RAMUS

Rheumatoid Arthritis and MUScle

An observational, single-arm study of skeletal muscle metabolism in patients with rheumatoid arthritis receiving Tofacitinib

Protocol

Sponsor: The Newcastle upon Tyne Hospitals NHS Foundation Trust, Freeman Hospital, Freeman Road, High Heaton, Newcastle upon Tyne, NE7 7DN.

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Protocol Contacts

Chief Investigator

Professor John Isaacs
Institute of Cellular Medicine 4th Floor, Leech Building Medical School Framlington Place
Newcastle upon Tyne NE2 4HH
Tel: 0191 2085337
Email:john.isaacs@ncl.ac.uk

Co-Investigators

Dr. Arthur G. Pratt
NIHR Academic Clinical Lecturer
Institute of Cellular Medicine (Musculoskeletal Research Group), Newcastle University
Floor 3, Leech Buidling, Framlington Place
Newcastle upon Tyne NE2 4HH
Tel: 0191 222 5337
Email: arthur.pratt@ncl.ac.uk

Dr. Avan Avan Aihie Sayer
Director, NIHR Newcastle Biomedical Research Centre, NIHR Senior Investigator and Professor of Geriatric Medicine
NIHR Newcastle Biomedical Research Centre
3rd Floor Biomedical Research Building Campus for Ageing and Vitality
Newcastle University, Newcastle upon Tyne NE4 5PL
Tel: 0191208 6000
Email: avan.sayer@ncl.ac.uk

Dr. Kieren Hollingsworth
Senior Lecturer in Magnetic Resonance Physics
Newcastle Magnetic Resonance Centre
Newcastle University-Campus for Ageing and Vitality
Newcastle upon Tyne NE4 5PL
Tel: 0191 208 1169
Email: kieren.hollingsworth@ncl.ac.uk

Dr. Maha S. Egail
Clinical Research Associate
Institute of Cellular Medicine (Musculoskeletal Research Group), Newcastle University
Floor 3, Leech Buidling, Framlington Place
Newcastle upon Tyne NE2 4HH
Email: maha.egail@newcastle.ac.uk

Synopsis

Trial Title	An observational , single-arm study of skeletal muscle metabolism in patients with rheumatoid arthritis receiving Tofacitinib	
Internal ref. no. (or short title)	Rheumatoid Arthritis and MUScle (The RAMUS study)	
Trial Design	This is an observational, single arm study designed to provide preliminary information on changes in muscle structure, biochemistry and function in patients receiving a JAK inhibitor. Data from this study will be used to power a formal controlled trial.	
Trial Participants	Rheumatoid arthritis patients in whom the decision has been taken to prescribe Tofacitinib, in accordance with the NICE criteria and the drug's license, and who have an elevated acute phase response.	
Planned Sample Size	15	
Treatment duration	6 months	
Follow up Duration	Nil beyond the end of study treatment	
Planned Trial Period	24 months	
	Objective	Outcome Measures
Primary	Change in muscle bulk during the course of therapy	Baseline, 1 month and 6 months investigations: • Accelerated MRI of selected limb/compartment
Secondary	a- To compare muscle function	Baseline, 1 month and 6 months : Grip strength and timed rise from chair
	b- Change in serum biochemistry	Baseline, 1 month and 6 month investigations: • Serum creatinine • Serum creatinine phosphokinase • Serum Aldolase

		• Serum Myoglobin • Serum Cystatin C
	c- Change in Muscle biochemistry	• Magnetic resonance spectroscopy of skeletal muscle: ratio of total creatine to water; content of ATP, phosphocreatine and inorganic phosphate • T2 relaxation time of muscle to measure muscle inflammation.
	d- Change in muscle histology and biochemistry	• Muscle Biopsy (Baseline and 6 months)
	e- To relate changes in muscle to reduction in systemic inflammation	Baseline, 1 month and 6 month investigations: • Serum CRP, IL-6, TNF, INF, IL1, IL17
	f- To seek any overall changes in body composition	• DEXA scan (Baseline and 6 months)

GLOSSARY OF TERMS AND ABBREVIATIONS

Acronym	Meaning
ACR	American College of Rheumatology
AST	Aspartate Aminotransferase
CK	Creatine Kinase
CRP	C-Reactive Protein
DAS	Disease Activity Score
DEXA	Dual Energy X-Ray
DMARDS	Disease Modifying Anti Rheumatic Drugs
ECG	electrocardiography
eCRF	Electronic Case Report Form
ESR	Erythrocyte Sedimentation Rate
GCP	Good Clinical Practice
GP	Glycoprotein
HAS	Health Assessment Questionnaire
IFN	Interferon
IL	Interleukin
JAK	Janus Kinase
mGFR	Measured Glomerular Filtration Rate
MRI	Magnetic Resonance Imagine
MRS	Magnetic resonance spectroscopy
RA	Rheumatoid Arthritis
RC	Rheumatoid Cachexia
Scr	Serum Creatinine
TCZ	Tocilizumab
TNF	Tumor Necrosis factor
VAS	Visual Analog Scale

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1 Introduction

1.1 Background

Rheumatoid arthritis (RA) is an immune mediated inflammatory disease that causes chronic, painful inflammation of mainly peripheral joints. Left untreated it causes joint destruction, disability and, because of systemic manifestations such as accelerated cardiovascular morbidity, premature death. Affecting approximately 1% of adults worldwide and with a peak incidence during the 5th decade of life, it is an important cause of work instability: at least 50% of patients in developed countries are unable to continue in full-time employment within 10 years of disease onset[i].

In addition to joint damage, changes in body composition have been observed in patients with RA. Typically this manifests as reduced fat-free mass (FFM), of which muscle mass is the major component, with relatively little loss of fat mass (FM), resulting in no or limited changes in body mass index[ii]. This condition has been referred to as rheumatoid cachexia, RC. Despite great advances in the treatment of RA, it appears that rheumatoid cachexia persists even after joint inflammation improves**,** potentially impeding rehabilitation of patients and contributing to fatigue.

Understanding the molecular mechanisms responsible for muscle wasting is necessary to develop targeted therapies for patients. It is assumed that sarcopenia of ageing is a consequence of hormonal and immunological changes. Pro-inflammatory cytokines, particularly interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), are believed to increase muscle loss[iii] . Given that both are elevated in RA, the development of this disease could cause and accelerate the progression of sarcopenia[iv]. In addition, a decrease in physical activity[v],increased energy expenditure during rest, immobility secondary to stiffness, and pain intensify the risk of sarcopenia

A previous study investigated the effects of Tocilizumab (TCZ), a humanized anti-interleukin 6 receptor antibody, on body composition and metabolic profile in patients treated for RA. Among the study population, 28.6% had a skeletal muscle mass index below the cut-off point for sarcopenia (4.8% of controls). After 1 year of treatment with TCZ, there was a significant weight gain without changes in fat mass In contrast, an increase in lean mass was observed with a significant gain in appendicular lean mass and skeletal muscle mass index between 6 and 12 months[vi]. While antirheumatic therapies such as DMARDs help suppress joint deterioration, they could also help prevent sarcopenia; further studies are needed to understand the underlying mechanisms and to develop effective therapies.

1.2 Rationale

Sarcopenia, has been defined as an age related, involuntary loss of skeletal muscle mass and strength. It begins as early as the 4th decade of life[vii], constituting an independent and vital threat for dexterity and independence. Sarcopenia is also associated with acute and chronic disease states, increased insulin resistance, fatigue, falls, and mortality[viii],[ix],[x]. Of the chronic disease states, sarcopenia has been especially associated with rheumatologic conditions, especially rheumatoid arthritis (RA) in women[xi].

As our understanding of RA has advanced, intracellular signaling pathways such as Janus kinase (JAK) pathways have emerged as key hubs in the cytokine network and, therefore, important as therapeutic targets. For example, IL-6 classically binds to gp130-coupled IL-6R on target cells, leading to activation of JAKs which, in turn, activate *signal transduction and activator of transcription-3* (STAT3), thereby initiating a transcriptional programme - linking an event at the cell membrane with downstream events in the nucleus[xii]. Importantly, however, this same pathway has also been found to be activated in clinical and experimental studies of cachexia. Indeed, ex vivo and murine studies indicate that STAT3 activation is both necessary and sufficient for muscle wasting, whether directly downstream of IL-6 or in a mixed cytokine model of C26 tumor-induced cachexia[xiii].

Tofacitinib is a non-specific JAK inhibitor for the treatment of RA. It is a targeted small molecule (jakinib), and an innovative advance in RA therapy. By targeting JAKs 1,2 and 3, it modulates the effects of multiple cytokines critical to the progression of immune and inflammatory responses [xiv].

During the Tofacitinib RA clinical development program, rises in mean serum creatinine (SCr) were observed in patients. This was not associated with nephrotoxicity in preclinical and healthy volunteer studies[xv],[xvi] ,[xvii]. However, changes in GFR (mGFR) are not the sole determinant of SCr. SCr is derived from muscle creatinine, and therefore many factors affect

SCr independently of GFR; these include, but are not limited to, age, gender, race, medications, diet, illness, muscle mass and muscle turnover[xviii],[xix],[xx],[xxi],[xxii]. As above, patients with RA present multiple factors that are associated with sarcopenia and, hence, a reduced SCr[xxiii],[xxiv],[xxv]. Consequently, a potential explanation for the rise in SCr seen with Tofacitinib is a reversal of rheumatoid sarcopenia, via the drug's interruption of pro-infammatory cytokine signaling. In support of this hypothesis, a previous study analyzed the relationship between Scr, CRp and CK. Data were pooled from five Phase 3 studies (in DMARD-inadequate responders) and two ongoing long-term extension (LTE) studies investigating Tofacitinib (CP-690,550; Pfizer Inc, Groton, CT, USA) in RA. There was a trend toward greater increases in SCr in those patients who had greater increases in CK along with reduction of the inflammation burden, assessed by CRP[xxvi].

1.3. Hypothesis

Modest rises of both creatine phosphokinase and creatinine levels were noted in clinical trials of Tofacitinib and were not attributable to nephrotoxicity[xxvii],[xxviii],[xxix]. These rises along with the observed weight gain following treatment with Tofacitinib makes a strong case for exploiting the hypothesis that Tofacitinib reverses rheumatoid cachexia which is a fundamental mechanism of sarcopenia. In turn, it will have a positive effect on treatment outcomes in terms of strength, activity and potentially even survival.

2 Study Objectives

2.1 Primary objective

To identify a change in muscle bulk when RA patients with active inflammation are treated with Tofacitinib.

2.2 Secondary objectives

The secondary objectives are:

a. To compare muscle function/strength at baseline, and 1 and 6 months after commencing Tofacitinib.

b. To compare muscle biochemistry at baseline, and 1 and 6 months after commencing Tofacitinib.

c. To seek histological and molecular changes in muscle that relate to changes seen in muscle bulk, biochemistry or function (eg hyperplasia or hypertrophy of muscle fibres; or molecular changes relating to activation of anabolic or down-regulation of catabolic pathways).

d. To relate changes in serum biochemistry (serum creatinine, serum creatine phosphokinase) to biochemical, structural, functional and histological/molecular changes in muscle

e. To relate any changes noted in muscle to reduction in systemic inflammation

f. To seek any overall changes in body composition using DEXA scanning.

2.3 Primary endpoint/outcome

a. Change in skeletal muscle bulk within both lower limbs between baseline, 1 month and 6 months, using compressed sensing (accelerated) MRI.

2.4 Secondary endpoints/outcomes

a. Muscle function (grip strength and timed rise from a chair) at baseline, 1 month and 6 months, assessed by hand grip and timed- raised from chair.

b. Serum creatinine, serum creatine phosphokinase, serum aspartate transaminase, serum Aldolase, serum Myoglobin and serum Cystatin C at baseline, 1 month and 6 months.

c. Magnetic resonance spectroscopy of skeletal muscle: content of ATP, phosphocreatine and inorganic phosphate at baseline, 1 month and 6 months, assessed by magnetic resonance spectroscopy.

d. Muscle histology and biochemistry at baseline and 6 months

e. Circulating CRP and pro-inflammatory cytokines at baseline, 1 month and 6 months.

f. DEXA scanning at baseline, 1 month and 6 months (body composition).

In each case, individual patient data (intra-patient) and pooled group data will be examined, to allow for expected heterogeneity in response.

2.5 Table of endpoints/outcomes

Objectives	Outcome Measures	Time point(s) of evaluation of this outcome measure (if applicable)
Primary Objective To identify a change in muscle bulk	**Primary outcome** Compressed sensing (accelerated) MRI.	Baseline, 1 month and 6 months
Secondary Objectives to identify a change in: a. muscle function/strength	**Secondary outcomes** Grip strength, timed rise from chair	Baseline, 1 month and 6 months

b. muscle biochemistry	Magnetic resonance spectroscopy of skeletal muscle: total creatine to water ratio, content of ATP, phosphocreatine and inorganic phosphate of the soleus	Baseline, 1 month and 6 months
c. muscle histology and biochemistry	Muscle Biopsy	Baseline and 6 months
d. To relate changes in serum biochemistry with effects on muscle	Serum creatinine, creatine phosphokinase, aspartate transaminase. Serum CRP, IL-6, TNF, IFN, IL1, IL17.	Baseline, 1 month and 6 months
e. To relate changes in muscle to reduction in systemic inflammation		Baseline, 1 month and 6 months
f. To seek any overall changes in body composition.	DEXA scan	Baseline, 1 month and 6 months

3 TRIAL DESIGN

This is an observational, single arm study designed to provide preliminary information on changes in muscle structure, biochemistry and function in patients receiving a JAK inhibitor. Data from this study will be used to power a formal controlled trial.

4 TRIAL SETTING

This will be a single centre study, in secondary care. Due to the nature of the endpoints, study visits will take place at the Newcastle NIHR Clinical Research Facility and Newcastle Magnetic Resonance Centre.

5 Subject Selection

5.1 Subject population

This is an observational, single arm study designed to provide preliminary information on changes in muscle structure, biochemistry and function in patients receiving a JAK inhibitor. Data from this study will be used to power a formal controlled trial. Within this context sample size was determined pragmatically in terms of feasibility over a 12 month recruitment period at a single centre.
These are Rheumatoid Arthritis patients in whom the decision has been taken to prescribe Tofacitinib, in accordance with the NICE criteria and the drug's license, and who have an elevated acute phase response.

5.2 PARTICIPANT ELIGIBILITY CRITERIA

5.2.1 Inclusion criteria

Participants will be individuals with active RA who have been prescribed Tofacitinib for treatment of their disease, and are prepared to participate in the study:

- 2010 ACR/ EULAR classification criteria for a diagnosis of Rheumatoid Arthritis
- At least 6 months disease duration
- Inadequate response to intensive therapy with synthetic disease-modifying anti-rheumatic drugs (DMARDs) alone, or inadequate response to at least one biologic DMARD, thereby qualifying for treatment with Tofacitinib according to local guidelines.
- Age> 18 years
- Willing and able to provide written informed consent.
- ACR Functional Class I-III
- Willing to undergo muscle biopsy on 2 occasions.
- Willing to undergo MRI and MRS and Dexa scan on 3 occasions
- Active systemic disease, as exemplified by a C-reactive protein of at least 10 mg/L.

5.2.2 Exclusion criteria

- Serum creatinine that is above the upper limit of normal at baseline.
- Patients receiving glucocorticoids
- Patients will be excluded if they have any contraindications to Tofacitinib which include:
    - Pregnancy and lactation

    - Women of Childbearing Potential (WOCP) who are not prepared to use effective contraception during treatment with Tofacitinib and for at least 4 weeks after the last dose.
    - Severe Hepatic impairment (Child Pugh C)
    - Active TB, serious infections such as sepsis or opportunistic infections as detailed in the SmPC
    - Chronic infections (HIV, Hepatits B, Hepatitis C)
- Participants will be excluded if they have any contraindications to muscle biopsies. These include:
    - Participants on anticoagulant therapy. These include Vitamin K antagonists, Thrombin inhibitors, and Heparin and Low Molecular Weight Heparin preparations.
    - Participants on antiplatelet. *Participants on Aspirin for primary prevention will be included in this study. However, Aspirin will be held for 7 days prior to the muscle biopsies and recommenced 48 hours after.
    - Participants who are known to have bleeding disorders. These include, but are not limited to, Haemophilia, Factor II, V, VII, X, or XII deficiencies and Von Willebrand's disease
    - Previous reactions to local anesthetics
    - Platelets count < 100 x 10^9/L
- Participants will be excluded if they have any contraindications to MRI. These include:
    - limb metal pins, plates, rods of screws that were placed less than 6 weeks from scanning day
    - heart pacemaker or replacement valves
    - neuro-stimulator or programmable intra-cerebral shunt, cerebral aneurysm clips
    - metallic foreign body in their eye
    - internal hearing devices, ocular prosthesis
    - weight >190 kg
    - claustrophobia

6 Trial Events

6.1TRIAL flowchart and Schedule of Visits

Schedule of events (all will take place either in the clinical research facility or at the Newcastle Magnetic Resonance Centre):

	Visit1	Visit 2 a-c	Visit3	Visit4	Visit5
Test	Screening		Baseline (within 4 weeks of visit 1)	1 month from visit 3 ± 3 days	6 months from visit 3 ± 7 days
Informed consent	X				
Inclusion and exclusion criteria	x				
Medical History	X		X	X	X
Concomitant medications	X		X	X	X
Adverse Events			X	X	X
Physical examination	X		X	X	X
Vital Signs	X		X	X	X
Urinalysis (dipstick)	X		X	X	X
CPK			X	X	X
AST			X	X	X
Myoglobin			X	X	X
Aldolase			X	X	X
Cystatin C			X	X	X
LDH			X	X	X
ESR	X		X	X	X
CRP	X		X	X	X
CBC	X				X
creatinine	X		X	X	X
INR	X				X
Pregnancy test	X				

Circulating cytokines			X	X	X
66/68 joint counts	X		X	X	X
Health assessment questionnaire Disability index			X	X	X

Patient global assessment VAS (ACR)			X	X	X
Physician global assessment VAS (ACR)			X	X	X
Pain VAS (ACR)			X	X	X
Patient general health VAS (DAS28)	X		X	X	X
Muscle strength testing			X	X	X
consent for procedure		X		X	X
DEXA		X		X	X
Magnetic resonance spectroscopy *		X		X	X
Accelerated MRI of selected limb*		X		X	X
Muscle biopsy		X			X
clinical consumables	X		X	X	X
Patient's meal			X		X

* MRS and MRI will take place in Newcastle Magnetic Resonance Centre

All Participants will follow the visit schedule summarised in section 6.1 If participants cannot attend on the due date, flexibility- window on either side of the assessment due date will be permitted

6.2 Identification and Recruitment

Potential participants will be identified and recruited at routine out-patient clinics at the Freeman Hospital. Identification may be extended to Patient Identification Centres (PIC sites) at surrounding hospitals. Potential participants will initially be identified by clinical or nursing staff responsible for routine care. They will be patients with active RA, in whom a decision has been taken to prescribe Tofacitinib for their rheumatoid arthritis, in accordance with NICE guidelines; they will also have a CRP of at least 10mg/L at the most recent reading. If they are potentially interested in research participation then they will be contacted by a member of the research team and provide a participant information sheet (PIS). Potential participants who decline participation will have anonymised information recorded in a screening log (age, gender, ethnicity, the reason not eligible for trial participation, or if they are eligible but declined). Potential participants identified at PIC sites will be contacted by a research nurse by telephone in the first instance and have a PIS mailed to them; they will be offered the opportunity to discuss the study face-to-face. Potential participants who remain interested in the study after receiving a PIS, and having had an opportunity to discuss the study, will be given an appointment to attend the clinical research facility for a screening visit (see schedule of events).
Participants will be reimbursed for reasonable travel expenses for all visits that take place outside of their routine care.

6.3 Screening

At the screening visit the PI or a delegated co-investigator or sub-investigator will discuss the trial with the patient in detail; potential participants will be encouraged to ask any questions in relation to the study and their concerns will be addressed and clarified. Patients may take additional time to consider their participation following this discussion and return at a later date to provide written, informed consent if they wish. Following this process, patients wishing to participate in the trial will provide written, informed consent by signing and dating the trial Consent Form. 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. Informed consent will be taken by an appropriate member of the study team. 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.

As per the study visit schedule, screening will entail evaluation of:

- Inclusion and exclusion criteria
- Demographic data including age, gender and race
- Medical history, comorbidities and concomitant medications
- Physical examination: vital signs, 66/68 joint count

- Urine sample will be taken for dipstick testing (glucose, blood and protein)
- Blood tests (ESR, CRP, CBC, serum creatinine, INR)
- Females will be required to carry out a pregnancy test
- Patient general health VAS (DAS28)

6.4 Visit 2a-c

Following the confirmation of the participant's eligibility, Visits 2a, 2b and 2c will take where DEXA scan, MRI and MRs, and muscle biopsy will take place respectively. Depending upon local arrangements and participant's preference, visits 2 a-c and baseline may be combined into 3, 2 or a single visit. However, muscle biopsy must be done after imaging has been completed.

6.5 Baseline visits – Visit 3

The baseline visit will take place within 4 weeks of the screening visit in the CRF. At the end of this visit, patient will be instructed that they can start to take their NHS-prescribed Tofacitinib.

Eligibility will first be confirmed, in particular incorporating the results of blood tests taken at the screening visit. In addition, the participant will undergo the following assessments:

- Medical history will be revisited including adverse events since screening
- Physical examination
- CPK , AST, myoglobin, aldolase, cystatin C , LDH and circulating cytokines in addition to ESR and CRP
- Questionnaires will be completed either by the participant or by the physician as appropriate

- Muscle strength testing
- Consent for procedure

6.6 Visit 4

The 4th Visit will take place 1 month after baseline visit at the CRF. In this visit:

- Medical history will be updated, including adverse events, intercurrent illnesses and medications check
- Physical examination will be repeated including vital signs and 66/68 joint counts.
- Questionnaires will be completed either by the participant or by the physician as appropriate
- A urine sample will be collected for urinalysis (dipstick).
- Blood samples will be collected as per schedule of events
- DEXA scan will be performed at the CRF. Magnetic resonance imaging and spectroscopy will be repeated at Newcastle Magnetic Resonance Centre.

6.7 Visit 5

The final study visit, visit 5 at 6 months after baseline, essentially recapitulates the baseline visit. Participants will return to the CRF where:

- Medical history will be updated, including adverse events, intercurrent illnesses and medications check
- Physical examination will be repeated, including vital signs and 66/68 join counts.
- Questionnaires will be completed either by the participant or by the physician as appropriate
- Blood samples will be collected as per schedule of events
- DEXA scan and accelerated MRI and MRS will be performed.
- The second muscle biopsy will be taken.

After visit 5 the participant's consultant rheumatologist will be contacted by letter and by telephone to inform them that the patient has completed study participation. Tofacitinib, if effective, will continue to be prescribed within the NHS.

6.6 Withdrawal criteria

Participants may withdraw from the study at any point. If a participant wishes to withdraw from certain aspects of follow-up (eg repeat muscle biopsy) but to continue with other study assessments, this will be permitted and encouraged. The PI may also withdraw patients from the study for medical reasons.

Participants who fail screening will be replaced but participants who drop out after the baseline visit will not be replaced. Where there is appropriate consent, their data will be used within the study report.

7 Study procedures

7.1 Consent

Written consent must be obtained prior to any study-specific procedures being performed, including any study specific screening procedures prior to randomisation. 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.

To ensure effective communication, in the presence of a language barrier, interpreters will be made available through the hospital translation service. 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.

Written consent will be taken by a clinician, who has signed / dated the staff authorisation / delegation log.

7.2 Muscle Biopsy:

Muscle Biopsies will be performed on all subjects participating in the trial. These are scheduled to take place at visits 2 and 5. It is a day case procedure that is performed by

trained personnel using aseptic techniques and under local anaesthesia. Patients will be instructed to observe the surgical site for signs of infection or bleeding, but no biopsy-specific follow-up visits are normally necessary.
The obtained muscle specimens will be transferred to the labs where muscle fiber size and number will be studied along with fat content, anabolic and catabolic pathways. Analysis of the aforementioned will be done through a combination of methodologies, immunohistochemistry, qRT-PCR and Western Blot,

Obtaining a muscle specimen for histopathologic study is an important component of this trial. It provides evidence of muscle changes as a consequence of treatment at the tissue level, for example hypertrophy or hyperplasia of muscle fibres, activation of anabolic pathways and down-regulation of catabolic pathways.

7.3Imaging assessments

7.3.1 DEXA Scan

All subjects will undergo a total body DEXA scan (Lunar iDXA scan) before treatment commences, 1 and 6 months later. Fat, lean, and bone masses for the total body and per region (arms, legs, and trunk) will be measured and analyzed by using the manufacturer's validated software. Quality control and calibration procedures will be performed by using the manufacturer's standard.

Body fat percentage will be calculated as the proportion of total fat mass to total mass. Appendicular fat and lean masses will be computed as the sum of the tissue compartment (fat or lean) of both arms and legs. Skeletal muscle mass index (SMI) will be calculated as appendicular lean mass divided by height $(m)^2$, fat mass index as total fat mass divided by height $(m)^2$, and fat-free mass index as total body mass without total fat mass divided by height $(m)^2$. The trunk-peripheral fat ratio, a measure of 'android' fat, will be calculated by using fat of the body trunk divided by the peripheral (legs and arms) fat. Separation of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) will be performed by two blinded readers (SMG and CG) inside a region of interest by using a new software developed on DXA with a validated method.[xxx],[xxxi] Lin's concordance correlation coefficient for the inter-reader concordance was 0.96 for VAT and 0.99 for SAT. The ratio of VAT area/SAT area will be calculated. An SMI lower than two SDs below the mean SMI of young male and female reference groups is defined as the gender-specific cut point for sarcopaenia (Baumgartner's criteria: men 7.26 kg/m^2, women 5.5 kg/m^2).[xxxii]

7.3.2 Accelerated MRI and MRS

The Newcastle Magnetic Resonance Centre has developed advanced methodology for measuring tissue energetics (31P MRS) and metabolism (1H MRS) as well as conventional images to measure structure and muscle fat infiltration. MRS is a companion technique to the more familiar magnetic resonance imaging (MRI) scan. It is noninvasive and capable of measuring a broad range of biological compounds across a variety of tissues. In other words, it allows us to study muscle metabolism, which is a point of interest in this trial.

In this trial, the total skeletal muscle volume in the upper and lower leg, covering both legs will be measured using a custom fat-water decomposition MRI, accelerated by compressed sensing methodsxxxiii. A custom 3-point Dixon sequence will be used to automatically segment the signals from skeletal muscle, bone and subcutaneous fat. The muscle volume of the upper leg will be delimited from the superior aspect of the greater trochanter to the superior aspect of the patella. The muscle volume of the lower leg will be defined from the superior aspect of the talus to the tibial plateau. This method, developed at Newcastle, is not only faster than conventional T1 weighted imaging but can also be analysed more rapidly.
To assess muscle inflammation, we will measure the T2 relaxation time by multi-echo spin-echo imaging at mid-lower and mid-upper leg, defined with respect to the bony landmarks above.
We will use PRESS single voxel spectroscopy to examine the relative concentration of creatine to water in the soleus at each time point (voxel size approx. 40 mm x 40 mm x 20 mm)xxxiv .
We will use ISIS single voxel spectroscopy to examine the concentration of phosphocreatine, ATP and inorganic phosphate in the soleus.
All MRI assessments will be made at the Newcastle MR Centre on a 3T Philips Achieva dStream Scanner using a 28 channel array coil and a 14cm surface coil for 31P MRS. Measurements obtained at base line will be compared to those taken 1 and 6 months later.

There is a small possibility that an unexpected abnormality could be observed of which the patient and their doctors are unaware. The MRI scans will be reviewed by a radiologist in the Newcastle upon Tyne Hospitals NHS Foundation Trust to look for any such findings. The Principal Investigator will be informed of any abnormal findings and will contact the patient's clinical care team or GP to make recommendations about any further investigations which it may be appropriate for them to arrange. Text agreed with the Sponsor to this effect is contained in the Participant Information Sheet.

8 Risks and Benefits of the proposed interventions:

We will study rheumatoid arthritis patients who have been prescribed Tofacitinib (5mg bd) by their clinical team, in accordance with its license and NICE criteria. From this perspective the risks are no more than for standard care. In terms of additional investigations, patients will provide a slightly larger volume of blood during the 6 months of observation than they would in standard care but the difference is modest and should not be associated with significant risk. Magnetic resonance imaging and spectroscopy are safe, non-invasive procedures that do not use ionising radiation; DEXA scanning involves exposure to a very low dose of x-irradiation. Muscle function testing involves simple tasks (eg rising from a chair without using hands) that are not associated with significant risk. Biopsies will be performed by trained personnel under aseptic techniques to ensure the lowest risk of infection. The majority of patients have no adverse reaction to the procedure and it is generally very well tolerated. Possible complications are significant bleeding or bruising (risk of 1 in 100) and nerve damage resulting in localised paraesthesia (risk of 1 in 1000)

The proposed research has the potential to contribute work of significant improvement in our understanding of sarcopenia that's associated with RA. It will have a positive impact on providing targeted future therapy that's beyond joint inflammation albeit having no direct benefits to this study's participants.

9 Statistical analysis

9.1 Sample Size

With a sample size of N=15 patients we should be able to detect changes of around the same order of magnitude as the variability in those differences (delta/sigma=1) with greater than 90% power on a one sided test at the 5% level of statistical significance.

9.2 Primary Endpoint Analysis

The MRI studies will provide continuous data for the muscle volume (in cm^3) of both legs. There will be two volume measurements, one for the lower leg and one for the upper legs at the three timepoints. There will be one spectroscopic measurement which is the ratio of creatine to water in the soleus for all three time points. There are therefore three outcome measures at three time points for each of 15 individuals.
Each outcome measure will be tested for suitability for a one-way repeated ANOVA assessment (checks for outliers, normality, homogeneity and sphericity). If the ANOVA is significant, post-hoc testing will be carried out using Bonferroni correction to identify which timepoints are significantly different. Effect sizes will be calculated.
In the event that the data do not satisfy the criteria for one-way repeated ANOVA, then Friedman's ANOVA will be performed, followed by step-wise post-hoc tests to identify which timepoints are significantly different. Effect sizes will be calculated. All statistics will be performed using IBM SPSS Statistics 24.
Should we establish significant changes in both muscle volume and creatine/water ratio, then we will further investigate the relationship between these changes, bearing in mind the size of this pilot study

10 Ethics & Regulatory Issues

The conduct of this trial will be in accordance with the ethical principles for medical research involving human subjects determined by the World Medical Association Declaration of Helsinki of the 64th General Assembly in October 2013. All members of the research team and the investigators will be trained in the statutory instrument and those aspects of Good Clinical Practice appropriate to their role in the trial. Favourable ethical opinion and Clinical Trial Authorisation from relevant Competent Authority(ies) will be sought prior to commencement of the trial. Local approvals will be sought before recruitment may

commence at each site. Information sheets will be provided to all eligible subjects and written informed consent obtained prior to any trial procedures

11 Confidentiality

11.1 Safeguarding confidentiality

Personal data will be regarded as strictly confidential. To preserve anonymity, any data and laboratory samples leaving the site will identify participants by their initials and a unique trial identification code only. The trial will comply with the UK Data Protection Act, 2018. All trial records and Investigator Site Files will be kept at site in a locked filing cabinet with restricted access.

11.2 Long term data storage

At the end of the trial, Case Report Forms and Consent Forms will be securely archived for 5 years following publication of the last paper or report from the trial, in line with Sponsor policy and Standard Operating Procedures. This will allow any queries or concerns about the data, conduct or conclusions of the trial to be resolved.

12 Insurance and Finance

The Newcastle upon Tyne Hospitals NHS Foundation Trust has liability for clinical negligence that harms individuals toward whom they have a duty of care. NHS Indemnity covers NHS staff and medical academic staff with honorary contracts conducting the trial for potential liability in respect of negligent harm arising from the conduct of the trial. The Newcastle upon Tyne Hospitals NHS Foundation Trust is Sponsor and through the Sponsor, NHS indemnity is provided in respect of potential liability and negligent harm arising from trial management. Indemnity in respect of potential liability arising from negligent harm related to trial design is provided by NHS schemes for those protocol authors who have their substantive contracts of employment with the NHS and by Newcastle University Insurance schemes for the management of the study and for those protocol authors who have their substantive contract of employment with Newcastle University. This is a non-commercial trial and there are no arrangements for non-negligent compensation.

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