Protocol Title: **S**tratification of Biologic **T**herapies for **RA** by **P**athobiology (**STRAP-EU**): A randomised, open-labelled biopsy-driven stratification trial in DMARD inadequate responder patients randomised to Etanercept, Tocilizumab or Rituximab.

Version:	3.0
EudraCT reference:	2017-004079-30
Ethics committee reference:	
Dated:	25/03/2020

1 TITLE OF THE PROTOCOL:

Stratification of Biologic **T**herapies for **RA** by **P**athobiology (**STRAP-EU**): A randomised, open-labelled biopsy-driven stratification trial in DMARD inadequate responder patients randomised to Etanercept, Tocilizumab or Rituximab.

Short title/Acronym: STRAP-EU

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2 Chief Investigator Agreement Page

The clinical study as detailed within this research protocol **(Version 3.0, dated 25th March 2020),** 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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5 STUDY SUMMARY/SYNOPSIS

TITLE	Stratification of Biologic Therapies for RA by Pathobiology (STRAP-EU): A randomised, open-labelled biopsy-driven stratification trial in DMARD inadequate responder patients randomised to Etanercept, Tocilizumab or Rituximab.
SHORT TITLE	STRAP-EU
Protocol Version Number and Date	Protocol V3.0, 25/03/2020
Methodology	Type of study: open-label, randomised controlled clinical trial, multi-centre.
Total Study Duration	Estimated duration: 2 years Recruitment: 1 year Treatment: 48 weeks Post-treatment visit/call: 30+ days after visit 15
Objectives	This study will aim to test the utility of analysing synovial B-cell infiltrates as a potential biomarker to guide therapeutic decisions in RA patients failing DMARD therapy and started on Rituximab, Tocilizumab or Etanercept therapy. We hypothesise that the stratification of patients into the B-cell rich or B-cell poor pathotype by combining histopathology [35] and a gene expression data [33-34] will help predict response to targeted drugs and help rationalise therapeutic choices. Specifically, we hypothesise that Tocilizumab and Etanercept (treated together for analysis) are superior to Rituximab in "B-cell-poor" patients.
Phase of the Trial	III
Number of Subjects/Patients	210 patients (219 allowing for 5% dropout) (this recruitment target combines both STRAP-EU (EudraCT number: 2017-004079-30) and the UK STRAP trial (EudraCT number: 2014-003529-16). Target for STRAP-EU trial: maximum of 60 patients.
Inclusion Criteria	Patients will be recruited with active Rheumatoid Arthritis: 1. 2010 ACR / EULAR classification criteria for a diagnosis of Rheumatoid Arthritis* 2. Patients with DMARD failure and eligible for anti-TNF therapy according to UK NICE guidelines** 3. Patients must have a minimum of 3 swollen joints – the joint selected for biopsy and a minimum of 2 from 28 joint count set, as assessed at biopsy visit

	4. Selected joint for biopsy must be minimum grade 2 synovial thickening, as assessed at the biopsy visit 5. 18 years of age or over 6. Patients must be capable of giving informed consent and the consent must be obtained prior to any screening procedures 7. Willingness and ability to comply with scheduled visits, treatment plans and laboratory tests and other study procedures *The ACR/EULAR classification for a diagnosis of RA could have been at any time in the patient's disease history; the score does not need to be 6 or more at screening. **According to the UK National Institute for Health and Care Excellence (NICE) guidelines, the TNF-α inhibitors are recommended as options for the treatment of adults who have both of the following characteristics: 1) Disease is severe, that is, a disease activity score (DAS28) greater than 5.1 2) Disease has not responded to intensive therapy with a combination of conventional disease-modifying antirheumatic drugs (DMARDs).
Statistical Methodology and Analysis	1. For the randomised comparison of Rituximab versus Tocilizumab and Etanercept, treated together, in B-cell poor patients, the primary endpoint will be analysed (by intent-to-treat) adjusting for MTX using logistic regression for the difference between two proportions. 2. A test of interaction between treatment and B-cell status (rich versus poor) will be based on a likelihood ratio tests between nested logistic regression models.

6 Glossary of Terms and Abbreviations

ACR	American College of Rheumatology
AE	Adverse Event
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
Anti-CCP	Anti-Cyclic Citrullinated Peptide antibody
Anti-TNF	Anti-Tumour Necrosis Factor
AR	Adverse Reaction
ASR	Annual Safety Report
AST	Aspartate Aminotransferase
CA	Competent Authority
CDAI	Clinical disease activity index
CF	Consent Form
CI	Chief Investigator
CIOMS	Council for International Organizations of Medical Sciences
CRF	Case Report Form
CRO	Contract Research Organisation
CRP	C-Reactive Protein
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CXCR4	C-X-C chemokine receptor type 4
DAS28	Disease Activity Score 28
DMEC	Data Monitoring Committee
EC	European Commission
EMEA	European Medicines Agency
EMR	Experimental Medicine and Rheumatology
ESR	Erythrocyte Sedimentation Rate
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Union Drug Regulating Authorities Clinical Trials
EudraVIGILANCE	European Union Drug Regulating Authorities Pharmacovigilance
FACIT	Functional Assessment of Chronic Illness Therapy
FACS	Fluorescent Activated Cell Sorting
FANTOM-5	Functional ANnoTation Of Mammalian Genome
FBC	Full Blood Count
GAfREC	Governance Arrangements for NHS Research Ethics Committees
GCP	Good Clinical Practice
GH	General Health
GMP	Good Manufacturing Practice
HLA-DR	Human Leukocyte Antigen-D related
IB	Investigator Brochure
IgD	Immunoglobulin D class
IgM	Immunoglobulin M Class
IP	Interphalangeal
IGRA	Inteferon Gamma Release Assay
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISRCTN	International Standard Randomised Controlled Trial Number
JRO	Joint Research and Development Office

LFT	Liver Function Tests
MA	Marketing Authorisation
Main REC	Main Research Ethics Committee
MCP	Metacarpophalangeal Joints
MHRA	Medicines and Healthcare products Regulatory Agency
MAF	Multidimensional Assessment of Fatigue
mm^3	Cubic Millimeter
MS	Member State
MTP	Metatarsophalangeal
MTX	Methotrexate
NHS R&D	National Health Service Research & Development
NICE	National Institute for Health and Clinical Excellence
NIHR	National Institute of Health Research
NRES	National Research Ethics Service
OMERACT	Outcome Measures in Rheumatoid Arthritis Clinical Trials
Participant	An individual who takes part in a clinical trial
PEAC	Pathology of Early Arthritis Cohort
p.o.	Per Os (by mouth)
PI	Principle Investigator
PIP	Proximal Interphalangeal Joints
PIS	Patient Information Sheet
PML	Progressive Multifocal Leukoencephalopathy
QC	Quality Control
QMUL	Queen Mary University of London
QP	Qualified Person for release of trial drug
RA	Rheumatoid Arthritis
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
REFLEX	Randomized Evaluation of Long-Term Efficacy of Rituximab in RA
RF	Rheumatoid Factor
RTX	Rituximab
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SC	Subcutaneous
SDV	Source Document Verification
SF-36v2©	Short Form (36) version 2
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
STRAP	Stratification of Biologic Therapies for Rheumatoid Arthritis by Pathobiology
SUSAR	Suspected Unexpected Serious Adverse Reaction
TJC	Tender Joint Count
TMG	Trial Management Group
TSC	Trial Steering Committee
U+E	Urea and Electrolytes
WLQ-25	Work Limitations Questionnaire

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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]. 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 (anti-CCP), strongly suggesting an autoimmune pathogenesis. According to the UK 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[2-4]. RA costs the UK NHS around £560 million annually. Estimates of the total cost of RA to the UK economy, including NHS costs as well as carer costs, the costs of nursing homes, private expenditure, sick leave and work-related disability are £3.8 to £4.8 billion a year.

Biologics have transformed the treatment of Rheumatoid Arthritis. However, the annual cost to the UK NHS is ~ £160 million and ~40% of patients do not respond (<20% improvement, ACR20). This leaves a major unmet clinical need and a considerable health and economic burden to the UK NHS: 4,500 new patients /year are started on biologics at the cost of approximately £10K/patient/year. Thus, the identification of the 40% non-responders prior to “blind” therapy would provide: a) better care (avert delay starting a more effective biologic), b) prevent unnecessary exposure to potentially toxic drugs and c) conceivably save £13-18 million per annum.

The main aim of this study is to integrate disease tissue (synovium) pathology into clinical & imaging algorithms while, at the same time, searching for blood surrogate markers. Indeed factors such as the standardisation of synovial sublining macrophages as biomarkers of response to treatment[5-7] and the increased accessibility of synovial tissue, following the advent of novel biopsy techniques[8, 9], have made synovial biopsy a standard intervention in an increasing number of clinical trials. The need for synovial tissue analysis is also emphasized by evidence in the literature indicating that disease biomarkers are enriched 50-100 fold in the synovial tissue compared with the blood[10]. Further, the need for a quantitative integration between tissue biomarkers and blood biomarkers has been obvious for many years in multiple fields of medicine. For instance, abnormal creatinine or liver enzymes[11] represent important blood biomarkers of tissue damage, 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 the tissue level (e.g. ER, HER), hence the consensus recommendations of incorporating molecular markers from diseased tissue into breast cancer therapy[12].

The researchers will capitalise on powerful evidence both from the Pathobiology of Early Arthritis Cohort (PEAC) initiative - http://www.peac-mrc.mds.qmul.ac.uk/index.php (funded in the UK by Medical Research Council (MRC), and sponsored by Queen Mary University of London, UK) and established RA that patients' synovial tissue can be classified into at least three histo-morphological patterns i.e. Fibroblast (F), Myeloid (M) and Lymphoid (L) to test the overarching hypothesis that different synovial pathotypes (the strata) are associated with diverse treatment response to targeted biologic therapies. This will build on evidence from research collaborators at Genentech, who have demonstrated that the L pattern is rich in B cells, whilst the F & M patterns are B cell poor and are associated with different transcriptomic signatures (Patent W02011/028945 A1). Of most relevance to this programme, strong pilot data in 27 established anti-TNF inadequate responder (ir) RA patients suggesting that low/absent B cells in the biopsy (approximately 50% patients) are an independent predictor of no-response to RTX (80% chance of no-response). In addition, in an independent RA cohort n=20, Genentech Scientists (Townsend personal communication – paper in press) have confirmed that the F pattern (B cell poor) is associated with poor RTX outcome and conversely, synovial M1 macrophages and pro-inflammatory cytokine processes, but not B cells, predict response to anti-TNFα. Importantly, the above pilot data has formed the basis for a biopsy-based randomised clinical trial (RCT) funded by NIHR under the Efficacy and Mechanism Evaluation (EME) programme in the UK and recruiting patients in both the UK and a number of other European countries, in which anti-TNF-ir patients are randomized to standard therapy (RTX) or Tocilizumab (TOC): "A Randomised, open labelled study in anti-TNFa inadequate responders to investigate the mechanisms for Response - Resistance to Rituximab versus Tocilizumab in RA (R4-RA)" R4-RA. In that study the hypothesis to be tested is that in patients with a B-cell poor biopsy, by definition, active synovitis is driven by other cell types and these patients are less likely to respond to RTX and more likely to respond to TOC.

However, an even more important question is whether a synovial biopsy can provide valuable information to enrich for response to first biologic therapy and identify the ~40% of patients who are unlikely to respond to anti-TNF therapy[8-10]. Thus, in this study, it is proposed to use synovial

pathobiology to stratify DMARD-ir patients to first line biologics in a RCT: "Stratification of Therapy for RA by Pathobiology (STRAP)" using a multi-arm, design.

1.2 Investigational Medicinal Products

1.2.1 Rituximab

Within the remit of this study Rituximab is being used off-label as a first-line biologic. Rituximab (MabThera®, Roche) 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 RA compared to traditional DMARDs or TNF-α 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 is administered intravenously, following premedication with an anti-pyretic and antihistaminic such as paracetamol 1g per os (p.o.), the antihistaminic chlorpheniramine 10mg I.V. and methyl prednisolone 100mg I.V. These drugs are standard care for patients having an infusion of rituximab. Premedication will be administered 30 minutes prior to Rituximab, as per the Rituximab SmPC.

The dosage of Rituximab is 1000mg by iv infusion on days 1 and 15; this cycle is repeated every 24 weeks.

1.2.2 Tocilizumab

Within the remit of this study Tocilizumab is being used in accordance with its 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 RA. 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.

The recommended dosage of Tocilizumab in the UK is 162 mg administered subcutaneously every week.

1.2.3 Etanercept (anti-TNFα)

Within the remit of this study Etanercept is being used in accordance with its licence. Etanercept (Enbrel ®, Pfizer) is a human tumour necrosis factor receptor p75 Fc protein, genetically engineered by fusing the extracellular ligand binding domain of human tumour necrosis factor receptor-2 (TNFR2/p75) to the Fc domain of human IgG1. This Fc component contains the hinge, CH2 and CH3 regions, but not the CH1 region of IgG1. Etanercept is a competitive inhibitor of TNF binding to its cell surface receptors and thereby inhibits the biological activity of TNF. TNF and lymphotoxin are pro-inflammatory cytokines that bind to two distinct cell surface receptors (TNFRs). The mechanism of action of etanercept is thought to be its competitive inhibition of TNF binding to cell surface TNFR, preventing TNF-mediated cellular responses by rendering TNF biologically inactive. Etanercept may also modulate biologic responses controlled by additional downstream molecules (e.g., cytokines, adhesion molecules, or proteinases) that are induced or regulated by TNF.

Etanercept is administered subcutaneously. The recommended posology is 50mg once weekly.

1.3 Preclinical data

N.B. Based on SmPC data as of September 2014.

1.3.1 Rituximab

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 MTX. 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 [11].

1.3.2 Tocilizumab

The pharmacokinetics of Tocilizumab were determined using a population pharmacokinetic analysis on a database composed of 29 patients with RA following SC administration (by syringe) of 162 mg weekly or 162 mg fortnightly for 12 weeks (study NP22623)[17]. In the 162 mg QW (weekly administration) group (N = 14) the median Tmax (time to maximum concentration) was 2−3 days. The mean Cmax (peak concentration), Cmin (minimum concentratrion), and AUC0-168h at Week 12 were 39.4 ± 18.1 µg/mL, 27.9 ± 14.7 µg/mL, and 5.5 ± 2.6 mg • hr/mL. The median accumulation ratio (Rac) (Week 12/Week 1) was 5.7 for AUC0-168hr, 5.5 for Cmax, and 5.2 for Cmin. In the 162 mg Q2W group (N = 15) the median Tmax was 3 days. The mean Cmax, Cmin, and AUC0-336h at Week 11 were 10.7 ± 6.57 µg/mL, 2.3 ± 3.2 µg/mL, and 2.3 ± 1.7 mg·hr/mL. The median accumulation ratio (Rac) (Week 11/Week 1) was 1.8 for AUC0-336hr, 1.7 for Cmax, and 9.0 for Cmin. In study WA22762, compared with 162 mg SC QW dosing, steady-state Cmax and AUC over 4 weeks following 8 mg/kg IV Q4W dosing were approximately 3−4-fold and approximately 1.2−1.4-fold higher, respectively. The observed steady-state Ctrough values were approximately 40 µg/mL following SC QW dosing and approximately 18 µg/mL following IV Q4W dosing, which are both higher than historical IV data[15].

1.3.3 Etanercept

Etanercept is slowly absorbed from the site of subcutaneous injection, reaching maximum concentration approximately 48 hours after a single dose. The absolute bioavailability is 76%. With twice-weekly doses, it is anticipated that steady-state concentrations are approximately twice as high as those observed after single doses. After a single subcutaneous dose of 25 mg the average maximum serum concentration observed in healthy volunteers was 1.65 ± 0.66 µg/ml, and the area under the curve was 235 ± 96.6 µg•hr/ml. Mean serum concentration profiles at steady state in treated RA patients were Cmax of 2.4 mg/l vs. 2.6 mg/l, Cmin of 1.2 mg/l vs 1.4 mg/l, and partial AUC of 297 mgh/l vs. 316 mgh/l for 50 mg Etanercept once weekly (n=21) vs. 25 mg twice weekly (n=16), respectively. In an open-label, single-dose, two-treatment, crossover study in healthy volunteers, Etanercept administered as a single 50 mg/ml injection was found to be bioequivalent to two simultaneous injections of 25 mg/ml. A bi-exponential curve is required to describe the concentration time curve of etanercept. The central volume of distribution of etanercept is 7.6 l, while the volume of distribution at steady-state is 10.4 l. Etanercept is cleared slowly from the body. The half-life is long, approximately 70 hours. Clearance is approximately 0.066 l/hr in patients with rheumatoid arthritis, somewhat lower than the value of 0.11 l/hr observed in healthy volunteers[16].

1.4 Clinical Data

1.4.1 Rituximab

Rituximab in combination with methotrexate (MTX) 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 TNF-α inhibitor therapy.

1.4.1.1 Clinical outcomes

The efficacy and safety of Rituximab in alleviating the symptoms and signs of RA in patients with an inadequate response to TNF-inhibitors was demonstrated in a pivotal randomized, controlled, double-blind, multicentre study (REFLEX)[17].

REFLEX evaluated 517 patients that had experienced an inadequate response or intolerance to one or more TNF inhibitor therapies. Eligible patients had active RA, 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.

1.4.1.2 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 MTX demonstrated significantly less radiographic progression than patients originally receiving MTX alone at 56 weeks. Of the patients originally receiving MTX 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

1.4.1.3 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 MTX 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 MTX alone.

1.4.2 Tocilizumab

Tocilizumab, in combination with MTX, is indicated for the treatment of moderate to severe active RA in adult patients who have either responded inadequately to, or who were intolerant to, previous therapy with one or more DMARDs or TNF-α antagonist. 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 MTX.

1.4.2.1 Clinical outcomes

In a number of studies, patients treated with Tocilizumab IV had statistically significant higher ACR 20, 50, 70 response rates at 6 months compared to controls. 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[18]. 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 on-going open label extension of a number of clinical trials – AMBITION[18], LITHE[19], OPTION[20], TOWARD[21] and RADIATE[22]. Patients in the aforementioned 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 was 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.

In study WA22762 treatment with 162 mg SC QW TCZ was not-inferior to 8 mg/kg of IV Q4W TCZ with regard to the difference in the proportion of patients who achieved an ACR20 response at Week 24, using a non-inferiority margin of 12%. SC TCZ was also non-inferior to IV TCZ at a more restricted non-inferiority margin of 10%[15]. Analysis of the secondary endpoints of ACR50 responders, ACR70 responders, DAS28 remission, decrease in HAQ-DI, and withdrawal for lack of therapeutic response all supported the hypothesis that the SC TCZ regimen was non-inferior to the IV TCZ regimen. In the OLE period of the study, results demonstrated that the efficacy of TCZ was maintained. Switching from the IV to SC regimen appeared to have no effect on efficacy outcomes up to Week 73. In study NA25220 162 mg SC Q2W TCZ was superior to placebo with regard to the difference in the percentage of patients who achieved an ACR20 response at Week 24. Analysis of the key secondary endpoints (ACR50, ACR 70, DAS28 < 28 [DAS28 remission], decrease in HAQ-DI, and progression of joint damage) supported the hypothesis that the SC TCZ regimen was superior to the placebo regimen. In the OLE period of the study, results demonstrated that the efficacy of TCZ was maintained[23].

1.4.2.2 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 IV compared to control. In the open-label extension of this study the inhibition of progression of structural joint damage in Tocilizumab IV 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 IV plus MTX ($p<0.0001$) compared with patients who were randomised to placebo plus MTX[19].

Progression of joint damage in patients in Study NA25220 was assessed by X-ray. The results show that the progression of joint damage between baseline and Week 24 measured by the change in mTSS was on average smaller in the SC TCZ arm compared with the SC placebo arm[23].

1.4.2.3 Quality of life outcomes

Tocilizumab IV-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[19]. In the WA22762 study similar improvements in quality of life (QOL) endpoints (HAQ-DI score and SF-36) between baseline and Week 24 were also observed in both treatment arms[15]. In study NA25220 162 mg SC Q2W TCZ was superior to placebo with regard to the difference in the percentage of patients who achieved decrease in HAQ-DI at Week 24. Similar improvements in QOL endpoints (HAQ-DI score, and SF-36) were also observed between baseline and Week 24[23].

1.4.3 Etanercept

Etanercept in combination with MTX is indicated for the treatment of moderate to severe active RA in adults, when the response to DMARDs, including MTX (unless contraindicated), has been inadequate. Etanercept can be given as monotherapy in case of intolerance to MTX or when

continued treatment with MTX is inappropriate. Etanercept is also indicated in the treatment of severe, active and progressive RA in adults not previously treated with MTX. Etanercept, alone or in combination with MTX, has been shown to reduce the rate of progression of joint damage as measured by X-ray and to improve physical function.

1.4.3.1 Clinical outcomes

The efficacy of Etanercept was assessed in a randomised, double-blind, placebo-controlled study[16]. The study evaluated 234 adult patients with active RA who had failed therapy with at least one but no more than four DMARDs. Doses of 10 mg or 25 mg Etanercept or placebo were administered subcutaneously twice a week for 6 consecutive months. ACR 20 and 50 responses were higher in patients treated with Etanercept at 3 and 6 months than in patients treated with placebo (ACR 20: Etanercept 62% and 59%, placebo 23% and 11% at 3 and 6 months respectively: ACR 50: Etanercept 41% and 40%, placebo 8% and 5% at months 3 and 6, respectively; $p<0.01$ Etanercept vs. placebo at all timepoints for both ACR 20 and ACR 50 responses). Among patients receiving Etanercept, the clinical responses generally appeared within 1 to 2 weeks after initiation of therapy and nearly always occurred by 3 months. A dose response was seen; results with 10 mg were intermediate between placebo and 25 mg. Etanercept was significantly better than placebo in all components of the ACR criteria as well as other measures of RA disease activity not included in the ACR response criteria, such as morning stiffness.

The efficacy of Etanercept was compared to MTX in a randomised, active-controlled study with blinded radiographic evaluations as a primary endpoint in 632 adult patients with active RA (<3 years duration) who had never received treatment with MTX. Doses of 10 mg or 25 mg Etanercept were administered SC twice a week for up to 24 months. Methotrexate doses were escalated from 7.5 mg/week to a maximum of 20 mg/week over the first 8 weeks of the trial and continued for up to 24 months. Clinical improvement including onset of action within 2 weeks with Etanercept 25 mg was similar to that seen in the previous trials, and was maintained for up to 24 months[16].

1.4.3.2 Radiographic response

In this later study, structural joint damage was assessed radiographically and expressed as change in Total Sharp Score (TSS) and its components, the erosion score and Joint Space Narrowing (JSN) score. Radiographs of hands/wrists and feet were read at baseline and 6, 12, and 24 months. The

10 mg Etanercept dose had consistently less effect on structural damage than the 25 mg dose. Etanercept 25 mg was significantly superior to MTX for erosion scores at both 12 and 24 months. The differences in TSS and JSN were not statistically significant between MTX and Etanercept 25 mg.

In another active-controlled, double-blind, randomised study, clinical efficacy, safety, and radiographic progression in RA patients treated with Etanercept alone (25 mg twice weekly), MTX alone (7.5 to 20 mg weekly, median dose 20 mg), or a combination of Etanercept and MTX initiated concurrently were compared in 682 adult patients with active RA. Patients in the combination therapy group had significantly higher ACR 20, ACR 50, ACR 70 responses and improvement for DAS and HAQ scores at both 24 and 52 weeks than patients in either of the single therapy groups.

Radiographic progression at 12 months was significantly less in the Etanercept group than in the MTX group, while the combination was significantly better than either monotherapy at slowing radiographic progression[16].

1.4.3.3 Quality of life outcomes

A Health Assessment Questionnaire (HAQ), which included disability, vitality, mental health, general health status, and arthritis-associated health status subdomains, was administered every 3 months during the first trial (see “Clinical outcomes”). All subdomains of the HAQ were improved in patients treated with Etanercept compared to controls at 3 and 6 months.

In the study comparing Etanercept to Methotrexate MTX, at baseline, patients had a moderate degree of disability, with mean HAQ scores of 1.4 to 1.5. Treatment with Etanercept 25 mg resulted in substantial improvement at 12 months, with about 44% of patients achieving a normal HAQ score (less than 0.5). This benefit was maintained in Year 2 of this study.

1.5 Rationale and Risks/Benefits

This is an open-labelled randomised controlled clinical trial investigating the use of synovial B cell assessment by histopathology and gene expression as a potential diagnostic biomarker to stratify RA patients’ response to first-line biologic therapy. Currently, NICE guidelines in the UK suggest the use of anti-TNF-α in all patients following inadequate response to conventional DMARD therapy. Inadequate response to TNF-α inhibitors is observed in approximately 40% of these DMARD-ir patients. Identification of these patients might facilitate the use of Rituximab (anti-CD20

monoclonal antibody) or Tocilizumab (IL-6 receptor monoclonal antibody) and enrich response. In this study patients will be randomised to receive Etanercept, Rituximab or Tocilizumab. No placebo arm has been included, as withholding an approved potentially beneficial therapy would not be ethical. In the UK, Etanercept is an option for the treatment of active RA, in patients who have inadequate response to DMARD therapy. Tocilizumab is licenced in the UK for use in adult patients with moderate to severe active RA who have either responded inadequately to, or who were intolerant to, previous DMARD therapy or TNF antagonists. Rituximab is an option for the treatment of adults with severe active RA who have had an inadequate response to, or are intolerant of, other DMARDs, including at least one TNFα inhibitor.

All patients will undergo synovial biopsies, either US-guided or arthroscopic. Since these biopsies would not necessarily be considered routine clinical care, the main risks to patients enrolled would be associated with this interventional procedure. Through the MRC-funded Pathobiology of Early Arthritis Cohort (PEAC) initiative (http://www.peac-mrc.mds.qmul.ac.uk/index.php) a National Training Centre for the performance of US guided synovial biopsies has been developed. The procedure itself has excellent safety and tolerability and can be applied to both large and small joints in most patients. Arthroscopic biopsies, whilst being technically more complicated and requiring theatre time, have been extensively validated with respect to tissue quality in therapeutic intervention studies[27]. Participating centres will use either one of the above techniques depending on experience, resources and facilities. The type of biopsy technique performed will be documented for all participants. Sites with sufficient training and experience in synovial biopsies, confirmed by the site questionnaire, will be selected to participate.

The researchers have previously suggested that one mechanism for treatment resistance in Rituximab may be the survival of self-sustaining, B cell niches within the synovium[28]. Recent histological data has demonstrated a correlation of clinical response at 16 weeks following Rituximab and levels of synovial membrane B-cell depletion[29]. Likewise, there is evidence (though limited by the small number of patients in these studies) that synovial tissue biomarkers are associated with anti-TNF response [30, 31]. 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[10]. In addition, pharmacological response signatures in the blood are not

helpful as, for example, downstream pathway to TNF is modulated in all anti-TNF treated patients, irrespective of clinical response[32]. 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) [12].

There is strong evidence emerging from the PEAC initiative that RA patients can be classified into at least 3 histo-morphological patterns i.e. Fibroblast (pauci-immune), Lymphoid (B cell rich) and Myeloid (rich in monocytes but poor in B cells). There is also evidence that the PEAC histopathology patterns correspond to different transcriptomic signatures. More important still, there is 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; 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 either by US guided approach or arthroscopic biopsy technique, both from large and small joints, through a minimally invasive approach, thus, potentially benefiting all patients from stratified medicines. We have pioneered the US guided biopsy 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 (B-cell assessment by immunohistochemical analysis and gene expression in synovial tissue) for patient stratification into responsive/non-responsive categories with respect to first-line biologic therapy. The proposed research also has the potential to contribute work of significant clinical advantage for the treatment of 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

2.1.1 Primary objective

The main aim of this study is to test the utility of analysing synovial B cell infiltrates as a potential biomarker to guide therapeutic decisions in patients failing DMARD therapy. We hypothesise that stratification of patients according to their synovial B cell infiltrate into the B cellpoor/rich pathotype by histomorphology [35] and/or a B cell specific gene expression module derived from FANTOM5 (Functional ANnoTation Of Mammalian Genome) [33-34] will better define response rates. Specifically, we hypothesise that the other two treatment options (Tocilizumab and Etanercept, treated together for analysis) are superior to Rituximab in B-cell-poor patients.

2.1.2 Primary Endpoint

The primary end point will assess the difference in the ACR20 response between Rituximab and other treatments (Tocilizumab and Etanercept therapy treated together for analysis) at 16 weeks from baseline in the B-cell poor pathotype sub-group.

2.1.3 Secondary Endpoints

1. Patients deemed treatment failures at 16 weeks, will be switched to the other therapeutic option. Such patients will be considered a new patient starting at week 0 with treatment response assessed again at 16 weeks for ACR20 response.

2. For the B-cell-rich synovial pathotype sub-group, we aim to compare the treatment effects of Rituximab to the other two treatment options (Tocilizumab and Etanercept, treated together for analysis)
3. To examine the interaction between treatments and B-cell status (rich and poor).
4. Percentage of patients in remission (DAS28 < 2.6) at 16 weeks.
5. Percentage of patients with ACR 50, and 70 response rates at 16 weeks.
6. Percentage of patients with a low clinical disease activity index score (CDAI≤ 10) at 16 weeks.
7. Mean % change in CDAI score at 16 weeks.

2.1.4 Exploratory endpoints

1. Percentage of patients with low disease activity (DAS28 < 3.2) at 16, 24, 36, and 48 weeks.

2. Percentage of patients in remission (DAS28 < 2.6) at 24, 36, and 48 weeks.
3. Percentage of patients with ACR20, 50, and 70 response rates at 24, 36, and 48 weeks from baseline.
4. Percentage of patients with a low clinical disease activity index score (CDAI) at 24, 36 and 48 weeks from baseline.
5. Mean % change in DAS28, HAQ, FACIT, and WLQ-25 score at 16 weeks from baseline.
6. Mean % change in DAS28, CDAI, HAQ, FACIT, and WLQ-25 score at 24, 36, and 48 weeks from baseline.

7. The association between synovial histology and ultrasound measures of inflammation, and drug response rates, disease outcome and disability.
8. SAEs from 0 to 48 weeks or 0 to 24 weeks (+ 30 days –see section 7.3) for all patients.
9. Mean change in the van der Heijde/Sharp scores at 16 and 48 weeks from baseline.
10. Change in 12-max summary measure of US 2D synovial thickness (ST)and power Doppler (PD) signal from baseline. at 4, 16, 24, and 48 weeks.
11. Changes from baseline to week 16 in the total histopathological synovitis score.
12. Correlation between the differential expression of key pathobiological markers of vascularity/inflammation/joint damage and US inflammation assessments at baseline and 16 weeks.
13. Correlation between Ultrasound and X-ray assessments at baseline and 16 weeks.

The above endpoints will be explored within the B cell poor and rich sub-groups.

2.2 Trial Design

This study represents a multi-site, prospective, open-labelled, randomised, controlled phase III clinical trial in a population of patients who have an inadequate response to DMARDs and have fulfilled UK NICE guidelines for the commencement of anti-TNF therapy. The STRAP trial (differences between STRAP and STRAP-EU outlined in Appendix 2) has been approved by the UK Competent Authority (MHRA) and was deemed to be a Type B trial as per MHRA risk assessment.

Patients recruited to this study will undergo a synovial biopsy prior to randomisation. Possible synovial biopsy sites are the knee, elbow, wrist, shoulder, ankle, MCP, PIP, and MTP joints. Patients will subsequently be stratified according to their synovial histomorphological phenotype:

- B cell Poor (Myeloid - M & Fibroblast – F)
- B cell Rich (Lymphoid - L)

This result will be recorded centrally prior to randomisation of the patient. All participating site staff will be blinded to the pathotype (B Cell Poor, B Cell Rich). In a small number of cases, patients may be randomised to a third "unknown" strata if a biopsy result is not yet obtained, or the biopsy cannot be classified at the time of randomisation. Reasons for unknown strata will be recorded as either un-gradable tissue, expedited treatment required or processing delays. However, any biopsies that are later classified will be included in analysis of the trial data. Patients with a pre-randomisation 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.

All patients will be randomised to receive Rituximab, Etanercept or Tocilizumab (1:1:1 ratio) with a clinical assessment of disease activity at 16 weeks as the primary end point. The treatment will end with the 48-weeks assessment, with a post-treatment visit/call scheduled 30+ days after visit 15.

2.3 Response criteria

Responders – patients who achieve ACR20 response at week 16, as further described in section 5.14.1

Non-responders – patients who do not achieve ACR20 response at week 16, as further described in section 5.14.1.

Patients who achieve an initial ACR20 response may still be switched to their second line therapy at the physician's discretion if a high level of disease activity persists at week 16 or subsequent visits after their primary endpoint assessment. These patients will however be treated as responders in the statistical analysis.

Primary failures (i.e. by week 16) to first biologic:

- Patients who have an inadequate response at 16 weeks to Etanercept will be switched to Rituximab at that visit or the following visit.

- Patients with an inadequate response at week 16 to Rituximab will be switched to Etanercept at that visit or the following visit.
- Patients with an inadequate response at week 16 to Tocilizumab will be switched to Etanercept at that visit or the following visit.

Secondary failures (i.e. after week 16) to first biologic:

Patients showing initial clinical response by 16 weeks will be subsequently classified as a secondary failure at subsequent study visits, if their ACR response is less than 20% at any subsequent follow up visit. Such patients should switch treatment either at that visit or the following visit.

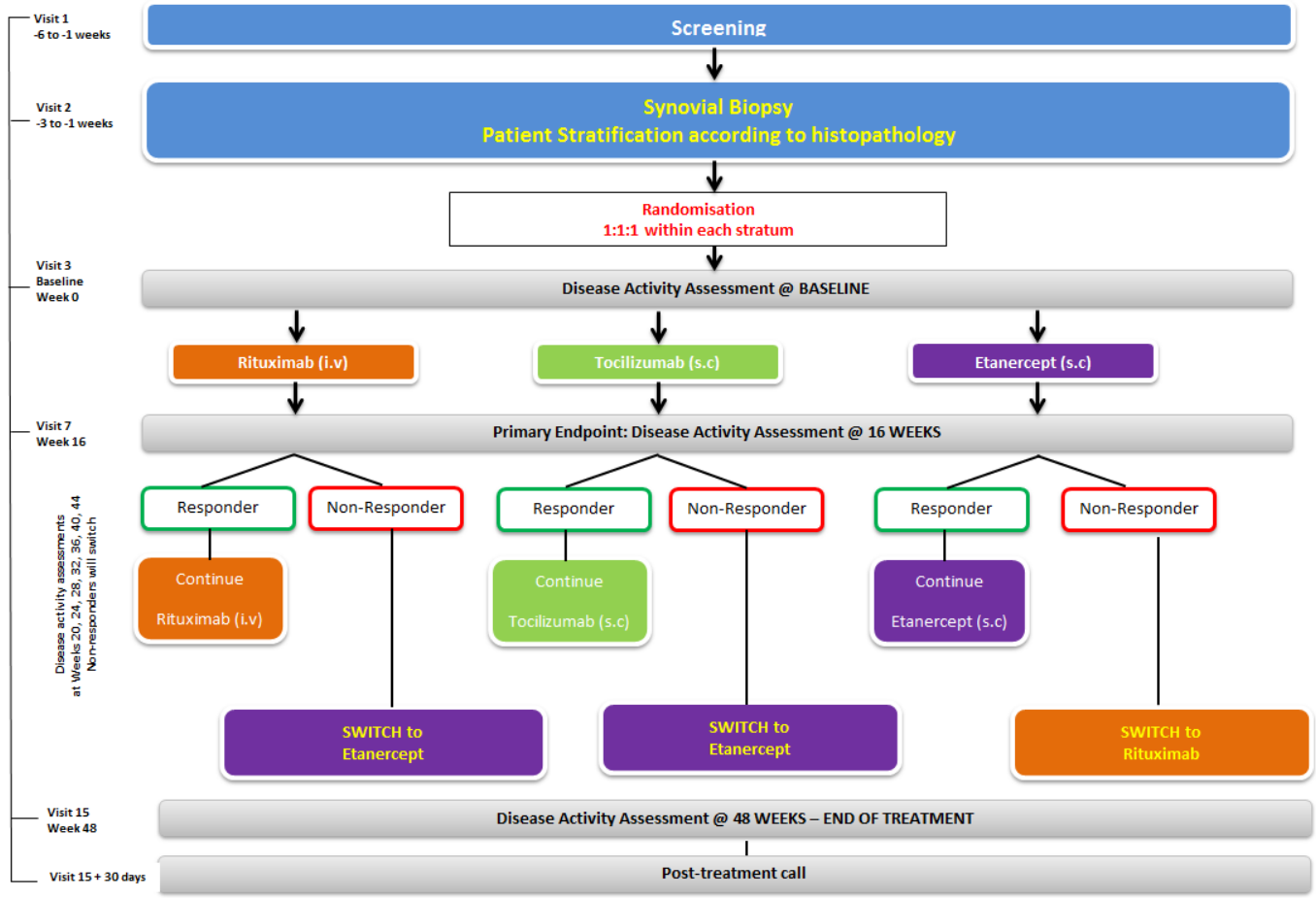
A patient initially randomised to Rituximab and deemed a responder at 16 weeks will be retreated at 24 weeks. If their ACR response falls below 20% at any subsequent visit after week 24 they can have their treatment switched to Etanercept (at the physician's discretion).

Failure (at any time) to a second biologic agent:

Failure of a second biological therapy at any time would permit the patient to receive therapy at the physician's discretion (patients do not have to complete a full 16 weeks on the second biologic). With regards to primary endpoint analysis, patients classified as a failure to second biologic will continue in the trial but the data collected (and any further treatment changes) will form an observational component of the trial. If the third biological therapy is not one of the trial IMPs, ongoing treatment for such patients should be as per local procedures.

2.4 Study scheme diagram

Figure 1. Study scheme diagram



3 SUBJECT SELECTION

3.1 Number of subjects and subject selection

Number of subjects to be enrolled = 210 patients (219 patients to allow for 5% dropout). Please note that this is a combined recruitment target with the STRAP trial taking place in the UK. This study will take place in secondary care settings throughout Europe. Patients will be invited to participate as they present to the biologics initiation and monitoring clinic. Patients will be recruited from within the Rheumatology departments who have been referred by their consultant Rheumatologists for a first-line biological agent following failure of DMARDs

3.2 Inclusion criteria

Patients will be recruited with active RA:

1. 2010 ACR / EULAR Rheumatoid Arthritis classification criteria for a diagnosis of RA *
2. Patient with DMARD failure eligible for anti-TNF-α therapy as per UK NICE guidelines**
3. Patients must have a minimum of 3 swollen joints – the joint selected for biopsy and a minimum of 2 from 28 joint count set, as assessed at biopsy visit
4. Selected joint for biopsy must be minimum grade 2 synovial thickening, as assessed at the biopsy visit
5. 18 years of age and over
6. Patients must be capable of giving informed consent and the consent must be obtained prior to any screening procedures

* The ACR/EULAR classification for a diagnosis of RA could have been at any time in the patient's disease history; the score does not need to be 6 or more at screening.

**According to the UK National Institute for Health and Care Excellence (NICE) guidelines, the TNF-α inhibitors are recommended as options for the treatment of adults who have both of the following characteristics:

1) Disease is severe, that is, a disease activity score (DAS28) greater than 5.1
2) Disease has not responded to intensive therapy with a combination of conventional disease-modifying antirheumatic drugs (DMARDs).

Current NICE guidelines available at the following link: http://www.nice.org.uk/guidance/ta375.

Exclusion criteria

Patients will be excluded if they have any contraindication to Etanercept, Rituximab or Tocilizumab therapy:

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 an effective method of contraception (recommend double contraception) throughout the trial and beyond the end of trial treatment for the duration as defined in the relevant SmPC; 12 months for Rituximab, at least 3 weeks for Etanercept, and at least 3 months for Tocilizumab.
3. History of or current primary inflammatory joint disease or primary rheumatological autoimmune disease other than RA (if secondary to RA, then the patient is still eligible).
4. Prior exposure to Rituximab, any anti-TNF, Tocilizumab, or any other biologic for treatment of RA
5. Treatment with any investigational agent ≤ 4 weeks prior to baseline or < 5 half-lives of the investigational drug (whichever is the longer)
6. Intra-articular or parenteral corticosteroids ≤ 4 weeks prior to screening visit.
7. Oral prednisolone more than 10mg/d or equivalent ≤ 4 weeks prior to baseline synovial biopsy.
8. Active infection
9. Known HIV, active Hepatitis B/C infection. Hepatitis B screening test must be performed at or in the preceding 3 months of screening visit.
10. Septic arthritis of a native joint within the last 12 months
11. Septic arthritis of a prosthetic joint within 12 months or indefinitely if the joint remains in situ
12. Latent TB infection unless they have completed adequate antibiotic prophylaxis
13. Malignancy (other than basal cell carcinoma) within the last 10 years
14. New York Heart Association (NYHA) grade III or IV congestive heart failure
15. Demyelinating disease
16. Known allergy to latex, Rituximab, Tocilizumab or Etanercept

17. Any other contra-indication to the study medications as detailed in the applicable SmPC including low IgG levels, at physician's discretion
18. Receipt of live vaccine <4 weeks prior to first IMP infusion or dose
19. Major surgery in 3 months prior to first IMP infusion or dose
20. Presence of a transplanted organ (with the exception of a corneal transplant >3 months prior to screening).
21. Known recent substance abuse (drug or alcohol).
22. Poor tolerability of venepuncture or lack of adequate venous access for required blood sampling during the study period
23. Patients unable to tolerate synovial biopsy or in whom this is contraindicated including patients on anti-coagulants. Oral anti-platelet agents are permitted.
24. Patients currently recruited to other clinical trials.
25. 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 their 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.

3.3 Criteria for Early withdrawal

A subject may withdraw from the study at any time at his/her own request without prejudice, or may be withdrawn at any time at the discretion of the investigator. At the time of withdrawal of consent, a full efficacy and safety evaluation should be performed, if the patient agrees. Withdrawn trial subjects will not be replaced. See sections 5.12 and 5.13 for further details.

4 INVESTIGATIONAL MEDICINAL PRODUCTS (IMP)

4.1 List and definition of each IMP

4.1.1 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, which depletes the B-cell population by targeting cells bearing the CD20 surface marker.

4.1.2 Tocilizumab

Tocilizumab is a humanised IgG1 monoclonal antibody against the human interleukin-6 (IL-6) receptor produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology. The final drug product has intravenous (IV) and subcutaneous (SC) formulations.

4.1.3 Etanercept

Etanercept is a human tumour necrosis factor receptor p75 Fc fusion protein produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian expression system.

The SmPCs will be checked 6 monthly by the STRAP-EU clinical trials office for new safety information.

4.2 Formulation of IMP

4.2.1 Rituximab

Rituximab (MabThera® 500mg concentrate for infusion, Roche Products Ltd) is available as 50ml single-use vials containing 500mg Rituximab for infusion (10mg/ml). Rituximab is a clear, colourless liquid. Rituximab will be sourced from local hospital stocks.

4.2.2 Tocilizumab

Tocilizumab (RoActemra SC® 162mg solution for injection in pre-filled syringe, Roche Products Ltd) subcutaneous (SC) formulation is a sterile, yellowish, preservative-free liquid solution of approximately pH 6.0 for SC injection. It is supplied at a concentration of 180 mg/mL in syringe/autoinjector (AI) forms with a nominal amount of 162 mg of Tocilizumab in 0.9 mL of arginine, methionine, and histidine buffered solution. Tocilizumab will be sourced from local hospital stocks.

4.2.3 Etanercept

Etanercept (Enbrel® 50mg solution for injection in pre-filled pen, Pfizer) is available in pre-filled pens containing 50mg of Etanercept in a clear, and colourless or pale yellow solution. Pfizer is providing Etanercept for use in the STRAP-EU trial.

4.3 IMP supply

4.3.1 Rituximab

Rituximab will be prescribed off label and will be supplied from hospital stocks.

4.3.2 Tocilizumab

Tocilizumab will be prescribed as per license and thus will be supplied from hospital stocks.

4.3.3 Etanercept

Etanercept will be prescribed as per license and thus will be supplied from commercial stock from Pfizer commercial care team (who would supply hospitals with normal non-trial stock). Ordering would occur by each site requesting a drug order via the STRAP-EU trials office who will in turn order through Pfizer. Further details of this procedure is described in the STRAP-EU IMP Manual.

4.4 Prescription of IMP

4.4.1 Rituximab

Rituximab will be prescribed by a physician as a member of the study team, using trial specific prescription forms (either Sponsor or local template may be used). 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.

4.4.2 Tocilizumab

Tocilizumab will be prescribed by a physician as a member of the study team, using trial specific prescription forms (either Sponsor or local template may be used). 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.

4.4.3 Etanercept

Etanercept will be prescribed by a physician as a member of the study team, using trial specific prescription forms (either Sponsor or local template may be used). 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.

4.5 Preparation and administration of IMP

4.5.1 Rituximab

Patients randomised to receive rituximab therapy will be given treatment on days 1 and 15, which is one infusion cycle, after premedication with paracetamol, antihistaminic, and methyl-prednisolone (see *Method and Rate of Administration*). All treatment infusions will be prepared, administered and monitored according to the guidance included in the SmPC. The paracetamol, antihistaminic, and methyl-prednisolone will be sourced locally. Preparation and administration (including pre-medications) will be performed by a suitably trained member of the local study team as per local policy.

Rituximab is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dihydrate, and Sterile Water for Injection (pH 6.5). It is supplied in sterile preservative-free non-pyrogenic single-use pharmacopeial Type I glass vials at a concentration of 10 mg rituximab per mL solution.

Instructions for Dilution and Suitable Dilutent:

- Aseptically withdraw 100ml (2 X 50ml vials), which is 1000mg of Rituximab.
- Slowly add the total volume of Rituximab (100mls) to a 500ml bag of sterile pyrogen free 0.9% sodium chloride. To mix the solution, gently invert the bag in order to avoid foaming. Do not shake. The final concentration of the drug in this case it will be 1.67mg/ml.
- **If, according to local policy, a different dilution is used** (i.e. different volume of sodium chloride 0.9% solution for injection or 5% dextrose in water); **the final rituximab concentration must in any case be between 1-4mg/ml**.
- Since the drug product does not contain any antimicrobial preservative or bacteriostatic agents, aseptic technique must be observed. Care must be taken to ensure sterility of prepared

solutions. Inspect the bag visually for any particulate matter and discolouration prior to administration – discard the solution if this is observed.

- Any unused product or waste material should be disposed of in accordance with local requirements

Method and Rate of Administration:

Pre-medication should be prescribed and administered 30 minutes prior to the start of infusion. Variation to the below as per local Trust policy is permitted:

Table 1: Pre-medication

Drug	Dose	Route	Frequency
Methylprednisolone	100mg	IVI	Stat (statim: immediately)
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.

Example 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

The following infusion rates are based on a final concentration of 1.67mg/ml of rituximab. In case a different dilution is used according to local policy (see above), the following rates in <u>mg/hour</u> must be respected (i.e. the rates in ml/hour will be different).

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). This rate corresponds to an administration time of 4.25 hours.

Table 2. RTX infusion rates

	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

Second infusion of each course

As per the Rituximab SmPC, subsequent doses can be infused at an initial rate of 100 mg/hr, and increased by 100 mg/hr increments at 30 minute intervals, to a maximum of 400 mg/hr. This rate corresponds to an administration time of 3.5 hours.

Alternative 120-minute subsequent infusions with the concentration of 4 mg/mL in a 250 mL volume as per the Rituximab SmPC:

If patients did not experience a serious infusion-related adverse event with their previous infusion administered over the original administration schedule, a 120-minute infusion can be administered for subsequent infusions. Initiate at a rate of 250 mg/hour for the first 30 minutes and then 600 mg/hour for the next 90 minutes. If the 120-minute infusion is tolerated, the same alternative 120-minute infusion rate can be used when administering subsequent infusions and courses.

Patients who have clinically significant cardiovascular disease, including arrhythmias, or previous serious infusion reactions to any prior biologic therapy or to rituximab, should not be administered the more rapid infusion.

4.5.2 Tocilizumab

The recommended dose of Tocilizumab for adult patients with RA is 162mg administered as a weekly subcutaneous injection and it will be self-administered by patients. Prior to use, the prefilled syringe (0.9 mL) must be removed from the refrigerator (2°C and 8°C) and allowed to sit at room temperature outside of the box for 30 minutes, out of the reach of children. It should not be warmed in any other way. Once removed from the refrigerator, Tocilizumab must be administered within 8 hours and must not be stored above 30°C. Comprehensive instructions for administration are given in the package leaflet. Patient will return unused product to the site. Any unused product or waste material should be disposed of as detailed in the STRAP-EU IMP Manual, and in accordance with local requirements. Suitable sites for injection include the thigh or abdomen. Patients will be asked to complete a diary to confirm the timely administration of Tocilizumab therapy that will be returned at every clinic visit along with any used syringes. Administration of Tocilizumab is weekly. In the event that patients deviate from this they are advised on correct action as detailed in the package leaflet.

4.5.3 Etanercept

The recommended dose of Etanercept for adult patients with RA is 50 mg (given as a subcutaneous injection) once a week and it will be self-administered by patients. Before injection, Etanercept (Enbrel®) single-use pre-filled pens must be allowed to reach room temperature (approximately 15 to 30 minutes). The needle cover must not be removed while allowing the pre-filled pen to reach room temperature. By looking through the inspection window, the solution should be clear to slightly opalescent, colourless or pale yellow and may contain small translucent or white particles of protein. Comprehensive instructions for administration are given in the package leaflet "Using the MYCLIC pre-filled pen to inject Enbrel". Any unused product or waste material should be disposed of as detailed in the STRAP-EU IMP Manual and in accordance with local requirements. Suitable sites for injection include the thigh or abdomen. Patients will be asked to complete a diary to confirm the timely administration of Etanercept therapy. Administration of Etanercept is weekly. In the event that patients deviate from this they are advised on correct action as detailed in the package leaflet. Patients will be issued with a patient diary at the baseline visit. Patients will record the use of trial medication in the diary that will be returned at every clinic visit along with any used

pens. Adherence to the prescribed treatment will be checked at every clinic visit. This will serve as a measure of compliance.

4.6 Packaging and labelling of IMPs

4.6.1 Rituximab

The IMP will be labelled as clinical trial material when being dispensed from local pharmacy. The method that details the packaging and labelling of the IMP is in the STRAP-EU IMP manual.

4.6.2 Tocilizumab

The IMP will be labelled as clinical trial material when being dispensed from local pharmacy. The method that details the packaging and labelling of the IMP is in the STRAP-EU IMP manual.

4.6.3 Etanercept

The IMP will be labelled as clinical trial material when being dispensed from local pharmacy. The method that details the packaging and labelling of the IMP is in the STRAP-EU IMP manual.

4.6.4 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.

4.7 Dispensing of IMP

A Drug Dispensing Log at Pharmacy will be kept current and will contain the following information:

- Pharmacy dispensed medication.
- 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.

Rituximab, may be reconstituted under conditions approved by the hospital pharmacy and the local site team as part of site initiation procedures.

4.8 IMP stability

4.8.1 Rituximab

Prepared infusion solutions of Rituximab are biologically and chemically stable at 2° − 8°C (36° − 46°F) for 24 hours or at room temperature for 12 hours. However, since Rituximab solutions do not contain a preservative, diluted solutions should be stored refrigerated (2-8°C). Any deviations from the necessary temperature range will be documented and appropriate action taken. The product should not be used beyond the expiration date stamped on the carton.
Rituximab vials should be protected from direct sunlight. No incompatibilities between Rituximab and polyvinylchloride or polyethylene bags have been observed.

4.8.2 Tocilizumab

The recommended storage conditions for Tocilizumab drug product (IV and SC formulations) are between 2°C and 8°C (36°F and 46°F), protected from light. Once outside the refrigerator, the drug should be administered within 8 hours. Syringes should be kept dry. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If visibly opaque particles, discoloration or other foreign particles are observed, the solution should not be used. A temperature log must be kept, on which the storage temperature of the Tocilizumab is recorded at least once a day. Any deviations from the necessary temperature range will be documented and appropriate action taken.

4.8.3 Etanercept

Etanercept will be stored at a controlled temperature of 2-8°C. Etanercept may be stored at temperatures up to a maximum of 25°C for a single period of up to 4 weeks; after which, it should not be refrigerated again. Etanercept should be discarded if not used within 4 weeks of removal from refrigeration. The pre-filled pens will be kept in the outer carton in order to protect from light. A temperature log will be kept on which the storage temperature of Etanercept is recorded at least once a day. Any deviations from the necessary temperature range will be documented and appropriate action taken.

4.8.4 Temperature Deviation, Faults-Device and Complaints

Rituximab/Tocilizumab (to be provided from routine hospital stocks)

Sites will report temperature deviations to the STRAP-EU Trial Office using the 'STRAP-EU temperature deviation reporting form'. Temperature deviations arising at trials sites after Rituximab and Tocilizumab has been dispensed/ring fenced for use in the STRAP-EU trial are the responsibility of the trial site to notify to the STRAP-EU trials office. Further details can be found in the STRAP-EU IMP manual.

Etanercept (Pfizer IMP)

Sites will report temperature deviations and any faults or complaints to the STRAP Trial Office using the STRAP-EU temperature deviation reporting form. Pfizer will only be responsible for the IMP during delivery of IMP to the trial site. Temperature deviations arising at trials sites after drug delivery are the responsibility of the trial site to notify to the STRAP trials office, and subsequently the Sponsor's responsibility to take appropriate action in line with contractual agreement with Pfizer. Further details can be found in the STRAP IMP Manual.

4.9 Prior and concomitant therapies

4.9.1 Prior DMARD therapy

Patients eligible for anti-TNF-α therapy will have previously not responded to intensive therapy with a combination of DMARDs, including MTX (unless contraindicated).

4.9.2 Methotrexate

Patients receiving methotrexate (MTX) will be continued during the course of the study at the maximum tolerated dose (≥ 2.5 mg / week). Patients will be stable on their current dose of MTX for at least 4 weeks prior to the first biopsy visit. In the event that a patient is unable to tolerate their current dose of MTX due to toxicity or intolerance whilst participating in the trial, then the highest tolerable dose should be maintained, and the reason for this clearly documented in the source documents and CRF. Patients may receive their MTX orally or subcutaneously, and in combination with oral folic or folinic acid during the course of the study.

4.9.3 Continued DMARD therapy

Patients may also continue to receive DMARDs at their physician's discretion. All DMARD therapy should have been commenced a minimum of 4 weeks prior to the biopsy visit. Patients must be on a stable dose of DMARDs for at least 4 weeks prior to the biopsy visit.

4.9.4 Non-steroidal anti-inflammatory drugs

The patient will be permitted to be on NSAIDs at any time throughout the duration of the study.

4.9.5 Corticosteroids

Nasal, cream or oral inhaled steroid preparations may be used throughout the study duration. Patients may receive corticosteroids throughout the trial at the discretion of the treating clinician. However, within 4 weeks prior to visit 1 (screening), visit 2 (biopsy), visit 3 (baseline), visit 7 (week 16), and visit 15 (48 weeks), the corticosteroid dose should not be increased and should **not exceed** prednisolone 10mg/day (or equivalent). This also applies to the subsequent 16-week assessment (from second drug initiation) of patients who switch treatment following failure of their first therapy.

Intra-articular and parenteral corticosteroids should not be used in the 4 weeks prior to the screening visit (visit 1), biopsy visit (visit 2), baseline (visit 3), week 16 (visit 7), or in the 4 weeks prior to the week 48 visit if a biopsy is being performed. Any injected joints should be excluded from the joint assessment for 12 weeks following the injection. Patients may receive corticosteroids (po, intra-articular, or parenteral), if required, at the end of that study visit.

4.9.6 Other medications

Other medications (with the exception of investigational / unlicensed drugs) are permitted as required during the study and should be recorded on the applicable visit Case Report Form (CRF).

4.10 Dose modification/ reduction/ delay

Adherence to the planned dose regimen of study medication is required unless an adjustment is necessary for safety events as per applicable SmPC. Please refer to section 5.10 for further details.

4.11 Return/Recall or Destruction of IMP

All IMP that is to be destroyed will be documented and accounted for in accountability/drug destruction logs. This will be delegated to the local site pharmacy. Destruction of IMP will be as per local requirements.

The batch number of all IMP supplies will be recorded on the accountability log and recall by IMP suppliers will be managed by the Sponsor.

Disposition of unused study drug not dispensed to patients, or partially used/returned study drug will be recorded as described in the IMP manual. IMP destruction of product not dispensed/administered must be confirmed with and approved by the STRAP trials office.

5 STUDY PROCEDURES

The principal investigator (or other delegated person(s) in the local trial team) will be responsible for collecting, recording and reporting data on adverse events and drug therapy at each study visit. The PI must not perform the joint assessments. All joint assessments will be performed by a member of the local trial team who will be blinded to treatment allocation of all participants. The joint assessor should also complete the VAS physician assessment component of the VAS Pain Score questionnaire. Delegation of these responsibilities to ensure the blind is maintained will be documented on the site signature/delegation log.

Data will be collected as follows:

- demographic data including age, gender
- diagnostic information including the 1987 criteria of the ACR classification criteria for a diagnosis of RA, titre of rheumatoid factor and disease duration
- disease activity including ACR/EULAR core set, DAS28 and CDAI, and pain score using the VAS system
- physical function using the Health Assessment Questionnaire (HAQ)
- Fatigue using the FACIT score
- Assessment of health status using the SF-36 (version 2)
- On-the-job impact of RA using the Work limitations questionnaire (WLQ-25)

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

5.1 Informed consent procedures- Recruitment

Written informed consent will be obtained from each patient by the Principal Investigator or other medically qualified designee such as co-investigators or a Clinical Fellow with suitable experience or training. Informed consent forms will be prepared according to NRES and study sponsor requirements for informed consents. Patients who are potential candidates for the study will receive a Patient Information Sheet (PIS) Summary Sheet and the main PIS in the clinic which explains the purpose of the trial and highlights the benefits and risks of participation in the trial. Patients will be given adequate time (minimum 24 hours) to review the information and 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 clinical 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. The right of the patient to refuse consent without giving reasons will be respected.

If a potential patient cannot speak the native language of the country interpreters will be made available through the hospital translation service. The consent process should be clearly documented in the patient's medical notes and it must be clear that the trial was adequately explained and the patient was able to give informed consent. A translator should be available for each patient visit and this should be clearly documented in the patient notes. If a translator is not available the patient should not be recruited to the trial.

The original consent will be filed in the Investigator Site File; a copy of the consent will be given to the patient, and one filed in the hospital notes. The written consent will be taken by a clinician, who has signed / dated the staff authorisation / delegation log. The process of obtaining written consent will be clearly documented in the patient's medical notes.

5.2 Screening procedures (Visit 1)

During the screening visit, written informed consent will be obtained from all patients by the principal investigator or his/her designee before any protocol-specific procedure is performed. Study details, risks and benefits will all be reviewed and patients will be encouraged to ask questions and clarify any concerns. Demographic data (including age, gender and race) and medical history will be obtained. Patients, both men and women, will be reminded to use an acceptable and effective method of contraception during the study period and the appropriate time period after stopping study treatment.

Patients may be screened up to 6 weeks prior to baseline visit. As per the study visit schedule screening will entail evaluation of:

- Inclusion and exclusion criteria
- Demographic data including age, gender, and race
- 2010 ACR/EULAR RA classification criteria
- Systemic disease assessment (RA involvement)
- Medical history
- Procedures history
- Allergies
- Concomitant medication
- DMARD therapy
- Corticosteroid therapy
- Clinical examination
- Rheumatoid Factor (RF) and Anti-CCP antibodies (CCP) [a]
- Routine blood tests (ESR, CRP, Hb, WBC, Platelets, Neutrophils, Lymphocytes, ALT, AST, Creatinine)[b]
- Total cholesterol, HDL, LDL, and triglycerides
- Immunoglobulins/Immunodeficiency panel [c]
- Hepatitis serology, HIV and IGRA[d]
- Vital signs
- Chest X-ray [e]
- ECG

- Pregnancy test [f]
- Joint assessment
- ACR-core set measures
- DAS 28 assessment
- Clinical Disease Activity Index (CDAI)
- Physical function using the Health Assessment Questionnaire (HAQ)
- VAS Pain score
- US assessment [g]

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

[a] RF/CCP tests should be performed unless these tests have been done previously and do not need repeating as per local guidelines.

[b] Routine blood tests do not need to be taken if the patient has already had these performed within 10 days of the screening visit

[c] Serum immunoglobulins must be performed at screening unless previously performed within the last 8 weeks (to confirm trial eligibility). Maximum time between testing and infusion is 12 weeks. Retests to be considered if clinically required and documented in medical notes. Immunodeficiency panels are not mandatory and to be performed as per local guidance.

[d] Hepatitis B screening must be performed unless it has been done in the preceding 3 months of the screening visit. If core antibody result is positive but the surface antigen and the viral load result is negative it is assessed as indicating a 'negative' overall result and therefore result is deemed as negative. TB (IGRA), Hepatitis C, and HIV screening is not mandatory; however, all centres are expected to act according to local guidelines with respect to patient screening prior to Anti-TNFa, Rituximab, and Tocilizumab therapy.

[e] A chest x-ray must be performed as screening for tuberculosis prior to biological therapy. A chest x-ray must be done at the screening visit to confirm the patient's eligibility, unless a chest X-Ray has been done in the preceding 3 months of the screening visit and the patient must not have had any pulmonary symptoms since then.

[f] A pregnancy test will be performed at each study visit for female patients of child bearing age irrespective of the use of contraceptive methods.

[g] US assessment will be of the joint selected for biopsy to ensure that there is sufficient thickening to meet the following inclusion criterion: *selected joint for biopsy must be minimum grade 2 synovial thickening, as assessed at the biopsy visit*. This scan will not be reviewed centrally and is optional and not required if the site are confident that the joint will show sufficient inflammation at the biopsy visit.

5.3 Schedule of each visit

5.3.1 Biopsy visit and randomisation (visit number 2)

Patients will receive a synovial biopsy (refer to STRAP-EU Synovial Biopsy SOP) 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 blood tests (ESR, CRP, Hb, WBC, Platelets, Neutrophils, Lymphocytes, ALT, AST, Creatinine)[a]
- Study specific bloods
- Vital signs
- Pregnancy test in female patients of child bearing potential [b]
- Joint assessment [c]
- DAS 28
- CDAI
- ACR-core set measures
- VAS Pain Score
- Physical function using the Health Assessment Questionnaire (HAQ)
- Work Limitations Questionnaire (WLQ)-25
- Pre Biopsy Assessment Form
- Adverse events
- Ultrasound examination of the patients' joints prior to the synovial biopsy

- Pre-baseline synovial biopsy [d]
- Randomisation [e]

[a] Routine blood tests do not need to be taken if the patient has already had routine blood tests performed within 10 days of the biopsy visit.

[b] A pregnancy test will be performed at each study visit for female patients of child bearing age irrespective of the use of contraceptive methods.

[c] If possible the same assessor should perform the joint count at all (pre- and post-randomisation) visits to ensure consistency.

[d] A synovial biopsy prior to baseline is mandatory as part of the patient stratification process Subsequent synovial biopsies will remain optional in all other cases. Patients not receiving a subsequent biopsy at 16 or 48 weeks will continue within the study as per protocol.

[e] Randomisation into the study can only occur once the biopsy has been taken and biopsy result confirmed (B cell poor, B cell rich or unknown). The randomisation procedure can occur at the baseline visit (see 5.3.2), or prior, to allow pharmacy sufficient time to prepare the allocated treatment. Patients should remain blinded to their randomised treatment prior to undergoing the Baseline assessments and will be informed of their treatment allocation as soon as assessments are completed.

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 the 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, ACR core set measures. The database will accommodate this scenario and data will not need to be duplicated at the biopsy visit.

5.3.2 Baseline visit (visit number 3)

Patients will receive Etanercept, Tocilizumab or Rituximab at their baseline visit depending upon randomisation. **All assessments should be performed prior to commencement of infusion / injection of therapy.** Patients at baseline will have the following assessments:

- Concomitant Medication
- DMARD therapy

- Corticosteroid therapy
- Clinical examination
- Cardiovascular risk assessment
- Routine blood tests (ESR and CRP only)
- Vital signs
- Pregnancy test in women of childbearing potential [a]
- Joint assessment
- ACR core set measures
- Disease activity core data set (DAS28)
- Clinical Disease Activity Index (CDAI)
- VAS Pain score
- Physical function using the Health Assessment Questionnaire (HAQ)
- SF-36v2© (Licenced by QualityMetric Incorporated)
- FACIT - Fatigue Questionnaire
- Post Biopsy Assessment Form
- Adverse events
- Plain X-rays of hands and feet [b]
- Ultrasound assessment

All baseline assessments should be done within a +/- 7 day window.

[a] A pregnancy test will be performed at each study visit for female patients of child bearing age irrespective of the use of contraceptive methods

[b] Plain X-rays of hands and feet do not need to be redone if the patient has already had them within 8 weeks of the baseline visit.

5.4 Randomisation procedures

Randomisation will take place when all the screening procedures and the biopsy visit are complete and the patient is eligible for enrolment in the study. Patients will be stratified according to synovial histopathology (2 strata based on B cells, or a third strata where result is unknown) and

methotrexate use (yes/no), and randomised using ratio 1:1:1 to three treatments. The local principal investigator/research nurse must log-in to the secure 24-hour automated web-based STRAP-EU database and complete Screening and Biopsy visit (visits 1 and 2) electronic CRFs prior to randomisation. In the rare event that the STRAP-EU database is not accessible paper CRFs may be submitted in order to perform the randomisation. The STRAP-EU Trial Office will then confirm eligibility and perform randomisation. Subsequently, the assigned treatment and unique randomisation number (study ID) will be allocated automatically by the application. This ensures that neither the patient nor the clinician can choose whether or not to enter a trial depending on the next allocation. The application codes of hierarchical dynamic randomisation are securely embedded in the STRAP-EU database. Database Programmer and trial statisticians will have access to the system in order to monitor and check the randomisation during the trial. 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.

Randomisation procedures are detailed in the STRAP-EU Trial Randomisation Specification form.

5.5 Schedule of Treatment and visits

Patients randomised to Rituximab will have one infusion cycle consisting of two infusions given 2 weeks apart (Day 1 and Day 15). The cycle will be repeated at 24 weeks if patient is responding. All patients will continue to have 4-weekly visits (± 7 days) for assessment as per study visit schedule (Table 3). Patients randomised to Etanercept or Tocilizumab will have weekly subcutaneous injections.

Patients who are switching treatment, or whose assessments could not be done on one day, may attend a separate visit for the administration of the new biologic agent or completion of trial assessments. These visits must still remain within the +/- 7 day window around the scheduled visit due date, as already described.

Table 3. Study visit schedule

Visit Number		1		2	3	3a [n]	4	5	6		7	8	9	9a [n]	10	11	12	13	14	15	Post-treatment visit/call [0]	Treatment Cessation/ Safety Follow-Up visits
Timeline (weeks)		- 6 – -1 weeks		- 3 – -1 weeks	0	2	4	8	12		16	20	24	26	28	32	36	40	44	48	30+ days after visit 15	
Deviation window (days from scheduled visit)	RTX	N/A		N/A	N/A	±7	±7	±7	±7		±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	N/A	
	ETN/TCZ																					
Visit Type		Screening		Biopsy [m] & 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
Informed consent		X									x									x		
Inclusion/Exclusion criteria		X																				
Demographics		X																				
RA classification criteria		X																				
Systemic disease assessment		X																				
Medical history		X																				
Procedures history		X																				
Allergies		X																				
Concomitant medication		X		x	x		x	x	x		x	X	x		x	x	x	X	x	x		x
DMARD therapy		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
Clinical Examination		X			x		x	x	x		x	X	x		x	x	x	X	x	x		x
Cardiovascular risk assessment					x																	
RF/CCP [a]		x																				

Routine blood tests (ESR, CRP, Hb, WBC, Platelets, Neutrophils, Lymphocytes, ALT, AST, Creatinine,**)** [b]	x		x	x [b]		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		
Immunoglobulins/Immunodeficiency panel [c]	x									x											
Hepatitis serology, HIV, IGRA [d]	x																				
Vital signs	x		x	x		x	x	x		x	X	x		x	x	x	X	x	x		x
Chest X-ray[e]	x																				
ECG	x																				
Pregnancy test [f]	x		x	x		x	x	x		x	X	x		x	x	x	X	x	x		x
Joint assessment (66/68 Joint Count) [g]	x		x	x		x	x	x		x	X	x		x	x	x	X	x	x		x
VAS Pain score [g]	x		x	x		x	x	x		x	X	x		x	x	x	X	x	x		x
ACR core set measurements	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
CDAI	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		x	x	x	X	x	x		x
SF-36 health Survey				x				x				x				x			x		x
FACIT-Fatigue Questionnaire				x				x				x				x			x		x
WLQ- 25			x					x				x				x			x		x
Pre Biopsy Assessment Form			x							x											
Post Biopsy Assessment Form				x							X										
Adverse events			x	x		x	x	x		x	X	x		x	x	x	X	x	x	x	x
Synovial biopsy [h]			x							x									x		
X-ray hands and feet [i]				x						x									x		
Ultrasound assessment [j]	x[p]		x	x		x				x		x							x		
Randomisation [k]			x																		
ETN and TCZ dispensed				x		x	x	x		x	X	x		x	x	x	X	x			
RTX dispensed				x	x							x	x								

A - RF/CCP tests should be performed unless these tests have been done previously and do not need repeating as per local guidelines.

B - At screening visit - routine blood tests do not need to be taken if the patient has already had routine blood tests performed within 10 days of the screening visit.
- At biopsy visit – routine bloods do not need to be taken if the patient has already had routine bloods performed within 10 days of the biopsy visit
- At baseline visit – only ESR and CRP tests are required.

c - Serum immunoglobulins must be performed at screening unless previously performed within the last 8 weeks (to confirm trial eligibility). Maximum time between testing and infusion is 12 weeks. Retests to be considered if clinically required and documented in medical notes. Serum immunoglobulins then only required subsequently for patients receiving Rituximab. These should be repeated prior to every cycle of Rituximab. Maximum time between testing and infusion is 12 weeks. This includes patients who are inadequate responders to Etanercept and switch to Rituximab (and have not consented to participate in the R4-RA Trial). Immunodeficiency panel is not mandatory but should be carried out as per local guidance.

d - Hepatitis B screening must be performed unless it has been done in the preceding 3 months of the screening visit. TB (IGRA), Hepatitis C, and HIV screening are not mandatory however risk assessment must be carried out and documented. All centres are expected to act according to local guidelines, but screening does not need to be repeated if it has been performed in the preceding 3 months of the screening visit.

e - A chest x-ray must be performed as screening for tuberculosis prior to biological therapy. A chest x-ray must be done at the screening visit to confirm the patient's eligibility, unless a chest X-Ray has been done in the preceding 3 months of the screening visit and the patient must not have had any pulmonary symptoms since then.

f - A pregnancy test will be performed at each study visit for female patients of child bearing age irrespective of the use of contraceptive methods.

g - Joint assessments and VAS physician assessment to be completed by the nominated 'blinded joint assessor'.

h - A synovial biopsy prior to baseline is mandatory as part of the patient stratification process and mandatory (if possible) at week 16 for patients undergoing additional MRI scans. Subsequent synovial biopsies will remain optional in all other cases and patients not receiving a subsequent biopsy will continue within the study as per protocol.

i - Plain X-rays of hands and feet do not need to be taken if the patient has already had them done within 8 weeks of the baseline visit.

j - Some ultrasound assessments may be optional at some participating sites.

k - Randomisation can occur as soon as the biopsy result is obtained and prior to the scheduled baseline visit to allow sufficient preparation time for pharmacy.

-

m - 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. This data will not need to be duplicated at the biopsy visit.

n - 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.

o - The post-treatment visit/call should be scheduled for 30+ days after visit 15 in order to identify any adverse events. If a scheduled follow-up visit falls within this time frame AEs can be assessed as part of this visit, with no requirement for an additional visit/call.

p - The US assessment at screening will be of the joint selected for biopsy to ensure that there is sufficient thickening to meet the following inclusion criterion: *selected joint for biopsy must be minimum grade 2 synovial thickening, as assessed at the biopsy visit.* This scan will not be reviewed centrally and is optional and not required if the site are confident that the joint will show sufficient inflammation at the biopsy visit.

Notes:
- Patients on Etanercept who are switching treatment to Rituximab (and have not consented to participate in the R4-RA Trial) may attend a separate visit for the infusion (Visits 7 – 13). The subsequent study visit must still remain within the +/- 7 day window schedule.
- Patients randomised to receive Tocilizumab and Etanercept - routine blood tests should be carried out and checked at each study visit.
- F/U = Follow-up

- **Study specific bloods – visit schedule changes:
  - Participants' treatment schedule (as per protocol) with regards to study specific bloods may be subject to a change as a result of intentional deviation from the Study Visit Schedule. This applies to participants who are deemed to be a non-responder to Etanercept (ETN) at Week 16 or at any subsequent visit to week 44 and who are switching treatment to Rituximab (and have not consented to participate in the R4-RA Trial).For example, a patient who is deemed a non-responder to Etanercept at Week 16 and is to switch treatment to Rituximab, study specific bloods should be taken prior to the 1st infusion (week 16) and 2nd infusion (week 18), and 4 weeks post first infusion (week 20) and then week 24, 32, 40, (and then at week 42 and 44 assuming there is another RTX infusion at week 40).
  - ii) All patients will have study specific bloods at week 48 visit.
  - All scenarios are outlined below:

STRAP Trial - Study Specific Bloods Schedule - All scenarios																								
Visit Number	1	2	3	3a	4	5	6	7	7a	8	8a	9	9a	10	10a	11	11a	12	12a	13	13a	14	14a	15
Timeline (weeks)	<6 weeks	<2 weeks	0	2	4	8	12	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
		0		2	4	8		16				0	2	4				12						24
Visit Type	Screening	Biopsy	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	F/U	F/U	F/U	F/U
Scenario 1 - patients allocated RTX or TCZ		x		xRTX	x	x		x				x	xRTX	x				x						x
Scenario 2 - ETN patients switched to RTX at v7		x			x	x		x	xRTX	x		x				x				x	xRTX	x		x
								0	2	4		8				16				24	26	28		
Scenario 3 - ETN patients switched to RTX at v8		x			x	x		x		x	xRTX	x		x				x				x	xRTX	x
										0	2	4		8				16				24	26	
Scenario 4 - ETN patients switched to RTX at v9		x			x	x		x				x	xRTX	x		x				x				x
												0	2	4		8				16				
Scenario 5 - ETN patients switched to RTX at v10		x			x	x		x				x		x	xRTX	x		x				x		x
														0	2	4		8				16		
Scenario 6 - ETN patients switched to RTX at v11		x			x	x		x				x		x		x	x	x		x				x
																0	2	4		8				16
Scenario 7 - ETN patients switched to RTX at v12		x			x	x		x				x		x				x	xRTX	x				x
																		0	2	4		8		
Scenario 8 - ETN patients switched to RTX at v13		x			x	x		x				x		x				x		x	xRTX	x		x
																				0	2	4		
Scenario 9 - ETN patients switched to RTX at v14		x			x	x		x				x		x				x				x	xRTX	x
																						0	2	4
As per study visit schedule specified in protocol																								

5.6 Follow-up procedures

5.6.1 Follow-up visits (4-15)

Patients will be monitored on a 4-weekly basis as shown in the study visit schedule (Table 3, Section 5.5). Safety blood tests will be undertaken every 4 weeks. Additional blood tests for monitoring of toxicity/safety of therapy may be undertaken at the physician's discretion.

The ACR 20, a validated composite 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. The WLQ-25 will be used to measure work productivity.

Visits 3a and 9a are specific to Rituximab patients. Rituximab patients 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 6.

Visits 4 - 15 will be carried out every 4 weeks (±7 days) from the baseline 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.
Patients who consent to the optional biopsy procedure at Visit 7 (Week 16) will be asked to complete the Pre Biopsy Assessment Form at Visit 7 (week 16) and the Post Biopsy Assessment Form at Visit 8 (Week 20).

As per normal clinical practice, the GPs of participants with active RA and a cardiovascular risk profile will be informed and asked to review treatment.

All joint assessments will be performed by a member of the local trial team who will be blinded to treatment allocation. The joint assessor should also complete the VAS physician assessment component of the VAS Pain Score questionnaire.

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

5.6.2 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.

5.7 Laboratory assessments

Laboratory assessments are detailed in section 6.

5.8 Imaging assessments

Patients will have plain x-rays and ultrasound assessments of disease activity and joint damage and will be related to the exploratory outcome measures in this study. Arrangements will be made to facilitate the transfer of X-ray images of the hands and feet and US assessments from participating sites to the STRAP-EU Trial Office as detailed in the STRAP-EU Trial Operational Guide SOP.

5.8.1 Plain radiograms

Plain x-rays will be recorded of both hands and feet at baseline (unless performed within 8 weeks of the screening visit), 16, and 48 weeks follow-up. Each hand and foot (left and right) will be imaged individually. The standard hospital templates should be used. X-rays will be scored centrally by the van der Heijde/ Sharp scoring system.

A chest x-ray will be acquired as screening for tuberculosis prior to the screening visit as described in section 5.2.

5.8.2 Ultrasound

A US assessment will be performed by a trained member of the site team during the biopsy and randomisation visit, baseline, 4, 16, 24, and 48 weeks follow-up. Images will be acquired and centrally scored for Doppler signal and synovial thickness. The core US data set is described in the "STRAP-EU US Manual". Each joint will receive a score (0-3) for Power Doppler and (0-3) for Synovial thickening. Additional joints may be scanned at the local centres discretion. Details are provided in the STRAP-EU Trial US Manual.

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.

5.9 End of study definition

The end of the study will be triggered 6 months after the last patient completes their final study visit (Last Patient Last Visit LPLV; e.g. the post-treatment visit/call assessment). This additional 6 months will allow time for sample processing and image analysis. Note: at the end of the study

treatment at week 48, patients will continue to be followed in the Rheumatology clinics as part of their routine care. The end of trial declaration will be submitted within 90 days of the end of trial definition and this will mark the end of trial data and sample collection, sample and image analysis. The clinical study report will be submitted within 12 months of the end of trial definition.

5.10 Management of laboratory abnormalities and infections

Based on risk-benefit assessment, the investigator must decide what action should be taken to ensure the safety of the provided treatment. This includes temporary interruption or cessation of IMPs and/or concomitant treatment, repeat or unscheduled laboratory assessment, and reporting of AE/SAE when applicable.

5.10.1 Bone marrow toxicity

Neutropenia- Absolute neutrophil count (ANC) decrease to <1000/mm^3 should result in treatment interruption until ANC rise to >1000/mm^3. In the case of Tocilizumab, treatment is resumed, when ANC increases to >1000/mm^3, at 162mg every other week and is increased to 162mg weekly as clinically appropriate. Treatment should be ceased and patient withdrawn from the study, if ANC < 500/mm^3 (see section 5.12).

Thrombocytopenia- Platelet decrease to <100,000/mm^3 should result in treatment interruption until platelets increase to >100,000/mm^3. In the case of Tocilizumab, treatment is resumed, when platelets increase to >100,000/mm^3, at 162mg every other week and is increased to 162mg weekly as clinically appropriate. Treatment should be ceased and patient withdrawn from the study, if platelets < 75,000/mm^3 (see section 5.12).

5.10.2 Liver enzyme abnormalities

In ALT or AST elevations up to 3x ULN, the dose of the concomitant medication (e.g. Methotrexate) should be modified whenever appropriate and suitable investigations undertaken. In the case of Tocilizumab, persistent increases of ALT or AST in this range (1-3x ULN) should lead to dose frequency reduction to 162mg every other week or interruption, until resolution. Treatment is resumed with weekly or every other week injection as clinically appropriate. Dose interruption is recommended if ALT or AST >3x ULN, and the aforementioned recommendations are followed when ALT or AST decrease to 1-3x ULN. For persistent increases of ALT or AST >3x ULN, as well as ALT or AST elevation >5x ULN, treatment discontinuation is recommended (see section 5.12).

5.10.3 Infection

Patients must be monitored closely for signs and symptoms of infections including tuberculosis. Suspicion of infection should lead to treatment interruption if indicated. Study treatment should be resumed after resolution of the infection and regardless of when antibiotics have been stopped, at physician's discretion.

5.10.4 Reporting of laboratory abnormalities

Please record any abnormal laboratory results as AE if it is deemed clinically significant or requires intervention or interruption of the IMP. If it was considered serious please report the event as SAE/SUSAR.

5.11 Subject withdrawal

Participants may be withdrawn at any time during the trial if they are intolerant to the therapeutic product, experience toxicity related side-effects or inter-current illness necessitating cessation of the therapy or at their physician's discretion. Specifically, the following criteria will necessitate premature withdrawal of a study participant:

- Suspected progressive multifocal leukoencephalopathy (PML)
- ALT or AST > 5 x ULN (or persistently >3x ULN)
- ANC < 500/mm^3 (0.5 x 10^9 /l)
- Platelet count <75000/mm^3 (75 x 10^9/l)
- Receipt of live vaccines
- Pregnancy

Subjects may withdraw consent for any reason at any time without prejudice to their normal care, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. Withdrawal of consent may be regarded as a withdrawal from trial medication or any other components which form part of the Informed Consent Form (CF) signed by the participant. At the time of withdrawal of consent, a full efficacy and safety evaluation should be performed, if patient consents. Data and specimens collected prior to withdrawal will be kept unless the patient specifically request otherwise.

5.12 Data Collection and Follow up for Withdrawn Subjects

At the time of withdrawal, a full efficacy and safety evaluation should be performed if patient agrees. Treatment cessation and reason for discontinuation should be documented on the

applicable CRF and medical records. On-going data collection requirements for patients who withdraw from the trial prematurely are as follows:

• If the patient withdraws consent to any further participation in the trial (full withdrawal) the Treatment Cessation CRF should be completed immediately. A final assessment should be undertaken if the patient is present and consents to this as per the Treatment Cessation visit (see Study Visit Schedule, Section 5.5, Table 3) and the Treatment Cessation CRF should be completed. No further data is to be collected.

• If the patient ceases trial treatment before the Visit 7 (16-week assessment), the Treatment Cessation CRF should be completed immediately. The patient will revert to routine care treatment but continue to attend follow-up visits at 16, 24, 48, weeks. In these cases the post-treatment visit/call will not be necessary as AEs can be assessed as part of these follow-up visits. At each visit, assessments should be observational only as per the Safety Follow-up visit (see Study Visit Schedule, Section 5.5, Table 3) and the Safety Follow-up CRF should be completed each time.

• If the patient ceases treatment after visit 7 (16-week assessment), the Treatment Cessation CRF should be completed immediately. The patient will revert to routine care treatment but continue with protocol assessments and follow-up data collected according to protocol, unless participant withdraws consent for this. The applicable visit assessments and CRF should be completed at each subsequent visit. In these cases the post-treatment visit/call will not be necessary as AEs can be assessed as part of these follow-up visits.

Details of commencement of any further treatment outside of the trial will be recorded for all patients who withdraw prematurely from the trial.

Patients withdrawing from the study will continue to be monitored and managed within their routine Rheumatology clinic by their named consultant. Withdrawn trial subjects will not be replaced.

5.13 Completion of Trial Treatment

The duration of trial treatment is 48 weeks. Patients that complete trial treatment, and those that withdraw prematurely, will require further 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. Any future treatment plan will be discussed fully with the patient.

Patients will be reminded to use effective contraception throughout the trial and beyond the end of trial treatment for the duration as defined in the relevant SmPC; 12 months for Rituximab, at least 3 weeks for Etanercept, and at least 3 months for Tocilizumab.

Patients that are randomised to Rituximab first line 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 (applicable as per national guidelines for non-UK sites).

5.13.1 Post- treatment Visit/Call

The post-treatment visit/call should be scheduled for 30+ days after visit 15 in order to identify any AEs occurring during this washout period. If a scheduled follow-up visit falls within this time frame AEs can be assessed as part of this visit, with no requirement for an additional visit/call. **This follow-up can be either a visit, or alternatively can be conducted via telephone.**

5.14 Study Clinical Outcome Measures

Patients will be assessed for disease activity using clinical assessments at baseline and 4-weekly thereafter. All patients will be asked to complete a series of case report at baseline and during follow-up as per STRAP-EU Visit Schedule. Patients will be assessed clinically using the ACR 20/50/70, CDAI (Clinical disease activity index), DAS 28 (CRP and ESR), Health assessment questionnaire, the Short Form 36, FACIT-fatigue questionnaire and the Work Limitations Questionnaire as described below.

5.14.1 ACR 20/50/70

ACR response will utilise 66/68 joint count which evaluates 66 joints for swelling and 68 joints for tenderness and pain on motion. The hip joints can be assessed for tenderness, but not for swelling. The following joints are included, upper: temporomandibular, sternoclavicular, acromioclavicular, shoulder, elbow, wrist, metacarpophalangeal (MCP), proximalinterphalangeal (PIP), and distal interphalangeal; and lower: hip, knee, ankle, tarsus, metatarsophalangeal (MTP), and interphalangeal (IP) joints of the feet.

ACR20 response will be used to identify responders/non-responders to determine treatment switches. The following definition of improvement is used for ACR20: 20% improvement in tender

and swollen joint counts and 20% improvement in 3 of the 5 remaining ACR-core set measures: patient and physician global assessments (both 10 cm VAS), pain (VAS), disability as measured by HAQ, and an acute-phase reactant. Similar definitions are used for ACR50 (50% improvement) and ACR 70 (70% improvement). Replaced joints and joints injected with corticosteroids within the last 12 weeks will not be included in the calculation.

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

Failure of a second biological therapy would permit the patient to receive treatment option of the physician's discretion as described in section 2.3. Such patients will continue in the trial as per the study visit schedule (Table 3).

5.14.2 CDAI and DAS28

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 RA disease activity on a scale from 0-76.

CDAI scores

- High disease activity: >22
- Moderate disease activity: $10 < CDAI \leq 22$
- Low disease activity $2.8 < CDAI \leq 10$
- Remission ≤ 2.8

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

$$DAS28\ (CRP) = 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(SJC28)} + 0.36*\ln(CRP+1) + 0.014*GH\ (VAS) + 0.96$$

$$DAS28\ (ESR) = 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(SJC28)} + 0.7*\ln(ESR) + 0.014*GH$$

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

(10), wrists (2) (includes carpometacarpal, intercarpal, and radiocarpal), elbows (2), shoulders (2), and knees (2).

Examination of the upper extremities will be performed with the patient in the sitting position. Examination of the lower extremities will be performed with the patient supine. During the assessment of pain on passive motion, no concurrent pressure will be applied to the joint margin. During pain on passive motion testing, the joint will be moved through the full available range in order to detect any end range pain. Joint pain with palpation or pain on passive motion (either is sufficient) will be scored according to the following scale:

- No pain
- Patient states that there is pain

Assessment of Swelling: 28 joints will be assessed for the presence of swelling. The joints to be evaluated include those evaluated for tenderness. Joint swelling on palpation will be scored according to the following scale:

- No swelling
- Swelling

5.14.3 Health Assessment Questionnaire (HAQ)

The Health Assessment Questionnaire (HAQ) is usually self-administered, but can also be given face-to-face in a clinical setting. 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.

5.14.4 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.

5.14.5 FACIT-Fatigue

The FACIT-Fatigue scale is a 13-item, symptom-specific subscale of the FACIT scales. 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.

5.14.6 Work Limitations Questionnaire (WLQ) - 25

The WLQ measures the on-the-job impact of chronic health conditions and treatment with a focus on assessing limitations while performing specific job demands. Original 25-item version (WLQ-25) was published in 2001. Several shortened versions have been tested or applied in studies in workers with various musculoskeletal disorders such as the WLQ-25. The WLQ-25 is self-administered and can be organized into the four following domains (recall period is 4 weeks): physical demands (examines ability to perform job tasks that involve bodily strength, movement, endurance, coordination, and flexibility), mental-interpersonal demands (addresses cognitively demanding tasks and on-the-job social interactions), time management (addresses difficulty with handling a job's time and scheduling demands), and output demands (concerns reduced work productivity). Responses on the WLQ-25 are on a five-point scale (ranging from none of the time to all of the time), including ‘‘not relevant to my job’’ option. The various domains have differing numbers of items: physical demands (n = 4), time management (n = 2), output demands (n = 4), and mental/interpersonal (n = 6). Applying the OMERACT filter the WLQ-25 shows good face, content-, and construct- validity, feasibility and better responsiveness than other versions of the WLQ in musculoskeletal disorders.

6 LABORATORIES

6.1 Central/Local laboratories

Routine laboratory bloods for safety will be taken as per routine clinical care for patients receiving Etanercept, Rituximab or Tocilizumab and will be performed at the Local Site hospital laboratories. Study specific samples analysis will be performed as follows;

1) Synovial tissue analysis will be performed at Barts Health NHS Trust, Queen Mary University of London Blizard Institute - Core Pathology, Pathology and Pharmacy Building, Second Floor 80 Newark Street London, E1 2ES. Synovial samples will be transported by an approved courier company. Samples will be stored at Core Pathology until transfer to QMUL, EMR for long term storage.

2) Study specific bloods analysis will be performed at QMUL, Experimental Medicine and Rheumatology, Centre for Experimental Medicine and Rheumatology, 2nd Floor, John Vane Science Centre, William Harvey Research Institute Barts and the London School of Medicine and Dentistry Charterhouse Square London EC1M 6BQ. Blood samples will be transported by an approved courier company.

6.2 Sample collection/labelling/logging

Details on the process of sample collection/labelling and logging from the patient, as well as pseudo-anonymisation prior to expedition to the laboratories are provided in the STRAP-EU Trial Sample Collection and Shipment SOP.

6.3 Sample receipt/chain of custody/accountability

Details of sample receipt, chain of custody, and accountability are contained in the STRAP-EU Trial Sample Collection and Shipment SOP.

6.4 Sample analysis procedures

6.4.1 Peripheral blood analysis

Local site laboratory - The following blood tests will be performed at screening, biopsy and at each 4-weekly follow up visit: ESR, CRP, Hb, WBC, Platelets, Neutrophils, Lymphocytes, ALT, AST, Creatinine. These investigations will be performed at the local site laboratory. Lipid profile will be assessed at screening, weeks 8, 36, and 48. Immunoglobulins must be tested at screening (Visit 1) for all patients as described in section 5.2 and then subsequently prior to every cycle of rituximab.

This includes patients originally allocated to the Etanercept arm of the trial, who are deemed non-responders at Week 16 or any subsequent visit to week 44 and are consequently switched to Rituximab. Immunodeficiency panel is not mandatory, but should be carried out as per local guidance.

QMUL, Experimental Medicine and Rheumatology - Study specific bloods will be taken as follows:

Biopsy visit (visit 2), Visits 7, and 15

Samples drawn in the order:

1. Four (9.0ml) full Heparin (green top)
2. One (9.0ml) full serum (red top)
3. Two PAXgene RNA (8.5ml)

Visits 3a*, 4, 5, 9, 9a*,10, 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 (Table 3, section 5.5).

Important Note: Participants may be subject to variation to the study specific bloods schedule as detailed in the study visit schedule (Table 3, foot note section 5.5).

Further details of sample requirements, handling, transfer and storage are contained in the STRAP-EU Trial Sample Collection and Shipment SOP.

Blood and synovial tissue samples collected as part of the STRAP-EU trial will be used in the following types of analyses: FACS analysis, Functional B cell studies and peripheral blood autoantibody and cytokine production, ELISA/WB, Next Generation Sequencing & Validation and transcriptomic analysis in the Experimental Medicine and Rheumatology (EMR) Laboratory. Samples may be sent to other laboratories for analysis; it will be ensured that appropriate agreements are in place for this.

6.4.2 Synovial biopsies and tissue analysis

Synovial biopsies (either US-guided or arthroscopic) will be performed at baseline, 16, and 48 weeks. The biopsy prior to baseline is mandatory. Subsequent biopsies are optional. Synovial fluid - whenever available- 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

B Cell Score (used for patient stratification and randomisation):

Barts Healthcare NHS Trust, Pathology - The level of B cell infiltration in synovial tissues is based on a 5-point scale: 0-4 depending on the increasing number of positively stained cells calibrated against a standardized atlas (Figure 2). Accordingly, synovial biopsies will be categorized using a previously validated method in B Cell Poor (0-1), B Cell Rich (2-4). (GC will be further identified by the presence of CD21 positive follicular dendritic cell (FDC) networks). Biopsy grading is shown in Figure 2. The biopsy tissue processing, embedding, staining and slide scanning will be undertaken by an accredited NHS Histopathology Department at The Royal London Hospital. Samples will be stored at Core Pathology until transfer to QMUL, EMR for long term storage. Further details can be found in the separate workflow "STRAP Trial processing and cutting synovial biopsy SOP". In some circumstances, processing and review of biopsy samples may be undertaken at the EMR laboratory, QMUL. Analyses being conducted will be detailed in more specific laboratory analysis plans. Samples collected during this project will be stored in a tissue bank at the end of the project and will be used in future research.

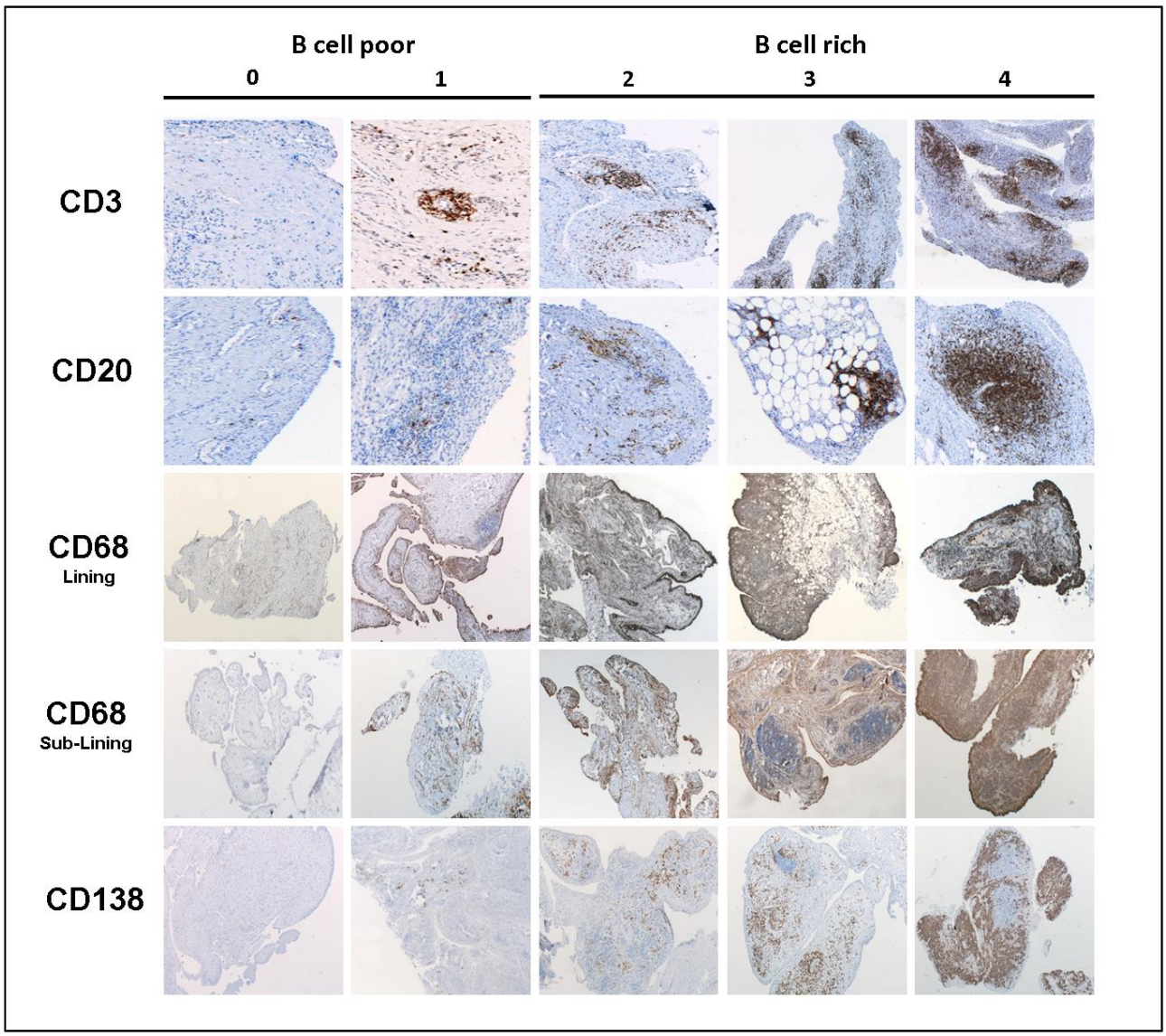


Figure 2. Atlas of representative images for scoring synovial tissue in NHS laboratories (Barts Health NHS Trust).

Representative images of immunohistochemical staining for B cells (CD20), T cells (CD3), plasma cells (CD138) and macrophages (CD68) for B cell poor (0-1) and B cell rich (2-4) synovial tissue.

6.5 Sample storage procedures

See STRAP-EU Trial Sample Collection and Shipment SOP.

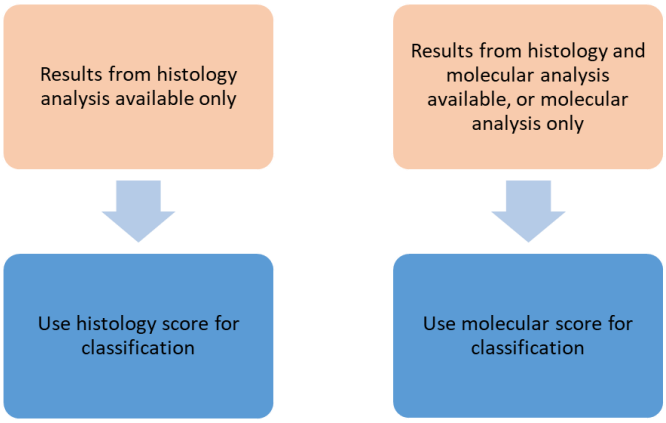
6.6 Molecular Analysis

RNA-Sequencing B cell module

RNA-sequencing will be performed on synovial biopsies as follows: library preparation will be performed on 500ng of total RNA. Generated libraries will be amplified by PCR and library size and concentration determined. Libraries will be sequenced using Illumina HiSeq technology to

generate 50 million paired-end 150 base-pair reads. Transcript abundance will be derived using Salmon and summarized over GENCODE transcript isoforms using tximport. Gene level counts will be subject to variance stabilizing transform (VST). RNA-Seq B cell module will be calculated from B cell-specific genes derived from analysis of FANTOM5 gene expression data[33], as previously described and validated[34]. Patients will be categorized as B cell poor or B cell rich using a predefined cut-off, namely the median RNA-Seq B cell module value in the R4RA clinical trial synovial RNA-Seq data.

6.7 Algorithm for determining pathotype for primary, secondary and exploratory endpoints



As pathotype will be categorised by both B cell specific gene expression and histomorphology, the above algorithm will be used for deciding the final pathotype to be used for the primary, secondary and exploratory analysis of the trial. It is expected that the majority of histomorphology and B cell specific gene expression scores will be concordant, but if results are discordant, the B cell specific gene expression scores will be used for the final pathotype.

7 PHARMACOVIGILANCE

7.1 General Definitions

7.1.1 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.

7.1.2 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.

7.1.3 Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

A **Serious Adverse Event (SAE)** fulfils at least one of the following 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 <u>not</u> considered as SAEs for the STRAP-EU trial:

- Pregnancy (however it is an event that requires monitoring and follow up) – see section 7.9
- 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

Note: The following definition **is** considered an SAE for the STRAP-EU trial:

- Elective surgery at any time, which is related to, or has resulted from, any new or worsening condition.

Serious Adverse Reaction (SAR)

An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.

An SAR is an adverse reaction which fulfils 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

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 SmPC.

7.1.4 Adverse Event of Special Interest (AESI)

An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the IMP provider, for which ongoing monitoring and rapid communication by the investigator to the IMP provider can be appropriate.

7.2 Investigators Assessment

7.2.1 Seriousness

The Principal Investigator (PI) or, in their 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 7.1.

7.2.2 Causality

The PI or -in their absence- an authorised medic within the research team who has been delegated this role must assess the causality of all serious adverse events/reactions in relation to the trial treatment and any previous trial treatment if the patient has switched during the trial treatment period. If the SAE is assessed as having a reasonable causal relationship, then it is defined as a SAR. Causality will also be assessed centrally by the CI or clinical delegate.

7.2.3 Expectedness

The PI or -in their absence- an authorised medic within the research team who has been delegated this role 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 reference safety information (the tabulated list of adverse reactions in section 4.8 of the current competent authority approved UK SmPC for the medication(s) administered to the patient during the trial treatment period). Expectedness will also be assessed centrally by the CI or clinical delegate.

7.2.4 Severity

The PI, or in their absence an authorised medic within the research team who has been delegated this role, 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

7.3 Notification and reporting Adverse Events or Reactions

All Adverse events (AEs) will be recorded, assessed for seriousness and severity, analysed and managed in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004 (as amended). Full details of all AEs including the event term, start and stop dates, severity, and outcome will be recorded in the subject's medical records and on the study record forms. This assessment will be undertaken by the Principal Investigator or an authorised medic within the research team who has been delegated this role and centrally by the CI or clinical delegate.

If the AE is not defined as SERIOUS, the AE is recorded in the CRF (and medical notes) and the participant is followed up by the research team. AEs will be monitored and followed up until satisfactory resolution or stabilisation. The STRAP-EU trials office will forward listings of non-serious AEs originating from the Study to Roche on a quarterly basis as per contractual requirements.

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.

AEs and SAEs should continue to be reported, following the same reporting procedures, throughout the patient’s time on the trial **and for a further 30+ days after visit 15**. Participants will be requested to report any AEs/SAEs during the 30+ days following visit 15. This data will be collected at a scheduled follow-up visit or via the post-treatment visit/call as described in section 5.13.1.

7.4 Notification and Reporting of Serious Adverse Events/SUSAR

7.4.1 SAE

All Serious Adverse Events (SAEs) will be recorded in the subjects' notes, the SAE CRF at the local trial site. Causality must be assessed and completed by the PI or an authorised medic within the research team who has been delegated this role (see section 7.3). The person assessing causality must sign a paper copy of the SAE CRF. The CRF must be sent to the STRAP-EU Trial Office as soon as reasonably practical and in any event within 24 hours of first becoming aware of the event. The STRAP-EU 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. The STRAP-EU Trial Office will then forward the signed SAE form to the Joint Research Management Office (JRMO), QMUL. All SAEs received at the STRAP-EU trial office must be reported to the Joint Research Management Office (JRMO) at QMUL **immediately and within 24 hours** of the site becoming aware of the event. Nominated co-investigators will be authorised to sign the SAE forms in the absence of the CI at the co-ordinating site. Where this co-investigator is also a site PI there is no requirement for a second clinical review. The JRMO, QMUL, as Sponsor, will be informed of these nominated co-investigators. The PI or an authorised medic within the research team who has been delegated this role shall submit further detailed information relating to such events, as the JRMO shall request this within 24 hours of it becoming available. SAEs will be reported to the IMP provider (Pfizer) within 24 hours of the site becoming aware of the event as per contractual requirements.

7.4.2 SUSAR

Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur during the trial should be dealt with in the same way as all other SAEs and will be reported to the STRAP-EU Trial Office immediately and in any case within 24 hours of the site becoming aware of the event. The STRAP-EU Trial Office will then report to the JRMO and IMP provider (if applicable) within the same time frame. Expedited safety reporting according to the relevant national guidelines will be delegated to the research team in the country in which the SUSAR or SAE occurred, with sponsor oversight.

SUSAR reporting to the IMP provider (Pfizer) will be within 7 days of the event (for fatal or life-threatening SUSARs) and any follow-up information within a further 8 days, or 15 days for all other SUSARs.

If warranted, an investigator alert may be issued, to inform all investigators involved in any study (sponsored by QMUL) 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 SAE CRFs must be kept with the local Investigator File at the study site.

7.4.3 Adverse Events of Special Interest (AESI)

AESIs (serious and not serious) for patients that have been administered either Tocilizumab or Rituximab at any point during the trial treatment period require expedited reporting and must be sent to the STRAP trial Office as soon as reasonably practical and in any event within 24 hours of the site becoming aware of the event. The following AESIs associated with Roche IMPs will be collected at the STRAP-EU trials office:

- Serious and/or medically significant infections
- MI or acute coronary syndrome
- GI perforations
- malignancies
- anaphylaxis/hypersensitivity reactions
- demyelinating disorders
- stroke
- Serious and/or medically significant bleeding events
- Serious and/or medically significant hepatic events

Potential Risk of Biopsy:

The majority of patients have no adverse reaction to the procedure and it is generally very well tolerated. Possible complications include infection of the joint or skin, bleeding, pain and rarely nerve or tendon damage (less than 1:10,000 risk).

Risk management:

Infection of joint or skin: biopsy techniques will be performed by trained experts under aseptic techniques to ensure the lowest risk of infection.

Bleeding and pain: Procedures are not performed on patients with known bleeding disorders or anticoagulation therapy. Only minor bleeding has been reported. Simple analgesics will be prescribed to take as required.

Nerve and tendon damage: biopsy techniques will be supervised by trained personnel. Given the ultrasound guided approach, nerve or tendon damage is extremely rare. In the event of nerve or tendon damage the patient will be appropriately treated as per standard hospital practice.

7.5 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 prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor.

The reporting of urgent safety measures to the national competent authority and local ethics committees (or equivalent) is delegated to the lead research team in each country (following Sponsor approval). The sponsor (JRMO) must be sent a copy of the correspondence with regards to this matter.

7.6 Development Safety Update Reporting and Annual Progress Report (APR)

The Development Safety Update Report (DSUR) will be sent by the CI to the sponsor for review and once agreed, will be submitted via CESP by the Trials Office to the European national competent authorities. Submission of the DSUR to each country's Ethics committee should be done by the lead research team in that country (if required).The CI will carry out a risk/benefit analysis of the IMPs encompassing all events having arisen on the trial.

Submission of Ethics annual progress reports (if required) to local Ethics committees has been delegated to the lead research team in each country.

7.7 Overview of the Safety Reporting Process/Pharmacovigilance responsibilities

The CI has the overall pharmacovigilance oversight responsibility. The CI and the STRAP-EU 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 'STRAP-EU Trial: SAE reporting for Investigators SOP' and 'SAE reporting responsibilities for the Sponsor'.

7.8 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 24 hours 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 interventions and may attend follow ups as per scenario for patients ceasing treatment prior to 16 weeks subject to continued consent. The procedures for early withdrawal should be followed as described in section 5.11.

The PI/CI also must follow up the pregnancy until delivery as well as monitoring the development of the new-born for 1 month 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 7.4.1, utilising the SAE CRF reporting form.

The IMP provider (Pfizer) must also be notified of any event of Pregnancy within 24 hours of the site becoming aware of the event as per the procedure described above.

8 STATISTICAL CONSIDERATIONS

8.1 Primary Endpoint Efficacy Analysis

Treatment response will be assessed using the ACR20 response at 16 weeks. Sections 2.3 and 5.14.1 define treatment response/failure criteria.

The intention to treat (ITT) will be applied, i.e. the analysis will be based on the initial treatment assignment. The primary analysis will focus on whether there is a superiority of Etanercept and Tocilizumab (treated together for analysis) over Rituximab in histologically and molecularly defined B-cell poor patients, adjusting for MTX use, using logistic regression.

8.2 Secondary Endpoint Efficacy Analysis

The statistical tests will use the significance level of 5%. The 95% confidence intervals will be provided for the estimates of interest.

1. For the B-cell rich synovial pathotypes, we aim to compare treatment effects (with 95% confidence intervals) of Rituximab to Tocilizumab and Etanercept (treated together for analysis).
2. The interaction between treatments and B-cell status (rich and poor) will be tested using the likelihood ratio test between nested logistic regression models. The model will use all the sample and will be adjusted for MTX.
3. 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.
4. The descriptive analysis using the percentage of patients will be carried out on DAS28 (<2.6), ACR response (50 and 70) and CDAI ≤ 10 (low disease activity) at 16 weeks.
5. The change in CDAI from baseline will be summarised using the mean with 95% confidence intervals at 16 weeks.

8.3 Exploratory Endpoints

All aforementioned measures of clinical improvement at all relevant timepoints, i.e. 12, 24, 36 and 48 weeks, will be analysed. Additional exploratory clinical measures will be assessed, such as RAMRIS and radiographic and ultrasound scores. Also, the difference in response to Rituximab between B cell rich and B cell poor patients will be tested. Finally, adverse events and serious

adverse events will be summarised in each treatment group. Further information on the analyses of exploratory endpoints will be detailed in the STRAP Statistical Analysis plan.

8.4 Safety Endpoints

All AEs and SAEs will be recorded throughout the trial duration both in relation to the IMP and trial conduct (e.g. synovial biopsy procedure). Safety analysis will be assessed using descriptive statistics. Pre- and post-biopsy questionnaires will also collect information on the safety and tolerability of the synovial biopsy procedure.

8.5 Sample Size

Test the primary hypothesis that Etanercept and Tocilizumab (treated as a single comparison for analysis) are superior to Rituximab in B-cell poor patients. Assuming a response rate of 30% and 60% for Rituximab and Etanercept + Tocilizumab respectively, and using a 2-sided chi-square test of proportion with 5% Type 1 error, 96 B-cell poor patients (32 for RTX and 64 for Etanercept + Tocilizumab) will be required to achieve 80% power and 126 B-cell poor patients (42 for RTX and 84 for Etanercept + Tocilizumab) will be required to achieve 90% power.

To ensure the power of testing primary hypothesis, assuming that the proportion of B cell poor patients is 60% in the population, the total sample size will be 159 for power of 80% and 210 for 90% power.

159 patients in total will give a power of 85% to test the interaction between treatments and B-cell status (rich and poor), assuming the response rates are 0.3, 0.6, 0.8 and 0.6 respectively for B-cell poor Rituximab, B-cell poor Eta+Toc, B-cell rich Rituximab and B-cell rich Eta+Toc. The same setting gives 93% power to test the interaction using 210 patients.

Allowing 5% dropout rate, 102 B-cell poor patients would be required to achieve 80% power while 132 B-cell poor patients would be required to achieve 90% power. The study will recruit 168 patients for 80% power and 219 for 90% power.

Please note that the data obtained from patients in the STRAP-EU trial will be combined with data from patients in the STRAP trial (running in the UK), so total sample size includes patients recruited in both the STRAP-EU trial and STRAP trial.

9 Data Handling & Record Keeping

9.1 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 UK Data Protection Act, UK NHS Caldicott Guardian, The UK Research Governance Framework for Health and Social Care and Research Ethics Committee Approval and any other applicable National Laws for non-UK sites.

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 will be allocated a unique screening number by the database before undergoing any screening procedures. 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 trial database.

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.

9.2 Study Documents

The STRAP-EU Trial Office will maintain a Trial Master File (TMF) containing all essential documents relating to the trial. All sites will be issued with an Investigator Site File (ISF) and Pharmacy Site File (PSF).

9.3 Data Collection and clinical study report

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. Patient questionnaires may be completed on paper or electronically (e.g. iPad). If completed electronically this should be documented in source notes.

Data from the STRAP and STRAP-EU trials will be analysed together for all endpoints and combined and submitted as one clinical study report at the end of the trial (both EudraCT numbers will be referenced in the report)

9.4 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. From the end of the trial, it is a requirement of the Research Governance Framework and QMUL Policy that the records are kept for a further 20 years. For trials sponsored by QMUL, site files must be archived by the participating site. Electronic data will be stored and archived for 20 years in compliance with QMUL's archiving SOP in encoded password-protected format.

9.5 Compliance

This trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice (GCP) as laid out in the EU directive, the UK Research Governance Framework, and The UK Medicines for Human Use (Clinical Trials) Regulation 2004, and its amendments, and any other applicable National Laws for non-UK sites

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.

9.6 Clinical Governance Issues

9.6.1 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 screening. 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 Research Ethics Committee (REC) in each EU country. 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 an independent Data Monitoring and Ethics Committee (DMEC). 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. See section 10.3.

9.7 Quality Control and Quality Assurance

The CI/PI will ensure that the trial will be appropriately monitored by ensuring that all the rights of the subjects are adequately protected, that the trial data are accurate, complete and verifiable from source documents and that the conduct of the trial is in compliance with the protocol and its subsequent amendments, with GCP and with applicable regulatory requirements.

The CI/PI will verify that for all patients a written informed consent was obtained before each subject's participation in the trial. The CI/PI will also ensure that all patients enrolled will be eligible according to the in- and exclusion criteria as defined in the protocol.

9.7.1 Summary Monitoring Plan

On-Site Monitoring will be carried out on this trial. The trial monitor will perform the first monitoring visit within approximately 1 month of the first patient being randomised at a site. Monitoring visits will be performed a minimum of twice a year during recruitment and treatment period at approximately 6-monthly intervals (+/- 4 weeks). The frequency of visits is detailed in the Monitoring Plan and 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 STRAP-EU 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

SDV on all data points will be described in the STRAP trial monitoring plan.

Note: The following UK 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
- The GeneWiz lab are performing the RNA sequencing for the study and will have one remote monitoring visit.

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 3 months.

9.7.2 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 (MHRA / Clinical Trials Unit). Investigators are obliged to cooperate in any inspection.

9.8 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.

It is the responsibility of the lead site in the country to prepare and submit local forms to report Serious Breaches to their local ethical and regulatory bodies, as directed by the Sponsor.

The Lead site in each country 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 affect 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 STRAP-EU trial office or CI for further information.

9.9 Non-Compliance

All deviations from GCP or the study protocol will be recorded at the STRAP-EU Trial Office and appropriate action will be taken.

10 TRIAL COMMITTEES

10.1 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 and Trial Unit Representative. 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.

10.2 Trial Steering Committee (TSC)

The role of a Trial Steering Committee will be to provide overall supervision on behalf of the Trial Sponsor and Trial Funder and to ensure that the trial is conducted to the rigorous standards set out in the Medical Research Council’s (MRC) Guidelines for Good Clinical Practice and the relevant regulations. In particular, the TSC should concentrate on progress of the trial e.g. recruitment, adherence to the protocol, patient safety and the consideration of new information of relevance to the research question. The TSC will be asked to comment in detail on extension requests or substantial changes to protocol. A standing item on the TSC agenda will be the possible commercialisation of the study and its findings.

The Trial Steering Committee (TSC) has membership from the TMG plus independent members, including the Chair as detailed in the TSC Terms of Reference. The TSC membership is for the duration of the study. If any members leave the TSC, the CI (in collaboration with the TSC Chair and TMG) will provide replacements promptly for appointment by the Chair.

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.

10.3 Data Monitoring and Ethics Committee (DMEC)

The role of a Data Monitoring and Ethics Committee will be to review the accruing trial data (both STRAP and STRAP-EU) 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 and Ethics 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. Once recruitment is complete and the study is in follow-up phase the DMEC may not meet formally to review the study. The decision will be made by the DMEC based on accumulating data.

11 FUNDING INDEMNITY AND INSURANCE

The STRAP-EU trial forms part of a larger UK-led consortium entitled: Maximising Therapeutic Utility in Rheumatoid Arthritis (MATURA) which is a jointly funded by MRC and Arthritis Research UK (ARUK).The JRMO, QMUL, as sponsor of this trial, will provide full *no fault* indemnification cover for this study.

12 PUBLICATION POLICY

This is an investigator led trial, sponsored by the CI's substantive employers, QMUL. Publication policy will not contradict the larger MATURA consortium agreement. It is anticipated that the results will be published in peer reviewed journals. Any investigator involved with this study is obliged to provide the Sponsor with complete test results and all data derived from the study on request. 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. No participant may present data from his/her centre separately from the rest of the trial results unless approved by the CI/STRAP-EU management group and the Sponsor.

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APPENDIX 1:

Protocol Amendment Summary

Amendment No.	Details of amendment	Date approved by Ethics Committee	Date approved by competent authority	Associated Documents
Original	N/A			
SA#1 *This amendment was submitted as SA#2 in Spain.	• Addition of legal representative • Minor correction to sample size calculation	Spain: N/A-approved by administrative silence. Portugal: 25.09.2019 Italy (Lead site: Novara): 06.09.2019 Belgium: 06.05.2019	Spain: 18.05.2019 Portugal: 27.06.2019 Italy: 08.08.2019 Belgium: Pending	
SA#2	• Change to PIS/consent forms only, it was an addition of Hepatoxicity risk to the PIS and consent form.	Spain: Pending Portugal: 25.09.2019 Italy (Lead site: Novara): 06.09.2019 Belgium: 27.01.2020	Spain: Pending Portugal: 27.06.2019 Italy: 08.08.2019 Belgium: Pending	
SA#3	• Integration of RNA-seq molecular analysis into the original biopsy histopathology classification method • 4 exploratory endpoints (week 16 timepoint) moved to secondary endpoints • Modification to exploratory endpoints • Roche has changed the legal representation from UK to Germany. The Marketing Authorization Holder has been changed from Roche Registration Ltd. Welwyn UK to Roche Registration GmbH (RRG), Grenzach, Germany. • Reference Safety Information has been updated for Etanercept and Tocilizumab.			

APPENDIX 2:

Differences between the STRAP trial (2014-003529-16) and STRAP-EU trial (2017-004079-30)

As explained in this protocol, the results from both the STRAP and STRAP-EU trials will be combined at the end of both studies. The STRAP trial (2014-003529-16) running in the UK has been approved by the UK Competent Authority (MHRA) and the UK Ethics Committee. The STRAP-EU trial ((2017-004079-30) will run at several European countries and will be approved by each country's Competent Authority and Ethics committee. The main differences between the 2 protocols have been outlined below:

STRAP Protocol	**STRAP-EU**
Etanercept is being provided by Pfizer and Tocilizumab and Rituximab is provided by Roche	Etanercept is being provided by Pfizer and Tocilizumab and Rituximab are being sourced from local hospital stocks
Has a MRI sub-study	This study does not include the MRI sub study
EudraCT number: 2014-003529-16	EudraCT number: 2017-004079-30