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Research Protocol

The Rheumatoid Arthritis and MUScle (RAMUS) Study: Protocol for an observational single-arm study of skeletal muscle in patients with rheumatoid arthritis receiving tofacitinib

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Abstract

People with rheumatoid arthritis (RA) are disproportionately affected by sarcopenia, the generalised loss of muscle strength and mass, consequently facing an increased risk of falls, functional decline and death. Currently, there are no approved pharmacological treatments for sarcopenia. RA patients who start tofacitinib (a Janus kinase inhibitor) develop small increases in serum creatinine that are not explained by renal function changes and could reflect sarcopenia improvement. The RAMUS Study is a proof of concept, single-arm observational study in which patients with RA who commence tofacitinib according to routine care will be offered participation according to eligibility criteria. Participants will undergo lower limb quantitative magnetic resonance imaging, whole-body dual energy x-ray absorptiometry, joint examination, muscle function testing and blood tests at three time points: prior to starting tofacitinib and 1 and 6 months afterwards. Muscle biopsy will be performed before and 6 months after starting tofacitinib. The primary outcome will be lower limb muscle volume changes following treatment initiation. The RAMUS Study will investigate whether muscle health improves following tofacitinib treatment for RA. Identifying a potential pharmacological treatment for sarcopenia could have important implications for individuals with RA and for older people in general. ISRCTN registry ID: 13364395.

Keywords: Body composition, Magnetic resonance imaging, Rheumatoid arthritis, Sarcopenia, Tofacitinib

Introduction

Extra-articular sequelae are well recognised in rheumatoid arthritis (RA). Sarcopenia, the accelerated loss of muscle strength and mass, disproportionately affects individuals with RA, with a prevalence of 25%[1]. By itself, sarcopenia is associated with adverse outcomes including falls, functional decline and mortality[2].

Effective evidence-based treatments for RA-associated sarcopenia (RS) are lacking. Utilising conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) with a treat-to-target approach does not restore muscle mass or strength[3]. Randomised control trials comparing tumour necrosis factor inhibitor drugs against csDMARDs showed no significant difference in lean mass change between treatment arms[4,5]. Resistance exercise improves muscle

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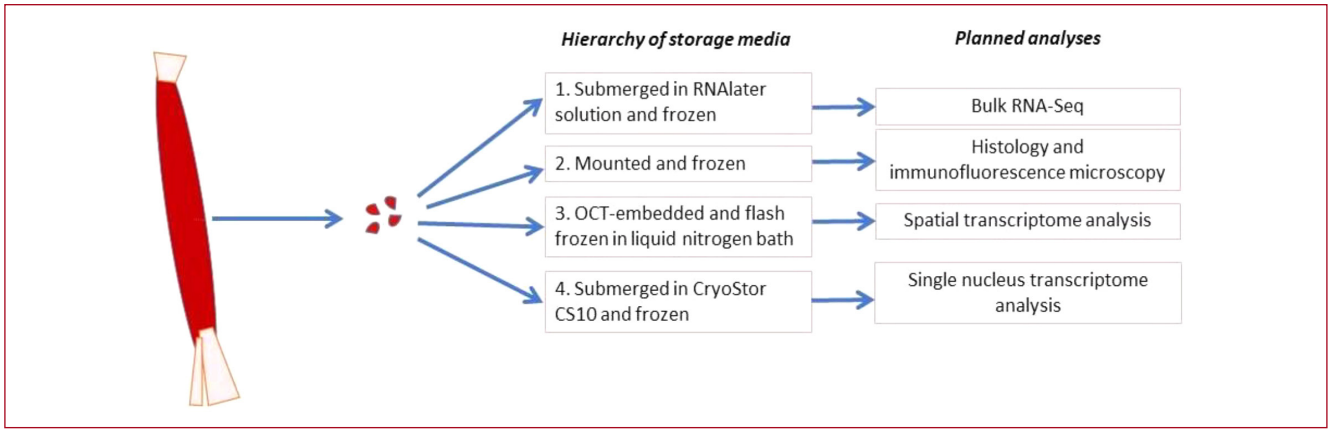

Figure 1. Processing and storage of muscle tissue. OCT, optimal cutting temperature compound.

PRIMARY OBJECTIVE	SECONDARY OBJECTIVES	EXPLORATORY OBJECTIVES
- To identify any lower limb muscle volume changes at 1 month and 6 months after starting tofacitinib by performing quantitative magnetic resonance imaging (MRI).	- To identify any changes at 1 and 6 months of tofacitinib therapy in: • muscle strength (measured by grip strength and FTSST), • muscle function (gait speed), • muscle biochemistry and inflammation (MRS and MRI), • body composition (DXA), • serum creatinine, serum muscle enzymes and systemic inflammation (as measured by CRP and pro-inflammatory cytokines). - To describe any molecular changes arising in skeletal muscle by analysing tissue acquired from vastus lateralis biopsies at baseline and 6 months after starting tofacitinib. - To correlate data endpoints to seek inter-relationships and dependencies.	- To explore, at baseline and 1 month, the relationship between the expression of pSTAT 1/3 in ex vivo peripheral blood mononuclear cells in response to tofacitinib and therapeutic efficacy at 6 months, seeking a response biomarker. - To identify changes in muscle quality by measuring the fat fraction of the lower limb muscle compartments at 1 and 6 months of tofacitinib therapy.

Table 1. Objectives of the RAMUS Study. CRP, C-reactive protein; DXA, dual energy x-ray absorptiometry; FTSST, five-times-sit-to-stand test; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; pSTAT, phosphorylated signal transducer and activator of transcription.

strength and physical performance in RA[6], although relevant studies mostly involve middle-aged adults only. No approved drug treatments exist for sarcopenia, meaning that there is a huge unmet need for a pharmacological adjunct to resistance exercise.

Tofacitinib is a licensed treatment for RA. During the tofacitinib clinical development program, small serum creatinine (SCr) increases were observed in RA patients that were not explained by changes in renal function[7]. Additionally, data from phase 3 and long-term extension studies of tofacitinib in RA found that patients with the greatest improvements in inflammation had the largest increases in SCr[7].

Muscle mass is the main determinant of variability in SCr production[8]. Given that inflammation contributes to the aetiology of sarcopenia[2] and may accelerate the progression of RS, one explanation for the rise in SCr seen with tofacitinib is reversal of the muscle wasting of RS as inflammation is reversed.

Materials and Methods

Aims and Objectives

The RAMUS Study is an observational, single-arm, experimental medicine study designed to test the hypothesis that treating RA with tofacitinib increases muscle mass.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Meets 2010 ACR / EULAR classification criteria for RA	Serum creatinine > 1.5 times the ULN
At least 6 months' disease duration	ALT >2.0 times the ULN
ACR Functional Class I-III	Previous or current JAK inhibitor treatment
Age> 18 years	Significant comorbidities, at the discretion of the Principle Investigator
Able to provide written informed consent	Systemic or intra-articular glucocorticoid treatment within 4 weeks of the Baseline Visit (inhaled, topical or intranasal glucocorticoids administered permitted)
Usual clinician has made decision to treat with tofacitinib according to NICE guidelines	Tofacitinib contraindications: A. Pregnancy and lactation B. Women of childbearing potential who do not wish to use effective contraception C. Severe hepatic impairment (Child Pugh C) D. Active tuberculosis, serious or opportunistic infections as detailed in the Summary of Product Characteristics E. Chronic infections (Human Immunodeficiency Virus, Hepatitis B, Hepatitis C)
Willing to undergo: • muscle biopsy on 2 occasions and • MR and DXA imaging on 3 occasions	Muscle biopsy contraindications: A. Anticoagulant or antiplatelet therapy (excluding aspirin for primary prevention) B. Bleeding disorders C. Adverse reaction to local anaesthetics D. Platelet count <100x10^9/L
One of the following three sarcopenia risk criteria: A. Active systemic disease, as exemplified by either: a. A baseline CRP of at least 10 mg/L or b. At least two CRP values of 5 mg/L or greater within the past 24 months, at least one month apart, that can reasonably be attributed to RA disease activity B.* DXA scan demonstrating ALMI <7.0 kg/m^2 in males or <5.5 kg/m^2 in females C.* Muscle weakness not limited by pain, as exemplified by either: a. Reduced grip strength (<27 kg for males or <16 kg for females) b. Five-times-sit-to-stand test >15 seconds	MR imaging contraindications: A. limb metal pins, plates, rods or screws placed less than 6 weeks from day of scan B. heart pacemaker or replacement valves C. neuro-stimulator or programmable intra-cerebral shunt, cerebral aneurysm clips D. metallic foreign body in or near to the eye E. internal hearing devices F. ocular prosthesis G. weight >190 kg H. claustrophobia

Table 2. Eligibility criteria. *Sarcopenia risk criteria B and C were added to criterion A partway through the recruitment stage of the study (see 'Protocol version' statement below). ACR, American College of Rheumatology; ALT, Alanine transaminase; ALMI, appendicular lean mass index; CRP, C-reactive protein; DXA, dual energy x-ray absorptiometry; EULAR, The European Alliance of Associations for Rheumatology; JAK, janus kinase; MR, magnetic resonance; NICE, National Institute for Health and Care Excellence; ULN, upper limit of normal.

Table 1 outlines the study objectives, which will be achieved by comparing baseline data with follow-up data at 1 or 6 months or both.

Participants

We aim to recruit 15 participants from a single centre (Rheumatology, Freeman Hospital, Newcastle upon Tyne, UK) over an 18-month period. Patients are eligible if they meet the criteria in Table 2.

Trial events

The RAMUS Study activities are detailed in Table 3. Each visit can be completed in one day or over several days, as contemporaneously as possible. Activities are completed in a dedicated Clinical Research Facility (based in the Royal Victoria Infirmary, Newcastle upon Tyne, UK), except for magnetic resonance (MR) imaging (performed in the Newcastle Magnetic Resonance Centre, Newcastle upon Tyne, UK) and dual energy x-ray absorptiometry (DXA) imaging (performed in the Freeman Hospital, Newcastle upon Tyne, UK).

Recruitment

After the decision to commence tofacitinib, patients are offered a study information sheet and are given at least 24 hours to consider being involved. Patients can undergo their Screening Visit immediately after receiving tofacitinib counselling.

		Screening Visit	Baseline Visit (within 4 weeks of Screening Visit completion)	1-month Follow-up Visit (1 month after Baseline ± 3 days)	6-month Follow-up Visit (6 months after Baseline ± 7 days)	Early Withdrawal Visit
Informed consent		X				
Inclusion and exclusion criteria		X				
Medical history		X	X	X	X	X
Concomitant medications		X	X	X	X	X
Demographics questionnaire		X				
Adverse events			X	X	X	X
Physical examination		X	X	X	X	X
66/68 joint count		X	X	X	X	X
Strength testing	Grip strength	X	X	X	X	X
	Five-Times-Sit-to-Stand Test	X	X	X	X	X
Gait speed testing		X	X	X	X	
Vital signs	Blood pressure	X	X	X	X	X
	Pulse rate	X	X	X	X	X
	Temperature	X	X	X	X	X
Urinalysis (dipstick)		X	X	X	X	X
Blood tests	Creatine kinase		X	X	X	X
	Aspartate transaminase		X	X	X	X
	Myoglobin		X	X	X	X
	Cystatin C		X	X	X	X
	Lactate dehydrogenase		X	X	X	X
	Erythrocyte sedimentation rate	X	X	X	X	X
	C-reactive protein	X	X	X	X	X
	Full blood count	X			X	X
	Creatinine	X	X	X	X	X
	Coagulation screen	X			X	X
	Pregnancy test	X				
	Circulating cytokines		X	X	X	X
	Flow cytometry based tofacitinib sensitivity assays		X	X		X
Patient reported outcome measures	HAQ-DI		X	X	X	X
	Patient global VAS (ACR score)		X	X	X	X
	Patient general health VAS (DAS28)	X	X	X	X	X
	Pain VAS (ACR score)		X	X	X	X
Physician global VAS (ACR)			X	X	X	X

Table 3. Study activities. *Each activity is repeated if >3 weeks has elapsed since the test was last performed. **The Screening Visit DXA also assesses femoral neck and lumbar spine bone mineral density. ACR, American College of Rheumatology; DAS-28, 28-joint disease activity score; HAQ-DI, Health Assessment Questionnaire Disability Index; RAPA, Rapid Assessment of Physical Activity; VAS, Visual Analogue Score.

	Screening Visit	Baseline Visit (within 4 weeks of Screening Visit completion)	1-month Follow-up Visit (1 month after Baseline ± 3 days)	6-month Follow-up Visit (6 months after Baseline ± 7 days)	Early Withdrawal Visit
RAPA questionnaire[9]	X		X	X	
Dual energy x-ray absorptiometry**	X		X	X	X*
Magnetic resonance spectroscopy		X	X	X	X*
Quantitative MRI of lower limbs		X	X	X	X*
Consent for procedure		X		X	X*
Muscle biopsy		X		X	X*
Clinical consumables	X	X	X	X	X
Patient's meal		X		X	X

Table 3. (Cont. from previous page).

Baseline Visit

Participants undergo muscle biopsy after all other activities are completed. Participants begin taking their NHS-prescribed tofacitinib in the evening after this visit.

Muscle strength and function testing

The five-times-sit-to-stand test and gait speed assessment are performed as described by Dodds et al[10], except the former is performed in padded, straight-backed chairs and gait speed is measured using a 4-metre course. Grip strength is measured using a Jamar dynamometer as per the Southampton protocol[11].

DXA

A Lunar iDXA scanner measures the proportions of whole-body bone, fat and lean mass. Appendicular lean mass index is determined by dividing appendicular lean mass by the square of the individual's height.

MR imaging and spectroscopy

A 3T Philips Achieva dStream whole-body scanner measures lower limb muscle mass (total for both legs) using quantitative 3-point Dixon imaging[12]. Bony landmarks are used in the lower and upper sections of the leg to define the muscle compartments to be acquired and measured. The water signal arising from lean muscle tissue (separated from lipid signals arising from bone and subcutaneous fat) is isolated, and the lean muscle volume calculated. Proton MR spectroscopy measures the concentrations of creatine and water in the soleus muscle and phosphorus MR spectroscopy measures phosphocreatine, inorganic phosphate and ATP in the soleus muscle and facilitates the inference of muscle pH. The T2 relaxation time of the muscle was evaluated using a multi-echo spin echo sequence at mid-thigh and mid-calf, measuring the effect of muscle inflammation, modelling for the possible presence of fat in the muscle[12].

Biopsy

This day case procedure is performed under sterile conditions with local anaesthesia, using the semi-open technique[13]. The sample is divided, allowing some to be transferred to the laboratory for processing and remaining tissue to be flash frozen in a liquid nitrogen bath (see Figure 1). Tissue is stored at -80°C.

Tofacitinib sensitivity assays (TSAs)

6 millilitres of blood are drawn into heparin anti-coagulant and processed according to a separately developed standard operating procedure using Smart Tube Proteomic Stabiliser buffer before freezing and storage at -80°C for downstream undertaking of TSAs.

Withdrawal

If a participant wishes to withdraw from certain activities but to otherwise continue with the study, this will be permitted and encouraged. The PI may also withdraw participants from the study for medical reasons. Participants who fail screening are replaced but any participants who drop out after the Baseline Visit will not be replaced.

Participants who discontinue tofacitinib after visit 4 will be invited to attend an Early Withdrawal Visit at the earliest opportunity. If a participant wishes to decline some activities but undergo others at that visit, this will be permitted and encouraged. Participants may also decline an Early Withdrawal Visit in toto.

Treatment changes

Tofacitinib is prescribed as 5 mg twice daily. If adverse effects occur, tofacitinib may be discontinued for 1 week before restarting at 5 mg once daily with subsequent escalation to 5 mg twice daily. Temporary suspension of tofacitinib (for instance for illness or surgery) will be managed as per standard clinical practice.

Participants can request additional visits for review of their RA. Adjustments to background csDMARD therapy are permitted at the PI's discretion. A total of two intra-articular glucocorticoid injections are permitted throughout the study (two on one occasion or one on two occasions). If additional escape therapy is required, including substitution of an alternative JAK inhibitor or biologic therapy, the participant will be withdrawn from the study and an Early Withdrawal Visit triggered.

Statistical analysis

In this pilot study, a sample of 15 patients was used. Based on reproducibility of the MRI muscle measurements, 15 subjects will provide greater than 90% power to detect a 1% increase in lower limb muscle mass across the intervention, based on a 1-sided test at the 5% level of statistical significance[14].

MR imaging will provide continuous data for upper and lower leg muscle volume, the primary outcome, at three time points. All outcome measures will be tested for suitability for one-way repeated analysis of variance (ANOVA). If the ANOVA is significant, post-hoc testing will identify which time points are significantly different. If the data do not satisfy the criteria for one-way repeated ANOVA, Friedman's ANOVA will be performed, followed by stepwise post-hoc tests to identify which time points are significantly different.

Discussion

Sarcopenia may manifest in otherwise healthy older adults (primary sarcopenia) or, as with RS, in association with chronic disease (secondary sarcopenia). Understanding how disease-modifying drugs affect muscle health in chronic disease is critical for clinicians to be able to manage secondary sarcopenia. Trials such as the RAMUS Study may reveal novel anti-sarcopenia indications for licensed drugs. There is also the potential for all people affected by sarcopenia to benefit if drugs like tofacitinib can be repurposed.

An important aspect of the RAMUS Study is the muscle biopsy, facilitating the interrogation of tissue for molecular changes associated with tofacitinib exposure. If improvements in muscle mass and function are observed, our planned histological, transcriptomic and protein analyses may suggest potential mechanisms. Little is known about the pathophysiology of RS, so data obtained from muscle tissue will significantly contribute to this field.

The RAMUS Study will provide insight into whether tofacitinib improves muscle health in RA. Longitudinal MRI measurements will establish whether lower limb muscle volume increases significantly. Muscle function may be influenced by RA disease activity, and this will be accounted for by comparing participants according to their EULAR response through data visualization tools and plots of the data.

A limitation is that we did not assess all factors that could influence muscle outcomes. For example, dietary assessments were considered but ultimately not incorporated to avoid unduly burdening participants in an already complex protocol.

We anticipate that the RAMUS Study will inform larger-scale studies of RS and may eventually broaden the armamentarium available for treating individuals living with sarcopenia. Furthermore, the resulting data will improve our understanding of sarcopenia in the context of RA.

Ethics approval

The RAMUS Study was reviewed by the South East Scotland Research Ethics Committee on 11th September 2019 (REC reference 19/SS/0100) and was granted a favourable opinion (subject to specified conditions being met). Good Clinical Practice standards are adhered to.

Clinical Trial Registry

The RAMUS Study was prospectively registered with the ISRCTN registry on 18th October 2019 (reference number 13364395).

Protocol version

On 10th May 2022, an update of the RAMUS Study protocol to version 1.9 was approved by the HRA. On 3rd November 2021, the HRA approved a substantial amendment that broadened the CRP inclusion criterion by also allowing individuals to be eligible through low muscle mass or low muscle strength.

Consent to participate

Participants sign a study-specific informed consent form in the Screening Visit and are also asked to sign their consent before each of the two muscle biopsies.

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JDI, AEA, RD, ME, KGH, AGP, GSG and AAS designed the study and prepared the successful funding application and the first draft of the study protocol. CF and GSG provided practical support and guidance for performing muscle biopsies. All authors have read and approved the manuscript.

References

1. Dao T, Kirk B, Phu S, Vogrin S, Duque G. Prevalence of Sarcopenia and its Association with Antirheumatic Drugs in Middle-Aged and Older Adults with Rheumatoid Arthritis: A Systematic Review and Meta-analysis. Calcif Tissue Int 2021;109(5):475-89.
2. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. The Lancet 2019;393(10191):2636-46.
3. Lemmey AB, Wilkinson TJ, Clayton RJ, Sheikh F, Whale J, Jones HSJ, et al. Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology 2016;55(10):1736-45.
4. Marcora SM, Chester KR, Mittal G, Lemmey AB, Maddison PJ. Randomized phase 2 trial of anti-tumor necrosis factor therapy for cachexia in patients with early rheumatoid arthritis. The American Journal of Clinical Nutrition 2006;84(6):1463-72.
5. Engvall I-L, Tengstrand B, Brismar K, Hafström I. Infliximab therapy increases body fat mass in early rheumatoid arthritis independently of changes in disease activity and levels of leptin and adiponectin: a randomised study over 21 months. Arthritis Research & Therapy 2010;12(5):R197.
6. Baillet A, Vaillant M, Guinot M, Juvin R, Gaudin P. Efficacy of resistance exercises in rheumatoid arthritis: meta-analysis of randomized controlled trials. Rheumatology 2011;51(3):519-27.
7. Isaacs JD, Zuckerman A, Krishnaswami S, Nduaka C, Lan S, Hutmacher MM, et al. Changes in serum creatinine in patients with active rheumatoid arthritis treated with tofacitinib: results from clinical trials. Arthritis Res Ther 2014;16(4):R158.
8. Stevens LA, Coresh J, Greene T, Levey AS. Assessing Kidney Function — Measured and Estimated Glomerular Filtration Rate. New England Journal of Medicine 2006;354(23):2473-83.
9. Topolski TD, LoGerfo J, Patrick DL, Williams B, Walwick J, Patrick MB. The Rapid Assessment of Physical Activity (RAPA) among older adults. Prev Chronic Dis 2006;3(4):A118-A.
10. Dodds RM, Murray JC, Granic A, Hurst C, Uwimpuhwe G, Richardson S, et al. Prevalence and factors associated with poor performance in the 5-chair stand test: findings from the Cognitive Function and Ageing Study II and proposed Newcastle protocol for use in the assessment of sarcopenia. J Cachexia Sarcopenia Muscle 2021;12(2):308-18.
11. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. Age Ageing 2011;40(4):423-9.
12. Wood CL, Hollingsworth KG, Hughes E, Punniyakodi S, Muni-Lofra R, Mayhew A, et al. Pubertal induction in adolescents with DMD is associated with high satisfaction, gonadotropin release and increased muscle contractile surface area. European Journal of Endocrinology 2021;184(1):67-79.
13. Patel H, Syddall HE, Martin HJ, Cooper C, Stewart C, Sayer AA. The feasibility and acceptability of muscle biopsy in epidemiological studies: findings from the Hertfordshire Sarcopenia Study (HSS). J Nutr Health Aging 2011;15(1):10-5.
14. Chow S-C, Shao J, Wang H. Sample size calculations in clinical research. 2nd ed. ed. Boca Raton: Chapman & Hall/CRC; 2008.