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Rituximab versus Tocilizumab in anti-TNF inadequate responder patients with Rheumatoid Arthritis (R₄RA): a stratified, biopsy-driven, multi-centre, randomised, open label, controlled clinical trial – 16 week outcomes

Keywords: Rheumatoid Arthritis, Biologic, Synovium, Synovial tissue, Histology, Ultrasound, Rituximab, Tocilizumab

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Summary

Background

Though biologic therapies have transformed the outlook for rheumatoid arthritis (RA), only 30% of patients achieve low disease activity. As over 50% of RA patients show low/absent synovial B-cell infiltration, in this first stratified, biopsy-driven, multi-centre, randomised-controlled-trial (RCT) we tested the hypothesis that, in synovial-biopsy B-cell poor patients tocilizumab (targeting IL-6) is superior to rituximab (targeting CD20+ve B-cells).

Methods

R4RA is a 48-week biopsy-driven phase-IV open-label RCT conducted in 19 European centres. Baseline/pre-treatment synovial tissue was obtained, classified histologically as B-cell poor/rich and anti-TNF inadequate responder RA patients randomised 1:1 to receive rituximab or tocilizumab. Molecular B-cell poor/rich synovial tissue classification was also undertaken. The study was powered to test superiority of tocilizumab over rituximab in the B-cell poor population at 16-weeks. The primary end-point was defined as Clinical-Disease-Activity-Index (CDAI) \geq 50% improvement from baseline. CDAI major treatment response (CDAI-MTR), defined as CDAI \geq 50% improvement and CDAI $<$ 10.1, was also analysed. Trial ISRCTN number: ISRCTN97443826.

Findings

Between 28/02/2013 and 17/01/2019 164 patients were randomised (83:rituximab, 81:tocilizumab). In histologically-classified B-cell poor patients, the primary outcome was not met (RR:1.25, 95% CI:0.8-1.96), while CDAI-MTR was met (RR:1.96, 95% CI:1.01:3.78). Moreover, in molecularly-classified B-cell poor patients both the primary end-point and CDAI-MTR were met (RR:1.72, 95% CI:1.02-2.91 and RR:4.12, 95% CI:1.55-11.01). Higher numbers of adverse events (327 vs 284) and serious adverse events (18 vs 8, $p < 0.05$) was observed in tocilizumab patients treated.

Interpretation

Molecular stratification of RA synovial tissue shows stronger correlations with clinical responses compared to histopathological classification demonstrating that, in patients with B-cell poor synovium, tocilizumab is superior to rituximab. This supports the notion that disease tissue target levels are important to inform treatment response. Confirmation in independent studies, together with refined stratification methods, may lead to better therapeutic choice in RA.

Funding

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Introduction

Rheumatoid arthritis (RA) is a chronic immune mediated inflammatory disease characterised by synovitis and joint damage that results in considerable morbidity and an increased mortality.(1) Although biologic 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 have underlined the need to define predictive markers of response that enrich for best therapeutic outcome and facilitate patient stratification prior to treatment.(2) B-cells are central to RA pathogenesis driving synovial inflammation through the production of local disease specific autoantibodies, (3) secretion of pro-inflammatory and osteoclastogenic cytokines(4) and acting as antigen-presenting cells. The pivotal role of B cells has been confirmed by the efficacy of the B cell depleting agent rituximab.(5) Rituximab is licensed for use following failure of conventional synthetic (cs)DMARDs and TNF inhibitor (TNFi) therapy. However in this more therapy-resistant patient cohort clinical response to rituximab is heterogeneous with only 30% of patients achieving an American College of Rheumatology (ACR) 50% response at 6 months(6). Given the mechanism of action of rituximab it was hypothesized that the level of pre and/or post-treatment circulating B cells could be used to predict treatment response. However, pre-treatment peripheral B cell numbers or depletion levels, measured by conventional flow cytometry, found no association with clinical outcome.(5–7) Moreover, although the depth of depletion measured by high-sensitive flow cytometry initially appeared to be more informative, small studies have reported contradictory results (8,9). Nevertheless, these studies have highlighted the importance of high levels of circulating plasma-blasts/pre-plasma cells (CD20-ve) associated with non-response. However, the critical still unanswered question remains why despite the profound depletion of peripheral blood B cells induced by rituximab in all patients, only approximately half of the patients respond to this therapy. Also unclear is how the number and/or the level of depletion of B cells in the disease tissue (synovium) related to rituximab response. In that context, variable results from small observational biopsy-based studies have been reported (10–12). In particular, the number of pre-treatment synovial CD79a+ B cells (12) and specific molecular signatures(11) as well as the reduction post-treatment of synovial plasma cells(10) have shown an association with response to rituximab. However, the observational nature of these studies, the small number of patients analysed and the use of different timepoints for assessment of treatment outcome

makes it difficult to draw firm conclusions. Importantly, and as reported in joint replacement tissue from late stage RA patients (13) we have recently demonstrated in active early RA (14) that over 50% of patients showed low/absent B-cell infiltration in the synovial biopsy, suggesting that in these patients joint inflammation is driven by other cell types. This prompted us to test the hypothesis, in a randomised controlled trial (RCT), in patients who are candidates for rituximab therapy according to NICE guidance (inadequate responders to TNF inhibitors) with low/absent B cells in the synovial biopsy, that an alternative biologic agent targeting different biological pathways such as tocilizumab (a specific biologic IL6R inhibitor) maybe more effective. Here we report the 16-week clinical outcomes from the first biopsy-driven, multi-centre, stratified RCT in RA (R4RA) evaluating whether patient stratification according to synovial B-cell poor/rich status influences clinical response to rituximab in comparison to tocilizumab.

Methods

Trial design

We conducted a phase IV open label randomised control trial in 19 European centres. 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 protocol, amendments and documentation of consent were approved by the institutional review board of each study centre or relevant independent ethics committees. The trial was supported by an unrestricted grant from the National Institute for Health Research (NIHR). The study protocol is available at <http://www.r4ra-nihr.whri.gmul.ac.uk>.

Patients

Patients aged 18 years or over, fulfilling 2010 ACR/EULAR classification criteria for RA(15) who were eligible for treatment with rituximab therapy according to UK NICE guidelines (failing or intolerant to csDMARD therapy and at least one biologic therapy (excluding trial IMPs)(16) 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 the appendix (table 1). All patients provided written informed consent.

Interventions

Synovial Biopsy: Patients underwent a synovial biopsy of a clinically active joint at entry to the trial performed according to local expertise as either US-guided or arthroscopic procedure, as previously described. (17,18)

Histological analysis: a minimum of 6 synovial biopsies were paraffin embedded en masse and sections stained for Hematoxylin and Eosin (H&E), and immune-histochemical markers CD20 (B cells), CD3 (T cells), CD138 (plasma cells) and CD68 (macrophages) as previously described. (3,19) Sections underwent semi-quantitative scoring (0-4) to determine levels of CD20+ B cells, CD3+ T cells, CD138+ plasma cells and CD68+ lining (l) and sub lining (sl) macrophages (appendix figure 1) adapted from a previously described score(19,20). H&E stained slides also underwent evaluation to determine the level of synovitis. (21) If CD20+ve cells were identified staining for CD21 (follicular dendritic cells) was also performed as previously described. (3) Patients were then classified as B-cell rich or B-cell poor if definite synovial tissue could be identified (appendix figure 2) in the NHS pathology laboratory of Barts Health NHS Trust by a consultant pathologist (HR) and independent histological classification in the research laboratories of QMUL by a second expert in synovial pathology (GT). Synovial tissue with a CD20 score ≥ 2 and with CD20+ B cell aggregates were classified as B cell rich as previously described (19). Synovial tissue with CD20 score < 2 were classified as B cell poor (19). Any discrepancies in classification were resolved through mutual agreement. Patients in which definite synovial tissue could not be identified were classified as “unknown”. B-cell rich samples were further classified as germinal centre (GC)+ve if CD21+ follicular dendritic cell (FDC) networks were subsequently identified (appendix figure 3). As predefined in the study protocol only patients classified as B-cell rich or B-cell poor were included in the primary analysis of the trial presented herein with examination of the GC+ve cohort to be undertaken as part of a subsequent exploratory analysis.

RNA-seq analysis: A minimum of 6 synovial samples per patient were immediately immersed in RNA-Later and RNA extracted using one of two protocols; either using Phenol/Chloroform isolation or via a Zymo Direct-zol™ RNA MicroPrep - Total RNA/miRNA Extraction kit as previously described. (19) All RNA samples were sent to Genewiz for RNA sequencing. 184

paired-end RNA-seq samples of 150 base pairs were trimmed to remove the Illumina adaptors using `bbduk` from the `BBMap` package version 37.93 using the default parameters. Transcripts were then quantified using `Salmon` version 0.13.1(22) and an index generated from the Gencode release 29 transcriptome following the standard operating procedure. `Tximport` version 1.13.10 was used to aggregate the transcript level expression data to genes, counts were then subject to variance stabilising transform (VST) using the `DESEQ2` version 1.25.9 package(23). One sample was removed as an outlier with the assistance of principal component analysis. Patients were classified as B cell poor/rich according to a previously developed B cell-specific gene module derived from analysis of FANTOM5 gene expression data (24). As no pre-determined 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 (appendix. Figure 4)

Randomisation and masking

Patients were randomised to receive rituximab or tocilizumab stratified into 4 blocks according to histological classification of baseline synovial biopsy (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 6 and 4. 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 to all the clinical trial site staff by the R4RA trial office except the named joint assessor (research nurse/assistant) at each site who remained blinded to study drug allocation. Clinical trial staff remained blinded to histological subtypes throughout the duration of the study.

Trial procedures

Following synovial biopsy and subsequent randomisation, rituximab (Mabthera, Roche) as two 1000mg infusions at an interval of 2 weeks or tocilizumab (RoActemra, Roche) infused at a dose of 8mg/kg at 4 weekly intervals was administered at baseline. Both drugs were obtained from hospital stocks. Patients were followed up at 4 weekly intervals throughout the 48 week trial treatment period where RA disease activity measurements and safety data

were collected (appendix figure 5). Clinical outcomes up to week 16 only are presented herein.

Outcomes

The study was powered to test superiority of tocilizumab over rituximab in the B-cell poor population at 16 weeks. The primary end-point was defined as difference in Clinical Disease Activity Index (CDAI)(25) $\geq 50\%$ improvement at 16 weeks from baseline between tocilizumab and rituximab treated groups .

The study was not powered to evaluate comparative efficacy of either drug in the B-cell rich cohort, however, assessment of CDAI response (as defined for primary outcome analysis) at 16 weeks was also carried out as secondary outcome analysis where non-inferiority of rituximab compared to tocilizumab with a 0.2 margin on the relative risk of response was evaluated.

Primary efficacy analysis evaluated the number of patients meeting primary end-point (CDAI $\geq 50\%$ improvement from baseline). Supplementary efficacy analysis evaluated the number of patients meeting CDAI major treatment response (CDAI-MTR = CDAI $\geq 50\%$ improvement and CDAI < 10.1). In addition, both CDAI $\geq 50\%$ improvement and CDAI-MTR were evaluated in B-cell poor and B-cell rich patients classified according to the molecular methodology described above. Additional secondary efficacy analyses and other key secondary endpoints, such as rates of DAS28 CRP/ESR low disease activity and remission and patient reported outcomes such as fatigue are defined in the appendix table 2. The incidence and severity of treatment and procedure emergent adverse events were monitored throughout the study; adverse event coding was performed according to the Medical Dictionary for Regulatory Activities, version 22.

Statistical Analyses

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 met the primary endpoint (assuming 55% response in tocilizumab and 20% response in rituximab). The assumed proportions of B-cell-poor, B-cell-rich and GC+ recruited patients were 60%, 35% and 5% respectively. After estimating for 10% ungradable biopsy samples and a 5% dropout rate, we estimated a total

of 160 patients would be required to achieve 90% power for the study. No power calculation was conducted on the B-cell-rich population. The primary endpoint and other binary endpoints were analysed using a Chi-square or Fisher's exact test as appropriate. For continuous outcomes an Analysis of covariance (ANCOVA) was performed or non-parametric ANCOVA depending on normality of data distribution. Changes from baseline within groups were analysed through paired Wilcoxon test. A Generalized Linear Mixed Model (GLMM) with treatment as a fixed effect and baseline DAS28 as a covariate was run to test treatment effect. Although the study was not powered to evaluate comparative efficacy of either drug in the B-cell-rich group, we tested non-inferiority of rituximab over tocilizumab with a 0.2 margin on the relative risk of response as a supplementary analysis. The analysis of the interaction between treatments and pathotypes was conducted through 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.

All efficacy analyses were performed in the intention-to-treat (ITT) population and then on the per protocol (PP) set to assess the robustness of the results. The PP population included all subjects from ITT who did not have any major protocol violations. The list of deviations that exclude a subject from PP was reviewed at a classification meeting prior to data lock. 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), where patients were analysed according to their actual treatment in case this differed from the scheduled treatment (randomised or switched). Missing values when assuming MAR were imputed using Multiple Imputation by Chained Equations (MICE) and implemented using R package "Amelia" 1.7.5. All statistical analyses were carried using R, version 3.5.1. The trial was registered on the ISRCTN database (ref 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 there were any safety issues, and to make recommendations to the Trial Steering Committee.

Funding

Funding for this study was provided by the Efficacy and Mechanism Evaluation (EME) programme of the National Institute for Health Research (NIHR). The funder of the study had

no role in study design, data collection, data analysis, data interpretation or writing of the report. No industry funding was implicated in this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

212 patients were screened, 190 were consented and 164 patients underwent randomisation. The first patient visit was on 28th February 2013 and the last patient visit was on 17th January 2019. The trial ended as recruitment targets were reached. 83 patients were randomised to receive treatment with rituximab and 81 with tocilizumab. 161 patients (82 rituximab and 79 tocilizumab) received investigational medicinal product (IMP); 99% (81/82) of rituximab and 92% (73/79) of tocilizumab treated patients completed treatment to primary endpoint at week 16 (figure 1). The largest proportion of patients (38%, 62/164) was recruited at Barts Health NHS Trust (appendix table 3).

Baseline characteristics, disease activity and histological groups were balanced across the treatment groups (table 1 and appendix table 4). Most patients were female (80%) and the majority were sero-positive for rheumatoid factor (74%) or anti-citrullinated peptide antibodies (80%). Median disease duration was 9 years (IQR 4,19). Disease activity was high with a mean DAS28(ESR) of 5.8 (SD 1.2). 49% (79/161) patients were classified as B-cell poor, 40% (64/161) as B-cell rich, 6% (9/161) as GC+ and 6% (9/161) as unknown.

49% (79/161) patients who received IMP were classified as B-cell poor histologically. 48% (38/79) patients were randomised to rituximab and 52% (41/79) to tocilizumab (Figure 1). At 16 weeks in the B-cell poor population no significant difference was observed in primary outcome (Figure 2A), CDAI \geq 50% improvement from baseline response rates (RR 1.25 95% CI 0.8:1.96) between rituximab and tocilizumab treatment groups. A predefined supplementary analysis of CDAI MTR (Figure 2A), however, did reach statistical significance (RR 1.96 95% CI 1.01:3.78). In addition, throughout a number of secondary endpoints in the B cell poor population the response rates in the tocilizumab treated patients were higher (Figure 2A) including: CDAI remission (<10.1) (RR 1.6, 95%CI 0.88:2.91), DAS28(ESR) moderate/good EULAR response (RR 1.33 95%CI 1.03:1.72), DAS28(ESR) low disease activity (RR 1.67 95%CI 0.88:3.15), DAS28(ESR) remission (RR2.32 95% CI 1:5.35), DAS28 (CRP) moderate/good EULAR

response (RR1.35, 95% CI 0.98:1.85), DAS28(CRP) low disease activity (RR 1.47 95%CI 0.83:2.6) and DAS28 (CRP) remission (RR1.72 95%CI 0.77:3.85) (figure 2A).

Other week-16 secondary endpoints also favoured tocilizumab including trends for greater falls in DAS28(ESR/CRP) and CDAI (appendix table 5). Quality of life outcome measures (FACIT and SF36 scores) also demonstrated higher levels of improvement between baseline and 16 weeks in tocilizumab treated patients (appendix table 5). We observed little difference in HAQ scores between IMP groups (appendix table 5). Importantly per protocol analyses were consistent with the ITT outcomes (appendix table 6).

Synovial tissue from 162 patients was available for RNA extraction and was subsequently sent for RNAseq analysis. Following exclusion of patients classified histologically as GC+ (n=9) 153 patients remained. One patient was withdrawn before IMP was administered and 28 were excluded following RNAseq quality control or due to poor mapping. Therefore 124 patients had RNAseq data available for subsequent analysis. 65/124 patients were classified as B cell poor using the median B cell module value. We next examined clinical outcomes comparing treatment groups in patients categorized as B cell poor according to RNAseq based B cell-specific gene module derived from analysis of FANTOM5 gene expression data. (24) Using the molecular classification, we observed a significantly higher response rate in the tocilizumab versus rituximab group both for CDAI \geq 50% improvement, the primary outcome measure (RR 1.72, CI 1.02:2.91) and CDAI-MTR (RR 4.12 95% CI 1.55:11.01). A number of secondary outcomes including EULAR DAS28 (ESR and CRP) good/moderate response, DAS28 (ESR and CRP) low disease activity (\leq 3.2) and DAS28 (ESR) remission (\leq 2.6) (figure 2B) also favoured tocilizumab. Similarly, to the histopathological classification, we also observed trends for larger falls in CDAI and DAS28 ESR/CRP between baseline and 16 weeks in the tocilizumab vs rituximab groups (appendix table 7) and trends for greater improvements in quality of life measures (FACIT and SF36 MCS and PCS) in tocilizumab treated patients (appendix table 7).

Finally, we analysed patients who were classified as B-cell rich, again using both the histopathological 40% (64/161) and RNA-seq 47% (59/124) classification. 52% (33/64) patients were randomised to rituximab and 48% (31/64) to tocilizumab (**Error! Reference source not found.**). Although the study was not powered for the comparative analysis of the B-cell-rich group week 16 response rates between the two biologic agents, we observed similar response rates for the majority of endpoints analysed including CDAI \geq 50%

improvement from baseline response rate (RR 1.31, 95% CI 0.76:2.26) and CDAI-MTR (RR 2.34, 95% CI 0.92:5.97) (Figure 3A). Similar effects were seen through a number of additional secondary endpoints (appendix table 8). Importantly, in comparison to the analysis in the B-cell poor cohort we saw minimal difference in quality of life measures (FACIT and SF36) between rituximab and tocilizumab treated groups (appendix table 8). Per protocol analyses were consistent with the ITT results (appendix table 9).

The 16 week outcomes were then evaluated between treatment groups in patients classified as B cell rich according to RNAseq classification criteria (n=59). No significant difference between rituximab and tocilizumab treated patients was observed for the primary endpoint (CDAI \geq 50% improvement), CDAI-MTR and the majority of primary and secondary efficacy endpoints evaluated (figure 3B and appendix table 10).

Logistic regression analysis showed no evidence of an interaction between IMP and histologically defined B-cell subgroups for primary endpoint but a statistically significant interaction between RNA-seq defined B-cell subgroup and IMP (p=0.049) was observed, suggesting that the difference between rituximab and tocilizumab was statistically different between RNA-seq B-cell rich and B-cell poor stratified groups. When we evaluated differences in CDAI \geq 50% improvement response rates to rituximab between patients classified histologically as B cell rich or B cell poor, however, we saw no significant differences in outcome (Fishers exact test p=0.81).

In patients treated with rituximab (n=82) we saw no significant difference in CDAI 50% response rates between those classified as ACPA positive and those negative (responders 44.8% 30/67 and 46.3% 7/15, respectively, p-value=0.89) and no significant difference between patients classified as RF positive and as negative (responders 43.8% 28/64 and 50% 9/9, respectively, p-value=0.63) (appendix table 11). We also saw no significant difference in response rates according to sero positivity for ACPA or RF in patients treated with tocilizumab (appendix table 11).

Safety data up to week 48 are summarised in Table 2 and appendix table 12, it can be seen that a higher number of adverse events (327 vs 284) and serious adverse events (18 vs 8, p<0.05) was observed in patients treated with tocilizumab vs rituximab. One patient in the rituximab treated group (corneal melt reported as a serious unexpected serious adverse

reaction) and three patients in the tocilizumab group (pleural effusion, chest pain and cytokine release syndrome) discontinued IMP because of serious adverse events. Of the serious adverse events three infections were reported in each IMP group and four ischaemic cardiac events were reported in the tocilizumab group vs one in the rituximab group (appendix Table 11). One death due to suicide was reported in the rituximab group. No malignancies were reported within the 48 week trial period. There were three patients who underwent randomisation but did not receive study drug and no serious adverse events were reported in these patients. Importantly, there were no serious adverse events reported related to synovial biopsy.

Discussion

Rituximab remains a pivotal therapeutic option for RA patients, however response to therapy remains heterogeneous with only 30% of patients achieving an American ACR50 response rates at 6 months (6). Thus, understanding the mechanism of response/non-response is critical to avoid unnecessary exposure to a potentially toxic and expensive drug. Though B cells are considered key players in RA pathogenesis, particularly in relationship to the development of systemic autoimmunity in RF/ACPA positive patients that may precede clinical manifestations by years, their contribution in sero-negative RA is less clear. (26) In addition, at the disease tissue level (synovium), synovial B cell infiltration is highly heterogeneous being low/absent in approximately 50% of patients despite high disease activity.(13,14) This suggests that, in these patients, synovial inflammation is sustained by alternative cell types and that if the numbers of CD20+ B cells, the target for Rituximab, are low/absent in the disease tissue (synovium), therapeutic response to this targeted therapeutic may be poor, while an alternative therapy with a mode of action not exclusively dependent on B cell depletion maybe more effective. The R4RA study, was designed and independently supported by the UK National Institute for Health Research (NIHR) with the aim to determine whether specific cellular (CD20+ B-cells) and molecular signatures (B-cell associated) in synovial tissue can mechanistically explain specific disease outcomes. In this first biopsy-based, multi-centre, randomised control trial in RA, the primary endpoint (CDAI \geq 50% improvement) between IMP groups was not met using the histological classification of patients as B cell poor (CD20 -ve/low), however a supplementary analysis evaluating a pre-specified definition of non-response as patient not meeting CDAI \geq 50%

improvement and CDAI<10.1, (defined as CDAI-MTR) did reach statistical significance. In addition, when patients were classified as B-cell poor/rich according to the Fantom 5-derived(24) B cell molecular module (73 genes), which became available at trial end, as based on RNA-sequencing of the synovial biopsy, both primary endpoint (CDAI \geq 50%) and CDAI-MTR (CDAI \geq 50% and CDAI<10.1) reached statistical significance. Moreover, although logistic regression analysis showed no evidence of an interaction between IMP and histologically defined B cell subgroups for primary endpoint, a statistically significant interaction between RNA-seq defined B-cell subgroup and IMP (p=0.049) was observed, confirming that the difference between rituximab and tocilizumab was statistically different between molecularly defined RNA-seq B-cell rich and B-cell poor stratified groups.

The reasons for the histological and molecular differences are likely to relate to the sensitivity of the classification technique. CD20 staining was evaluated at 3 cutting levels on a minimum of 6 biopsies as recommended for use in clinical trials and reported to be representative of the whole joint tissue. (27) However, though the semi-quantitative score used for balanced stratification prior to randomization had been validated both against digital image analysis (DIA) and the transcript levels determined using the FANTOM 5-derived gene set, (19,24) as no published “gold standard” was available, the cut-off of 0-1 for B-cell poor and 2-4 for B-cell rich was set arbitrarily and potentially not at an optimal level.

The molecular B-cell poor / rich classification, on the other hand, was determined by applying a FANTOM 5-derived module to include 73 genes associated with B-cells (19,24) to the RNA-seq of 6 pooled homogenized biopsies that provide a more integrated measure (expression of 30,000 genes) of pathobiological processes within 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 e.g. plasma blast/pre-plasma cells, subsets that both in the peripheral blood and synovial tissue have been shown to influence response to rituximab (9-12). Thus, the application of molecular classification overcame a number of limitations of the histological classification including the relatively subjective assessment of synovial B cell infiltration by histopathology with an objective method using the transcript expression levels median value of a B-cell gene set module.

Notably, in the B cell poor molecularly classified patients, tocilizumab was significantly superior to rituximab not only in relation to the primary endpoint (CDAI \geq 50% improvement) and CDAI-MTR but also in most of the secondary endpoints considered, indicating a closer

correlation with a broad range of outcome measures. Vice versa, in the B cell rich molecularly classified population the efficacy of rituximab overlapped with tocilizumab supporting the concept that target expression levels associate mechanistically with response/non-response. Namely, as both tocilizumab and rituximab modulate B cell function both drugs are efficacious in B cell rich patients, while in the B cell poor patients tocilizumab is more efficacious as able to work on non-B cell dependent pathways e.g. IL-6.

This study also highlighted the importance of the synovial biopsy in relationship to the RF/ACPA status, as no significant difference in clinical response rates to rituximab or tocilizumab were observed between RF and/or ACPA positive and those negative.

In terms of safety data, although we report a higher number of serious adverse events and adverse events in patients treated with tocilizumab these appeared largely unrelated to study drug but may suggest in this first head to head trial of rituximab and tocilizumab that tocilizumab is less well tolerated. Importantly there were no serious adverse events related to synovial biopsy supporting previously published data relating to safety of minimally invasive synovial biopsy techniques performed by rheumatologists.(17)

Being the first randomised controlled biopsy-based trial in RA, the study had some limitations. These include, firstly, the important issue of the binary B-cell poor/rich classification discussed above. This will require further analysis of the trial to determine a more accurate cut-off or may lead in the future to a different quantification method using for example continuous variable data (e.g. transcript levels), this being a sensitive tool to predict clinical response as reported for other therapeutic targets e.g. PD1. (28) Second, the choice of tocilizumab as an active comparator to rituximab, might not have been optimal, as tocilizumab itself modulates B cell function and survival.(29) Thus, if a biologic lacking direct B cell modulatory effects had been selected (e.g. a TNFi) a more pronounced treatment difference between pathotype groups may have been observed. Third, and 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 relative late primary time point (16 weeks). Similarly, the lack of double blinding for IMP (considered impractical and extremely inconvenient for patients) may have amplified the clinical response to tocilizumab, which was given as a monthly infusion compared to rituximab at 6 monthly intervals. However, as this was an NIHR funded trial the selection of tocilizumab was a pragmatic choice based largely on the accessibility of NHS trusts to funds to support biologic treatment beyond

2nd line therapy. Fourth, it is conceivable that despite a washout period for previous biologic as well as standardisation of steroid and csDMARD therapy prior to trial entry, that prior and/or concomitant therapy modulated baseline synovial pathobiology. Future studies evaluating synovial pathobiological markers and clinical response to biologic treatment in patients naïve to therapy are certainly warranted to address this issue. Finally, the choice of CDAI \geq 50% improvement as a primary binary outcome rather than for example EULAR/DAS28 ESR response illustrates the lack of precision of current assessments methods, as the choice of the latter would have led to meeting the primary outcome even by the histopathological classification.

In conclusion, we report herein the results from the first pathobiology-driven, multi-centre randomised controlled trial in rheumatoid arthritis which, taking together the histological and molecular classification of patients in B-cell poor/rich, indicates that in RA patients with B cell poor synovial biopsy tocilizumab is significantly more likely to induce clinical response than rituximab. In patients presenting with a B-cell rich synovium, on the other hand, rituximab is as effective as tocilizumab. Due to the limitations of the study discussed above, these findings cannot justify change in clinical practice, however, their confirmation in independent studies, together with the refinement of the molecular pathology classification (e.g. using continuous variable data rather than a binary classification) may lead to the development of tests able to stratify patients and determine treatment allocation of specific targeted biologic therapies according to the expression levels of their corresponding target in the disease tissue. The ability to target biologic therapies to the right patients, rather than continue current practice of trial and error, may enrich for clinical response with the potential to impact significantly 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 where stratification of patients according to tissue expression of drug target has been adopted in routine clinical practice.(30)

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