Barts Health NHS
NHS Trust

Queen Mary
University of London

Protocol Title : A **R**andomised, open labelled study in anti-TNFa inadequate responders to investigate the mechanisms for **R**esponse - **R**esistance to **Rituximab** versus Tocilizumab in RA (R4-RA)

Version: **4**

EudraCT reference: **2012-002535-28**

MREC reference: **12/WA/0307**

Dated: **13 / March / 2013**

TITLE OF THE PROTOCOL:

Developing a novel, biopsy-based diagnostic for patient stratification: "A **R**andomised, open labelled study in anti-TNFa inadequate responders to investigate the mechanisms for **R**esponse - **R**esistance to **Rituximab** versus Tocilizumab in RA".

Short title/Acronym: R4-RA

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Chief Investigator Agreement Page

The clinical study as detailed within this research protocol **(Version 4, dated 13/ March /2013)**, or any subsequent amendments, involves the use of an investigational medicinal product and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

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Statistician Agreement Page

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STUDY SUMMARY/SYNOPSIS

TITLE	Developing a novel, biopsy-based diagnostic for patient stratification: "A **R**andomised, open labelled studyin anti-TNFa inadequate responders to investigate the mechanisms for **R**esponse - **R**esistance to **Rituximab** versus Tocilizumab in RA (R4-RA)".
SHORT TITLE	R4-RA
Protocol Version Number and Date	Protocol V4, 13/03/2013
Methodology	Type of study: open-label, randomised controlled clinical trial
Total Study Duration	4 years
Objectives	This study will aim to develop a diagnostic tool (immunohistochemical analysis of synovial tissue) for patient stratification into responsive/non-responsive categories with respect to Rituximab therapy in patients who have had an inadequate response to anti-TNF therapy. Specifically, can a diagnostic synovial biopsy showing a B-cell "rich/poor pathotype" define specific disease responsive/resistant subsets for patient stratification and help rationalize biologic drug choice.
Phase of the Trial	Phase IV study
Number of Subjects/Patients	180
Main Inclusion Criteria	Patients will be recruited with active RA: 1. Patients who have failed anti-TNF therapy (inadequate responders – ir). Note: this includes patients that have failed anti-TNF therapy because of reactions. 2. Who are eligible for Rituximab therapy according NICE guidelines 3. Patients should be receiving a stable dose Methotrexate for at least 4 weeks prior to biopsy visit. 4. 2010 ACR / EULAR Rheumatoid Arthritis classification criteria for a diagnosis of Rheumatoid Arthritis. 5. Over 18 years of age 6. Patient must be capable of giving informed consent 7. Willingness and ability to comply with scheduled visits, treatment plans and laboratory tests and other study procedures

Statistical Methodology and Analysis	1. For the randomized comparison of Rituximab versus Tocilizumab in B-cell poor patients, the primary endpoint will be analysed (by intent to treat) using the chi-squared test for the difference between two proportions. Patients switching treatment before 6 months because of lack of response will be considered as non-responders at 6 months. 2. For non-randomised comparisons between subgroups identified by B-cells in synovial biopsies, we will use the Fisher exact test comparing (i) response to Rituximab in B-cell poor patients compared to B-cell richThe definition of B-cell status will be clearly defined before the start of the trial. 3. A test of interaction between treatment and B-cell status (rich versus poor, excluding germinal centre) will be based on a likelihood ratio tests between nested logistic regression models.

Glossary of Terms and Abbreviations

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CTPU	Cancer Prevention Trial Unit
DMARD	Disease Modifying Anti-Rheumatic Drug
DMC	Data Monitoring Committee
EC	European Commission
EMEA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Union Drug Regulating Authorities Clinical Trials
EudraVIGILANCE	European Union Drug Regulating Authorities Pharmacovigilance
GAfREC	Governance Arrangements for NHS Research Ethics Committees
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISRCTN	International Standard Randomised Controlled Trial Number
JRMO	Joint Research and Management Office
MA	Marketing Authorisation
MHRA	Medicines and Healthcare products Regulatory Agency
MS	Member State
Main REC	Main Research Ethics Committee
NHS R&D	National Health Service Research & Development
PI	Principle Investigator
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person for release of trial drug
Participant	An individual who takes part in a clinical trial
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Document Verification
SmPC	Summary of Product CharacteristicsRituximab
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group

TSC	Trial Steering Committee
US	Ultrasound

Contents

1 INTRODUCTION

1.1 Background

Rheumatoid arthritis (RA) is one of the most important chronic inflammatory disorders in the UK. The diagnosis of RA leads to considerable morbidity and an increased mortality[1, 2]. According to the National Audit Office (2009 - http://www.nao.org.uk/) there are 26,000 new cases of RA each year with 582,000 prevalent cases in England. 45% of these people are of working age and within 1 year of diagnosis 30% are unemployed. RA is characterized by a symmetrical, erosive polyarthritis, resulting from chronic synovitis, and the presence of circulating autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (ACPA), strongly suggesting an autoimmune pathogenesis. Although biological therapies have revolutionized the treatment of RA, a sizable group of patients (30-40%) are "resistant"[3, 4].

Recently there has been a greater understanding of the importance of B cells in driving the inflammatory processes involved in RA. B cells may drive synovial inflammation by production of autoantibodies, acting as effective antigen-presenting cells and may promote synovial inflammation by producing pro-inflammatory cytokines[5]. Thus, depletion of B cells could interfere with important mechanisms involved in the perpetuation of the inflammatory response in RA. Rituximab is a chimeric monoclonal antibody directed against the CD20 antigen expressed by B cells, has been approved by the US Food and Drug Administration and by the European Medicines Agency in Europe for the treatment of patients with RA who have had an inadequate response (ir) or were intolerant to tumour necrosis factor alpha (TNF) inhibitors. Current evidence on the efficacy of Rituximab relates primarily to rheumatoid factor positive patients, although even within this population clinical responses are heterogeneous with only 60% achieving an ACR20 response at 6 months[6, 7]. Recent synovial-based studies suggest that the heterogeneous clinical response may in part be explained by variable B cell depletion within the synovial tissue rather than simply in the peripheral blood[8-10]. A growing body of evidence would suggest that a more rational approach to Rituximab therapy and a stratified approach to patients may be required[11-13]. Despite this, NICE guidelines have recommended that all patients with inadequate response to anti-TNF therapy should receive Rituximab (NICE, http://www.nice.org.uk/CG79). A "blind" implementation of these guidelines will result in many patients, unlikely to respond, receiving a B Cell depleting agent with the associated risks with none of the potential benefits. A tailored approach to this intervention with patient stratification is required to better identify both responders and non-responders. In this proposed study we will test the hypothesis that the presence or absence of B cells and B cell-associated signatures within the joint will enrich for response/non-response to the B cell depleting agent Rituximab. We also hypothesize that in patients with a B-cell poor synovial biopsy, alternative biologics such as the IL-6 receptor blocker Tocilizumab will be more effective. This study is considered a type A clinical study according to MHRA risk.

1.2 Investigational Medicinal Products

1.2.1 Rituximab

Within the remit of this study Rituximab is being used in accordance with its UK licence. Rituximab is a chimeric antibody consisting of a human immunoglobulin G1 (IgG1) kappa constant region with a variable region derived from a murine anti-CD20 antibody. Rituximab selectively targets CD20, a cell surface antigen that is uniquely expressed on a subset of B cells during the maturation process. Rituximab has a high binding affinity for the CD20 antigen, with specificity for the CD20 antigen residing in the variable murine regions. This represents a novel biological strategy for the treatment of rheumatoid arthritis (RA) compared with traditional disease-modifying anti-rheumatic drugs or tumour necrosis factor (TNF) inhibitors. Rituximab can disrupt a number of different events in the inflammatory process owing to the central role and multiple actions of B cells in the pathogenesis of RA. The synovial fluid of a joint affected by RA contains an abundance of B cells, and it is now recognised that the B lymphocyte plays three key roles in the pathogenesis of RA: antigen presentation leading to T cell activation, autoantibody production and cytokine production

Rituximab in combination with methotrexate is licensed for the treatment of adults with severe active rheumatoid arthritis who have had an inadequate response to or intolerance of other DMARDs, including one or more tumour necrosis factor α (TNF-α) inhibitor therapies.

Pharmacokinetic properties

Rituximab binds specifically to the transmembrane antigen, CD20, a non-glycosylated phosphoprotein, located on pre-B and mature B lymphocytes. CD20 is found on both normal and malignant B cells, but not on haematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissue. This antigen does not internalise upon antibody binding and is not shed from the cell surface. CD20 does not circulate in the plasma as a free antigen and, thus, does not compete for antibody binding. Peripheral B cell counts declined below normal following completion of the first dose of Rituximab. In rheumatoid arthritis patients, immediate depletion of B cells in the peripheral blood was observed following two infusions of 1000 mg Rituximab separated by a 14 day interval. Peripheral blood B cell counts begin to increase from week 24 and evidence for repopulation is observed in the majority of patients by week 40, whether Rituximab was administered as monotherapy or in combination with methotrexate. Following two intravenous infusions of Rituximab at a dose of 1000 mg, two weeks apart, the mean terminal half-life was 20.8 days (range, 8.58 to 35.9 days), mean systemic clearance was 0.23 l/day (range, 0.091 to 0.67 l/day), and mean steady-state distribution volume was 4.6 l (range, 1.7 to 7.51 l). Population pharmacokinetic analysis of the same data gave similar mean values for systemic clearance and half-life, 0.26 l/day and 20.4 days, respectively. The gender- related pharmacokinetic differences are not considered to be clinically relevant and dose adjustment is not required. No pharmacokinetic data are available in patients with hepatic or renal impairment.

1.2.2 Tocilizumab

Within the remit of this study Tocilizumab is being used in accordance with its UK licence. Tocilizumab (RoActemra, Roche) is a humanised monoclonal antibody that inhibits cytokine interleukin-6 (IL-6). Reducing the activity of IL-6 may reduce inflammation in the joints, prevent long-term damage, improve quality of life and function, and relieve certain systemic effects of rheumatoid arthritis. Tocilizumab binds specifically to both soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R). Tocilizumab has been shown to inhibit sIL-6R and mIL-6R-mediated signalling. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, monocytes and fibroblasts. IL-6 is involved in diverse physiological processes such as T-cell activation, induction of immunoglobulin secretion, induction of hepatic acute phase protein synthesis and stimulation of haemopoiesis.

Tocilizumab in combination with methotrexate (MTX), is indicated for the treatment of moderate to severe active rheumatoid arthritis (RA) in adult patients who have either responded inadequately to, or who were intolerant to, previous therapy with one or more disease-modifying anti-rheumatic drugs (DMARDs) or tumour necrosis factor (TNF) antagonists. In these patients, Tocilizumab can be given as monotherapy in case of intolerance to MTX or where continued treatment with MTX is inappropriate. Tocilizumab has been shown to reduce the rate of progression of joint damage as measured by X ray and to improve physical function when given in combination with methotrexate.

Pharmacokinetic properties

The pharmacokinetics of Tocilizumab were determined using a population pharmacokinetic analysis on a database composed of 1793 RA patients treated with a one-hour infusion of 4 and 8 mg/kg Tocilizumab every four weeks for 24 weeks. The following parameters (predicted mean±SD) were estimated for a dose of 8 mg/kg Tocilizumab given every four weeks: steady- state area under curve (AUC)=35000±15500 h µg/ml, trough concentration (Cmin)=9.74±10.5 µg/ml and maximum concentration (Cmax)=183±85.6 µg/ml, and the accumulation ratios for AUC and Cmax were small: 1.22 and 1.06, respectively. Following intravenous administration, Tocilizumab undergoes biphasic elimination from the circulation. The linear clearance was estimated as a parameter in the population pharmacokinetic analysis and was 12.5 ml/h. The concentration-dependent non-linear clearance plays a major role at low Tocilizumab concentrations. Once the

non-linear clearance pathway is saturated, at higher Tocilizumab concentrations, clearance is mainly determined by the linear clearance. The t1/2 of Tocilizumab was concentration-dependent. At steady-state following a dose of 8 mg/kg every four weeks, the effective t1/2 decreased with decreasing concentrations within a dosing interval from 14 days to 8 days.

1.3 Clinical Data

1.3.1 Rituximab

Clinical outcomes
The efficacy and safety of Rituximab in alleviating the symptoms and signs of rheumatoid arthritis in patients with an inadequate response to TNF-inhibitiors was demonstrated in a pivotal randomized, controlled, double-blind, multicenter study (REFLEX).
REFLEX evaluated 517 patients that had experienced an inadequate response or intolerance to one or more TNF inhibitor therapies. Eligible patients had active rheumatoid arthritis, diagnosed according to the criteria of the American College of Rheumatology (ACR). Rituximab was administered as two IV infusions separated by an interval of 15 days. Patients received 2 x 1000 mg intravenous infusions of Rituximab or placebo in combination with MTX. The primary endpoint was the proportion of patients who achieved an ACR20 response at week 24. Patients were followed beyond week 24 for long term endpoints, including radiographic assessment at 56 weeks and at 104 weeks. During this time, 81% of patients, from the original placebo group received Rituximab between weeks 24 and 56, under an open label extension study protocol.

Radiographic outcomes
Structural joint damage was assessed radiographically and expressed as change in modified total Sharp Score (mTSS) and its components, the erosion score and joint space narrowing score.
In the REFLEX study, conducted in patients with inadequate response or intolerance to one or more TNF inhibitor therapies, receiving Rituximab in combination with methotrexate demonstrated significantly less radiographic progression than patients originally receiving methotrexate alone at 56 weeks. Of the patients originally receiving methotrexate alone, 81 % received Rituximab either as rescue between weeks 16-24 or in the extension trial, before week 56. A higher proportion of patients receiving the original Rituximab/MTX treatment also had no erosive progression over 56 weeks

Quality of life outcomes
Significant reductions in disability index (HAQ-DI) and fatigue (FACIT-Fatigue) scores were observed in patients treated with Rituximab compared to patients treated with methotrexate alone. The proportions of Rituximab treated patients showing a minimal clinically important difference (MCID) in HAQ-DI (defined as an individual total score decrease of >0.22) was also higher than among patients receiving methotrexate alone

1.3.2 Tocilizumab

Clinical outcomes

In a number of studies, patients treated with Tocilizumab had statistically significant higher ACR 20, 50, 70 response rates at 6 months compared to control. In The AMBITION study, superiority of Tocilizumab was demonstrated against the active comparator MTX.

The treatment effect was similar in patients independent of rheumatoid factor status, age, gender, race, number of prior treatments or disease status. Time to onset was rapid (as early as week 2) and the magnitude of response continued to improve with duration of treatment. Continued durable responses were seen for over 3 years in the ongoing open label extension of a number of clinical trials - AMBITION, LITHE, OPTION, TOWARD and RADIATE. Patients in the afore mentioned studies had a mean Disease Activity Score (DAS28) of 6.5–6.8 at baseline. Significant reduction in DAS28 from baseline (mean improvement) of 3.1–3.4 were observed in Tocilizumab-treated

patients compared to control patients (1.3-2.1). The proportion of patients achieving a DAS28 clinical remission (DAS28 < 2.6) was significantly higher in patients receiving Tocilizumab (28–34%) compared to 1–12% of control patients at 24 weeks. In study II, 65% of patients achieved a DAS28 < 2.6 at week 104 compared to 48% at 52 weeks and 33% of patients at week 24.

Radiographic response

In the LITHE study, patients with an inadequate response to MTX, inhibition of structural joint damage was assessed radiographically and expressed as change in modified Sharp score and its components, the erosion score and joint space narrowing score. Inhibition of joint structural damage was shown with significantly less radiographic progression in patients receiving Tocilizumab compared to control. In the open-label extension of this study the inhibition of progression of structural joint damage in Tocilizumab plus MTX-treated patients was maintained in the second year of treatment. The mean change from baseline at week 104 in total Sharp-Genant score was significantly lower for patients randomised to Tocilizumab plus MTX ($p<0.0001$) compared with patients who were randomised to placebo plus MTX.

Quality of life outcomes

Tocilizumab-treated patients reported an improvement in all patient-reported outcomes (Health Assessment Questionnaire Disability Index - HAQ-DI), Short Form-36 and Functional Assessment of Chronic Illness Therapy questionnaires. Statistically significant improvements in HAQ-DI scores were observed in patients treated with Tocilizumab compared with patients treated with DMARDs. During the open-label period of LITHE study, the improvement in physical function has been maintained for up to 2 years.

Rationale and Risks/Benefits

This is an open labelled randomised controlled clinical trial investigating the use of synovial B cell histo-pathology as a potential diagnostic biomarker to stratify patients response to Rituximab therapy. Currently, NICE guidelines suggest the use of Rituximab in all patients following inadequate response to anti -TNF therapy. Inadequate response to Rituximab would allow for the use of Tocilizumab (IL-6 receptor monoclonal antibody). In this study patients will be randomised to receive either Rituximab or Tocilizumab. No placebo arm has been included, as withholding an approved potentially beneficial therapy would not be comparable with good standards of clinical practice. Tocilizumab has been approved by NICE for the use in patients with moderate to severe RA (NICE, http://www.nice.org.uk/CG79). Thus, there will be no greater risk from administered pharmacotherapy during this study than would be expected in routine clinical care. All patients will have a synovial biopsy which would not necessarily be considered routine clinical care and thus the main risks to patients enrolled would be associated with this interventional procedure. Through the MRC-funded Pathobiology of Early Arthritis Cohort (PEAC) initiative (see below and http://www.peac-mrc.mds.gmul.ac.uk/index.php) we have developed a National Training Centre for the performance of minimally invasive ultrasound (US) guided synovial biopsies. The procedure itself has excellent safety and tolerability and can be applied to both large and small joints in most patients.

Our group has previously suggested that one mechanism for treatment resistance in Rituximab may be the survival of self-sustaining, B cell niches within the synovium[14]. Recent histological data has demonstrated a correlation of clinical response at 16 weeks following Rituximab and levels of synovial membrane B-cell depletion[15]. Likewise, there is evidence (though limited by the small number of patients in these studies only 8-10 patients) that synovial tissue biomarkers are associated with anti-TNF response[16, 17]. No data is available with regard to IL-6 receptor blockade therapy. The need for synovial tissue analyses compared to peripheral blood is also emphasized by recent work indicating that disease biomarkers are enriched 50-100 fold in the synovial tissue compared with the blood[18]. In addition, pharmacological response signatures in the blood are not helpful as, for example, downstream pathway to TNF are modulated in all anti-TNF treated patients, irrespective of clinical response[19]. Finally, the need for a quantitative integration between tissue biomarkers and blood biomarkers has been obvious for many years in multiple fields of

medicine e.g., abnormal creatinine or liver enzymes represent important blood biomarkers of tissue pathology, but they are not informative of the respective specific renal or liver pathology. More importantly, as seen in breast cancer, biomarkers of prognosis and therapeutic response are expressed only at tissue level (e.g. ER, HER)[20].

We have strong evidence emerging from the MRC-funded Pathobiology of Early Arthritis Cohort (PEAC) initiative (220 recruited, target 300 by April 2012 - http://www.peac-mrc.mds.qmul.ac.uk/index.php) that RA patients can be classified into at least 3 histo-morphological patterns e.g. Fibroblast (pauci-immune), Lymphoid (B cell rich) and Myeloid (rich in monocytes but poor in B cells). We have also evidence that the PEAC histopathology patterns correspond to different transcriptomic signatures. More important still, we have strong pilot data in a biopsy-based study of 21 RA patients (anti-TNF-ir) that a significantly higher proportion of patients with synovial B cell-rich pattern respond to Rituximab compared with a synovial B cells-poor pattern and vice versa no-response is associated with absence/scarce B cells (chi squared $p<0.05$). Crucially, as mentioned above, synovial tissue can nowadays be obtained from most patients, both from large and small joints, through a minimally invasive ultrasound-guided approach, thus, potentially benefiting all patients from stratified medicines. We have pioneered such minimally invasive approach in the UK and the diagnostic tool emerging from this proposal could be adapted for execution in all NHS accredited clinical pathology Units. Thus, this study will develop a diagnostic tool (immunohistochemical analysis of synovial tissue) for patient stratification into responsive/non-responsive categories with respect to Rituximab therapy. The proposed research also has the potential to contribute work of significant clinical advantage for the treatment of rheumatoid arthritis (RA) and provide a measurable positive impact on health economics for patient benefit and the wider NHS.

2 TRIAL OBJECTIVES AND DESIGN

2.1 Trial Objectives

The main aim of this project is to test the hypothesis that the presence or absence of specific synovial cellular and molecular signatures (B cells and B cell-associated signatures), assessed following a synovial tissue biopsy, will enrich for response/non-response to the B cell depleting anti-CD20 monoclonal antibody (mAb) Rituximab. In addition, we will examine if clinical response is associated with inhibition of B cell-linked pathways within the synovium and dependent on local B cell lineage depletion and whether survival of auto-reactive B cells within “protected” synovial niches are responsible for B-cell joint re-population and disease resistance-relapse?

Therefore, the overarching hypothesis is whether a diagnostic synovial biopsy showing a B-cell “rich/poor pathotype” define specific disease responsive/resistant subsets for patient stratification and help rationalize biologic drug choice.

Therefore, while this study can be thought of as taking place in three distinct and separate synovial histomorphological phenotypes (B cell rich, B cell poor and Germinal centers);
(i) The primary aim of this project is to show that in patients failing anti-TNF therapy, with a B cell poor synovial pathotype, Rituximab is inferior to Tocilizumab therapy.
(ii) For the B-cell rich synovial pathotypes, we aim to show non-inferiority of Rituxumab compared to Tocilizumab.
(iii) Germinal Centre pathotypes will constitute an exploratory component to the trial as insufficient power will be generated to show a significant difference in clinical response between each treatment. Following a sub-analysis of the pilot data we established that patients showing a germinal centre pathotype are more likely to resistant to biological therapy (3 of 4 non-responders as seen in the pilot data) thus we will take a mechanistic approach to this population of likely resistant patients and explore the predominant histological pattern and whether destruction of these structures relates to clinical response.

2.2 Trial design

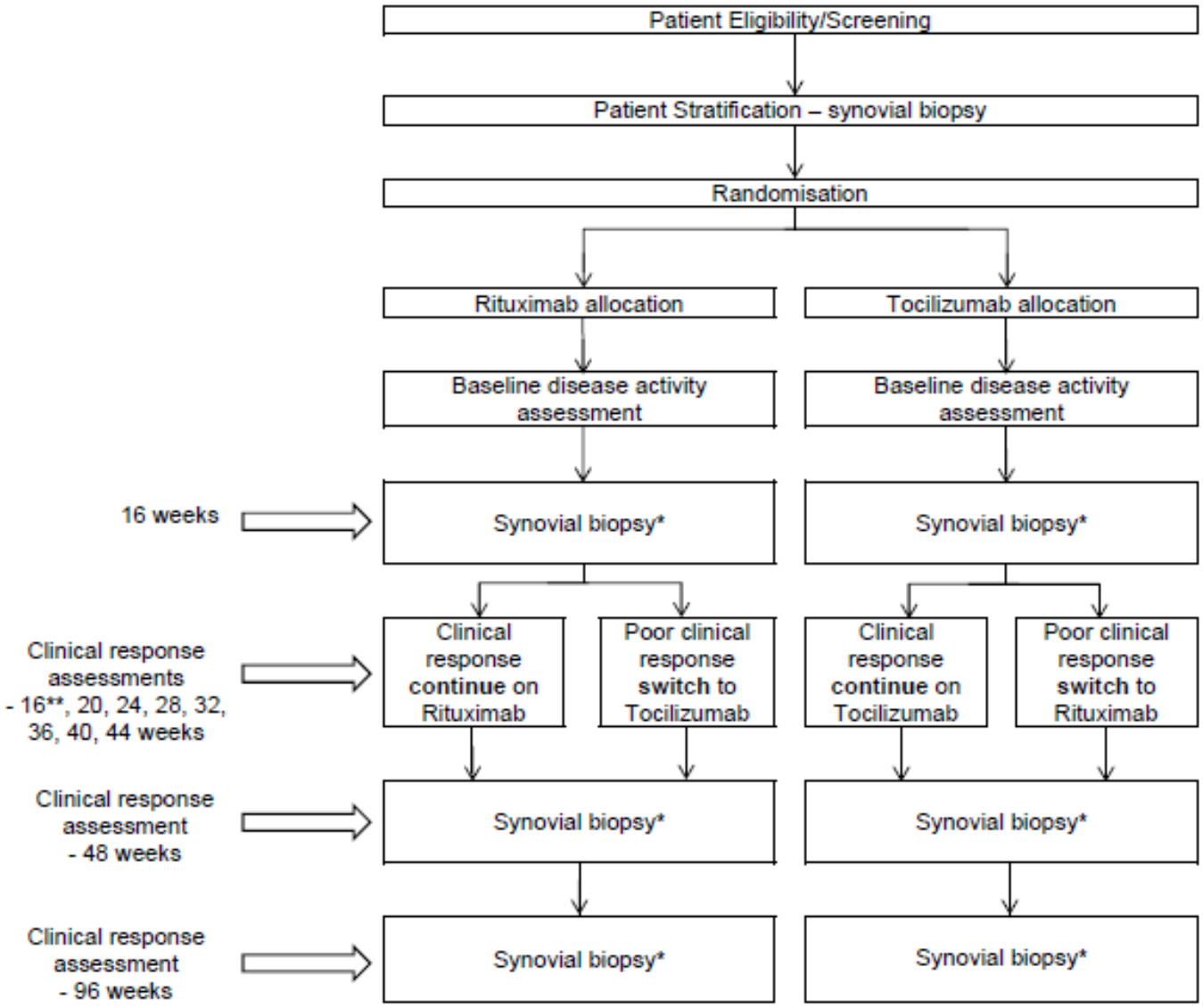
This is an open-label, randomised clinical trial. Patients recruited to this study will undergo a synovial biopsy at baseline, prior to randomisation.

Patients will subsequently be stratified in to 3 groups (B Cell Poor, B Cell Rich, Germinal Centres (GC) Rich) according to the following B-cell score prior to therapeutic intervention. All participating site staff will be blinded to the pathotype (B Cell Poor, B Cell Rich, Germinal Centre). This result will be recorded centrally prior to randomisation of the patient.

Note:
In a small number of cases, patients may be randomised to a fourth 'unknown' strata if a biopsy result is not yet obtained, or the biopsy cannot be classified at the time of randomisation, however, any biopsies that are later classified will be included in analysis of the trial data.

Patients with a baseline biopsy of unknown, and where a classification cannot be obtained should remain in the study as the data collected from blood biomarkers and other trial assessments will contribute to data analysis.

2.3 Study Scheme Diagram



*Synovial biopsy at 16, 48, and 96 weeks are optional.

**A patient initially randomised to Rituximab and deemed a responder can only be retreated at 24 weeks.

2.4 Study Population

2.4.1 Number of Subjects and Subject Selection

Number of subjects to be enrolled = 180 patients. Patients will be recruited from within the Rheumatology department who have been referred by their consultant Rheumatologists for a second line biological agent following failure of at least 1 anti-TNF agent.

Active R4-RA trial sites may utilise Participant Identification Centres (PICs) to identify potential participants and refer them to active R4-RA trial sites for participation in the trial and conduct of all research activities relating to the trial.

2.4.2 Inclusion Criteria

Patients will be recruited with active RA:

1. Patients who have failed anti-TNF therapy (inadequate responders – ir). Note; this includes patients who have failed anti-TNF therapy because of reactions.
2. Who are eligible for Rituximab therapy according NICE guidelines
3. Patients should be receiving a stable dose Methotrexate for at least 4 weeks prior to biopsy visit.
4. 2010 ACR / EULAR Rheumatoid Arthritis classification criteria for a diagnosis of Rheumatoid Arthritis.
5. Over 18 years of age
6. Patient must be capable of giving informed consent
7. Willingness and ability to comply with scheduled visits, treatment plans and laboratory tests and other study procedures

2.4.3 Exclusion Criteria

1. Women who are pregnant or breast-feeding
2. Women of child-bearing potential, or males whose partners are women of child-bearing potential, unwilling to use effective contraception during the study and for at least 12 months after stopping study treatment.
3. History of or current inflammatory joint disease or autoimmune disease other than RA.
4. Treatment with any investigational agent ≤ 4 weeks prior to baseline (or < 5 half lives of the investigational drug, whichever is the longer).
5. Intra articular or parenteral corticosteroids ≤ 4 weeks prior to baseline.
6. Active infection.
7. Septic arthritis within a native joint within the last 12 months.
8. Sepsis of a prosthetic joint within 12 months or indefinitely if the joint remains in situ.
9. Known HIV or hepatitis B/C infection.
10. Latent TB infection unless they have completed adequate antibiotic prophylaxis.
11. Malignancy (other than basal cell carcinoma) within the last 10 years
12. New York Heart Association (NYHA) grade 3 or 4 congestive cardiac failure.
13. Demyelinating disease.
14. Latex allergy or allergy to any excipients of Rituximab
15. Any other contra-indication to the study medications as detailed in their summaries of product characteristics
16. Receipt of live vaccine <4 weeks prior to first infusion
17. Surgery in 3 months prior to first infusion
18. Presence of a transplanted organ (with the exception of a corneal transplant >3 months prior to screening)
19. Known recent substance abuse (drug or alcohol)
20. Poor tolerability of venepuncture or lack of adequate venous access for required blood sampling during the study period.
21. Patients unable to tolerate synovial biopsy or in whom this is contraindicated (e.g. patients on anti-coagulants).
22. Patients current recruited to other clinical trials.
23. Other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration, or which, in the judgment of the investigator, would make the patient inappropriate for entry into this study

The PI reserves the right to exclude patients at his centre if they have concerns regarding compliance with the study procedures or any other aspect of the study eligibility not necessarily limited to the above exclusion criteria.

2.4.4 Criteria for Premature Withdrawal

A subject may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral or administrative reasons.

3 INVESTIGATIONAL MEDICINAL PRODUCT

3.1 List and definition of each IMPs

Rituximab
Rituximab is a genetically engineered chimeric mouse/human monoclonal antibody representing a glycosylated immunoglobulin with human IgG1 constant regions and murine light-chain and heavy-chain variable region sequences.

Tocilizumab
Tocilizumab humanised IgG1 monoclonal antibody against the human interleukin-6 (IL-6) receptor produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology

3.2 Formulation of IMP

Rituximab
Rituximab is available as 50ml single-use vials containing 500mg Rituximab for infusion (10mg/ml)
Rituximab is a clear, colourless liquid
Tocilizumab
Tocilizumab is available in 20mg/ml vials in a concentrate for intravenous infusion. The vials are available in 4ml (80mg), 10ml (200mg), and 20ml (400mg).

3.3 IMP Supply

Rituximab
Rituximab will be prescribed according as per license and thus will be sourced from the NHS supply at each site. The IMP will be labelled as clinical trial material.
Tocilizumab
Tocilizumab will be prescribed according as per license and thus will be sourced from the NHS supply at each site. The IMP will be labelled as clinical trial material.

3.4 Prescription of IMP

Rituximab
Rituximab will be prescribed by a physician as a member of the study team, using trial specific prescription forms. Prescription forms should be stored in the trial Pharmacy File and must be available for review for the purposes of monitoring visits/audit inspections throughout the study duration.
Tocilizumab
Tocilizumab will be prescribed by a physician as a member of the study team, using trial specific prescription forms. Prescription forms should be stored in the trial Pharmacy File and must be available for review for the purposes of monitoring visits/audit inspections throughout the study duration.

3.5 Preparation and Administration of IMP

3.5.1 Rituximab

Preparation will be performed by a suitably trained member of the local study team as per local policy.

Instructions for Dilution and Suitable Diluent:

- Aseptically withdraw 1000mg (100ml) of Rituximab.
- Slowly add the total volume of Rituximab (100mls) to a 500ml bag of sodium chloride 0.9%. To mix, gently invert the bag in order to avoid foaming. Do not shake.
- The final concentration of the drug should be between **1-4mg/ml** (in this case it will be 1.67mg/ml).
- Care must be taken to ensure the sterility of the prepared solution – aseptic technique must be observed.
- Inspect the bag visually for any particulate matter and discolouration prior to administration – discard the solution if observed.
- The prepared infusion should be used immediately.

Method and Rate of Administration:

Pre-medication should be prescribed and administered 30 minutes prior to the start of infusion:

Drug	**Dose**	**Route**	**Frequency**
Methylprednisolone	100mg	IVI	Stat
Chlorphenamine	10mg	IVB	Stat
Paracetamol	1000mg	PO	Stat

Observations e.g. temperature, blood pressure, pulse and respiratory rate should also be carried out prior to the start of infusion.

Using IV Volumat Pump

The Rituximab entry in the pump library defaults to 1000mg in 600ml (as described above) and defaults to an initial rate of 50mg/hr (30mls/hour).

First infusion of each course

IV infusion: Initial rate: 50 mg/hr (30mls/hour); increase rate by 50 mg/hr (30mls/hour) every 30 minutes if tolerated, to a maximum of 400 mg/hr (240mls/hour).

	Rate – mg/hour	**Rate – mls/hour**
Initial rate	50mg/hour	30mls/hour
After 30mins (if tolerated)	100mg/hour	60mls/hour
After 30mins (if tolerated)	150mg/hour	90mls/hour
After 30mins (if tolerated)	200mg/hour	120mls/hour
After 30mins (if tolerated)	250mg/hour	150mls/hour
After 30mins (if tolerated)	300mg/hour	180mls/hour
After 30mins (if tolerated)	350mg/hour	210mls/hour
After 30mins (if tolerated)	400mg/hour	240mls/hour

3.5.2 Tocilizumab

Preparation will be performed by a suitably trained member of the local study team as per local policy.

Instructions for Dilution and Suitable Diluent:

- The volume of Tocilizumab concentrate required for the patients dose should be calculated.
- Using aseptic technique the required volume of sodium chloride 0.9% should be removed from a 100ml infusion bag.
- The required volume of Tocilizumab should be withdrawn from the vial and placed in the 100ml infusion bag to give a final volume of 100ml
- To mix the solution, gently invert the infusion bag to avoid foaming. Do not shake.

Method and Rate of Administration:

IV infusion:

The final 100ml infusion bag should be administered by IV infusion over a one hour period[1].

Using IV Volumat Pump:

The pump does not have a preset setting for Tocilizumab.

Using Injectomat Syringe driver:

The syringe driver does not have a preset setting for Tocilizumab.

Example Calculation: (Adult Patients)

For a 70kg, the dose is usually 560mg every 4 weeks (70kg x 8mg/kg). This dose requires 28ml of Tocilizumab concentrate – ie 20ml from a 1 x 400mg vial and 2 x 4ml (2 x 80mg) doses. Twenty eight ml is withdrawn from the 100ml infusion bag and the 28ml form the vials added to the infusion bag. The resulting solution is then given as an IV infusion over a one hour period.

Flushes Compatible:

Sodium Chloride 0.9%[1]

Adverse effects which may be caused by IV administration and suggested monitoring:

Common side effects include abdominal pain, mouth ulceration, gastritis, raised hepatic transaminases, dizziness, peripheral oedema, hypertension, hypercholesterolaemia, headache and infection.

Special Handling Precautions:

The reconstituted solution should be used immediately due to sterile concentrate not containing any preservatives.

The Tocilizumab will be administered at room temperature by controlled infusion into an arm vein over a one hour period. In exceptional cases this time may be extended to up to 6 hours. The infusion speed must be 10 mL/hour for 15 minutes and then increased to 130 mL/h to complete the dosing over 1 hour. The entire 100 mL content of the infusion bag must be administered. 20 mL of normal saline will be administered following the infusion of study medication to flush the remaining study medication through the intravenous set.

3.6 Accountability/Receipt /Storage and Handling of IMP

The local Principal Investigator is responsible for the control of drugs under investigation at their site. Adequate records for the receipt (e.g. Drug Receipt Record) and disposition (e.g. Drug Dispensing Log) of the study drug will be maintained. Accountability will be assessed by maintaining adequate drug dispensing and return records. This will be delegated to the local site pharmacy. The IMP will be stored by the local pharmacy.

Accurate records will be kept for each study drug provided. These records will contain the following:

• Documentation of drug shipments received from the sponsor (date received and quantity)

• Disposition of unused study drug not dispensed to patient

Dispensing of IMP

A Drug Dispensing Log will be kept current and will contain the following information:
• the identification of the patient to whom the study medication was dispensed
• the date[s], quantity of the study medication dispensed to the patient
• the date[s] and quantity of the study medication returned by the patient.
All records and drug supplies must be available for the purpose of monitoring visits/audit inspections

3.7 IMP Stability

Rituximab
RIituximab solutions for infusion will be stored at a controlled temperature 2 -8^{o}C (36-46^{o}F) for 24 hours. Rituximab solutions for infusion have been shown to be stable for an additional 24 hours at room temperature. However, since Rituximab solutions do not contain a preservative, diluted solutions should be stored refrigerated (2-8^{o}C). A temperature log will be kept on which the storage temperature of Rituximab solutions is recorded at least once a day. No incompatibilities between Rituximab and polyvinylchloride or polyethylene bags have been observed. Any deviations from the necessary temperature range will be documented and appropriate action taken.

Tocilizumab
All Tocilizumab vials must be stored at a controlled temperature of 2-8°C. The infusion bag of Tocilizumab in saline may be made up within 24 hours of dosing and must be stored in a refrigerator at 2-8°C, provided the site takes precautions to prepare the infusion aseptically. A temperature log must be kept, on which the storage temperature of the Tocilizumab and infusion bags is recorded at least once a day. Any deviations from the necessary temperature range will be documented and appropriate action taken.

3.8 Prior and Concomitant Anti-Rheumatic Therapies

Patients Enrolled into this study will have received anti-TNF therapy in accordance with NICE guidelines. Previous anti-TNF agents will be recorded. There must be a minimum washout period of minimum 4 weeks for Infliximab and a minimum 2 weeks for all other anti-TNFs prior to the biopsy visit.

Patients may also have received or continue to receive Disease Modifying Anti-Rheumatic Drugs (DMARDs). Patients must be stable on Methotrexate (and other DMARDs as applicable) for at least 4 weeks prior to the biopsy visit.

Patients may also receive corticosteroid therapy, at the discretion of the treating clinician, but at a steady does for at least 4 weeks prior to the biopsy visit.

Oral corticosteroids may be prescribed at a dose not exceeding prednisolone 10mg/day (or equivalent), but the dose must remain stable throughout the trial. Intra-articular and intra-muscular triamcinolone may be used, but not within 4 weeks of synovial biopsy at baseline, within four weeks of the 16 week assessment visit or within 4 weeks of the 48 week assessment post initiation of either Rituximab or Tocilizumab.

Dose modification/reduction/ delay
Adherence to the planned dose regimen of study medication is required unless an adjustment is necessary for safety reasons. For patients receiving Tocilizumab 8 mg/kg during the period of this study, the dose may be lowered to 4 mg/kg to manage safety events.

4 STUDY PROCEDURES

4.1 Informed Consent Procedures

Written informed consent will be obtained from each patient by the Principal Investigator or designee. Informed consent will be prepared according to NRES and study sponsor requirements for informed consents. Patients who are candidates for the study will receive a Patient Information Sheet (PIS) which explains the purpose of the trial and highlights the benefits and risks of participation in the trial. Patients must be given adequate time (minimum 24 hours) to review the information and must have the opportunity to ask the Principal Investigator or designee any questions relating to the trial. Following this, the patient must sign an informed Consent Form (CF) in the presence of the Principal Investigator or designee who must then countersign the CF. Written consent must be obtained prior to any study-specific procedures being performed, including any study specific screening procedures prior to randomisation. At the time of consent, participants must be informed that they have the right to withdraw their participation in the trial at any stage and that doing so will not prejudice their future clinical management and care.

4.2 Screening (Visit number 1)

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Patients may be screened 1- 6 prior to recruitment.. As per the study visit schedule screening will entail evaluation of:

- Inclusion and exclusion criteria
- Demographic data including age, gender
- 2010 ACR/EULAR RA classification criteria
- Systemic disease assessment (RA involvement)
- Medical history
- Procedures history
- Concomitant medication
- Anti-TNF therapy
- DMARD therapy
- Corticosteroid therapy
- Clinical examination
- Rheumatoid Factor and Anti-CCP antibodies (RFCCP)
- Routine blood tests (FBC, UE, LFT, ESR, CRP)
- Total cholesterol, HDL, LDL, and triglycerides
- Immunoglobulins/Immunodeficiency panel
- Hepatitis serology, HIV and IGRA* (not mandatory, however all centres are expected to act according to local guidelines with respect to patient screening prior to Rituximab and Tocilizumab therapy)
- Vital signs
- Chest X-ray**
- ECG
- Pregnancy test
- Joint assessment
- DAS 28 assessment
- Clinical Disease Activity Index (CDAI)
- VAS Pain score

Note: Anti-TNF therapy screening to include TB screening (IGRA), Hepatitis screening and HIV according to local guidelines.

* If Hepatitis serology, HIV and IGRA have already been performed in the preceding 3 months of the screening visit these do not need to be repeated.

** Similarly, a chest radiograph will be performed prior to starting any biological therapy, in keeping with local guidelines unless a chest x-ray had been done in the preceding 3 months of the screening visit and the patient has not had any pulmonary symptoms since then.

4.3 Randomization Procedures

Randomisation will take place when all the screening procedures are complete and the patient is eligible for enrolment in the study. Patients will be stratified (3 strata based on B cells, or a fourth strata where result is unknown) and randomised within blocks (1:1), with random block size of 6 and 4. Allocated treatment will be revealed by database once eligibility has been confirmed and the patient is entered into trial. The local principal investigator/research nurse will log-in to a secure web application and confirm patient eligibility before they can randomise. The web application will check eligibility and subsequently allocate a unique randomisation number (study number). This ensures that neither the patient nor the clinician can choose whether or not to enter a trial depending on the next allocation.. The randomisation list will be prepared by the Barts CTU Statistician and securely embedded with the application code so that it is not accessible to end users or anyone other than the Database Programmer and a limited number of information support staff who have access to all systems. Once a participant has been allocated a treatment, there is an audit trail that prevents anyone from changing the allocation or pretending that no allocation had been made.

4.4 Biopsy visit (Visit number 2)

Patients will receive a synovial biopsy between 1 to 3 weeks prior to their baseline visit. Patients will have the following assessments recorded prior to the synovial biopsy at this visit:

- Concomitant medication
- DMARD therapy
- Corticosteroid therapy
- Routine clinical bloods
- Study specific bloods
- Vital signs
- A pregnancy test performed will be performed in female patients of child bearing age
- Joint assessment
- DAS 28
- CDAI
- VAS Pain Score
- Physical function using the Health Assessment Questionnaire (HAQ)
- A Ultrasound examination of the patients joints will be performed prior to the synovial biopsy
- Baseline synovial biopsy*

*A baseline synovial biopsy is mandatory as part of the patient stratification process however subsequent synovial biopsies will remain optional. Patients not receiving a subsequent biopsy at 16 weeks, 48 weeks or 96 weeks will continue within the study as per protocol.

The initial biopsy visit may be combined with completion of the study screening visit if all screening procedures are available and the patient is eligible for enrolment into the study. If the completion of the screening and biopsy visit occur on the same day, there is no need to repeat the procedures for vital signs, Pregnancy test, DAS28, CDAI, VAS pain score. The database will accommodate this scenario and data will not need to be duplicated at the biopsy visit.

Randomisation into the study can only occur once the biopsy has been taken and biopsy result confirmed (B cell poor, B cell rich, GC or unknown). The randomisation procedure can occur at the baseline visit (see 4.5), or prior, to allow pharmacy time to prepare the allocated treatment.

4.5 Baseline visit (Visit number 3)

Patients at baseline will have the following assessments:

- Medical history
- Procedures history
- Concomitant Medication
- DMARD therapy
- Corticosteroid therapy
- Clinical examination
- Cardiovascular risk assessment
- Routine bloods
- Vital signs
- Pregnancy test in women of child bearing age.
- Joint assessment
- Disease activity core data set (DAS28)
- Clinical Disease Activity Index (CDAI)
- VAS Pain score
- Physical function using the Health Assessment Questionnaire (HAQ)
- SF-36
- FACIT - Fatigue Questionnaire
- Adverse reactions
- Plain X-rays of hands and feet
- Ultrasound joint examination

Patients will receive either Tocilizumab or Rituximab at their baseline visit depending upon randomisation. All assessments should be performed prior to commencement of infusion of therapy.

4.6 Follow up visits

Patients will be monitored on a monthly basis as shown in the study visit schedule (Section 4.10).

The CDAI, a validated composited end point, will be used to assess response to therapy as the primary outcome measure. The Health Assessment Questionnaire and SF-36 will be used to gauge functional ability and improvement in other aspects of the patients life e.g. vitality, emotional role functioning, social role functioning and mental health.

Visits 3a and 9a are specific to Rituximab patients only must attend at these time points to receive the second Rituximab infusion. Extra study specific bloods will be taken from these patients as detailed in section 4.11.3.1.

Visit 4 - 15 will be carried out 4 weekly (+/- 7 days) from the baseline visit. Visit 16 will be carried out at 72 weeks from baseline and Visit 17 at 96 weeks from baseline. All follow up visits (Visits 4-17) will have a +/- 7 day window in which to perform the required visit. The Principal Investigator will need to review any non-compliance with a view to withdrawing patients at their discretion if drug schedule is adversely affected.

All joint assessments will be performed by a member of the local trial team who will be blinded from all other trial data.

The principal investigator (or other delegated person(s) in the local trial team) must not perform the joint assessments, and instead will be responsible for collecting, recording and reporting data on adverse events, drug therapy and direct NHS costs (investigations, routine blood monitoring, community and outpatient appointments, and inpatient stays) at each study visit.

Delegation of these responsibilities to ensure the blind is maintained will be documented on the

site signature/delegation log.

Data will be entered in the electronic CRF by the Investigator or designee who will also co-ordinate data validation checks and query resolution.

As per normal clinical practice, patients with active Rheumatoid arthritis with an unfavourable lipid / cholesterol profile will be initiated on a statin prior to commencing a biological agent.

4.7 Unscheduled Visits

While patients will be encouraged to attend for the normal visit schedule, unscheduled visits will be undertaken if the patient is unwell or there are any concerns as to the patient's progress. If necessary, the patient may be withdrawn from the trial and the Early Withdrawal CRF must be completed.

4.8 Early Withdrawal Visit

All patients have the right to withdraw consent to participation in the trial for any reason and at any time without prejudice

At the time of withdrawal of consent, a full efficacy and safety evaluation should be performed if patient consents. The Early Withdrawal visit should be completed and the reason for discontinuation documented. Assessments required at the Early Withdrawal visit constitute data collection as per Visit 3, except study specific bloods which should not be taken.

Withdrawn trial subjects will not be replaced. As analysis is intention to treat, participants who have withdrawn should continue to attend the next due follow-up visit at 24, 48, 72, 96 weeks from baseline visit date.

4.9 Schedule of Treatment for each visit

Patients randomised to Tocilizumab will have 4 weekly infusion (+/- 7 days) at each of the study visits alongside their routine assessment. Patients randomised to Rituximab will have infusions every 24 weeks (+/- 7 days), or later when re-treatment is necessary, but will continue to have 4 weekly visits for disease assessment and study specific bloods as per the study visit schedule.

4.10 Study visit schedule

Visit Number	1	2	3	3a[f]	4	5	6	7	8	9	9a[f]	10	11	12	13	14	15	16	17	Early withdrawal
Timeline (weeks)	- 6 – 0 weeks	- 2 – 0 weeks	0	2	4	8	12	16	20	24	26	28	32	36	40	44	48	72	96	-
Visit Type	Screening	Biopsy and randomisation	Baseline	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U	F/U
Informed consent	x									x							x		x	
Inclusion/Exclusion criteria	x																			
Demographics	x																			
RA classification criteria	x																			
Systemic disease assessment	x																			
Medical history	x		x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Procedures History	x		x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Concomitant medication	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Anti-TNF therapy	x																			
DMARD therapy	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Corticosteroid therapy	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Clinical Examination	x		x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Cardiovascular risk assessment			x																	x
RF/CCP	x																			
Routine blood tests (U&Es, LFTs, FBC, CRP, ESR)	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Total cholesterol, HDL, LDL and triglycerides	x					x								x					x	
Study specific blood tests		x		x	x	x		x		x	x			x			x		x	
Immunoglobulin/Immunodeficiency panel [g]	x									x[g]							x[g]	x[g]	x[g]	
Hepatitis serology, HIV, IGRA[b]	x																			
Vital signs	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Chest X-ray	x																			
ECG	x																			
Pregnancy test [d]	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Joint assessment	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
DAS28	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
CDAI	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
VAS Pain score	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
HAQ score		x	x				x			x				x			x	x	x	x
SF-36 Fatigue Questionnaire			x				x			x				x			x	x	x	x
FACIT-Fatigue Questionnaire			x				x			x				x			x	x	x	x
Adverse events		x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Synovial biopsy [c]		x						x									x		x	
X-ray hands and feet			x							x							x		x	x
US assessment		x			x	x	x	x		x				x			x	x	x	
Randomisation [h]		x																		

Notes:
a - routine study bloods do not need to be taken if the patient has already had routine safety bloods performed within 7 days of the screening visit.
b - Hepatitis serology, HIV and IGRA are not mandatory, however all centers are expected to act according to local guidelines with respect to patient screening prior to Rituximab and Tocilizumab therapy
c - a baseline synovial biopsy is mandatory as part of the patient stratification process however subsequent synovial biopsies will remain optional. Patients not receiving a subsequent biopsy will continue within the study as per protocol.
d - a pregnancy test will be performed at each study visit for female patients of child bearing age irrespective of the use of contraceptive methods.
e - The patients biopsy visit may be performed at the same time as the screening visit provided all screening procedures have been performed and the patient is eligible to be randomized. Vital signs, Pregnancy test, DAS28, CDAI and VAS scores do not need to be repeated in this scenario. The database will accommodate this scenario and data will not need to be duplicated at the biopsy visit.
f – extra study specific bloods are required for patients randomized to receive rituximab therapy as these patients are already attending for their second infusion of Rituximab
g - immunoglobulins to be repeated prior to every cycle of rituximab
h – randomization can occur as soon as the biopsy result is obtained and prior to the scheduled baseline visit to allow sufficient preparation time for pharmacy
i – US assessments may be optional at some participating sites
Note: if a patient receives a cycle of rituximab therapy within the first 48 weeks of the study and it is not in line with the visit schedule detailed above (e.g. if patient remained in remission until week 28 and then flared and was retreated at week 32 then study visit bloods should be taken with the 2nd infusion, at the 2 subsequent study visits, and alternate months thereafter until week 48)

4.11 Completion of Trial Treatment

The duration of trial treatment is 48 weeks. The follow-up period is between 48 weeks and 96 weeks.

Patients that complete trial treatment, and those that withdraw prematurely, will require ongoing treatment for their condition. The decision for ongoing treatment will be made by the treating clinician, following assessment of response to previous treatments, as per local guidelines. The ongoing treatment plan will be discussed fully with the patient.

Patients that are randomized to Tocilizumab and who are responding to this treatment must be informed that they may not be able to continue on this treatment after completion of the trial.

4.12 Study Outcome Measures

4.12.1 Clinical outcomes

Patients will be assessed clinically using the CDAI (Clinical disease activity index), DAS 28 (CRP), Health assessment questionnaire and the Short Form 36 as described below.

4.12.1.1 CDAI

The components of the CDAI (Clinical disease activity Index) are tender joints (28 joint count), the number of swollen joints (28 joint count), a Patient global health index (10 cm VAS) and physician global health index (10 cm VAS). This provides an assessment of Rheumatoid disease activity on a scale fr0m 0-76.

CDAI scores

High disease activity:	>22
Moderate disease activity:	10.1 - 22
Low disease activity	2.8 - 10
Remission	< 2.8

Non-responders – CDAI improvement of less than 50% from baseline. Patients may be deemed non-responders at the discretion of the treating physician if they have achieved a CDAI response of > 50% from baseline but have not reached low disease activity (CDAI of 10 or less)

Patients deemed treatment failures at 16 weeks, will be switched to the other therapeutic option.

Treatment response will be assessed at 16 weeks and at all subsequent 4 weekly assessments up to 48 weeks. Patients showing initial clinical response by 16 weeks may be subsequently classified as a secondary failure at subsequent study visits (up to 48 weeks) if their change in CDAI from baseline is < 50% at any subsequent follow up visit. This will also prompt a switch to the other therapeutic option.

Note: A patient initially randomised to Rituximab and deemed a responder can only be retreated at 24 weeks.

Failure of a second biological therapy after another 16 weeks follow-up, would permit the patient to receive treatment option of the physicians discretion. With regards to primary endpoint analysis, patients classified as a secondary failure will continue in the trial but the data collected (and any further treatment changes) will form an observational component of the trial.

Small response - CDAI ≥ 50% improvement form baseline assessment
Moderate response - CDAI ≥ 75% improvement form baseline
Good response – CDAI ≥ 85% improvement form baseline

4.12.1.2 Disease Activity Score

The components of the DAS28(CRP) are the number of tender joints (28 joint count), the number of swollen joints (28 joint count), a Global Health index (100 mm VAS), and the CRP (in mg/L). The formula for determining the DAS28(CRP) is as follows:

DAS28(CRP) = 0.56*√(TJC28) + 0.28*√(SJC28) + 0.36*ln(CRP+1) + 0.014*GH (VAS) + 0.96

The following 28 joints will be assessed for tenderness in response to pressure or passive motion: Finger Proximal Interphalangeal Joints (8), thumb Interphalangeal joint (2), metacarpophalangeal (MCP) (10), wrists (2) (includes carpometacarpal, intercarpal, and radiocarpal), elbows (2), shoulders (2), and knees (2).

DAS 28 Response criteria

Non-responders – patients will be designated non-responders if their DAS28 has not improved by > 1.2 from baseline to 16 weeks after treatment; patients will not be re-treated with their current therapy and will be switched to the other agent (e.g. Rituximab to Tociluzimab or vice versa). At the discretion of the patient's rheumatologist, a patient may be designated a non-responder if their DAS28 has improved by >1.2, but their DAS28 remains above 5.1.

Partial responders – patients will be designated partial responders if their DAS28 improves by >1.2 from baseline but remains >3.2.

Good responders – patients will be designated good responders if their DAS28 falls by >1.2 to a level <3.2 (i.e. into a 'low disease activity state' [LDAS])

Remission – patients will be designated as in remission if their DAS28 falls to <2.6.

Toxicity – patients with drug-related toxicity (as defined by the treating rheumatologist) will be switched to Tocilizumab or Rituximab if/when the investigator deems it safe and appropriate.
The indications for re-treatment are as follows:

- **'Treat-to-target'** – the treatment strategy is designed to achieve LDAS. To this end, patients will be re-treated every 6 months for Rituximab and monthly for Tocilizumab, if their DAS28 remains above 3.2, six months after each course of treatment. Patients in LDAS will have their treatment deferred until such time as their DAS28 rises above 3.2.
- **Flare** – a flare is defined as a rise in DAS28 of >1.2 between monthly assessments. Patients who flare may be re-treated provided that a minimum of 20 weeks has elapsed from their previous treatment; e.g., a patient with baseline DAS28 of 6.0 who improves to a DAS28 of 3.2 after 3 months but whose disease flares, with a rise in DAS28 to 4.5 after 4 months, would be re-treated after 20 weeks.

Table 1: Summary of response criteria using validated composite outcome measure DAS 28

DAS28 improvement from baseline	Post-treatment DAS28	Category of response
< 1.2	Any	Non-responder. Switch to alternative.
> 1.2	> 5.1	Patient may be designated a non-responder at the discretion of the rheumatologist
> 1.2	> 3.2 but < 5.1	Partial responder.
> 1.2	< 3.2 but > 2.6	Good responder.
> 1.2	< 2.6	Remission

•

4.12.1.3 Health Assessment Questionnaire (HAQ)

The HAQ is usually self-administered, but may also be given face-to-face in this clinical study. The Disability Index consists of eight categories assessed by the Disability Index are 1) dressing and

grooming, 2) arising, 3) eating, 4) walking, 5) hygiene, 6) reach, 7) grip, and 8) common daily activities. For each of these categories, patients report the amount of difficulty they have in performing two or three specific activities. Patients usually find the HAQ Disability Index entirely self-explanatory.

4.12.1.4 The Short Form (36) Health Survey - SF-36

The Short Form (36) Health Survey is a survey of patient health. The SF-36 is a measure of health status and is commonly used in health economics as a variable in the quality-adjusted life year calculation to determine the cost-effectiveness of a health treatment. The SF-36 consists of eight scaled scores, which are the weighted sums of the questions in their section. Each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight.
The eight sections are: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning and mental health.

4.12.1.5 FACIT-Fatigue

The FACIT-Fatigue scale is a 13-item, symptom-specific subscale of the FACIT scales.13 Lower values of the FACIT-Fatigue score denote higher fatigue (score range, 0 to 52). Cella et. al. validated a brief measure of fatigue in rheumatoid arthritis (RA), the Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue Scale. The FACIT Fatigue was tested along with measures previously validated in RA: the Multidimensional Assessment of Fatigue (MAF) and Medical Outcomes Study Short-Form 36 (SF-36) Vitality. The FACIT Fatigue showed good internal consistency (alpha = 0.86 to 0.87), strong association with SF-36 Vitality (r = 0.73 to 0.84) and MAF (r = -0.84 to -0.88), and the ability to differentiate patients according to clinical change using the American College of Rheumatology (ACR) response criteria (ACR 20/50/70). This suggests that the FACIT Fatigue is a brief, valid measure for monitoring this important symptom and its effects on patients with RA.

4.12.2 Imaging Assessments

Patients will have plain x-rays and ultrasound assessments of disease activity and joint damage and will be related to the secondary outcome measures in this study.

X-rays

Plain radiographs of the hands and feet will be recorded at baseline, 24, 48 and 96 weeks follow up as per routine clinical practise.
A chest x-ray will be acquired as per routine screening for TB prior to biological therapy.

Ultrasound

An Ultrasound assessment will be performed by a trained Rheumatologist at biopsy, 4, 8, 12, 24, 36, 48, 72 and 96 weeks follow up. Images will be acquired and scored for Doppler signal and synovial thickness within a limited joint set. The core US data set is described in the US manual. Additional joints may be scanned at the local centres discretion.

Due to variation amongst Rheumatology departments with regards to resources and expertise to perform ultrasound assessments these will be optional for the purpose of this trial. Any opt outs will be documented as part of the site set-up and initiation procedures for all participating sites.

4.12.3 Laboratory Assessments

Routine laboratory bloods for safety will be taken as per routine clinical care for patients receiving Rituximab or Tocilizumab.

4.12.3.1 Peripheral Blood analysis

Lab: Local site laboratory - The following blood tests will be performed at screening and at each visit: FBC, urea, creatinine, electrolytes, liver function tests, ESR, CRP, total cholesterol, HDL, LDL, triglycerides. These investigations will be performed at the local site laboratory. Immunoglobulins/immunodeficiency panel will be taken at screening and then subsequently prior to every cycle of rituximab.

Study specific bloods
Lab: QMUL, Experimental Medicine and Rheumatology
In addition, study specific bloods will be taken as follows;

Biopsy visit (visit 2), Visits 7, 15, 17
Samples drawn in the order:
1. Four (9.0ml) Heparin (green top)
2. One (9.0ml) serum (red top)
3. One PAXgene RNA* (8.5ml)
(Note: RNA taken at biopsy visits)

Visits 3a*, 4, 5, 9, 9a*, 12
Samples drawn in the order:
1. Four (9.0ml) Heparin (green top)
2. One (9.0ml) serum (red top)

Extra study specific bloods will be taken at visits 3a and 9a for Rituximab patients only as indicated on study visit schedule (section 4.10).

Note: If bloods are missed at the scheduled visits they may be taken at a subsequent visit.

Further details of sample requirements, handling, transfer and storage are contained in the R4-RA study Laboratory Manual.

FACS analysis
Lab: QMUL, Experimental Medicine and Rheumatology - Whole blood FACS analysis will be carried out at scheduled visits using a FORTESSA (BD, 4 laser) flow cytometer as routinely done in this laboratory. Typical analysis will include B-cells: CD19/CD27/CD38/IgD/IgM; T-cells: CD3/CD4/CD8/CD25; Monocytes: CD14/CD16/HLA-DR. In addition B cells will be analysed for expression of chemokine receptors (CXCR5, CXCR4, CCR7) and FCRLs expression profile. Once B cells start to repopulate in peripheral blood and/if relapse occurs (the former usually precedes the latter) further characterisation studies of B cells will be performed.

Functional B cell studies
Lab: QMUL, Experimental Medicine and Rheumatology - after sorting, B cells will be placed in culture in complete RPMI medium with the presence of IL4/BAFF cytokines. Culture supernatant will be collected after 7 days for measurement of RF and anti-CCP antibody production as well as antibodies to recall antigens such as tetanus toxoid.

Peripheral blood autoantibody and cytokine production.
Serum levels of RF and anti-CCP antibodies and cytokine measurement (e.g. BAFF, APRIL) will be determined by ELISA.

4.12.4 Synovial biopsies and tissue analysis

Synovial biopsies under ultrasound guidance (minimally invasive) will be performed at baseline,16 weeks, 48 weeks and 96 weeks. (Only the baseline biopsy is mandatory whilst subsequent biopsies are optional). Synovial fluid will also be collected and stored concurrently with each biopsy. Tissue will be processed for paraffin embedding, snap frozen for histological analysis and immersed in RNA-Later for later RNA extraction.

Histopathological characterisation

Lab: Barts Healthcare NHS Trust, Pathology and QMUL, Experimental Medicine and Rheumatology - The tendency and the acquisition of the typical features of secondary lymphoid organs (SLOs) and total number of B-cells by immunohistochemistry (IHC) and digital image analysis as described in 5.2.1 above and previously reported[14,21]. Infiltrating B-cells will be quantified by CD20, CD79a staining and further characterised for the expression of IgD (naïve) and CD27 (memory) markers. In addition we shall determine the number of plasma cells (CD138), FDCs (CD35-CD21L) and activation-induced-cytidine-deaminase (AID) in order to identify the presence of functional Germinal Centre (GC) supported by FDC networks.

B Cell Score:

Lab: Barts Healthcare NHS Trust, Pathology - The level of B cell infiltration in synovial tissues is base on a 5-point scale: 0-4 depending on the increasing number of positively stained cells calibrated against a standardized atlas (Appendix II). Accordingly synovial biopsies will be categorized in B Cell Poor (0-1), B Cell Rich (2-3) and Germinal Centre (GC) Rich (4). GC will be further identified by the presence of CD21 +ve follicular dendritic cells (FDC) networks. The biopsy tissue processing, embedding, staining and slide scanning will be undertaken by an accredited NHS histopathology department at The Royal London Hospital. Further details can be found in the separate workflow for processing and cutting synovial biopsy SOP.
In some circumstances, processing and review of biopsy samples may be undertaken at the EMR laboratory, QMUL.

Gene expression profiling

Lab: QMUL, Experimental Medicine and Rheumatology -To determine whether clinical response to Rituximab therapy is associated with modulation of specific molecular pathways involved in lymphoid organization and B-cell growth and differentiation we will investigated by QT-PCR expression levels of the following genes: LTα, LTβ, TNFα, RANKL, CXCL13, CCL19, CCL21, BAFF, APRIL, PBEF, TSLP, SLPI and AID. Similar considerations will apply with regard to pathways involved in response to IL-6 receptor blockade and IL-12, IL-17, IL-23, IL-21 amongst others will be also analysed by QT-PCR. In addition, a more comprehensive micro-array based gene expression profiling of synovial tissue before and after Rituximab and Tocilizumab therapy will be performed using the Illumina micro-array platform at WHRI-Genome-Centre that will also provide bio-informatic analyses expertise. Previous studies, performed with a variety of micro-array technologies, have revealed the validity of large-scale transcriptional analysis[22].

Local autoantibody and cytokine production

Lab: QMUL, Experimental Medicine and Rheumatology -Synovial fluid levels of autoantibodies (RF, anti-CCP) and cytokines (i.e. BAFF, APRIL) will be determined pre and post-Rituximab by ELISA. To assess whether the survival of autoreactive B-cells within “protected” synovial niches is responsible for B-cell joint re-population and disease resistance/relapse we will determine clonal relationship analysis pre- and post-treatment. This will be carried out in collaboration with Barts Cancer Institute, where all the techniques for clonal analysis of B-cell lymphomas have been optimised. Together we will construct B-cell immunoglobulin VH genes libraries pre- and post-treatment for exhaustive repertoire and clonality analysis evaluated using Roche 454 next generation sequencing (NGS) technology. Clonal identity pre- and post-treatment would clearly indicate that repopulation occurs from escaped clones within the synovial tissue and the need for disrupting-destroying such survival niches aiming for remission using alternative therapeutics currently being developed in cancer such us BTK and/or proteasome inhibitors.

4.13 End of Study Definition

The end of the study will be triggered when the last patient completes their final study visit (Last Patient Last Visit LPLV) at the 48 month assessment.

4.14 Subject Withdrawal

Participants may be withdrawn if they are intolerant to the therapeutic product, experience toxicity related side-effects or inter-current illness necessitating cessation of the therapy within 6 months of recruitment.

Specifically, the following criteria will necessitate premature withdrawal of a study participant:

- Suspected progressive multifocal leukoencephalopathy (PML)
- ALT or AST > 5 x ULN
- ANC < 1.5 x 10^9/ l
- Platelet count < 75 x 10^3/l
- Receipt of live vaccines
- Pregnancy

Subjects may withdraw consent for any reason at any time without prejudice to their normal care. Patients withdrawing from the study will continue to be monitored and managed within their routine Rheumatology clinic by their named consultant. Withdrawal of consent may be regarded as a withdrawal from trial medication or any other components which form part of the Informed Consent Form signed by the participant.

5 PHARMACOVIGILANCE

5.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of an Investigational Medicinal Product (IMP), whether or not considered related to the IMP or to trial related procedures.

5.2 Adverse Reaction (AR)

An AR is any untoward and unintended response in a subject to an Investigational Medicinal Product (IMP), which is related to any dose administered to that subject. All adverse events judged by either the reporting investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

5.3 Serious Adverse Event (SAE)

An SAE must fulfil at least one of the following seriousness criteria:
Is fatal – results in death
Is life-threatening
Requires inpatient hospitalisation or prolongation of existing hospitalisation
Results in persistent or significant disability/incapacity
Is a congenital anomaly/birth defect
Results in any other medically important event

Note: The following events are not considered as SAEs for the R4-RA trial

- Pregnancy (however it is an event that requires monitoring and follow up) – see section 5.5.6
- Elective surgery at any time which is not related to, or has not resulted from, any existing condition

- Procedures that were planned prior to the screening visit (although this does not exclude any complications post-procedure)
- Pre-existing conditions prior to the screening visit unless the condition has worsened

Serious Adverse Reaction (SAR)
An SAR is an adverse reaction that is classed as serious and which is consistent with the information about the medicinal product as set out in the Summary of Product Characteristics (SmPC) or Investigator's Brochure (IB) for that product.

Suspected Unexpected Serious Adverse Reaction (SUSAR)
The definition of a SUSAR is any serious adverse event related to an IMP that is both suspected to be related to the IMP and unexpected. In this case the event is not outlined in the Summary of Product Characteristics (SmPC) or Investigator's Brochure (IB) for that product.

5.4 Investigators Assessment

Seriousness
The Chief/Principal Investigator responsible for the care of the patient, or in his absence an authorised medic within the research team who has been delegated this role, is responsible for assessing whether the event is serious according to the definitions given in section 5.3.

Causality
The Investigator must assess the causality of all serious adverse events/reactions in relation to the trial treatment according to the definition given. If the SAE is assessed as having a reasonable causal relationship, then it is defined as a SAR.

Expectedness
The PI must assess the expectedness of all SARs according to the definition given. If the SAR is unexpected, then it is a SUSAR. Expectedness must be assessed with reference to the relevant SmPC (Rituximab or Tocilizumab) for the medication being administered to the patient.

Severity
The Investigator must assess the severity of the event according to the following terms and assessments. The intensity of an event should not be confused with the term "serious" which is a regulatory definition based on patient/event outcome criteria.

Mild: Some discomfort noted but without disruption of daily life
Moderate: Discomfort enough to affect/reduce normal activity
Severe: Complete inability to perform daily activities and lead a normal life

Notification and reporting Adverse Events or Reactions
If the AE is not defined as SERIOUS, the AE is recorded in the study file and the participant is followed up by the research team. The AE is documented in the participants' medical notes (where appropriate) and the CRF.

AEs and SAEs should be recorded from the time that the first trial specific assessment/procedure is undertaken (screening visit), and then subsequently at follow-up visits throughout duration of trial treatment. Participants should be advised to notify the trial site of any untoward medical events as soon as possible, even if this is outside of their normal visit schedule.

SAEs should continue to be reported following the same procedures up to 48 week visit where trial treatment ceases **and for a further 30 days post-treatment**. AEs and SAEs will continue to be reported after this time and until the patient reaches their final visit at 96 weeks. Events will be documented in the participants' medical notes (where appropriate) and the CRF, however such events will not be reported in an expedited fashion.

5.5 Notification and Reporting of Serious Adverse Events/SUSAR

5.5.1 Serious Adverse Events

All Serious Adverse Event (SAEs) will be recorded on the SAE eCRF at the local trial site.

Causality must be assessed and completed by the PI or other delegated person(s). The person assessing causality must sign a paper copy of the SAE CRF. The CRF must be sent to the R4RA trial office **immediately and within 24 hours** of becoming aware of the event. The R4RA trial office will review the SAE CRF for data completeness. The CI will assess the SAE and may send queries back to the reporting local site as applicable. All SAEs received at the R4-RA trial office must be reported to the Joint Research and Development Office (JRMO) at QMUL immediately and within 24 hours of the site becoming aware of the event. Nominated co-investigators will be authorised to assess causality on the SAE forms in the absence of the CI at the co-ordinating site. The JRMO office, QMUL, as sponsor will be informed of the nominated investigators who may sign in the absence of the CI.

5.5.2 Suspected Unexpected Serious Adverse Reactions

Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur during the trial should be dealt with in the same way as all other SAEs. The JRMO, QMUL, as the sponsor, has a legal obligation to report this to the MHRA within 7 days of the event (for fatal or life-threatening SUSARs) or 15 days for all other SUSARs. The reporting PI will need to complete the CIOMS form in conjunction with the SAE CRF to be sent to the MHRA by the sponsor. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with the same drug (or therapy) that this serious adverse event has been reported.

The first SAE CRF for an event and any subsequent follow up of Serious Adverse Event CRFs and CIOMS forms (where applicable), together with the fax/email confirmation sheet must be kept with the local Investigator File at the study site.

5.5.3 Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the clinical trial subjects from any immediate hazard to their health and safety, in accordance with Regulation 30. The measures should be taken immediately. In this instance, the approval of the Licensing Authority Approval prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor, Main Research Ethics Committee (via telephone) and the MHRA (via telephone for discussion with the medical assessor at the clinical trials unit) of this event immediately.

The CI (or delegated persons) has an obligation to inform both the MHRA and Main Ethics Committee in writing within 3 days, in the form of a substantial amendment. The sponsor (JRMO) must be sent a copy of the correspondence with regards to this matter.

5.5.4 Development Safety Update Reporting (DSUR) and Annual Progress Report

The Development Safety Update Reporting (DSUR) will be sent by the CI to the sponsor, the MREC and MHRA (the date of the anniversary is the date on the “notice of acceptance letter” from the MHRA) using the DSUR form. The CI will carry out a risk benefit analysis of the IMPs encompassing all events having arisen on the trial.

The CI will send the Annual Progress Report to the main REC using the NRES template (the anniversary date is the date on the MREC “favourable opinion” letter from the MREC) and to the sponsor.

5.5.5 Overview of the Safety Reporting and Pharmacoviligance responsibilities

The CI has the overall pharmacovigilance oversight responsibility. The CI and R4-RA trial office have a duty to ensure that pharmacovigilance monitoring and reporting is conducted in accordance with the sponsor's requirements.
Further details of the process for reporting SAEs/SUSARs are detailed in the separate 'R4-RA Trial: SAE reporting for Investigators SOP.

5.5.6 Pregnancy

If a patient becomes pregnant whilst involved in a CTIMP, it is not considered to be an SAE or an AE. However, it is an event that requires monitoring and follow-up. If a patient, or his partner, becomes pregnant whilst enrolled in a CTIMP in which the foetus has been exposed to an investigational medicinal product, immediate reporting to the sponsor is required (within one working day of the PI/CI becoming aware of the event) using a JRMO pregnancy template form. The CI/PI has the responsibility to ensure that the pregnancy form is completed and sent to the sponsor within the agreed timelines.

In the event of pregnancy, the patient must be withdrawn from the trial and the procedures for premature withdrawal should be followed as described in section 5.15.

The PI/CI also must follow up the pregnancy until delivery as well as monitoring the development of the newborn for the appropriate time (please indicate for this IMP) after birth. Any events that occur during this time that could be considered to be a SAE must be reported to the sponsor in line with section 6.2.1, utilising the sponsor SAE reporting form.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint Efficacy Analysis

Treatment response will be assessed using the Clinical Disease Activity Index (CDAI) at 16 weeks. Section 5.12, defines treatment response/failure criteria.

Patients deemed treatment failures at 16 weeks, will be switched to the other therapeutic option. Such patients will be considered a new patient starting at week 0 with treatment response assessed again at 16 weeks for primary response.

The primary analysis will focus on whether there is a superiority of Tocilizumab over Rituximab in histologically defined 'B cell poor patients'.

6.2 Secondary Endpoint Efficacy Analysis

1. For the B-cell rich synovial pathotypes, we aim to show non-inferiority of Rituxumab compared to Tocilizumab.
2. Germinal Centre pathotypes will constitute an exploratory component to the trial as insufficient power will be generated to show a significant difference in clinical response between each treatment.
3. Area under the curve (AUC) of mean improvement in DAS28 over time between 0, 16 and 48 weeks.
4. Percentage of patients with low disease activity (DAS28 < 3.2) at 12, 24, 36, 48, 96 weeks
5. Percentage of patients in remission (DAS28 < 2.6) at 16, 48 and 96 weeks
6. Percentage of patients with ACR 20, 50 and 70 response rates at 16, 48 and 96 weeks
7. Percentage of patients with a low clinical disease activity index score (CDAI)
8. Mean % change in DAS28 between baseline and 16, 48 and 96 weeks
9. Mean % change in clinical disease activity index score (CDAI) between baseline and 16, 48 and 96 weeks
10. Mean change in HAQ score between baseline and 16, 48 and 96 weeks
11. Change in Fatigue score between baseline and 16, 48 and 96 weeks

12. Serious adverse events over 12 months; the rate of serious adverse events in the 16 week period following a switch from one technology to the other will be compared

6.3 Exploratory end point

1. The effect of synovial immuno-histology on drug response rates and disease outcome.

6.4 Safety Endpoints

To provide specification, methods and timing for assessing and recording safety parameters and how the outcomes is measured.

6.5 Sample Size

In a cohort of 27 anti-TNF resistant patients, 67% were B cell poor, 18% B cell rich, and 15% germinal centre positive. Clinical trial data suggests ACR20 response proportions of around 50%-60% for both Rituximab (REFLEX study, Arthritis and Rheumatism 2006) and Tocilizumab (RADIATE study, Ann Rheum Disease 2008). Our pilot study further demonstrates response rates of 25% in germinal centre positive patients, 80% in the B cell rich group and 22% in the B cell poor group.

1.For the B cell poor patients the aim is to show whether or not Tocilizumab is superior to Rituximab assuming response rates of 55% and 20% respectively. In order to have 90% power using a two-sided test of proportions with alpha of 0.05, one would need 82 patients in total (41 per arm). Should the true response rates be 55% and 25%, 82 patients would give 79% power. Hence the RADIATE study and our pilot data suggest that we will have between 80% and 90% power.

2. Based on previous experience, it is assumed that the dropout rate will be extremely low. Nevertheless the trial will continue to recruit until the sum of patients evaluated at 16 weeks and those who have been randomised but have not yet completed 16 weeks in the trial is 82. By this time we predict that we will have recruited about 20 - 40 B-cell rich patients (67% B cell poor and 18% B cell rich would yield 22 patients, 60% B cell poor and 25% B cell rich would yield 34, negative binomial sampling mean that the actual numbers could easily be outside of this range). We will include all B cell rich patients recruited. If there are fewer than 32 we will continue recruitment of B-cell rich and germinal centre patients until we have randomised 32 B cell rich patients.

3. Thirty-two patients will provide insufficient power to show non-inferiority even assuming that the true efficacy of Rituximab is slightly better Tocilizumab in B-cell rich patients. (If the true response rates are 80% for Rituximab and 55% for Tocilizumab, then there would be 66% power to show that the lower bound of the relative risk was at least 0.8. Rather this will be a pilot for a larger trial in these patients). The plan is to test to see whether the relative effects of Rituximab and Tocilizumab differed between the B cell rich and B cell poor participants. Under the assumptions above, the power to detect an interaction is about 87%.

4.For Germinal Centre, we hypothesize that patients will do poorly in both arms. The plan is to study the change in biomarker in synovial biopsies before and after treatment. If it is true that patients with Germinal Centre do poorly, and if one treatment breaks up the Germinal centre making patients B cell rich, then those patients may do rather better subsequently. By the time we have recruited 82 B-cell poor patients and 32 B cell rich patients, we should have recruited at least 22 Germinal Centre patients. If the proportions of Germinal Centre patients that had become B-cell rich at six months were 5% and 60% in the two arms then we would have 79% power to detect such a difference. The proportions 5% represents little or no change in germinal centres which is what we assume would happen in the absence of treatment. The 60% change represents a drug which is able to break up just over half of the germinal centres which is something one would not wish to miss. We would also use this to provide pilot data on the clinical response to treatment at 12 months comparing: “Tocilizumab followed by Rituximab for patients who do not respond to Tocilizumab “to “Rituximab followed by Tocilizumab for patients who do not respond to Rituximab”

In summary we will recruit 180 patients during the course of this study which will provide sufficient power to address all of the hypotheses discussed above.

6.6 Statistical Analysis

All statistical tests will be two-sided and use alpha of 5%; 95% confidence intervals will be provided for estimated quantities.
1.The primary outcome measure will be the binary clinical endpoint based on the change in the mean CDAI score over 16 weeks. Non-response will be a CDAI response of less than 50% in comparison to baseline.
2.For the randomised comparison of Rituximab versus Tocilizumab in B-cell poor patients, the primary endpoint will be analysed (by intent to treat) using the chi-squared test for the difference between two proportions. Patients switching treatment before 16 weeks because of lack of response will be considered as non-responders at 16 weeks.
3.For non-randomised comparisons between subgroups identified by (presence or absence of) B-cells in synovial biopsies, we will use the Fisher exact test comparing (i) response to Rituximab in B-cell poor patients compared to B-cell rich (without germinal centre). There will be no adjustment for potential risk modifiers because, a priori, we know of no such factors in the trial population. (Inclusion and exclusion criteria to this trial ensure that the patients are clinically homogeneous). The definition of B-cell status will be clearly defined before the start of the trial.
4.A test of interaction between treatment and B-cell status (rich versus poor, excluding germinal centre) will be based on a likelihood ratio tests between nested logistic regression models.
5.Patients who fail to respond during the first 16 weeks and cross-over treatment will also provide evidence regarding the efficacy of the two treatments and the predictive significance of B-cells in synovial biopsies. The post cross-over results will be combined with the pre-cross over results in a secondary analysis stratified by pre/post cross-over. Such analyses will be particularly important for comparison of treatments in B-cell rich patients (where the difference in treatment efficacy is hypothesised to be modest) and in germinal centre patients (in whom it is hypothesised that initial treatment may break up the germinal centre and allow a second biological to be effective). The additional power obtained from such a combined analysis has not been taken into account here.

7 DATA HANDLING & RECORD KEEPING

7.1 Confidentiality

The CI and participating trial sites have a responsibility to ensure that patient anonymity is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. Information with regards to study patients will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

The CI and trial sites must adhere to these parameters to ensure that the Patient's identity is protected at every stage of their participation within the study. To ensure this is done accordingly, each patient, at time of consent must be allocated an unique screening number by either the PI or a member of the trial team before undergoing any screening procedures. The patients initials (the first letter of their first name and the first letter of their last name) should be used as a means of pseudo-anonymising parameters. This information should be kept on a screening log, which should be updated accordingly throughout the study. Once the patient has completed screening procedures and is enrolled onto the study, the patient will be allocated a randomisation number by the PI (from a master randomization list)

The co-ordinating site will not hold any patient identifiable data. All clinical data will be stored in an encrypted format on the database, only viewable in a readable format by local trial staff and only for participants recruited at their site. The Chief Investigator is the 'Custodian' of the data collected. Patients will be consented and will not own the results generated using the sample/s and data collected and in addition will not be entitled to any interest in or share of any profit that might arise from research using the sample/s or data. The patients will be anonymised with regards to any future publications relating to this study.

7.2 Case Report Form

Data collection will be in the form of completing electronic CRFs via the trial database to record all the required assessments at each study visit.

7.3 Record Retention and Archiving

During the course of research, all records are the responsibility of the Chief Investigator and must be kept in secure conditions. When the research trial is complete, it is a requirement of the Research Governance Framework and Trust Policy that the records are kept for a further 20 years. For trials involving Barts Health NHS Trust patients, undertaken by Trust staff, or sponsored by Barts Health NHS Trust or QMUL, the approved repository for long-term storage of local records is the Trust Modern Records Centre. Site files from other sites must be archived at that external site and cannot be stored at the Modern Records Centre.

7.4 Compliance

This trial will be conducted in accordance with the principles of Good Clinical Practice (GCP) as laid out in the EU directive and The Medicines for Human Use (Clinical Trials) Regulation 2004, and its amendments.

In addition, internal auditors and Competent Authority inspectors will be allowed access to CRFs, source documents and other trial files to evaluate the trial. Audit reports will be kept confidential.

7.5 Clinical Governance Issues

7.5.1 Ethical Considerations

The trial will be performed in accordance with the recommendations guiding ethical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 48th General Assembly, Somerset West Republic of South Africa, October 1996. Informed written consent will be obtained from the patients prior to randomisation/registration into the study. The right of a patient to refuse participation without giving reasons must be respected. The patient must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment.

The study will be submitted to and approved by a main Research Ethics Committee (REC). Changes in protocol that may increase the exposure to risk or present new risks to the patient, or may adversely affect the validity of the study, must be approved in writing by the sponsor and then the REC before the change is implemented. These changes are usually presented in the form of an amendment.

The study will be regularly reviewed by the in-house monitoring and ethics committee. This will be done to verify that data is being accurately recorded and documented. Further, the committee will routinely review study documents with an eye towards ensuring that the study protocol is accurately followed and GCP compliant.

7.6 Quality Control and Quality Assurance

7.6.1 Summary Monitoring Plan

On-Site Monitoring will be carried out on this trial. The trial monitor will perform the first monitoring visit within 1 month of the first patient being enrolled at a site.

Monitoring visits will be performed a minimum of twice a year during recruitment and treatment period.

The frequency visits may change (increase or decrease) depending on the issues raised during the trial (death, SAE, audit or inspections, site not recruiting). Any decrease in monitoring at a site will be approved by a member of the R4-RA trial office and the Sponsor.

Source Data Verification
100 % SDV will be performed on informed consent
100 % SDV will be performed on inclusion / exclusion criteria

100% SDV on all data points will be performed for a minimum of one patient per site or approximately 5% of all patients, whichever is greater.

If for some reason on-site monitoring cannot be completed as per the above schedule the Sponsor's 'Self Monitoring Form' will be sent out to sites for completion at these timelines. Reasons for not performing on-site monitoring will be agreed with the Sponsor and fully documented in the TMF.
The following central facilities are utilised in this trial and will undergo yearly monitoring visits for the duration of their participation in the trial:

* EMR Laboratory
* Barts Health NHS pathology lab

Please See Monitoring Plan for further details of monitoring procedures. A summary of all monitoring activity for this study will be provided to the Sponsor every 6 months.

7.6.2 Audit and Inspection

Auditing: Definition "A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s)."

This trial may be audited by the Sponsor, or the Competent Authority. Investigators are obliged to cooperate in any inspection.

7.6.3 Serious Breaches in GCP or the Trial Protocol

All investigators participating in the trial will promptly notify the Chief Investigator or Sponsor of a serious breach (as defined in Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928) that they become aware of. The CI is then responsible for notifying the JRMO (the sponsor) within 24 hours of becoming aware of a serious breach.
The Sponsor is responsible for notifying the licensing authority in writing of any serious breach of:
(a) The conditions and principles of GCP in connection with that trial; or
(b) The protocol relating to that trial, as amended from time to time in accordance with regulations 22 to 25, within 7 days of becoming aware of that breach.

A "serious breach" is a breach which is likely to effect to a significant degree:-
The safety or physical or mental integrity of the subjects of the trial; or the scientific value of the trial.

Participating centres should contact the R4-RA trial office or CI for further information.

7.7 Trial Committees

7.7.1 Trial Management Group (TMG)

The Trial Management Group normally includes those individuals responsible for the day-to-day management of the trial, such as the chief investigator, statistician, trial manager, research nurse,

data manager. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. The TMG will meet monthly.

7.7.2 Trial Steering Committee (TSC)

The role of a Trial Steering Committee will be to provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of GCP and the relevant regulations. The Trial Steering Committee will be chaired by:

Prof Robert Moots, Professor of Rheumatology, Institute of Ageing and Chronic Disease
Faculty of Health & Life Sciences, University of Liverpool, 4th Floor, UCD Building, Liverpool, UK

Decisions about continuation or termination of the trial or substantial amendments to the protocol will be the responsibility of the Trial Steering Committee. The TSC will meet every 6 months and may take the form of a teleconference or face-to-face meetings.

7.7.3 Data Monitoring and Ethics Committee (DMC)

The role of a Data Monitoring Committee will be to review the accruing trial data and assess whether there are any safety issues that should be brought to participants' attention or any reasons for the trial not to continue. The Data Monitoring Committee is independent of both the investigators and the funder/sponsor. It will meet 6 monthly which may take the form of a teleconference or face-to-face meetings. It will make recommendations to the Trial Steering Committee. The independent chair is:

Professor Max Parmar, Director, MRC Clinical Trials Unit, Aviation House, 125 Kingsway, London, WC2B 6NH

7.8 Publication Policy

This is an investigator led trial; sponsored by the CI's substantive employers, QMUL. The data collected will not be used to license/ register any pharmaceuticals. Authorship of the final manuscript(s), interim publications, or abstracts will be decided according to active participation in the study design, trial management group and accrual of eligible patients. Contributing centres (and participating Investigators) will be acknowledged in the final manuscript. Representatives for the Sponsor will be added, as appropriate, as co-authors. No participant may present data from his/her centre separately from the rest of the trial results unless approved by the CI/R4-RA management group and the Sponsor.

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