in order to ensure concealment. The named joint assessor was masked to study drug allocation, and all staff at the recruiting sites were masked to the B cell classification throughout the study.

Procedures

Patients underwent a synovial biopsy of a clinically active joint (clinically swollen and with ultrasound synovial thickening ≥ 2) before starting trial therapy. The biopsy was performed according to the expertise of the local centre as an ultrasound-guided or as an arthroscopic procedure, as previously described.^{5,6}

For histological analyses, a minimum of six synovial fragments were paraffin-embedded at Queen Mary University of London (London, UK) Core Pathology Laboratory. Tissue sections with 3–5 μm thickness were stained for haematoxylin and eosin, and immunohistochemical markers, as previously described.⁷ Following immunohistochemical staining, sections underwent semiquantitative scoring (0–4) to determine levels of CD20 and CD79a B cells, CD3 T cells, CD138 plasma cells, and CD68 lining and sublining macrophages (appendix p 13). Haematoxylin and eosin-stained slides also underwent assessment of synovitis (Krenn synovitis score), as previously described.⁸ After confirming the presence of synovial lining, patients were classified as B cell rich or B cell poor in the pathology laboratory of Barts Health NHS Trust (London, UK) by a consultant pathologist masked to randomly assigned therapy, according to a validated semiquantitative score:⁹ samples

with a CD20 score of 2 or higher and with CD20 B cell aggregates were classified as B cell rich, those with a CD20 score of less than 2 were classified as B cell poor, samples with no visible lining or characteristic synovial vessels or stroma were classified as ungraded (appendix p 14). The histological classification was replicated at Queen Mary University of London by an independent expert and discrepancies in classification were resolved through mutual agreement.

RNA was extracted from 216 synovial tissue samples (including baseline and 16-week samples) and sequenced at Genewiz (Bishop's Stortford, UK) according to the previously described standard operating procedure (appendix p 15).^{3,10} Following quality control, eight samples (five baseline and three follow-up) were excluded due to poor mapping rate.

Patients were classified as B cell poor or rich according to a previously developed B cell-specific gene module derived from analysis of FANTOM5 gene expression data¹¹ that had been validated using RNA sequence of drug-naïve early rheumatoid arthritis synovial biopsy samples.^{9,10} Module scores were calculated using scaled data. Scaled gene expression of the baseline samples was used for scaling and centring of the expression data from follow-up visits. Then, patients were classified as B cell poor or rich according to predetermined cutoff points for B cell transcript classification.² The final classification of patients into B cell rich or poor combined histological and molecular classification is shown in the appendix (p 15).

Following randomisation, patients received rituximab as two 1000 mg intravenous infusions at week 0 and 2, or 162 mg tocilizumab or 50 mg etanercept as weekly subcutaneous injections. Patients were followed up every 4 weeks (± 1 week) up to 48 weeks. At each visit, rheumatoid arthritis disease activity measurements and safety data were collected (appendix p 16). An optional repeat synovial biopsy was carried out at 16 weeks. Following a protocol amendment on Dec 24, 2018, the follow-up of STRAP patients recruited in the UK after Jan 1, 2019, ended at 24 weeks from baseline; however, there were no differences in the trial procedures up to that point, including the primary endpoint at week 16. Clinical outcomes up to week 16 are presented herein.

Outcomes

The primary objective was to ascertain whether etanercept or tocilizumab (grouped together) were superior to rituximab in patients with a B cell-poor synovial pathotype. The primary endpoint was a binary outcome of treatment response using the ACR20 at 16 weeks in the B cell-poor population. ACR20 response was defined as 20% improvement in tender and swollen joint counts and 20% improvement in three of the five remaining ACR core set measures: patient and physician global assessments on a visual analogue scale (VAS),

pain VAS, health assessment questionnaire (HAQ), and an acute-phase reactant (erythrocyte sedimentation rate [ESR] or C-reactive protein [CRP]).

Prespecified secondary outcomes were 16-week ACR20 response in the B cell-rich population; DAS28 remission (<2.6); ACR50 and ACR70 response; low Clinical Disease Activity Index (CDAI) score (≤ 10); mean percentage change from baseline in CDAI score at 16 weeks; the treatment effects of etanercept and tocilizumab versus rituximab in the B cell-rich population; the interaction between treatments and B cell status. Prespecified exploratory outcomes were response in patients stratified according to histological pathotypes,⁷ post-treatment changes in histopathological and molecular scores, and the progression of structural damage measured by the change from baseline in the radiographic Sharp–van der Heijde scores at 16 weeks.¹²

The incidence and severity of treatment-emergent and procedure-emergent adverse events were monitored throughout the study; adverse event coding was done according to the Medical Dictionary for Regulatory Activities (version 22). The causality and expectedness of all serious adverse events in relation to the trial treatment was assessed by the principal investigator (or delegated medic) according to the severe adverse event definition. If a severe adverse event related to the treatment was unexpected, it was considered a suspected unexpected serious adverse reaction. All severe adverse events up to week 48 (and up to 30 days later) were reported by relatedness using the Medical Dictionary for Regulatory Activities lowest level term classification. All adverse events up to week 48 (and up to 30 days later) were reported using the Medical Dictionary for Regulatory Activities system organ class classification. Recurrent events (ie, those that occurred more than once in the same participant) were considered as one event.

Statistical analysis

A sample size of 96 B cell-poor patients was planned to achieve 80% power and 126 B cell-poor patients to achieve 90% power to test the difference in response rates between treatment groups for the primary endpoint. This was based on assuming a response rate of 30% for the rituximab group and 60% for etanercept and tocilizumab groups, with a two-sided 5% type 1 error rate and a dropout rate of 10%. The primary analysis assessed the difference in the ACR20 response between rituximab and etanercept and tocilizumab grouped together at 16 weeks in the B cell-poor population. For the primary endpoint (ACR20 response) and secondary binary endpoints (DAS28 remission, low CDAI score, ACR50 response, and ACR70 response), we used logistic regression adjusting for treatment, baseline methotrexate use, and study (STRAP and STRAP-EU) as factors to calculate odds ratio (OR) and 95% CI. For continuous endpoints, we used ANCOVA, with treatment, methotrexate use, and study as factors and baseline score as covariate, to

calculate least-squares mean changes from baseline and 95% CI. In B cell-rich patients, although the study was underpowered for non-inferiority analysis, we aimed to ascertain whether rituximab was as effective as etanercept and tocilizumab (grouped together), by calculating the OR of response and its 95% CI through logistic regression and assessing whether the lower limit of the CI was above 0.8 (prespecified). The interaction between treatments and B cell status (rich *vs* poor) was tested using the likelihood-ratio test comparing the fit of two nested logistic regression models (full model with the interaction term *vs* a reduced model without) to determine the significance of the interaction term (package `lmtest`, version 0.9–40) and adjusted for baseline methotrexate use and seropositivity for rheumatoid factor or anticitrullinated protein antibody (ACPA). The primary and secondary analyses were based on the intention-to-treat population, defined as all randomly assigned patients. No correction for multiplicity was applied. Missing values were assumed to be missing at random and were imputed using multiple imputation (package `Amelia`, version 1.7.6). As for the prespecified exploratory analyses, we used the Mann-Whitney *U* test to compare baseline semiquantitative immune cell scores, Fisher's exact test to compare the proportion of patients with ACR20, ACR50, and ACR70 response stratified according to synovial pathotype, and paired-samples Wilcoxon test to compare immune cell scores at baseline and 16 weeks. Adverse events were analysed using Fisher's exact test.

In addition to the prespecified primary, secondary, and exploratory outcomes, we analysed individual outcome components (28 tender and swollen joint counts, patient and physician global assessments on VAS, ESR, and CRP) collected every 4 weeks up to week 16 (post-hoc outcomes). Least-squares mean was used to compare changes from baseline and 16 weeks, two-way repeated measures ANCOVA to assess the interaction between medications and time, and Mann-Whitney *U* test with Bonferroni correction for multiple comparisons to compare treatments at each timepoint. Synovial RNA sequencing, processed as described previously, underwent deconvolution using MCP-counter,¹³ and synovial signatures at baseline and 16 weeks were compared using paired-samples Wilcoxon test. A list of the genes included in each cell signature is provided in the appendix (p 10).

All analyses were carried out on R (version 3.6.3 or higher), using the `stats` package (version 4.2.2) unless differently specified.

These trials are registered with the EU Clinical Trials Register, 2014-003529-16 (STRAP) and 2017-004079-30 (STRAP-EU).

Role of the funding source

The funders of the study and the donors of the study drugs had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

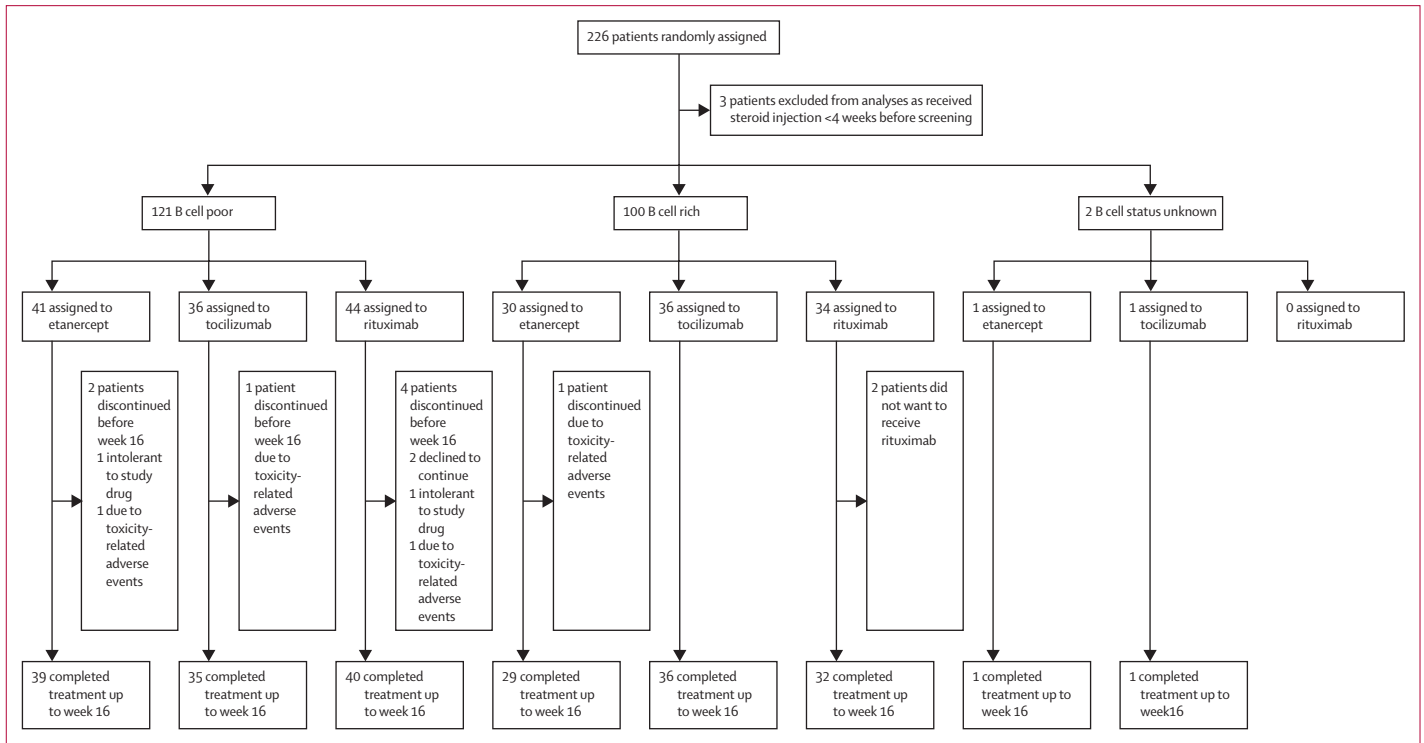


Figure: Trial profile

Results

Between June 8, 2015, and July 4, 2019, 331 patients were screened, of whom 294 (89%) gave consent, and 226 (68%) were randomly assigned (figure). Three patients were excluded because, after randomisation, it was found that they did not meet the inclusion criteria (appendix p 1), having received parenteral steroids less than 4 weeks before screening. After synovial biopsy and stratification according to B cell status, 78 patients were randomly assigned to rituximab (44 B cell poor and 34 B cell rich), 73 to tocilizumab (36 B cell poor, 36 B cell rich, and one with unknown status), and 72 to etanercept (41 B cell poor, 30 B cell rich, and one with unknown status); of these patients, 72 (92%) of 78 assigned to rituximab, 72 (99%) of 73 assigned to tocilizumab, and 69 (96%) of 72 assigned to etanercept, completed treatment to the primary endpoint at week 16 (figure). 168 (75%) of 223 patients in the intention-to-treat population were women and 170 (76%) were White. Recruitment per site can be found in the appendix (p 3). Baseline characteristics, disease activity, and synovial B cell stratification are reported in table 1 and the appendix (p 4).

At 16 weeks, the ACR20 response in the B cell-poor population (primary endpoint) was not significantly different between etanercept and tocilizumab grouped together and rituximab (46 [60%] of 77 vs 26 [59%] of 44; OR 1.02 [95% CI 0.47–2.17], p=0.97; table 2). In a post-hoc analysis of tocilizumab and etanercept separately, an ACR20 response was seen in 26 (72%) of 36 patients

assigned to tocilizumab and 20 (49%) of 41 patients assigned to etanercept (table 2).

Among the secondary outcomes, ACR50 response (OR 2.24 [95% CI 1.01–5.18]), DAS28(CRP)-based remission (2.61 [1.09–6.88]), and DAS28(ESR)-based remission (3.84 [1.52–11.17]) showed a significant difference between rituximab and etanercept and tocilizumab grouped together, in favour of the latter, whereas no differences were observed for CDAI (table 2).

In the B cell-rich population, ACR20 response was not different between etanercept and tocilizumab grouped together and rituximab (49 [74%] of 66 vs 23 [68%] of 34; OR 1.57 [95% CI 0.59–4.18]; these results show that non-inferiority of rituximab in the B cell-rich population was not met, although the study was underpowered for a non-inferiority analysis. Response to the individual medications showed no differences (table 3). Among the secondary outcomes, ACR50 response (OR 3.09 [95% CI 1.23–8.41]), ACR70 response (3.49 [1.09–13.89]), DAS28(ESR)-based remission (6.15 [2.20–19.89]), and changes in CDAI (least-squares mean 4.5 [95% CI 0.2–8.7]) favoured etanercept and tocilizumab grouped together over rituximab (table 3).

The interaction analysis between treatment and B cell status, adjusted for baseline methotrexate use and seropositivity for rheumatoid factor or ACPAs, was not significant (appendix p 5).

When considering the entire population (B cell rich and poor; appendix p 6) in a post-hoc analysis, no difference between rituximab and etanercept and tocilizumab

	Whole population*				B cell poor				B cell rich			
	Overall (n=223)	Rituximab (n=78)	Etanercept (n=72)	Tocilizumab (n=73)	Overall (n=121)	Rituximab (n=44)	Etanercept (n=41)	Tocilizumab (n=36)	Overall (n=100)	Rituximab (n=34)	Etanercept (n=30)	Tocilizumab (n=36)
Sex												
Male	55 (25%)	18 (23%)	18 (25%)	19 (26%)	32 (26%)	10 (23%)	10 (24%)	12 (33%)	22 (22%)	8 (24%)	8 (27%)	6 (17%)
Female	168 (75%)	60 (77%)	54 (75%)	54 (74%)	89 (74%)	34 (77%)	31 (76%)	24 (67%)	78 (78%)	26 (76%)	22 (73%)	30 (83%)
Age, years	53.7 (13.4)	55.1 (13.1)	52.2 (13.1)	53.5 (14.0)	53.5 (13.4)	55.1 (13.4)	51.1 (11.7)	54.4 (15.2)	53.5 (13.3)	55.2 (12.8)	52.9 (14.7)	52.3 (12.8)
Ethnicity												
White†	170 (76%)	63 (81%)	51 (71%)	56 (77%)	90 (74%)	35 (80%)	28 (68%)	27 (75%)	78 (78%)	28 (82%)	22 (73%)	28 (78%)
Asian or Asian British‡	34 (15%)	10 (13%)	12 (17%)	12 (16%)	17 (14%)	7 (16%)	6 (15%)	4 (11%)	17 (17%)	3 (9%)	6 (20%)	8 (22%)
Black or Black British§	7 (3%)	1 (1%)	4 (6%)	2 (3%)	5 (4%)	0	3 (7%)	2 (6%)	2 (2%)	1 (3%)	1 (3%)	0
Mixed¶	6 (3%)	1 (1%)	2 (3%)	3 (4%)	5 (4%)	0	2 (5%)	3 (8%)	1 (1%)	1 (3%)	0	0
Other	6 (3%)	3 (4%)	3 (4%)	0	4 (3%)	2 (5%)	2 (5%)	0	2 (2%)	1 (3%)	1 (3%)	0
Disease duration, years	3.0 (1.0-8.0)	3.0 (1.0-8.0)	3.0 (2.0-8.2)	3.0 (1.0-8.0)	4.0 (1.0-8.0)	3.0 (1.0-8.0)	5.0 (2.0-8.0)	4.0 (1.0-10.2)	3.0 (1.0-8.0)	2.0 (0.2-7.8)	6.5 (2.2-8.8)	2.5 (1.0-7.2)
Tender joint count	12.0 (7.0-19.0)	10.0 (7.0-17.8)	13.5 (8.0-20.0)	11.0 (7.0-19.0)	14.0 (8.0-18.5)	13.5 (8.2-18.8)	14.0 (8.0-21.0)	11.0 (7.0-17.0)	11.0 (7.0-19.0)	9.0 (5.8-14.8)	12.5 (8.0-16.5)	12.0 (7.8-23.2)
Swollen joint count	7.0 (4.0-10.5)	7.0 (4.0-11.0)	7.0 (4.0-10.2)	7.0 (5.0-10.0)	7.0 (4.0-10.0)	6.5 (4.0-11.0)	6.0 (3.0-9.0)	7.0 (5.0-10.0)	7.0 (5.0-10.8)	7.5 (4.0-10.0)	7.0 (5.2-11.0)	7.0 (5.0-10.2)
Erythrocyte sedimentation rate, mm/h	27.0 (16.0-42.0)	27.0 (16.0-45.8)	25.0 (16.0-37.0)	28.0 (15.0-45.0)	25.0 (15.5-40.0)	28.0 (17.2-44.2)	22.0 (12.0-31.0)	25.0 (11.6-36.5)	28.5 (16.0-44.5)	24.5 (14.0-46.5)	29.0 (17.5-38.5)	30.5 (19.5-45.5)
C-reactive protein, mg/L	11.5 (4.0-27.0)	11.5 (4.5-29.2)	10.0 (3.5-26.0)	13.0 (5.0-28.0)	10.0 (3.0-26.8)	12.0 (5.0-29.0)	8.0 (0.0-27.0)	10.0 (3.4-17.2)	13.0 (6.0-29.0)	9.0 (4.0-28.5)	11.0 (6.0-16.0)	17.5 (7.0-38.5)
Patient global assessment	73.0 (55.0-87.5)	71.5 (46.2-84.8)	72.5 (60.8-87.2)	75.0 (55.0-89.0)	73.0 (53.5-87.0)	74.5 (50.0-89.0)	73.0 (64.0-87.0)	67.5 (40.8-81.2)	73.0 (57.0-88.0)	67.5 (46.0-81.0)	71.0 (54.8-87.5)	78.0 (71.5-90.0)
Physician global assessment	62.0 (44.0-78.0)	55.5 (38.2-75.5)	66.5 (45.8-79.2)	61.0 (46.0-82.0)	57.0 (41.0-76.5)	53.5 (36.8-73.8)	70.0 (44.0-80.0)	53.0 (41.8-77.0)	64.0 (50.0-79.0)	58.0 (44.0-76.2)	64.5 (52.0-78.8)	68.5 (51.5-86.5)
CDAI	32.5 (25.9-42.6)	31.3 (24.6-41.2)	33.8 (26.0-44.2)	33.8 (25.9-41.8)	34.7 (25.6-43.0)	35.0 (27.2-43.5)	35.4 (25.7-44.1)	30.9 (22.6-39.2)	32.2 (26.1-42.2)	29.2 (22.2-40.4)	32.1 (28.1-41.7)	34.8 (27.9-44.2)
DAS28(E5R)	5.9 (1.2)	5.8 (1.3)	6.0 (0.9)	6.0 (1.3)	5.9 (1.1)	6.0 (1.1)	5.9 (0.8)	5.7 (1.4)	5.9 (1.2)	5.6 (1.4)	5.9 (1.0)	6.2 (1.2)
DAS28(CRP)	5.5 (1.1)	5.4 (1.1)	5.5 (1.0)	5.6 (1.1)	5.5 (1.0)	5.6 (1.0)	5.5 (1.0)	5.3 (1.1)	5.6 (1.2)	5.2 (1.2)	5.5 (1.0)	5.9 (1.2)
RF or ACPA positive	188 (84%)	69 (88%)	60 (83%)	59 (81%)	97 (80%)	37 (84%)	32 (78%)	28 (78%)	90 (90%)	32 (94%)	28 (93%)	30 (83%)
RF positive	154 (69%)	57 (73%)	49 (68%)	48 (66%)	78 (64%)	31 (70%)	26 (63%)	21 (58%)	75 (75%)	26 (76%)	23 (77%)	26 (72%)
ACPA positive	174 (78%)	63 (81%)	54 (75%)	57 (78%)	88 (73%)	32 (73%)	28 (68%)	28 (78%)	85 (85%)	31 (91%)	26 (87%)	28 (78%)
Haemoglobin, g/L	120.5 (111.2-130.0)	123.5 (117.2-134.8)	125.5 (119.0-130.0)	113.0 (101.2-120.5)	119.0 (108.0-128.8)	121.0 (116.0-134.0)	125.0 (118.8-128.5)	108.0 (101.0-118.0)	124.0 (116.0-133.5)	133.0 (118.0-135.0)	128.0 (120.0-136.8)	116.0 (115.0-125.0)
Methotrexate	142 (64%)	54 (69%)	41 (57%)	47 (64%)	78 (64%)	28 (64%)	24 (59%)	26 (72%)	62 (62%)	26 (76%)	16 (53%)	20 (56%)
Steroids	37 (17%)	15 (19%)	14 (19%)	8 (11%)	15 (12%)	5 (11%)	6 (15%)	4 (11%)	21 (21%)	10 (29%)	7 (23%)	4 (11%)
Additional DMARDs												
0	71 (32%)	30 (38%)	22 (31%)	19 (26%)	33 (27%)	16 (36%)	12 (29%)	5 (14%)	38 (38%)	14 (41%)	10 (33%)	14 (39%)
1	108 (48%)	32 (41%)	36 (50%)	40 (55%)	65 (54%)	18 (41%)	24 (59%)	23 (64%)	41 (41%)	14 (41%)	11 (37%)	16 (44%)
2	41 (18%)	15 (19%)	13 (18%)	13 (18%)	22 (18%)	9 (20%)	5 (12%)	8 (22%)	19 (19%)	6 (18%)	8 (27%)	5 (14%)
3	3 (1%)	1 (1%)	1 (1%)	1 (1%)	1 (1%)	1 (2%)	0	0	2 (2%)	0	1 (3%)	1 (3%)

Data are n (%), mean (SD), or median (IQR). ACPA=anticitrullinated protein antibody. CDAI=Clinical Diseases Activity Index. CRP=C-reactive protein. DAS28=disease-modifying antirheumatic drugs. E5R=erythrocyte sedimentation rate. RF=rheumatoid factor. *Two patients were classified as unknown B cell status. †Includes British, Irish, or any other White background. ‡Includes Indian, Pakistani, Bangladeshi, or any other Asian background. §Includes Caribbean, African, or any other Black background. ¶Includes White and Black Caribbean, White and Black African, White and Asian, or any other mixed background.

Table 1: Baseline characteristics of patients, stratified by histological classification and treatment group

	Rituximab (n=44)	Tocilizumab and etanercept (n=77)	Treatment effect	Adjusted p value*	Etanercept (n=41)	Tocilizumab (n=36)
Primary endpoint						
ACR20 response at week 16	26 (59%)	46 (60%)	1.02 (0.47 to 2.17)	0.97	20 (49%)	26 (72%)
Secondary endpoints						
ACR50 response at week 16	12 (27%)	35 (45%)	2.24 (1.01 to 5.18)	0.052	16 (39%)	19 (53%)
ACR70 response at week 16	6 (14%)	18 (23%)	1.92 (0.73 to 5.73)	0.21	9 (22%)	9 (25%)
CDAI ≤10 at week 16	16 (36%)	35 (45%)	1.45 (0.67 to 3.22)	0.35	17 (41%)	18 (50%)
DAS28(ESR) ≤2.6 at week 16	6 (14%)	29 (38%)	3.84 (1.52 to 11.17)	0.0072	8 (20%)	21 (58%)
DAS28(CRP) ≤2.6 at week 16	8 (18%)	28 (36%)	2.61 (1.09 to 6.88)	0.039	12 (29%)	16 (44%)
CDAI, least-squares mean change at week 16 (95% CI)	-18.1 (-22.6 to -13.6)	-21.3 (-25.1 to -17.4)	3.2 (-1.2 to 7.5)	0.15	-19.8 (-24.1 to -15.5)	-23.5 (-28.5 to -18.6)

Data are n (%) for primary and binary secondary endpoints and least-squares mean (95% CI) for CDAI changes. Treatment effect is expressed as odds ratio (95% CI) for primary and secondary binary endpoints and as least-squares mean difference (95% CI) for CDAI change. ACR=American College of Rheumatology. CDAI=Clinical Disease Activity Index. CRP=C-reactive protein. DAS28=disease activity score in 28 joints. *Adjusted for treatment, baseline methotrexate use, and study (STRAP and STRAP-EU).

Table 2: Clinical outcomes at 16 weeks in the B cell-poor population by intention to treat

	Rituximab (n=34)	Tocilizumab and etanercept (n=66)	Treatment effect	Adjusted p value*	Etanercept (n=30)	Tocilizumab (n=36)
ACR20 response at week 16	23 (68%)	49 (74%)	1.57 (0.59 to 4.18)	0.37	21 (70%)	28 (78%)
ACR50 response at week 16	9 (26%)	33 (50%)	3.09 (1.23 to 8.41)	0.02	14 (47%)	19 (53%)
ACR70 response at week 16	4 (12%)	20 (30%)	3.49 (1.09 to 13.89)	0.049	7 (23%)	13 (36%)
CDAI ≤10 at week 16	16 (47%)	38 (58%)	2.03 (0.80 to 5.39)	0.14	18 (60%)	20 (56%)
DAS28(ESR) ≤2.6 at week 16	8 (24%)	36 (55%)	6.15 (2.20 to 19.89)	0.0011	14 (47%)	22 (61%)
DAS28(CRP) ≤2.6 at week 16	11 (32%)	30 (45%)	2.22 (0.85 to 6.28)	0.12	12 (40%)	18 (50%)
CDAI, least-squares mean change at week 16 (95% CI)	-19.3 (-23.1 to -15.6)	-23.8 (-26.5 to -21.1)	4.5 (0.2 to 8.7)	0.038	-25.2 (-29.0 to -21.3)	-22.8 (-26.3 to -19.4)

Data are n (%) for primary and binary secondary endpoints and least-squares mean (95% CI) for CDAI changes. Treatment effect is expressed as odds ratio (95% CI) for primary and secondary binary endpoints and as least-squares mean difference (95% CI) for CDAI change. ACR=American College of Rheumatology. CDAI=Clinical Disease Activity Index. CRP=C-reactive protein. DAS28=disease activity score in 28 joints. *Adjusted for treatment, baseline methotrexate use, and study (STRAP and STRAP-EU).

Table 3: Clinical secondary outcomes at 16 weeks in the B cell-rich population by intention to treat

grouped together was seen in ACR20 response (OR 1.20 [95% CI 0.66–2.16]), whereas ACR50 response (2.52 [1.39–4.72]), ACR70 response (2.47 [1.17–5.63]), DAS28-based remission (4.27 [2.18–8.92]), and changes in CDAI (least-squares mean 4.0 [95% CI 1.0–6.9]) favoured etanercept and tocilizumab grouped together over rituximab.

A post-hoc analysis of individual outcome components showed that tocilizumab, in line with its known mechanism of action,¹⁴ led to a greater improvement in ESR and CRP at 16 weeks (appendix p 7). However, compared with rituximab, tocilizumab also showed significant improvements in disease activity in both patient global assessment and physician global assessment, and in pain. The longitudinal analysis of outcomes at monthly follow-up visits showed a significant interaction between treatment and time by

analysis of covariance for ESR, CRP, pain VAS, global disease activity, DAS28(ESR) and DAS28(CRP), and CDAI (appendix p 17). In particular, tocilizumab had a significantly greater effect on inflammatory markers, which were already significantly reduced after the first administration and remained significantly lower up to week 16. Accordingly, tocilizumab had a significantly greater effect on DAS-based outcomes, whereas the differences were less marked for CDAI. By contrast, rituximab was associated with a slower onset of action on several outcomes, including inflammatory markers, DAS outcomes, but also CDAI and swollen joints.

When analysing baseline synovial immune cell scores, we found no differences between responders and non-responders to each medication (appendix p 18). However, when patients were stratified according to synovial pathotypes,⁷ patients with a pauci-immune

pathotype—ie, patients without synovial immune cells, and, accordingly, lower autoantibody positivity (20 [59%] of 34 ACPA positive)—showed significantly lower responses to rituximab than to etanercept and tocilizumab. Conversely, the response rates were similar in patients with a lymphomyeloid pathotype, dominated by the presence of B cells in addition to myeloid cells, and in those with a diffuse-myeloid pathotype, with myeloid lineage predominance but scant B cells (appendix p 18).

Next, we examined the effects of medications on synovial immune cell infiltration by analysing matched pre-treatment and post-treatment synovial biopsies, which were available for 65 patients (appendix p 19). As the post-treatment biopsy was an optional procedure, patients who underwent a repeated biopsy had lower 16-week response rates, which is expected because responders are usually less likely to consent to a second biopsy (appendix p 8). Rituximab induced a significant reduction of synovial CD20 B cells, but also CD138 plasma cells, CD3 T cells, and the total synovitis Krenn score. Etanercept also reduced CD20 B cells (albeit less strongly than rituximab), CD138 plasma cells, and the total synovitis Krenn score, but also CD68 sublining macrophages. Tocilizumab induced a significant reduction of CD20 B cells, CD138 plasma cells, CD3 T cells, the total synovitis score, and CD68 sublining macrophages (appendix p 19).

To further dissect the specific modulation of synovial cells, we applied a post-hoc molecular deconvolution analysis (MCP-counter),³ showing a significant reduction of B cells and monocyte-macrophage signatures in patients who received rituximab and of monocytes and monocyte-macrophages in patients who received etanercept (appendix p 18). Tocilizumab affected multiple immune cell lineages, as it reduced B cells, macrophage-monocytes, myeloid dendritic cells, total and CD8 or cytotoxic T cells, and natural killer cells, but induced an increase in endothelial cells and fibroblast signatures.

Finally, we analysed structural disease outcomes by evaluating radiographic progression assessed using the Sharp–van der Heijde score on radiographs of hands and feet; data were available for 190 patients at baseline and 164 patients at week 16 (appendix p 9). We observed significantly worse radiographic progression with rituximab: the delta changes in the Sharp–van der Heijde score for rituximab was 0·9 (95% CI 0·5 to 1·3), as opposed to 0·3 (0·0 to 0·6) for etanercept and tocilizumab grouped together, with an effect size of 0·6 (0·2 to 1·0, $p=0\cdot0072$; appendix pp 9, 20 for probability plots). When stratifying patients according to synovial B cell status, B cell-poor patients showed a similar limited radiographic progression with all the drugs, whereas the presence of a B cell-rich infiltrate, previously linked with worse radiographic burden in patients with early rheumatoid arthritis,⁷ was associated with a significantly higher radiographic progression at 16 weeks in patients treated with rituximab (delta Sharp–van der Heijde score 1·3

	Total (n=283)	Rituximab (n=102)	Etanercept (n=108)	Tocilizumab (n=73)	p value
Serious adverse events*	18 (6%)	6 (6%)	9 (8%)	3 (4%)	0·53
Serious adverse events related to study drug	11 (4%)	4 (4%)	6 (6%)	1 (1%)	0·40
Circumscribed labyrinthitis	1 (<1%)	0	0	1 (1%)	..
Hypersensitivity pneumonitis	1 (<1%)	0	1 (1%)	0	..
Infected insect bite	1 (<1%)	0	1 (1%)	0	..
Influenza A virus infection	1 (<1%)	1 (1%)	0	0	..
Lower respiratory tract infection	1 (<1%)	0	1 (1%)	0	..
Lower respiratory tract infection viral	1 (<1%)	0	1 (1%)	0	..
Neutropenia	1 (<1%)	1 (1%)	0	0	..
Neutropenic sepsis	2 (1%)	1 (1%)	1 (1%)	0	..
Other appendicitis	1 (<1%)	0	1 (1%)	0	..
Other pyelonephritis or pyonephrosis, not specified as acute or chronic	1 (<1%)	0	1 (1%)	0	..
Postoperative wound infection	1 (<1%)	1 (1%)	0	0	..
Serious adverse events unrelated to study drug	11 (4%)	6 (6%)	3 (3%)	2 (3%)	0·54
Ovarian cancer	1 (<1%)	1 (1%)	0	0	..
Cardiac pacemaker insertion	1 (<1%)	0	1 (1%)	0	..
Cyst ovary	1 (<1%)	1 (1%)	0	0	..
Endometrial thickening	1 (<1%)	1 (1%)	0	0	..
Fall	1 (<1%)	1 (1%)	0	0	..
Fracture of spine	1 (<1%)	0	0	1 (1%)	..
Giant-cell arteritis	1 (<1%)	0	1 (1%)	0	..
Non-specific hepatitis	1 (<1%)	0	0	1 (1%)	..
Ovariectomy	2 (1%)	1 (1%)	1 (1%)	0	..
Xanthogranulomatous cholecystitis	1 (<1%)	1 (1%)	0	0	..
Serious adverse events resulting in study drug discontinuation	4 (1%)	2 (2%)	2 (2%)	0	..
Any non-serious adverse event†	241 (85%)	84 (82%)	95 (88%)	62 (85%)	0·51

Data are n (%). All events were reported after the first prescription of the study drug up to week 48 (+30 days). Some patients had more than one adverse event. Recurrent events (ie, those that occurred more than once in the same participant) were considered as one event. Events are classified using the Medical Dictionary for Regulatory Activities system classification, using the lowest level terms grouping. *Indicates the number of times any of the serious adverse events listed occurred in any patient, counted once per patient. †Indicates the number of times any non-serious adverse events occurred in any patient, counted once per patient.

Table 4: Safety data from baseline to 48 weeks (including treatment switch at 16 weeks)

[95% CI 0·5 to 2·1]) than in those treated with etanercept or tocilizumab (0·3 [−0·1 to 0·8], $p=0\cdot031$; appendix p 9). Accordingly, a significant interaction between treatment and B cell status on the 16-week total Sharp–van der Heijde score was found, independent of seropositivity (χ^2 value of 4·011, $p=0\cdot036$, corrected for rheumatoid factor or ACPA status).

A total of 22 serious adverse events (11 of which were related to the study drug and 11 unrelated to study drug; table 4) occurred in 18 patients throughout the trial. Of these patients, six were in the rituximab group, nine in the etanercept group, and three in the tocilizumab group, with no significant differences between the three drugs ($p=0\cdot53$; table 4). Non-serious adverse events occurred in 241 (85%) patients. Of these patients, 84 (82%) were in the rituximab group, 95 (88%) in the etanercept group, and 62 (85%) in the tocilizumab group, with no

significant differences. No deaths or suspected unexpected serious adverse reactions were reported. Two serious adverse events in the rituximab group (ovarian cancer and neutropenic sepsis) and two in the etanercept group (giant-cell arteritis and neutropenic sepsis) directly resulted in the permanent discontinuation of the study regimens. Of 288 synovial biopsy procedures performed in the trial, a coincidental tendon rupture in a patient with pre-existing evidence of tendon damage on MRI was reported, but no serious adverse events were directly related to the synovial biopsy.

Discussion

The STRAP trial aimed to tackle the current trial-and-error approach in the treatment of rheumatoid arthritis,¹⁵ building on evidence from the first biopsy-driven randomised, controlled trial in rheumatoid arthritis (R4RA), which showed that the absence of synovial B cell molecular signatures in a population of patients with an inadequate response to anti-TNF agents was associated with a lower response to rituximab than to tocilizumab.² STRAP sought to explore the utility of synovial biopsy analyses at an earlier disease stage—ie, in patients with an inadequate response to conventional synthetic DMARDs before their first biological DMARD. We hypothesised that patients with a low synovial histological or molecular B cell score would have a lower response to rituximab than to etanercept or tocilizumab. Contrary to expectation, the primary endpoint was not met, as the ACR20 response to rituximab in B cell-poor patients was not significantly different from the response to etanercept and tocilizumab. In other words, in this population of patients with an inadequate response to conventional synthetic DMARDs, the absence of synovial B cell signatures established using a prespecified dichotomic B cell-rich versus B cell-poor classification was not associated with a significantly different response to B cell depletion with rituximab compared with alternative treatments (etanercept or tocilizumab). It remains to be established whether these differences could be linked to the different disease stage (mean disease duration R4RA was 9 years, compared with 3 years in STRAP) and the potential plastic modification of disease pathology following exposure to anti-TNF therapy, or to the wrong choice of an arbitrary cutoff for the dichotomic synovial B cell-rich versus B cell-poor classification. For example, in an independent post-hoc analysis of the R4RA trial, using a different patient classification based on single-cell transcriptomics that defines specific cell-type abundance phenotypes in the synovial biopsy, cell-type abundance phenotypes were shown to be more accurate than the original R4RA B cell-poor and B cell-rich classification.¹⁶

When analysing the whole population, independently of the B cell status, etanercept and tocilizumab showed higher response rates than rituximab. This finding seems to contrast with previous non-biopsy studies that

showed the non-inferiority of rituximab to TNF inhibitors;¹⁷ however, in the ORBIT study,¹⁷ response was assessed at 6 months and 12 months, with a second administration of rituximab in patients flaring at 20 weeks, whereas in STRAP the response was recorded at 16 weeks. Our longitudinal analyses confirmed that, at this timepoint, rituximab is at a disadvantage because of its slower onset of action. In line with the known ability of tocilizumab to directly affect inflammatory markers,¹⁴ the analysis of individual outcome components showed their rapid and persistent reduction already evident at week 4, which could explain the higher response rates observed in the tocilizumab and etanercept group when using composite endpoints. However, we also identified a greater effect of tocilizumab than of rituximab on objective measurements, such as swollen joints, and, importantly, on patient-reported outcomes, such as patient global assessment of disease activity and pain. This is in line with previous reports showing a greater effect of tocilizumab on patient-reported outcomes than of conventional synthetic DMARDs and TNF inhibitors, particularly when used in monotherapy.¹⁸

Because B cells are only one of many players involved in driving synovial histopathology, by classifying patients into synovial pathotypes, as previously described in the Pathobiology of Early Arthritis Cohort,⁷ we observed that patients with a pauci-immune pathotype (scant or absent B cells, T cells, and macrophages) had a poor response to rituximab, with none of them reaching ACR50 or above. These observations are in line with previous studies linking the pauci-immune pathotype with no response to anti-TNF therapy¹⁹ and with the recent evidence of a fibroblast-stromal synovial signature associated with multidrug-resistant rheumatoid arthritis.³ Notably, in this population of biological DMARD-naïve patients with rheumatoid arthritis, the absence of synovial immune cells was associated quite specifically with no response to rituximab. Because the pauci-immune pathotype is also associated with a lower prevalence of autoantibody positivity (ACPA positivity 66% in the pauci-immune pathotype vs 83% in the diffuse-myeloid pathotype and 91% in the lymphomyeloid pathotype), these results could be in keeping with the established evidence showing better response to rituximab in seropositive patients.²⁰ However, the 66% of ACPA-positive patients with a pauci-immune pathotype did not reach at least a ACR50 response when treated with rituximab compared with patients with diffuse-myeloid and lymphomyeloid pathotype, suggesting that the absence of immune cells in synovia, even in the presence of circulating autoantibodies, is informative of non-response to B cell depletion. By contrast, the response of patients with a pauci-immune pathotype to etanercept and tocilizumab was equivalent to patients with a diffuse-myeloid or lymphomyeloid pathotype. This finding indicates that etanercept and tocilizumab have a broader effect on synovial inflammation, in addition to the direct effects on

immune cells. Accordingly, the analysis of post-treatment synovial biopsy samples showed that although rituximab has an exclusive effect on B cells, the wider effect of etanercept and, more strikingly, tocilizumab on myeloid and stromal cells could at least partially explain their higher efficacy in patients with lower synovial immune cell infiltration. For tocilizumab, in particular, this is in line with the pleiotropism of interleukin-6,¹⁴ a cytokine known to be produced by fibroblasts²¹ and able to induce their activation and differentiation.²²

Like most human studies, this biopsy-driven, randomised, head-to-head comparison of biological DMARDs has some limitations. First, the comparison of medications with different routes of administration and pharmacodynamics, in particular rapid versus slow mechanism of action. Second, grouping etanercept and tocilizumab for the primary analysis was a pragmatic choice, but individual medications were shown to have important differences that were probably diluted in the combined analysis. Third, the choice of a low threshold response rate (ACR20) as the primary endpoint might have reduced the chance of detecting a difference when comparing active treatments. In support of this notion, a meta-analysis, comparing biosimilars versus originator biological DMARDs, has shown that low threshold measures like ACR20 can be heavily affected by placebo response.²³ Finally, as the analyses at 16 weeks are based on a single cycle of rituximab (two 1000 mg infusions, 14 days apart), it remains to be established whether a second cycle of rituximab could have led to additive efficacy, as shown in previous studies.²⁴ Although STRAP extended beyond 16 weeks, patients without a response to rituximab at 16 weeks were switched to an alternative medication; therefore, the results past the primary endpoint cannot be informative on this issue. Importantly, however, our results show that rituximab has a much slower effect on various disease activity components, including swollen joints and patient-reported outcomes. Crucially, although in a relatively small number of patients, we observed a higher rate of structural damage progression at 16 weeks in B cell-rich patients treated with rituximab, an observation of potential clinical importance and in line with previous evidence showing higher risk of radiographic progression in patients with early rheumatoid arthritis and synovial lymphoid infiltrates.⁷ Notably, the interaction between B cell status and treatment on radiographic progression was independent of seropositivity, a well known risk factor for radiographic progression. Previous studies have shown that rituximab could reduce radiographic progression, but most of those studies included patients with an inadequate response to TNF inhibitors, who were thus at a more advanced disease stage, and did not compare rituximab with other biological DMARDs.^{25,26} In the IMAGE trial,²⁷ for example, rituximab inhibited structural damage in methotrexate-naïve patients, including at the 2-year follow-up; however, this was in

comparison with methotrexate, not with other biological DMARDs. Similar effects were observed by MRI, but, again, the comparator was methotrexate.²⁸ As the ORBIT trial¹⁷ did not include structural outcomes, to our knowledge, this is the first study comparing rituximab head-to-head with other biological DMARDs for structural outcomes, and our results emphasise the risk of damage progression when rituximab is used at a relatively early stage of the disease, particularly in patients with high degrees of synovial inflammation.

As expected, safety analyses indicated no differences in adverse events and serious adverse events between the three medications. Notably, however, this study confirms the safety and tolerability of minimally invasive synovial tissue analysis, together with the feasibility of its implementation in multicentre trials.

In conclusion, the STRAP trials showed that a dichotomic classification into synovial B cell poor versus rich is unable to predict treatment response in patients treated with rituximab compared with etanercept or tocilizumab, as in patients with a low or absent synovial B cell signatures the primary endpoint (ACR20 response) was not significantly different. However, the study shows that synovial tissue analysis could be of potential relevance for clinical practice. In particular, the lower response to rituximab in patients with a pauci-immune pathotype, together with the slower onset of action of rituximab and the higher risk of radiographic progression in B cell-rich patients, suggests that rituximab is less suitable in early disease, as the window of opportunity²⁹ for inhibiting structural damage requires medications that can rapidly control joint inflammation.

As for the future of precision medicine in rheumatoid arthritis, it is expected that predictive algorithms of response versus non-response will emerge from ongoing post-hoc deep molecular analyses, as previously shown in the R4RA trial.³ Additionally, given the well known heterogeneity of rheumatoid arthritis, rather than binary patient stratification, more comprehensive biomarker-driven approaches should be used, in which granular molecular and histological response signatures are combined to define predictive algorithms of treatment responses to individual medication.³⁰

Contributors

CP conceived of the trial, sought funding, finalised the protocol, and oversaw the trial and assumed overall responsibility for the trial and all the reported data. CP and PS conceptualised and designed the trial, and developed the protocol, and statistical analysis plan. FR, AN, GG, LW, and EJ collected and analysed the data. DvdH analysed and critically reviewed the radiographic data. LW and GG verified the underlying data. GG did the statistical analyses. FR and AN wrote the first draft of the manuscript. FR, AN, GG, LW, MJL, and CP contributed to the manuscript revision and final editing. FR, AN, FH, AGP, AF, NG, AC, EC, IM, PD, CJE, MHB, EG, PCT, NN, JDC, SR, NDM, DJ, PPS, RS, MRE, PH, JPP, BD, CG, JG, HC, and CP recruited the patients, collected patient data, and verified the clinical data. All authors contributed to the discussion and interpretation of the results, critically reviewed the manuscript, and approved the final version to be submitted. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

AF reports grants or contracts from Janssen, GSK, Mestag, Nascient, Bristol Myers Squibb (BMS), Roche, and UCB, and consulting fees from Janssen and Sonoma. AGP reports grants or contracts from GSK, Pfizer, and Gilead, and consulting fees from Inflection Biosciences. CJE reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Pfizer, Gilead, Galapagos, AbbVie, Eli Lilly, BMS, Biogen, Celgene, Roche, Sanofi, and UCB Pharma; consulting fees from Gilead, Galapagos, AbbVie, and Eli Lilly; and support for attending meetings or travel from Eli Lilly. CP reports grants or contracts from GSK, Pfizer, BMS, Sanofi, Novartis, Janssen, Exagen, Genentech, and Navidea; trial funding from the UK Medical Research Council and Versus Arthritis; provision of investigational medicinal products for the trial from Pfizer and Roche; consulting fees from AbbVie, Janssen, Exagen, and Kinikska; and support for attending meetings or travel from EULAR, British Society for Rheumatology, and ACR. DvdH reports consulting fees from GSK, Pfizer, Gilead, Galapagos, AbbVie, Eli Lilly, BMS, UCB Pharma, Novartis, Janssen, Argenx, Bayer, and Takeda; is an associate editor for *Annals of the Rheumatic Diseases*, an editorial board member for the *Journal of Rheumatology* and *RMD Open*, an adviser for Axial Spondyloarthritis International Society, and Director of Imaging Rheumatology. EC reports grants or contracts from Pfizer, Biogen, Sanofi, and Bio-Cancer; consulting fees from Gilead, AbbVie, Biogen, Sanofi, UCB Pharma, Janssen, Fresenius Kabi, and R-Pharm; payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Galapagos, AbbVie, Eli Lilly, Sanofi, Fresenius Kabi, and Chugai Pharma; support for attending meetings or travel from Galapagos and UCB Pharma. EG reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Pfizer, Galapagos, AbbVie, Eli Lilly, BMS, Novartis, and Janssen. FR reports consulting fees from Ono Pharmaceutical. HC reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from GSK and UCB Pharma; consulting fees from Pfizer, Galapagos, and Argenx; and participation on a Data Safety Monitoring Board or Advisory Board for AstraZeneca. IM reports grants or contracts from Pfizer, Gilead, AbbVie, Eli Lilly, BMS, UCB Pharma, Novartis, Janssen, AstraZeneca, Amgen, Causeway Therapeutics, Cabaletta, Sanofi Regeneron, Evelo, Compugen, and Moonlake, and is a trustee for Versus Arthritis. JG reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Pfizer, Galapagos, AbbVie, Eli Lilly, UCB Pharma, Janssen, and Vifor. JPP reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Pfizer, AbbVie, Eli Lilly; consulting fees from AbbVie; support for attending meetings or travel from AbbVie and Eli Lilly; and participation on a Data Safety Monitoring Board or Advisory Board for AbbVie. MRE reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from GSK, Galapagos, and AbbVie; support for attending meetings or travel from Eli Lilly, AbbVie, and Janssen; and consulting fees from GSK, Fresenius Kabi, and AstraZeneca. MHB reports grants or contracts from Gilead, and payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Galapagos, AbbVie, and Boehringer Ingelheim. NN reports support for attending meetings or travel from Janssen. PD reports payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Pfizer, Galapagos, AbbVie, and Eli Lilly, and support for attending meetings or travel from Galapagos, AbbVie, and Fresenius Kabi. PCT reports consulting fees from GSK, Pfizer, Gilead, Galapagos, AbbVie, Eli Lilly, Biogen, Sanofi, UCB Pharma, Janssen, Fresenius Kabi, and Nordic Pharma; grants or contracts from Galapagos; payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from AbbVie; support for attending meetings or travel from Eli Lilly; participation on a Data Safety Monitoring Board or Advisory Board from Moonlake, Kymab, and Immunovant. All other authors declare no competing interests.

Data sharing

The anonymised raw data will be stored in a publicly available web-based repository. At the current time all data requests should be submitted to

the corresponding author for consideration. Access to anonymised data may be granted following review.

Acknowledgments

The STRAP trial was part of the Maximising Therapeutic Utility in RA (MATURA) programme, jointly funded by the UK Medical Research Council (MRC) and Versus Arthritis (grant reference MR/K015346/1). Infrastructure support was also provided by Versus Arthritis (Experimental Arthritis Treatment Centre grant, number 20022). Financial support was provided as an Investigator Sponsored Research Grant from Pfizer and Roche Products through the donation of the study drugs. We thank the independent members of the Data Monitoring Committee: Max Parmar (Chair), John Gribben, and Chris Deighton for their expertise ensuring the trial was conducted to the highest standard. We also thank the independent members of the Trial Steering Committee: Patrick Kiely (Chair), Diana Skingle (Patient Representative), James Galloway, Adam Young, and David O'Reilly for their expertise and insightful advice given throughout the study; the Patient Advisory Group represented by Zoe Ide (Chair) for their continuous support in evaluating trial documentation and the linked research as well as in disseminating the importance of precision medicine among patients and through patient organisations, such as the National Rheumatoid Arthritis Society, and last, but not least, all patients participating in the trial for their generous contribution of time, patience and willingness to consent to a minimally invasive procedure that they would not have had as part of their routine care. Finally, we acknowledge our funders, the MRC and Versus Arthritis. We thank the UK National Institute for Health Research (NIHR) Manchester, Leeds, Newcastle, Birmingham, Cambridge, Oxford, Southampton, Barts, and University College London Hospital Biomedical Research Centres and NIHR Clinical Research Facilities for support; the views expressed are those of the authors and not necessarily the UK National Health Service, NIHR, or the Department of Health.

References

- 1 Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. *JAMA* 2018; **320**: 1360–72.
- 2 Humby F, Durez P, Buch MH, et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. *Lancet* 2021; **397**: 305–17.
- 3 Rivellesse F, Surace AEA, Goldmann K, et al. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial. *Nat Med* 2022; **28**: 1256–68.
- 4 Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; **69**: 1580–88.
- 5 Humby F, Kelly S, Bugatti S, et al. Evaluation of minimally invasive, ultrasound-guided synovial biopsy techniques by the OMERACT filter—determining validation requirements. *J Rheumatol* 2016; **43**: 208–13.
- 6 Just SA, Humby F, Lindegaard H, et al. Patient-reported outcomes and safety in patients undergoing synovial biopsy: comparison of ultrasound-guided needle biopsy, ultrasound-guided portal and forceps and arthroscopic-guided synovial biopsy techniques in five centres across Europe. *RMD Open* 2018; **4**: e000799.
- 7 Humby F, Lewis M, Ramamoorthi N, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Ann Rheum Dis* 2019; **78**: 761–72.
- 8 Krenn V, Morawietz L, Burmester G-R, et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* 2006; **49**: 358–64.
- 9 Rivellesse F, Humby F, Bugatti S, et al. B cell synovitis and clinical phenotypes in rheumatoid arthritis: relationship to disease stages and drug exposure. *Arthritis Rheumatol* 2020; **72**: 714–25.
- 10 Lewis MJ, Barnes MR, Blighe K, et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Rep* 2019; **28**: 2455–70.
- 11 Forrest AR, Kawaji H, Rehli M, et al. A promoter-level mammalian expression atlas. *Nature* 2014; **507**: 462–70.

- 12 van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000; **27**: 261–63.
- 13 Becht E, Giraldo NA, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol* 2016; **17**: 1–20.
- 14 Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR, Kishimoto T. Translating IL-6 biology into effective treatments. *Nat Rev Rheumatol* 2020 **16**: 335–45.
- 15 Smolen JS, Landewé RBM, Bergstra SA, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. *Ann Rheum Dis* 2023; **82**: 3–18.
- 16 Zhang F, Jonsson AH, Nathan A, et al. Cellular deconstruction of inflamed synovium defines diverse inflammatory phenotypes in rheumatoid arthritis. *bioRxiv* 2022; published online Feb 28. <https://doi.org/10.1101/2022.02.25.481990> (preprint).
- 17 Porter D, van Melckebeke J, Dale J, et al. Tumour necrosis factor inhibition versus rituximab for patients with rheumatoid arthritis who require biological treatment (ORBIT): an open-label, randomised controlled, non-inferiority, trial. *Lancet* 2016; **388**: 239–47.
- 18 Strand V, Michalska M, Birchwood C, et al. Impact of tocilizumab monotherapy on patient-reported outcomes in patients with rheumatoid arthritis from two randomised controlled trials. *RMD Open* 2017; **3**: e000496.
- 19 Nerviani A, Di Cicco M, Mahto A, et al. A Pauci-immune synovial pathotype predicts inadequate response to TNF α -Blockade in rheumatoid arthritis patients. *Front Immunol* 2020; **11**: 845.
- 20 Isaacs JD, Cohen SB, Emery P, et al. Effect of baseline rheumatoid factor and anticitrullinated peptide antibody serotype on rituximab clinical response: a meta-analysis. *Ann Rheum Dis* 2013; **72**: 329–36.
- 21 Baumann H, Kushner I. Production of interleukin-6 by synovial fibroblasts in rheumatoid arthritis. *Am J Pathol* 1998; **152**: 641–44.
- 22 Valin A, Del Rey MJ, Muncio C, et al. IL6/sIL6R regulates TNF α -inflammatory response in synovial fibroblasts through modulation of transcriptional and post-transcriptional mechanisms. *BMC Mol Cell Biol* 2020; **21**: 74.
- 23 Chadwick L, Zhao S, Mysler E, Moots RJ. Review of biosimilar trials and data on etanercept in rheumatoid arthritis. *Curr Rheumatol Rep* 2018; **20**: 84.
- 24 Vital EM, Dass S, Buch MH, Rawstron AC, Emery P. An extra dose of rituximab improves clinical response in rheumatoid arthritis patients with initial incomplete B cell depletion: a randomised controlled trial. *Ann Rheum Dis* 2015; **74**: 1195–201.
- 25 Cohen SB, Emery P, Greenwald MW, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* 2006; **54**: 2793–806.
- 26 Keystone E, Emery P, Peterfy CG, et al. Rituximab inhibits structural joint damage in patients with rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitor therapies. *Ann Rheum Dis* 2009; **68**: 216–21.
- 27 Tak PP, Rigby W, Rubbert-Roth A, et al. Sustained inhibition of progressive joint damage with rituximab plus methotrexate in early active rheumatoid arthritis: 2-year results from the randomised controlled trial IMAGE. *Ann Rheum Dis* 2012; **71**: 351–57.
- 28 Peterfy C, Emery P, Tak PP, et al. MRI assessment of suppression of structural damage in patients with rheumatoid arthritis receiving rituximab: results from the randomised, placebo-controlled, double-blind RA-SCORE study. *Ann Rheum Dis* 2016; **75**: 170–77.
- 29 Burgers LE, Raza K, van der Helm-van Mil AH. Window of opportunity in rheumatoid arthritis - definitions and supporting evidence: from old to new perspectives. *RMD Open* 2019; **5**: e000870.
- 30 Pitzalis C, Choy EHS, Buch MH. Transforming clinical trials in rheumatology: towards patient-centric precision medicine. *Nat Rev Rheumatol* 2020; **16**: 590–99.