

Rituximab for the treatment of fatigue in primary biliary cholangitis (formerly primary biliary cirrhosis): a randomised controlled trial

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Abstract

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Background: Primary biliary cirrhosis (PBC) is an autoimmune liver disease, and 50% of patients with this disease experience fatigue. This is a debilitating symptom affecting quality of life and resulting in social isolation, which is highlighted by patients as a research priority. PBC is characterised immunologically by the presence of high-titre autoantibodies that are directed at the pyruvate dehydrogenase complex (PDC) and are highly effective at blocking its energy generation function. We hypothesised that if anti-PDC antibodies were a driver of fatigue through bioenergetic dysfunction, then the B-cell-targeting biological agent rituximab (MabThera®, Roche Products Ltd, Welwyn Garden City, UK) might be a therapeutic option.

Objective: To assess whether or not rituximab safely improved moderate or severe fatigue in PBC patients.

Design: A Phase II, double-blind, randomised controlled trial comparing rituximab with placebo in fatigued PBC patients. Randomisation was conducted using a web-based system. Participants received two infusions on days 1 and 15 and were followed up at 3, 6, 9 and 12 months.

Setting: A single-centre UK study in Newcastle upon Tyne Hospitals NHS Foundation Trust.

Participants: Seventy-one participants aged ≥ 18 years with PBC and moderate or severe fatigue (score of > 33 on the PBC-40 fatigue domain) were screened. The PBC-40 questionnaire is a fully validated disease-specific health-related quality-of-life measure for use in patients with PBC. Fatigue, with a maximum score of 55, is one of its six domains. Fifty-seven participants were randomised to the trial, 55 of whom reached the primary end-point assessment.

Intervention: Participants were randomised in a 1 : 1 ratio to receive either rituximab (1000 mg) or a saline intravenous infusion (placebo) on days 1 and 15. The infusions were delivered in a double-blind manner using the same protocol.

Main outcome measures: The primary outcome measure was the PBC-40 fatigue domain at 3 months, assessed on an intention-to-treat basis. Secondary outcome measures included markers of bioenergetics

function (anaerobic threshold and post-exercise muscle pH assessed using magnetic resonance imaging) and physical activity levels. Impact on biochemical markers of liver disease severity was assessed as an experimental outcome.

Results: Rituximab therapy was safe, with no serious adverse events linked to the drug. There was no statistically significant difference in fatigue score at 3 months between the rituximab and placebo arms [adjusted mean difference -0.9 , 95% confidence interval (CI) -4.6 to 3.1]. However, improvement in fatigue was observed in both arms {mean score decreasing from 41.2 [standard deviation (SD) 5.5] to 36.2 (SD 8.4) in the rituximab arm and from 43.0 (SD 5.9) to 38.1 (SD 8.7) in the placebo arm}. There was little difference in any of the secondary outcomes between arms. However, anaerobic threshold improved significantly in the rituximab arm (adjusted mean difference at 3 months 1.41 , 95% CI 0.03 to 2.80). No change in muscle bioenergetics characteristics was seen. A suggestive improvement in liver biochemistry was observed.

Limitations: Recruitment was lower than the original target, leading to a reduction in study power. A clinically significant placebo effect on PBC-40 fatigue scores was seen.

Conclusions: Rituximab is ineffective for the treatment of fatigue in unselected PBC patients despite metabolic modulation through improvement of anaerobic threshold.

Future work: Results from the trial demonstrate that metabolic effect of rituximab is not translated into clinical benefit. This will help to guide us to design future trials and when looking at completely different targets.

Trial registration: Current Controlled Trials ISRCTN03978701, ClinicalTrials.gov identifier NCT02376335 and EudraCT number 2012-000145-12.

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List of supplementary material

Report Supplementary Material 1 Statistical analysis plan for the RITPBC trial

Supplementary material can be found on the NIHR Journals Library report project page (www.journalslibrary.nihr.ac.uk/programmes/eme/109003/#/documentation).

Supplementary material has been provided by the authors to support the report and any files provided at submission will have been seen by peer reviewers, but not extensively reviewed. Any supplementary material provided at a later stage in the process may not have been peer reviewed.

List of abbreviations

AE	adverse event	ITT	intention to treat
ALP	alkaline phosphatase	LDL	low-density lipoprotein cholesterol
ALT	alanine transaminase	MHRA	Medicines and Healthcare products Regulatory Agency
AMA	anti-mitochondrial antibody	MR	magnetic resonance
ANOVA	analysis of variance	NCTU	Newcastle Clinical Trials Unit
AR	adverse reaction	OGS	Orthostatic Grading Scale
AST	aspartate transaminase	PBC	primary biliary cirrhosis
AUC	area under the curve	PDC	pyruvate dehydrogenase complex
CI	confidence interval	PDH	pyruvate dehydrogenase
COGFAIL	Cognitive Failure Questionnaire	PI	principal investigator
CPET	cardiopulmonary exercise testing	PIC	participant identification centre
CRF	case report form	PROM	patient-reported outcome measure
CRP	C-reactive protein	PROMIS	Patient-Reported Outcomes Measurement Information System
DMEC	Data Monitoring and Ethics Committee	HAQ	Health Assessment Questionnaire
ENMO	Euclidean Norm Minus One	RA	rheumatoid arthritis
ESS	Epworth Sleepiness Scale	REC	Research Ethics Committee
GCP	Good Clinical Practice	RITPBC	B-cell-depleting therapy (RITuximab) as a treatment for fatigue in Primary Biliary Cirrhosis
GGT	gamma-glutamyl transpeptidase	SAE	serious adverse event
HADS	Hospital Anxiety and Depression Scale	SAR	serious adverse reaction
HDL	high-density lipoprotein cholesterol	SD	standard deviation
i.v.	intravenous	SUSAR	suspected unexpected serious adverse reaction
Ig	immunoglobulin	TSC	Trial Steering Committee
IgG	immunoglobulin G	U&E	urea and electrolytes
IgM	immunoglobulin M	UDCA	ursodeoxycholic acid
IMP	investigational medicinal product		
IQR	interquartile range		
IRR	infusion-related reaction		

Plain English summary

P primary biliary cholangitis [formerly primary biliary cirrhosis (PBC)] is a liver disease, and approximately half of the patients with this disease experience fatigue as one of the symptoms, which can significantly affect their quality of life. PBC patients have antibodies (which are molecules produced by their immune system), namely anti-mitochondrial antibodies. Mitochondria are parts of human cells in which energy-producing reactions take place. Studies have shown that abnormalities in the muscle energy regulation in fatigued PBC patients are linked to these antibodies. Rituximab (MabThera®, Roche Products Ltd) is a drug that works against these antibodies. This drug has been in use in other conditions for over two decades. Data from pilot studies using rituximab in fatigued PBC patients suggested some beneficial effects of the drug in symptom improvement. Based on this background, we designed a trial to assess whether or not rituximab improved moderate or severe fatigue in patients with PBC. We monitored safety and the tolerability of rituximab in patients with PBC. Fifty-seven adult participants (aged ≥ 18 years) with PBC and moderate or severe fatigue (assessed using a PBC-40 fatigue questionnaire) were recruited into the trial. They were randomised to receive either rituximab infusion (trial drug) or saline infusion (placebo) on two occasions. They were then followed up over a period of 12 months. Analysis of the results showed no significant difference in fatigue score at 3 months (primary outcome) between the rituximab and placebo groups. There was also little difference in any of the secondary outcomes between groups, although anaerobic threshold (a quantitative measure of exercise) improved significantly in the rituximab group compared with the placebo group. A suggestive improvement in some liver tests was observed. Rituximab was found to be safe, with no serious adverse events reported in the patients who received the drug. We concluded that rituximab is ineffective for the treatment of fatigue in unselected PBC patients.

Scientific summary

Background

B-cell-depleting therapy (RiTuximab) as a treatment for fatigue in Primary Biliary Cirrhosis (RITPBC) was a Phase II randomised placebo-controlled trial targeting moderate to severe fatigue in primary biliary cholangitis [formerly primary biliary cirrhosis (PBC)].

Primary biliary cirrhosis is an autoimmune liver disease characterised by loss of the intrahepatic bile ducts, accompanied by progressive cholestasis. Fifty per cent of patients experience significant fatigue, a particularly debilitating symptom affecting quality of life and resulting in social isolation. Fatigue can occur at any point in the disease course, and its severity is unrelated to liver disease activity or degree of liver damage. Unsurprisingly, in light of this, fatigue severity is not reduced by current first- and second-line therapy. Given its impact and lack of response to therapy, increasing understanding of, and treatment for, fatigue has been highlighted by patients as a priority for research. Patient support groups were actively involved in developing the concept underpinning this trial, contributing to the protocol and supporting recruitment for the trial. This was the first randomised controlled trial to investigate a treatment for fatigue in PBC.

Primary biliary cirrhosis is characterised immunologically by the presence of high-titre autoantibodies directed at pyruvate dehydrogenase complex (PDC), an enzyme complex that plays a critical role in cellular bioenergetics function-linking glycolysis and the Krebs cycle. Anti-PDC antibodies from PBC patients are highly effective, *in vitro* at least, at blocking PDC function. Clinically, PBC patients exhibit both central and peripheral elements to their fatigue. The peripheral component, likened by patients to feeling that their 'batteries are running down', is significant and associated with the inability to sustain repeat muscle contraction. Investigation of this phenomenon, using novel magnetic resonance (MR) spectroscopy approaches, revealed marked muscle acidosis with exercise, related to mitochondrial dysfunction, and a prolongation in the time taken for recovery of muscle acidosis following discontinuation of exercise, which was related to fatigue severity. The degree of mitochondrial dysfunction was also related to serum anti-PDC antibody level. In separate approaches, PBC patients have been shown to have lower anaerobic threshold levels than matched control subjects with normal or reduced bile flow (cholestasis). Taken together, these observations point to dysregulation of aerobic metabolism in muscle in PBC, with excessive or inappropriate utilisation of the anaerobic lactate dehydrogenase pathway. The link between anti-PDC antibody levels and mitochondrial dysfunction, and the capacity of anti-PDC antibodies to block PDC function (a key checkpoint in the progression from glycolysis to the Krebs cycle in aerobic metabolism), led us to postulate that anti-PDC antibodies are in fact the driver for the metabolic insult in PBC and that this insult underpinned fatigue. The potential for this approach was supported by a pilot study completed in Canada that demonstrated a beneficial action of rituximab (MabThera®, Roche Products Ltd) on fatigue in PBC.

If anti-PDC were a driver for fatigue in PBC, then the B-cell-targeting biological agent rituximab might be regarded as a plausible therapy option. This is a hypothesis that is supported by open-label pilot data, suggesting improvement in fatigue with this agent.

Objectives

- To assess if rituximab improved moderate or severe fatigue in patients with PBC and the sustainability of any improvement over time.
- To assess the safety and tolerability of rituximab in patients with PBC.
- To assess the effect of rituximab on anti-PDC antibodies in PBC and on bioenergetics abnormality potentially linked to fatigue.

Methods

Design

This was a Phase II, double-blind, randomised controlled trial comparing rituximab with placebo in fatigued PBC patients. Randomisation was conducted using a web-based system. Treatment allocation was kept blinded from the patients, study assessors and investigators until study completion. Participants received two infusions on days 1 and 15 and were then followed up at 3, 6, 9 and 12 months. The setting was a single centre in the UK.

Participants

Seventy-one patients aged ≥ 18 years with PBC and moderate or severe fatigue (as measured by a PBC-40 fatigue domain score of > 33) were screened and 57 participants were randomised to the trial.

The main inclusion criteria were that participants were aged ≥ 18 years with an established diagnosis of PBC and with stable or compensated liver disease. The major exclusion criteria were inability to give consent, alternative diagnosis of liver disease, advanced or decompensated disease, pregnancy or lactation, immune-compromised state, malignancy, active or severe infections, demyelinating disorder and psychiatric disorder.

Intervention

Participants in the study were randomised in a 1 : 1 ratio to receive either rituximab intravenous (i.v.) infusion (1000 mg) on days 1 and 15 ($n = 28$) or saline i.v. infusion (placebo) on days 1 and 15 ($n = 29$). The infusions were delivered in a double-blind manner to participants using the same protocol.

Outcome measures

The primary outcome measure was the PBC-40 fatigue domain score at 3 months, a disease-specific quality-of-life measure. The time course of the comparison between intervention and control groups over the 12-month follow-up period was also assessed. Secondary outcome measures included an extended panel of patient-reported outcome measures (PROMs) [including the other domains of the PBC-40 (cognitive, itch, social, emotional and other symptoms), and tools for depressions, anxiety, sleep disturbance and autonomic dysfunction], assessment of bioenergetics function [including anaerobic threshold assessed using conventional cardiopulmonary exercise testing (CPET) and post-exercise muscle pH assessed using MR] and physical activity monitoring.

Efficacy in terms of B-cell depletion was assessed using fluorescence-activated cell analysis and anti-PDC antibodies through enzyme-linked immunosorbent assay assessment of serum anti-PDC antibody levels.

The impact on biochemical markers of liver disease severity was assessed as an experimental analysis.

Results

Rituximab therapy was safe, with no serious adverse events linked to the drug and no difference in the adverse event profile between the rituximab and placebo groups.

Primary end point

There was no statistically significant difference in fatigue score at 3 months between the rituximab and placebo arms [adjusted mean difference -0.9 , 95% confidence interval (CI) -4.6 to 3.1]. However, improvement was observed in both arms {with mean score decreasing from 41.2 [standard deviation (SD) 5.5] to 36.2 (SD 8.4) and from 43.0 (SD 5.9) to 38.1 (SD 8.7) in the rituximab and placebo arms, respectively}. There was no significant difference between the two trial arms over the repeated assessments at 3, 6, 9 and 12 months ($F = 1.81$; $p = 0.18$).

Secondary end points

Immunological analyses suggested that rituximab was effective in mediating depletion of B-cells: its primary proposed mode of action. No depletion was seen in the placebo arm. Complete depletion was maintained at 6 months with gradual repopulation to 50% of the baseline level by 12 months. The kinetics of fatigue reduction in the rituximab group mirrored the kinetics of B-cell depletion and recovery. Reduction in the levels of anti-PDC antibody (the characteristic autoantibody of PBC) was also seen in the rituximab (but not placebo) group. Peak reduction was seen at 6 months and this was sustained at 9 months. Reduction in total immunoglobulin, and in particular the immunoglobulin M fraction, was seen. Again, reduction was incomplete in the rituximab-treated group and absent from the placebo group.

Additional PROMs were used to assess other aspects of the patient experience [the five non-fatigue domains of the PBC-40 (addressing itch, cognitive, emotional, social and other symptoms), Epworth Sleepiness Scale (daytime somnolence), Orthostatic Grading Scale (autonomic dysfunction), Cognitive Failure Questionnaire (cognitive symptoms) and Hospital Anxiety and Depression Scale (depression and anxiety)]. The unadjusted and adjusted differences in mean scores between trial arms showed little difference at 3 months. The 95% CIs were generally wide, but there was no real suggestion that the results were consistent with any clinically important differences. In contrast to fatigue, which showed improvement in both the active drug and the placebo arms, no notable improvement in the other measures was seen in either group. Any placebo effect was therefore restricted to fatigue, and was absent even from the closely linked cognitive symptom domain. Physical activity levels [Euclidean Norm Minus One (ENMO)] differed little between arms at 3 months: the adjusted mean ENMO levels were slightly lower in the rituximab arm, but there was little indication of a large and meaningful difference.

We used a combination of MR spectroscopy of muscle and anaerobic threshold assessment using CPET to calculate changes in pH and recovery time post exercise. In keeping with previous reports, anaerobic threshold at baseline was low in this PBC patient group. The mean values rose in the rituximab arm from baseline to 3-month follow-up, with no change in the placebo arm. The anaerobic threshold score at 3 months was higher in the rituximab arm than in the placebo arm (adjusted difference 1.41, 95% CI 0.03 to 2.80).

There was no apparent correlation between degree of change in anaerobic threshold and change in fatigue over 3 months from baseline.

In keeping with our previous reported findings, the minimum pH following the specific exercise task was highly variable among the PBC patients, with substantial acidosis seen in some patients. Minimum pH in muscle seen following exercise was higher and the fall in pH with exercise was lower following rituximab therapy compared with placebo. No reduction in the time taken to recover to baseline pH after exercise and no reduction in the 'area under the curve' for pH (a factor combining the degree of acidosis and the length of time taken to recover to the baseline level and an estimate therefore of degree of muscle intracellular acid exposure) was seen in either arm. Comparison of bioenergetics outcomes between trial arms shows that the adjusted differences in means at 3 months were all small, with relatively narrow 95% CIs for the pH parameters.

No significant association was seen between changes in any MR parameter and change in fatigue severity.

Experimental analysis

The RITPBC trial was not designed or powered to explore the impact of the drug on liver injury; however, the trial provided some insight into the impact of rituximab therapy in early disease, which could inform future trials of disease-modifying therapy. Ninety-three per cent of patients in the rituximab arm had normal alkaline phosphatase (ALP) levels at 3 months, whereas 65% had ALP levels in the normal range in the placebo arm at 3 months. All parameters progressively returned to the baseline level by 12 months of follow-up.

Conclusions

The following conclusions can be drawn from this study:

- There is no evidence to support the use of rituximab for the treatment of fatigue in otherwise unselected populations of PBC patients with moderate to severe fatigue. On average, the trial end point of a 5-point reduction in fatigue severity was met; however, this did not significantly exceed the effect seen with placebo.
- Trials of fatigue-modulating therapy are deliverable in PBC and are acceptable to patients (retention was 100%). However, there is a significant issue with placebo effects that are similar in scale to those seen in recent PBC trials of anti-itch therapy.
- Rituximab when used in PBC was safe with no disease-attributable serious adverse events.
- The bioenergetics abnormality reported previously in fatigued PBC patients and that underpinned the trial concept was also seen in this trial.
- Anaerobic threshold was significantly improved by rituximab. Muscle bioenergetics abnormality on MR spectroscopy was not, however, significantly improved. The capacity of muscle to handle protons and lactate may therefore play a more important role in fatigue expression than PBC autoantibody. Future trials of therapy for fatigue might address these aspects (exercise therapy and targeting autonomic dysfunction).
- Although the trial was not designed or powered to explore impact on liver biochemistry, our findings point to a positive impact of rituximab on liver function when used in patients not defined by ursodeoxycholic acid failure.

Trial registration

This trial is registered as ISRCTN03978701, ClinicalTrials.gov identifier NCT02376335 and EudraCT number 2012-000145-12.

Funding

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Chapter 1 Introduction

Scientific background and rationale

Primary biliary cirrhosis (PBC) is a chronic liver disease affecting approximately 20,000 patients in the UK (prevalence 35/100,000).¹ In 2015, the name of the disease was changed to primary biliary cholangitis at the request of international patient groups.² The revised name better reflects the disease process present in the majority of patients. The disease is characterised pathologically by inflammation within the portal tracts in the liver and progressive loss of small intrahepatic bile ducts.³ A relatively small proportion of patients progress, if untreated, to cirrhosis and end-stage liver disease requiring liver transplantation.⁴ Bile acid therapy in the form of the first-line agent ursodeoxycholic acid (UDCA) is effective at slowing or stopping progression to the end stage in the majority of patients, probably through a reduction of secondary liver injury as a result of retention of cytotoxic hydrophobic bile acids occurring in the context of bile duct loss.⁵⁻⁹ Up to 40% of PBC patients are under-responsive to UDCA and are at increased risk of progression of the disease to cirrhosis.¹⁰⁻¹² Second-line bile acid therapy in the form of obeticholic acid (a modified bile acid that has enhanced farnesoid X receptor agonist properties that lead to reduced endogenous bile acid synthesis) has recently been approved in the USA and Europe for use in patients showing an inadequate response to UDCA and was approved by the National Institute for Health and Care Excellence in early 2016.^{13,14}

The high levels of autoantibodies seen in PBC patients, strong associations with other autoimmune diseases and pattern of immunogenetic susceptibility (which points to a key role for immune dysregulation in the disease) all point to PBC being an autoimmune disease.¹⁵⁻¹⁸ It is increasingly clear, however, that the disease is multistage in nature, with initial immune injury to the target epithelial cells lining the small intrahepatic bile duct being followed by secondary cholestatic injury in which toxic hydrophobic bile acids retained in the liver as a consequence of early bile duct injury cause further bile duct injury. This cycle has the potential to be self-perpetuating.¹⁹

Fatigue: the commonest symptom in primary biliary cirrhosis

Historically, the main focus in terms of clinical impact and its treatment in PBC has been progression of the disease to cirrhosis and, ultimately, death. In recent years, with the advent of large-scale representative patient cohorts and validated patient-reported outcome measures (PROMs), it has become widely accepted that there is another important aspect to patient impact: that is quality-of-life impairment related to systemic symptoms.^{12,17,20-24} Although quality-of-life impairment can be significant in patients with decompensated cirrhotic disease, this state is very uncommon in the condition, meaning that the conventional complications of end-stage liver disease contribute relatively little to overall quality-of-life burden.^{25,26} In contrast, the disutility associated with the non-stage-specific symptoms is substantial.²⁴ Data from the very large UK-PBC cohort have also demonstrated that the burden of impaired quality of life is age related, with younger patients experiencing the worst symptoms and the greatest quality-of-life burden.²⁷

The symptom reported most frequently by PBC patients is chronic fatigue, which is present in > 50% and which has an often striking impact on patients.²⁸⁻³⁰ There are currently no effective treatments for fatigue in PBC, with international treatment guidelines recommending only supportive treatment.³¹ Unsurprisingly, given its prevalence, impact and the lack of effective treatments, fatigue is frequently identified by PBC patients as the principal priority area for new therapy development. There has been a significant recent increase in the understanding of the potential mechanisms that might contribute to fatigue in PBC, and significant advances in the development of clinical tools that are able to quantify the impact of that fatigue and that represent key tools for the study of fatigue pathogenesis and, critically, treatment. The key advance in this regard has been the development of the PBC-40, a patient-derived, disease-specific, quality-of-life measure that has become the standard measure used in all clinical trials.^{14,21,32}

Fatigue severity in PBC is not associated with the stage of the disease and is frequently seen in its most severe form in patients with early disease and a seemingly very good prognosis from the point of view of risk of death from their liver disease.^{12,28,33} This can result in a discrepancy between the physician's and patient's perception of how well the patient is doing, with the former seeing a level of liver injury suggesting a good prognosis and the latter focusing on their severe fatigue.³⁰ Furthermore, there is no evidence to suggest that either current first- (UDCA) or second-line therapy has any impact on fatigue severity.^{12,14} As prognostic management gets better in PBC, so the contrast between length of life and quality-of-life improvement will become increasingly marked.

The issue of the role played by depression in the expression of fatigue in PBC is a controversial one, with many patients being clinically labelled as having depression simply because they are fatigued, making the association self-fulfilling. Although depressive problems can clearly occur in fatigued PBC patients (frequently as a consequence of the considerable limitation to lifestyle that they experience), and positive coping strategies are an important part of managing fatigue, overt depressive illness is actually a rare cause of fatigue in PBC.^{34,35} In fact, in most fatigued PBC patients it is peripheral, activity-limiting fatigue that predominates.³⁶ Fatigue in PBC can be associated with significant fatigue disturbance (in particular daytime somnolence) and autonomic dysfunction (particularly in the form of baroreflex sensitivity loss). The extent to which these associations reflect causes or consequences of chronic fatigue is unclear at present.¹

The scale of the problem of fatigue in primary biliary cirrhosis

Population-based studies undertaken using the PBC-40, which contains a fully validated fatigue domain, have suggested that > 50% of PBC patients across the UK experience moderate or severe fatigue, with > 20% experiencing sustained severe fatigue (≈4000 patients nationally), with a profound impact on life quality.^{12,24,27} Fewer than 20% of severely fatigued patients are able to work, with the majority of this group dependent on state benefits for income. Severe fatigue also causes social isolation in people who lack the energy to leave their house and interact with others, a particular issue among younger patients.²⁴ Severely fatigued PBC patients also frequently have problems with their ability to care for others (90% of PBC patients are female and they often have young children and/or dependent elder relatives themselves), with cases of Meals on Wheels being provided for the children of socially isolated, severely fatigued PBC patients being far from uncommon. The management of severe fatigue in PBC, which is currently largely supportive, is a major draw on NHS budgets (the trial investigators are currently following up > 150 severely fatigued patients with, typically, monthly clinic follow-up).^{26,31} Severe fatigue is also a frequent driver for patients seeking liver transplantation, although the evidence to suggest that there is any benefit from transplantation for PBC fatigue is limited.^{25,37} Treatment able to reduce the impact of severe fatigue in PBC would transform the lives of patients and their families, substantially reduce the costs of NHS treatment (the costs of follow-up and supportive management of fatigued patients and the costs of transplantation where the indication is advanced liver disease but the patient driver is fatigue) and, importantly, the costs to society of this socially isolated, economically unproductive and dependent group of people.³⁸

Bioenergetic abnormality is frequent in primary biliary cirrhosis and is associated with fatigue

Primary biliary cirrhosis patients' descriptions of fatigue emphasise an inability to sustain physical activity and concepts related to 'energy' or 'power' depletion (a further group describe 'brain fog', which is associated with significant cognitive symptoms). Furthermore, on repeat testing, handgrip strength reduces rapidly in fatigued PBC patients and is associated with perceived fatigue severity.³⁶ More recently, using magnetic resonance (MR) spectroscopy approaches, we demonstrated (and replicated in a second cohort) the presence of significant peripheral muscle bioenergetic abnormality in PBC patients.^{39,40} Abnormality is also present in cardiac muscle, suggesting the presence of a broadly based bioenergetic abnormality.⁴¹ These studies have suggested the presence of abnormality in mitochondrial function (in particular, recovery response to the energetic burden of repeat exercise) and a tendency for excessive intramuscular acidosis during, and in the recovery from, exercise. Intramuscular acidosis is a well-recognised contributing factor to muscle fatigue in the general population.⁴²

In PBC, the length of time that significant acidosis is present in muscle and the rate of recovery from that acidosis are strongly associated with the severity of fatigue that patients experience.^{43–45} The cumulative effect of a pro-acidotic effect and delays in recovery is well demonstrated using an integrative approach exploring the ‘area under the curve’ (AUC) for pH during exercise and in the recovery from exercise, which gives a measure of the scale of excess proton exposure in PBC and its association with fatigue (*Figure 1*). Recent studies using metabolomics approaches have shown lactate accumulation within tissues in PBC.⁴⁶ Our own unpublished study of anaerobic threshold in PBC patients (compared with age- and sex-matched patients with primary sclerosing cholangitis and sedentary control subjects), assessed using an incremental exercise approach, shows significantly lower anaerobic threshold values in PBC that cannot be accounted for by either deconditioning or by the presence of cholestatic liver disease per se.⁴⁷ These observations further support the presence of bioenergetic abnormality in PBC and critically do so using complementary experimental approaches. Taken together, these studies all point to excessive utilisation of anaerobic metabolism pathways in muscle in PBC [metabolism of pyruvate to lactate via the actions of lactate dehydrogenase rather than to acetyl coenzyme A via the actions of pyruvate dehydrogenase (PDH)]. This leads, we hypothesised, to excessive and inappropriate lactic acid accumulation in muscle in PBC and, in the absence of an effective adaptive response to it, fatigue. In this model, capacity to adapt to acid load is a physiological variant that is naturally protective from fatigue in some people and that can be induced by exercise therapy. The critical question is ‘why do PBC patients seemingly overutilise anaerobic metabolism pathways and how can we use that knowledge to treat their fatigue?’.

Primary biliary cirrhosis is characterised by autoimmune responses directed at bioenergetic enzymes

The obvious link between PBC as an autoimmune disease and PBC as a disease characterised by overutilisation of anaerobic pathways and associated fatigue is the characteristic autoantibody response in PBC, which is directed at PDH, the enzyme that controls entry into aerobic metabolism pathways. High-titre anti-PDH antibodies are present in > 95% of PBC patients and are able to fully block PDH function during in vitro assays.^{48,49} The basis of this inhibitory function (which is so consistent that one of the assays for anti-PDH in PBC utilises inhibitory capacity as a readout) is the specific reactivity of anti-PDH antibodies with a lipoic acid cofactor present on the E2 and E3 binding protein components of PDH, to which the principal autoepitopes have been localised, which is essential for enzymatic function and which is blocked by antibody binding.⁵⁰

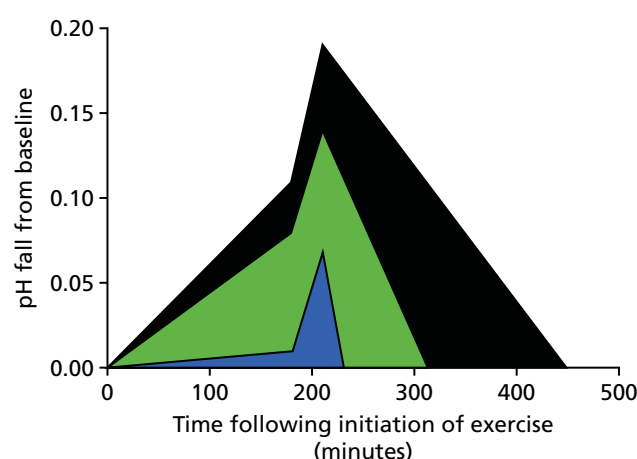


FIGURE 1 Area under receiver operating characteristic (AUROC) changes in pH vs. recovery time following exercise. Blue is normal, green is non-fatigued PBC patients and black is fatigued PBC patients.

Targeting autoreactive responses in primary biliary cirrhosis as a potential therapy for fatigue

Three strands of evidence support a potential direct role for anti-PDH antibody responses in bioenergetic abnormality and fatigue in PBC.

1. The mitochondrial bioenergetic abnormality in PBC is directly associated with the level of serum anti-PDH antibody, with median anti-PDH titre being > 200 -fold higher in patients with mitochondrial bioenergetic abnormality than in those without (9.9×10^5 vs. 4.1×10^3 ; $p < 0.05$).³⁹ At least part of the anti-PDH autoantibody response in PBC is directed at the lipoate component of PDH and is reactive with free lipoic acid in the circulation.⁵⁰ Given that free lipoic acid plays an important role in regulating PDH function (it activates PDH through inhibition of PDH-kinase), its consumption by autoantibody in PBC represents one potential mechanism for the bioenergetic effect of that antibody.⁵¹
2. Patients who are anti-PDH antibody positive but who have normal liver function ('pre-PBC' patients) experience fatigue of the same severity as 'classic' PBC patients, suggesting that fatigue is a feature of the presence of anti-PDH rather than PBC per se.⁵²
3. Fatigue is typically not improved following liver transplantation in PBC.^{38,53} Anti-PDH universally remains present at unchanged levels following liver transplantation in PBC.

A direct role for anti-PDH antibody in bioenergetic abnormality and fatigue was the justification for exploration of therapies able to deplete the B-cell compartment, exemplified by the anti-CD20 antibody rituximab (MabThera®, Roche Products Ltd) as a treatment for fatigue in PBC.

Rituximab in primary biliary cirrhosis

A pilot study performed in Canada exploring the use of rituximab in PBC (in 13 patients) provided proof of concept, showing that the agent is safe and well tolerated in patients and is associated with a clinically significant reduction in fatigue.⁵⁴ Fatigue severity was assessed using the Fatigue Severity Scale (potential range 9–63 points), with a fall being seen from pre treatment (median 36 points, range 11–59 points) to post treatment (median 29 points, range 12–55 points). Taking into account the floor value for the Fatigue Severity Scale, this represents a median fall in fatigue severity over 6 months of 26%. This compares with our own case–control study of fatigue in PBC, which suggests that fatigue severity in age- and sex-matched normal controls is 30% lower than in PBC patients, suggesting the potential for rituximab therapy to return PBC patients to close to normal with regard to their perceived fatigue.²⁸ However, this pilot study did not attempt to explore the mechanism of the effect and, as it did not use severe fatigue as an inclusion criterion, the extent of possible improvement for such patients is unclear. Moreover, the study was not optimised for the study of fatigue (fatigue was a secondary outcome and only some of the patients who participated had fatigue, potentially underestimating the clinical effect). Other evaluations of rituximab in PBC have focused exclusively on disease severity as an end point.⁵⁵ Both studies showed a sustained reduction in anti-PDH antibody levels of all isotypes, supporting the concept that rituximab has a beneficial effect on fatigue through depletion of PDH-reactive antibody. In all studies in PBC to date, rituximab has been found to be well tolerated.

Study objectives

The importance of severe fatigue in PBC and the current lack of treatments, the strong theoretical basis for the approach and the supportive pilot trial proof-of-concept data all, we believe, justified a formal clinical trial of rituximab targeting fatigue in PBC. Secondary outcome measures included an extended panel of patient-reported outcome measures (PROMs), [including the other domains of the PBC-40 markers (cognitive, itch, social, emotional and other symptoms), and tools for depressions, anxiety, sleep disturbance and autonomic dysfunction], assessment of bioenergetics function [including anaerobic threshold assessed using conventional cardiopulmonary exercise testing (CPET) and post-exercise muscle pH assessed using MR] and physical activity monitoring.^{17,56,57}

The primary objective of the study was to undertake a placebo-controlled trial to assess the efficacy of rituximab as a treatment for moderate to severe fatigue in PBC.

The secondary objectives of the study were to explore:

- the mechanism of any observed improvement in fatigue severity in relation to the identified associated mechanisms of fatigue related to bioenergetics abnormality in the cardiovascular system and peripheral muscle function in PBC
- the mechanisms underpinning physiological abnormality in PBC and their relationship to fatigue through the study of the baseline data for the study cohort
- any effects of rituximab therapy on biochemical parameters of disease severity in PBC.

Chapter 2 Methods

The text in this section is reproduced from the trial protocol we published in Jopson *et al.*³⁸ This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

Study conduct

This single-centre randomised controlled trial was conducted in the clinical research facility of the Newcastle upon Tyne Hospitals Foundation Trust, commencing 1 October 2012.³⁸ The final study visit was on 12 September 2016. The study was conducted in accordance with the principles of Good Clinical Practice (GCP) and received a favourable ethics opinion from the National Research Ethics Service Committee North East – Newcastle & North Tyneside 1 (12/NE/0095) on 16 May 2012 and a clinical trial authorisation from the Medicines and Healthcare products Regulatory Agency (MHRA) on 15 August 2012. The trial was managed through the Newcastle Clinical Trials Unit (NCTU) with a Trial Management Group, together with an independent Trial Steering Committee (TSC) and Data Monitoring and Ethics Committee (DMEC). Monitoring was performed by NCTU and the trial was audited by the sponsor, the Newcastle upon Tyne Hospitals NHS Foundation Trust.

The clinical trial protocol has been published (<https://njl-admin.nihr.ac.uk/document/download/2005806>; accessed September 2017) and the statistical analysis plan (see *Report Supplementary Material 1*) was finalised prior to the final analyses. All participants provided written informed consent prior to screening.

Patient and public involvement

Fatigue was identified by our patient group as a major research priority area. LIVERNORTH and the PBC Foundation were involved in the development of the trial concept and protocol. The final protocol was seen and approved by the patient groups. A patient representative (from LIVERNORTH) was a member of the TSC. The patient groups were updated throughout the course of the trial and played an important role in increasing awareness of the trial and thus in recruitment.

Trial design

This was a Phase II, single-centre, randomised controlled, double-blinded trial comparing rituximab with placebo in fatigued primary biliary cholangitis (cirrhosis) patients over 12 months.³⁸ Randomisation was in a 1 : 1 ratio.

Participants

Participants were all patients with a clinical diagnosis of definite or probable primary biliary cholangitis (cirrhosis); this was established using recognised epidemiological criteria:

- cholestatic liver biochemistry at disease outset [defined as elevation in the serum alkaline phosphatase (ALP) level or gamma-glutamyl transpeptidase (GGT)]
- associated autoantibody [anti-mitochondrial antibody (AMA) or PBC-associated anti-nuclear antibody by immunofluorescence or anti-PDH, anti-Gp210 or anti-Sp100] at a titre of $\geq 1 : 40$
- diagnostic or compatible liver biopsy.

Patients exhibiting all three criteria are defined as having definite PBC. Patients with two of the three criteria probably have PBC. Clinical practice has moved away from using liver biopsy to confirm diagnosis (because of the diagnostic accuracy of the combination of the other two parameters, which exceeds 95%), so the majority of patients in any clinical cohort fall into the probable disease category. Patients in whom ALP and GGT levels return to normal with therapy retain their diagnostic status, as current therapy approaches are disease suppressive not curative and biochemical deterioration is inevitable with universal discontinuation of therapy.

Inclusion criteria

- Patient had capacity to consent and provided written informed consent for participation in the study prior to any study-specific procedures.
- Moderate or severe fatigue as assessed using previously designated cut-off points of the PBC-40 fatigue domain (i.e. fatigue domain score of > 33).
- Presence of AMA (anti-PDH antibody) at a titre of $> 1 : 40$.
- Adequate haematological function (haemoglobin level of > 9 g/l), absolute neutrophil count of $> 1.5 \times 10^9$ /l and platelet count of $> 50 \times 10^9$ /l.
- Bilirubin level of ≤ 50 μ mol.
- International normalised ratio ≤ 1.5 .
- Child–Pugh score of < 7 .
- Eastern Cooperative Oncology Group performance status of < 2 .
- Adequate renal function: Cockcroft–Gault estimation of > 40 ml/minute.
- Women of childbearing potential had a negative pregnancy test prior to study entry and were using an adequate contraception method, which was continued for 3 months after completion of treatment. Acceptable forms of effective contraception included established use of oral, injected or implanted hormonal methods of contraception:
 - placement of an intrauterine device or intrauterine system
 - barrier methods of contraception – condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository
 - male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate)
 - true abstinence – when this was in line with the preferred and usual lifestyle of the subject
 - aged ≥ 18 years.

Exclusion criteria

- Advanced or decompensated disease (variceal bleed, hepatic encephalopathy or ascites).
- History or presence of other concomitant liver diseases (including hepatitis due to hepatitis B virus or hepatitis C virus or evidence of chronic viraemia on baseline screening), primary sclerosing cholangitis or biopsy-proven non-alcoholic steatohepatitis).
- Average alcohol ingestion of > 21 units per week (for males) or > 14 units per week (for females).
- Chronic sepsis or intercurrent condition likely to predispose the patient to chronic sepsis during the study.

- Previous treatment with B-cell-depleting therapy.
- Previous history of aberrant response or intolerance to immunological agents.
- Presence of significant untreated intercurrent medical condition itself associated with fatigue.
- Presence of significant risk of depressive illness [Hospital Anxiety and Depression Scale (HADS) score indicating cosiness].
- Current statin therapy or statin use within 3 months of enrolment.
- Ongoing participation in other clinical trials or exposure to any investigational agent 4 weeks prior to baseline or within five or fewer half-lives of the investigational drug.
- Major surgery within 4 weeks of study entry.
- Vaccination within 4 weeks of study entry; patients requiring seasonal flu or travel vaccines will be required to wait a minimum of 4 weeks post vaccination to enrol in the study.
- Pregnant or lactating women.
- Psychiatric or other disorder likely to have an impact on informed consent.
- Patient was unable and/or unwilling to comply with treatment and study instructions.
- Any other medical condition that, in the opinion of the investigator, would interfere with safe completion of the study.
- Hypersensitivity to the active substance (rituximab) or to any of the excipients [sodium citrate, polysorbate 80, sodium chloride, sodium hydroxide, hydrochloric acid and water (for infusion)] or to murine proteins.
- Active, severe infections (e.g. tuberculosis, sepsis or opportunistic infections).
- Known human immunodeficiency virus infection.
- Clinical history of latent tuberculosis infection unless the patient had completed adequate antibiotic prophylaxis.
- Aspartate transaminase (AST)/alanine transaminase (ALT) levels four times above the upper limit of normal.
- Severe immunocompromised state.
- Severe heart failure (New York Heart Association class IV) or severe uncontrolled cardiac disease.
- Malignancy (other than basal cell carcinoma) within the last 10 years.
- Demyelinating disease.
- Previous participation in this study.
- Any contraindication to rituximab therapy not covered by other exclusions.

Withdrawal criteria

The study protocol required that the study drug be discontinued if:

- the participant developed elevated serum ALT/AST levels four times above normal limits for each local laboratory
- the participant decided they no longer wished to continue
- cessation of study drug was recommended by the investigator.

Participants had the right to withdraw from the study at any time for any reason and without giving a reason. The investigator also had the right to withdraw patients from the study drug in the event of intercurrent illness, adverse events (AEs), serious adverse events (SAEs), suspected unexpected serious adverse reactions (SUSARs), protocol violations, cure, administrative reasons or other reasons. If a patient decided to withdraw from the study, all efforts were made to report the reason for withdrawal as thoroughly as possible. If a patient withdrew from the study drug only, efforts were made to continue to obtain follow-up data (with the permission of the patient).

Participants who wished to withdraw from study medication were asked to confirm whether or not they were still willing to provide:

- study-specific data at follow-up visits 5–19 [<https://njl-admin.nihr.ac.uk/document/download/2005806> (p. 33); accessed September 2017]
- end-of-study data as per visit 19, at the point of withdrawal
- questionnaire data collected as per routine clinical practice at annual follow-up visits.

If participants agreed to any of the above, they were asked to complete a confirmation of withdrawal form to document their decision.

Participants withdrawing from the study after receiving the second infusion were not replaced. In practice, all seven withdrawals from the study were replaced prior to study drug administration.

Setting

All screening, consent, treatment and follow-up assessments requiring patient attendance took place at the clinical research facility at the Royal Victoria Infirmary in Newcastle. Data recording took place in the same setting.

Screening and recruitment processes

- Information about the study was widely disseminated via all relevant patient groups as part of our patient and public involvement activity and through the Newcastle Clinical Service.
- Potential participants were initially identified through routine clinic outpatient appointments by their treating physician in the hosting NHS trust [the principal investigator (PI) or other member of the research team with documented, delegated responsibility]. Once the trial was under way, participant identification centres (PICs) were established across the region, allowing local-level identification of possible participants. During the course of the trial, the UK-PBC Stratified Medicine Programme (www.UK-PBC.com; accessed September 2015) was established; this developed a national database of PBC patients with phenotyping data and, if given, consent to be approached about follow-on studies (including trials), which were relevant given the patient phenotype. This approach identified potential participants, both in the host NHS trust and in PICs, aiding recruitment and opening up access to patients from across the UK. For patients who were identified through either PICs or the UK-PBC platform, all screening, recruitment and trial-related activity still took place at the single trial centre.
- In all cases, eligible participants were invited to participate by their consultant and the study was explained to them. A study patient information sheet was provided and the participants had a minimum of 48 hours to consider the information before giving written informed consent.
- The chief investigator or member of the research team with documented, delegated responsibility witnessed, signed and dated the written informed consent form.
- Patients were then invited to participate in the formal screening process.
- An eligibility screening form was completed by the investigator to document fulfilment of the entry criteria for all patients considered for the study and subsequently included or excluded. The log also ensured that potential participants were approached only once.
- The original signed consent form was retained in the investigator site file, with a copy in the clinical notes and a copy provided to the participant. The participant specifically consented for their general practitioner to be informed of their participation in the study.
- The right to refuse to participate without giving reasons was respected.
- Arrangements were in place for translation for potential participants who were non-English speaking. In practice, this option was not required.
- The screening assessments had to occur within the 4-week period prior to the baseline visit. If there was a delay beyond 4 weeks for any reason, patients underwent rescreening.

Intervention

The study intervention consisted of two treatments administered on study days 1 and 15, with patient conditioning immediately prior to each treatment. The protocol required a minimum interval of 2 weeks between the two treatments. A 2-day visit window was allowed for each dispensing visit. Study medication was prescribed by a study clinician in accordance with the protocol and administered to the patient by clinical staff according to local pharmacy policy. All interventions were administered in the presence of a

clinician. All participants had been encouraged to have adequate oral hydration in the 24 hours prior to attendance. Resuscitation equipment was immediately available during the infusion period. Blood pressure, heart rate and temperature were monitored during the infusions. Participants remained under observation in the clinical research facility for at least 2 hours after the infusion.

All unused study medication was stored in pharmacy until the end of the study, or until the trial manager had completed appropriate reconciliation. Documentation of prescribing, dispensing and return of study medication was maintained for study records.

Experimental intervention: rituximab therapy

The investigational medicinal product (IMP) used in the clinical trial was 1000 mg of intravenous (i.v.) rituximab (MabThera). This product was approved by the European Agency for the Evaluation of Medicinal Products (marketing authorisation number EU/1/98/067/002). The licence holder of MabThera is Roche Products Ltd. The product was supplied as vials containing 500 mg of rituximab at a concentration of 10 mg/ml. Supplies of rituximab were stored in a refrigerator at between 2 °C and 8 °C in a secure location in their original packaging in order to protect them from light. Supplies of rituximab were labelled as IMP in accordance with regulatory requirements. Storage and supply of the rituximab was delegated to the local pharmacy at the trial centre. Rituximab was released for use in the study once all the appropriate regulatory and governance approvals were in place. Patients randomised to receive rituximab therapy were given treatment at the infusion rates recommended for rheumatoid arthritis (RA) patients (i.e. for the first infusion at an initial rate of 50 mg/hour; after the first 30 minutes the rate could be increased in increments of 50 mg/hour every 30 minutes to a maximum rate of 400 mg/hour). Second doses were infused at an initial rate of 100 mg/hour and increased by 100 mg/hour at 30-minute intervals to a maximum of 400 mg/hour. Rituximab has a shelf life of 30 months. The prepared infusion solution of rituximab (MabThera) was physically and chemically stable for 24 hours at temperatures between 2 °C and 8 °C and subsequently for 12 hours at room temperature. Further details can be found in the MabThera Summary of Product Characteristics [<https://njl-admin.nihr.ac.uk/document/download/2005806> (p. 56)].

Control intervention: placebo infusion

Patients randomised to receive placebo received conditioning in addition to the control saline infusion. The control infusion was delivered in a double-blind manner to participants using the same placebo that was used in our RA studies, under the supervision of a clinician (as per the clinical research facility protocol).

Conditioning

In line with recommendations for the administration of rituximab in other conditions, patients received a conditioning regimen prior to the infusions of study medication on days 1 and 15. Given that this was a double-blinded trial, both arms received the conditioning regimen. This conditioning regimen was administered 30 minutes prior to infusion of study medication and to all patients, to maintain the double blind. The conditioning regimen comprised 1 g of paracetamol orally, 10 mg of i.v. chlorpheniramine and 100 mg of i.v. methylprednisolone. The paracetamol, chlorpheniramine and methylprednisolone were sourced locally.

Adverse reactions during treatment administration

During the infusion, patients were clinically monitored for the onset of clinical features of infusion-related reactions (IRRs), the most common AEs in rituximab usage.

The protocol stated that patients who developed evidence of severe IRRs, especially severe dyspnoea, bronchospasm or hypoxia, would have the infusion interrupted immediately, with restart of the infusion (at not more than half the rate at which the reaction took place) not taking place until complete resolution of all symptoms and normalisation of laboratory values and chest radiographic findings. If the same severe adverse reactions (ARs) occurred for a second time, the treatment would be stopped. Such reactions were not observed in the study and this protocol was therefore not invoked.

Mild or moderate IRRs were addressed by halving the rate of infusion. The infusion rate was increased again on improvement of symptoms. Paracetamol and/or non-steroidal anti-inflammatory drugs were allowed for the symptomatic management of IRRs.

Concomitant medication

Concomitant therapies were managed as per the MabThera Summary of Product Characteristics. A complete listing of all concomitant medication received during the treatment phase was recorded in the case report form (CRF).

For patients who were receiving UDCA therapy, the dose of UDCA could not be changed during the patients' 12-month participation in the study. No other disease-modifying agents could be introduced during the trial. Therapy aimed at reducing pruritus and its impact could be introduced if unavoidable at the discretion of the investigators; however, this proved unnecessary.

Live vaccines could not be administered during the study (see *Exclusion criteria*).

Outcomes

Primary outcome measure

The primary outcome variable was fatigue severity in PBC patients, assessed at 3 months using the fatigue domain of the PBC-40: a fully validated, psychometrically robust, disease-specific quality-of-life measure.²¹

Secondary outcome measures

The following secondary outcome measures were prespecified:

- The PBC-40 domain scores covering itch, cognitive, emotional, social and other symptom domains at 3, 6, 9 and 12 months.
- Physical activity assessed using 7-day physical activity monitoring.
- Perceived fatigue severity assessed using a self-completion fatigue diary.
- Daytime somnolence [as assessed using the Epworth Sleepiness Scale (ESS)⁵⁷], vasomotor autonomic symptoms [assessed using the Orthostatic Grading Scale (OGS)⁵⁸] and functional status [assessed using Patient-Reported Outcomes Measurement Information System Health Assessment Questionnaire (PROMIS HAQ), incorporating the Cognitive Failure Questionnaire (COGFAIL)²⁹]. Reduction in depressive and anxiety-related symptoms was assessed using the HADS.⁵⁹
- Serum anti-pyruvate dehydrogenase complex (PDC) antibody levels assessed using enzyme-linked immunosorbent assays (to confirm whether or not any clinical effect is directly related to antibody modulation).^{60,61}
- Peripheral muscle bioenergetic function on exercise (to confirm whether or not any clinical effect was directly related to effects on muscle bioenergetic function). Approaches used included MR spectroscopy assessment of muscle pH change during a fixed exercise protocol, time taken for recovery of pH to baseline following exercise, AUC for pH (a measure of total muscle pH exposure with exercise) and anaerobic threshold assessed using cardiopulmonary exercise testing (CPET).^{39,40,47}

Exploratory end points

- Biochemical parameters associated with severity of the liver disease in PBC (ALP), ALT, bilirubin and albumin.
 - Serum immunoglobulins (Igs) [total Ig, immunoglobulin G (IgG) and immunoglobulin M (IgM)].
- During the course of the study, a continuous variable model for the prediction of outcomes in PBC was described and validated. This used routinely collected clinical data and could be incorporated into the protocol as exploratory end points.⁶² Concern had also developed around second-line therapeutics in PBC and cardiovascular risk related to lipids. In the light of these changes, the following exploratory end point was added during the study:
- blood lipid levels.

Experimental assessments

Quality-of-life and symptom assessment tools

Fatigue severity for the primary end point was assessed using the PBC-40 fatigue domain. This is a validated, disease-specific and patient-derived quality-of-life measure. The fatigue domain score ranges from 11 to 55 (11 items scored 1–5), with higher values denoting worse fatigue.

Other symptom severity was assessed by the relevant domain of the PBC-40 (itch, cognitive, social, emotional and other symptoms). Functional status was assessed using the PROMIS HAQ to assess function. The PROMIS HAQ measures the functional and physical ability of the participants (covering, dressing, arising, eating, walking, hygiene, reach, grip and activity). The score is on a 0–100 scale with higher scores indicating worse functional ability. Anxiety and depression were assessed by HADS score (range 0–42). Daytime somnolence was assessed using the ESS (range 0–24), vasomotor autonomic symptoms using the OGS (range 0–20) and cognitive functionality using COGFAIL (range 0–100), where higher scores indicate worse outcome for all cases.

Fatigue diaries

Participant-held diaries were used to gather qualitative information on symptoms and functional ability. The diaries measured fatigue using a scale of 1–6 (1 = no fatigue and 6 = extreme fatigue). Participants were asked to complete the diaries six times during the study. They completed the diaries for a period of 1 week during the first week of each month at baseline and 1, 3, 6, 9 and 12 months. Participants returned the diaries at their final visit.

Magnetic resonance spectroscopy

Magnetic resonance data relating to muscle energetics and pH were acquired at baseline and at 12 weeks using a 3-T Intera Achieva scanner (Philips, Best and NL; Philips Medical System, Andover, MA, USA). The steps used for acquisition and analysis have been described, in detail, in published protocols.^{39,40} In summary, this involved controlled plantarflexion using a purpose-built exercise apparatus developed for operation within the MR imaging scanner. Participants performed two 180-second bouts of plantarflexion contractions at 25% and then at 35% of maximal voluntary contraction, with each bout preceded by 60 seconds of rest and followed by 390 seconds of recovery. Phosphorous spectra were collected at 10-second intervals. The minimum pH seen in the exercise and recovery period, the time required post exercise for pH to return to within 0.01 units of baseline levels (calculated as the sum for each individual for the three bouts to form a total pH recovery time) and the mean AUC for pH for the three exercise episodes, which reflected total acid exposure, were calculated.

Anaerobic threshold

Anaerobic threshold was assessed using conventional CPET. Participants cycled on a stationary ergometer (Lode BV, Groningen, the Netherlands) at between 60 and 70 revolutions per minute. The test was terminated either voluntarily by the participant or when they were unable to maintain a pedal frequency of 60 revolutions per minute. Expired air was collected at rest and during exercise using a breathing mask and analysed online using a gas analysis system (MetaLyzer II, CORTEX Biophysik GmbH, Walter-Kohn, Germany). Anaerobic threshold was determined using the computerised v-slope method at baseline and at the 12-week follow-up.

Physical activity levels

Participants completed physical activity monitoring using wrist-worn tri-axial GENEActiv accelerometers (Activinsights Ltd, Cambridgeshire, UK). The accelerometer was worn continuously on the right wrist for a period of 7 days in free-living conditions. Raw accelerometer data were processed in R studio (www.cran.r-project.org; accessed February 2017) using R-package GGIR version 1.2.8 (The R Foundation for Statistical Computing, Vienna, Austria). Patients were included in the analysis if they had worn the monitor for a minimum period of 5 days (with at least one of these days at the weekend). Only days with at least 22 hours of valid data were retained for further analysis. The first and last days of the raw

accelerometer measurement were excluded as they were influenced by the monitor distribution and collection procedure.

Following data processing and the exclusion of patients who did not meet the wear-time criteria, the average magnitude of wrist acceleration was calculated via metric Euclidean Norm Minus One (ENMO) as previously described.⁶³ The output from metric ENMO is in mg ($1 \text{ mg} = 0.001 \text{ g} = 0.001 \times 9.8 \text{ m/s}^2 = 0.001 \times \text{gravity}$). In addition, the average acceleration (mg) during the most active 5-hour period of each day was assessed pre versus post intervention.

Immunology

Quantification and phenotyping of total B-cell populations and B-cell subsets was undertaken using a standard fluorescence-activated cell analysis-based approach. Total B-cell levels in peripheral blood were evaluated using a direct immunofluorescence reagent (Fast Immune™ CD19/CD69/CD45, BD Biosciences, San Jose, CA, USA).

Anti-PDH antibody total and individual isotype levels were studied on day 0 and at the primary end point (12 weeks after therapy), using a well-established enzyme-linked immunosorbent assay developed within our research group.⁶¹

Biochemistry and haematology

Biochemical (liver biochemistry, electrolytes and lipids) and haematological (platelet count) assessments were undertaken using the routine clinical laboratories at the research centre. These are quality-assured clinical parameters. The UK-PBC risk score was calculated using the published formula.⁶² The values generated are percentage risks of death from liver-related causes or need for liver transplant at 10 years.

Randomisation

Randomisation was conducted by the NCTU web-based system in a 1 : 1 ratio using random-permuted blocks with random block length. The randomisation system generated a treatment number for each participant, which linked to the corresponding allocated study drug. The treatment number was then documented by the investigator on the trial prescription to ensure that the study pharmacist dispensed the correct study medication.

Patients in the study were randomised to receive either:

- rituximab therapy on days 1 and 15 (study drug) or
- placebo (normal saline) on days 1 and 15 (control).

Patients could be randomised into the study only by an authorised member of staff at the study research site, as detailed on the study delegation log. Patients could be randomised into the study once only.

Blinding

Assignment to either the rituximab or placebo arm was blinded to both the participant and investigators/assessor.

A code-break list was kept in the pharmacy and could be accessed only in an emergency (preferably with authorisation from the chief investigator). If the code was broken, details including the participant number, who broke the code, why and when were recorded and maintained in the site file. Code breaks were not routinely opened for participants who completed study treatment. One patient in the placebo arm was unblinded during the course of the study for emergency medical reasons (a SAE). The patient remained blinded.

Data management and monitoring

Study data were entered into a paper CRF and then uploaded by the data manager into the MACRO database system (Elsevier Ltd).

The study was managed through the NCTU. The Trial Management Group included the chief investigator/PI, senior trial manager, trial manager, trial statistician, database manager, clinical research fellow and other members of the trial team when applicable. NCTU provided day-to-day support for the site and provided training through the site initiation visit and routine monitoring visits. The PI was responsible for the day-to-day study conduct at the site.

Quality control was maintained through adherence to sponsor standard operating procedures, NCTU standard operating procedures, Newcastle Magnetic Resonance Centre standard operating procedures, the study protocol, the principles of GCP, the Research Governance Framework and clinical trial regulations.

Data Monitoring and Ethics Committee

An independent DMEC was appointed, the terms of reference of which were governed by a signed DMEC charter. It consisted of an independent chairperson, an independent expert clinician and an independent statistician and was convened to undertake independent review. The purpose of this committee was to monitor efficacy and safety end points. Only the independent DMEC members and the unblinded statistician had access to unblinded study data. The committee met eight times during the study.

Trial Steering Committee

A TSC was established to provide overall supervision of the study, the terms of reference of which were governed by a signed TSC charter. The TSC consisted of an independent chairperson, an independent clinician, an independent consumer representative, the chief investigator/PI, senior trial manager, trial manager and trial statistician. Representatives of the sponsor were invited to all TSC meetings. The committee met eight times during the study.

Study monitoring

Monitoring of study conduct and data collected was performed by a combination of central review and site monitoring visits to ensure that the study was conducted in accordance with GCP, the protocol and clinical trial regulations. Study site monitoring was undertaken by the trial manager. The main areas of focus included consent, SAEs, essential documents in the investigator site file and drug accountability and management.

Site monitoring included the following:

- All original consent forms were reviewed as part of the study file (the presence of a copy in the patient hospital notes was confirmed for 20% of participants).
- All original consent forms were compared against the study participant identification list.
- All reported SAEs were verified against treatment notes/medical records (source data verification).
- Essential documents were accessible in the investigator site file.
- Source data verification of primary end point data and eligibility data was undertaken for 20% of participants entered in the study.
- Drug accountability and management was checked; an unblinded monitor conducted pharmacy monitoring visits.

Central monitoring included the following:

- All applications for study authorisations and submissions of progress/safety reports were reviewed for accuracy and completeness, prior to submission.
- All documentation essential for study initiation was reviewed prior to site authorisation.

Statistical methods

Sample size

The study was planned to detect a mean change in PBC-40 fatigue domain score of 5 units at 3 months' follow-up (equating to an average of a 0.5-point change per question, a difference in PBC-40 score demonstrated in our population-based studies to be associated with significantly higher levels of social function).²⁴ Previous studies had shown a standard deviation (SD) of 8 units and a correlation of 0.6 between baseline and follow-up, so, using a power of 90% and a 5% significance level, this required outcome data from 35 participants per arm. A total of 78 participants (39 per arm) was planned to be recruited and randomised, assuming 10% attrition at 3-month follow-up.²¹

However, as recruitment was slower than expected, even after expanding recruitment beyond the north-east region of England, a revised sample size calculation was undertaken. Consequently, trial recruitment was extended by 6 months and the power of the trial was reduced to 80%, with a revised target sample size (with other estimates of parameters unchanged) of 58 participants (29 per arm). These changes to the design of the trial were agreed by the funder and submitted as a substantial amendment, which was accepted by both the Research Ethics Committee (REC) and MHRA during October 2015.

Statistical analysis

A complete statistical analysis plan was finalised and signed before datalock and unblinding (see *Report Supplementary Material 1*). It provides full details of statistical analyses, variables and outcomes.

All analyses were performed on the intention-to-treat (ITT) population. A pragmatic ITT approach was used in which patient outcomes were reported at their visit nearest the scheduled appointment date. Baseline characteristics of the study population were summarised separately within each randomised group.

The primary analysis was the PBC-40 fatigue domain. Descriptive statistics were reported at each time point (baseline and 3, 6, 9 and 12 months) in both arms. PBC-40 fatigue domain scores at 3 months were compared in the intervention and placebo groups using multiple linear regression with adjustment for baseline PBC-40 fatigue score, age in years, UK-PBC risk model score at 10 years and patient location (managed by the Newcastle Clinics for Research and Service in Themed Assessment centre for at least 1 year or not) at baseline. The results were reported as an adjusted difference in means with a 95% confidence interval (CI). Bootstrap estimation was used throughout. The time course of the comparison between intervention and control groups over the 12-month follow-up period using the time points above was assessed for PBC-40 fatigue domain using repeated measures analysis of variance (ANOVA).

The above analyses for PBC-40 fatigue score were repeated for secondary outcomes, namely:

- other PBC-40 domain scores (itch, cognitive, social, emotional and other symptoms)
- clinical symptom and functional capability scales (ESS, OGS, PROMIS HAQ, COGFAL, HADS and fatigue diary)
- metric ENMO activity monitoring (daily average and best 5 hours)
- bioenergetics outcomes (minimum pH post exercise, pH recovery half-time, pH fall with exercise, AUC for pH and anaerobic threshold)
- biological outcomes [serum immunoglobulin level (IgG and IgM)].

Euclidian Norm Minus One, bioenergetics and biological outcomes were collected only at baseline and 3 months, so no repeated measures analyses were performed.

Descriptive analyses were reported for other secondary outcomes listed below [additional repeated measures (ANOVA up to 12 months) were carried out for bilirubin and serum ALP outcomes]:

- full blood count (haemoglobin concentration, white blood cell count and platelet count)
- liver function test [prothrombin time, bilirubin level, ALP level, ALT level, AST level, albumin level, GGT level, activated partial thromboplastin time and C-reactive protein (CRP) concentration]
- lipid profile [low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglyceride]
- urea and electrolytes (U&E) (urea, creatinine, sodium and potassium concentrations)
- anti-mitochondrial antibody titre (expressed as number and percentage) and anti-PDC antibody level
- CD19 B-cell depletion.

Change in serum ALP level (at 3 and 12 months from baseline) was also measured and we classified a drop in level of > 15% or normalisation (within normal range) as being clinically significant. The number (%) of clinically significant results in each arm was reported.

Mechanistic analyses

Scatterplots and correlations between the change in PBC-40 fatigue domain score over 3 months and change in five bioenergetics outcomes were produced (see *Figure 4*).

All statistical analyses were performed using the statistical software package Stata® (version 14.1; StataCorp LP, College Station, TX, USA).

Safety

Monitoring for safety was undertaken in accordance with GCP. Full details and definitions of AEs, SAEs, ARs, serious adverse reactions (SARs), unexpected adverse reactions, suspected serious adverse reactions and SUSARs, as well as severity of AEs and expected ARs, are given in the trial protocol in section 19.1 (<https://njl-admin.nihr.ac.uk/document/download/2005806>). The local investigator responsible for the care of the participant was asked to assign causality, in accordance with the study protocol. Causality was defined as described in section 19.1 (<https://njl-admin.nihr.ac.uk/document/download/2005806>; accessed September 2017).

Protocol specifications

For purposes of the specific study protocol:

- All non-SARs were recorded at visits 2–19.
- Any SAEs were recorded throughout the duration of the trial until 12 months after trial therapy was stopped
- The SAEs excluded any pre-planned hospitalisations (e.g. elective surgery) that were not associated with clinical deterioration.
- The SAEs excluded routine treatment or monitoring of the studied indication that was not associated with any deterioration in condition.
- The SAEs excluded fatigue (primary outcome measure, already documented and monitored within study).

Adverse reactions during treatment administration

During the infusion, patients were monitored clinically for the onset of clinical features of IRRs (the most common AEs in rituximab usage). Patients who developed evidence of severe IRRs, especially severe dyspnoea, bronchospasm or hypoxia, would have had the reaction managed as outlined in *Adverse reactions during treatment administration*. In practice, this did not occur.

Recording and reporting of serious adverse events or reactions

All AEs were reported. Depending on the nature of the event, the reporting procedures below were followed (or would have been followed if relevant).

Adverse events (including adverse reactions)

All non-SAEs/SARs during drug treatment were reported on the study CRF and sent to the NCTU management team within 2 weeks to be entered into the MACRO database. Severity of AEs will be graded on a five-point scale (mild, moderate, severe, life-threatening, causing death). Any relationship between the AE and the treatment was assessed by the investigator at the site. The investigator at the site was responsible for managing all AEs/ARs according to the local protocol.

Serious adverse events and reactions (including suspected unexpected serious adverse reactions)

All SAEs, SARs and SUSARs during drug treatment were reported to the chief investigator within 24 hours of the site being notified of its occurrence. The initial report was made by via the secure fax to e-mail reporting system (Soho66, Sunderland UK), which generates a copy for the chief investigator, trial manager, senior trial manager and sponsor. In the case of incomplete information at the time of initial reporting, all appropriate information was provided as follow-up as soon as this became available. The relationship between the SAE and the treatment was assessed by the investigator at the site, as was the expected or unexpected nature of any SARs.

The MHRA and main REC were to be notified by the chief investigator or NCTU (on behalf of the sponsor) of all SUSARs occurring during the study in accordance with the following time lines: fatal and life-threatening within 7 days of notification, and non-life-threatening within 15 days. No SUSARs, however, occurred.

Chapter 3 Results

Participant flow

Figure 2 shows the Consolidated Standards of Reporting Trials (CONSORT) flow diagram giving details of recruitment, randomisation and retention in the trial.

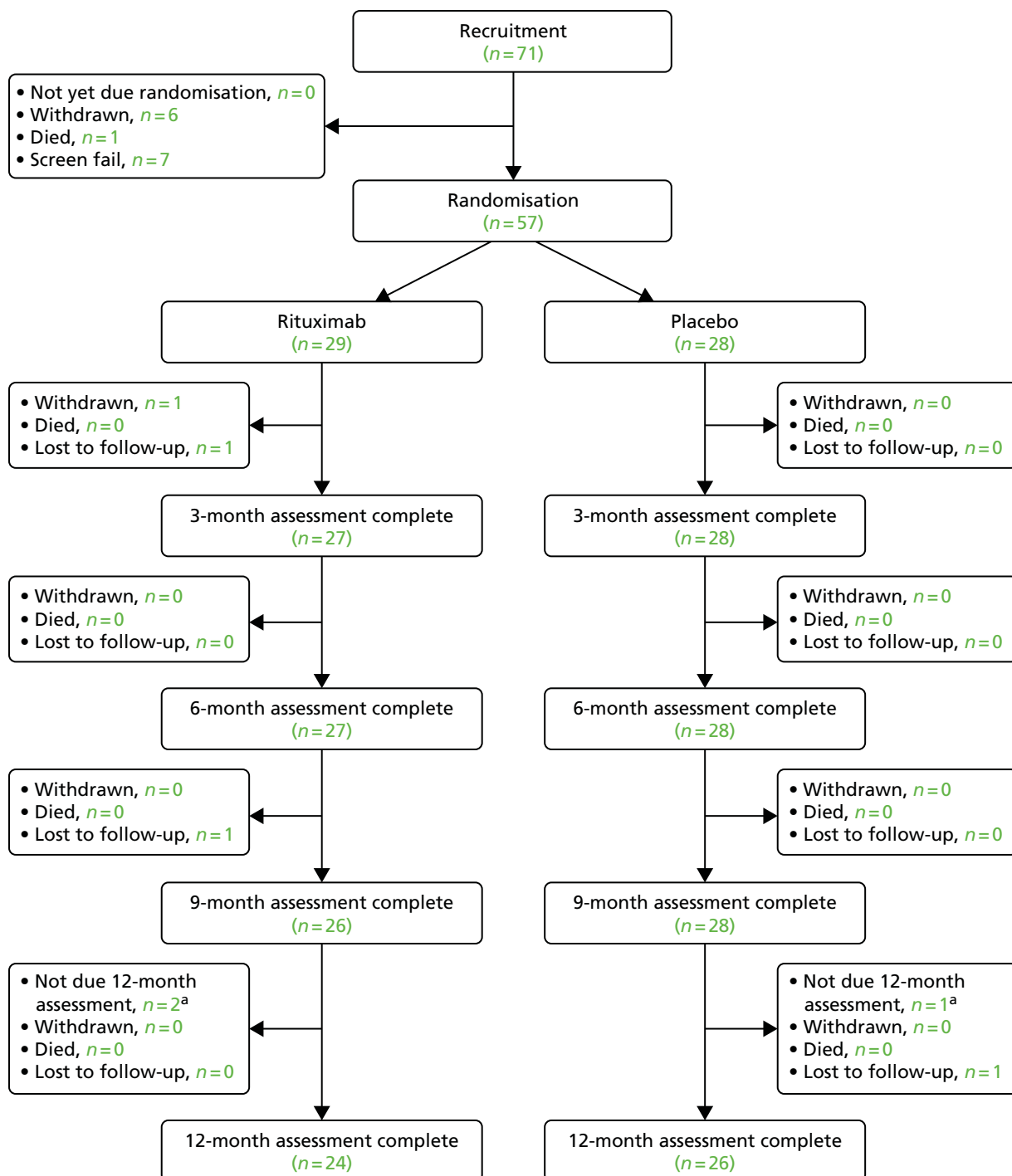


FIGURE 2 Recruitment flow chart. a, Trial was terminated early on 12 September 2016, so these patients forwent their 12-month follow-up visits that were due after this date.

Recruitment

The trial opened to recruitment on 1 October 2012, but due to slower than expected recruitment a no cost extension was obtained, resulting in the trial closing to recruitment on 1 October 2015 (*Figure 3*). The original target recruitment number was 78 participants, but this was later revised to 58 when recruitment was slower than expected. Seventy-one participants were recruited into the trial, but only 57 were randomised due to seven screen failures, six withdrawals and one death; the last patient was randomised on 18 November 2015. Rates of attrition were low, with 50 participants staying in the trial for the full 12 months' follow-up: 55 provided outcome data at 3 and 6 months and 54 provided outcome data at 9 months, with two participants being lost to follow-up and one withdrew. The trial was terminated early on 12 September 2016 following consultation with the MHRA and REC and with the endorsement of the DMEC and TSC. This early termination enabled the final analysis to start once all the 9-month follow-up data were complete, to meet the final draft report submission deadline. The early termination of the trial had no effect on the primary end point of the trial, which was measured at 3 months. It might have had an impact on the secondary objective of the trial, which was to look for any sustained effect of rituximab; however, the chief investigator did not foresee any impact as being significant, as it was anticipated that any sustained effects of rituximab would have worn off after 6 months, as seen in other indications (i.e. RA).³⁸ For this reason, three participants forwent their final 12-month follow-up visit and finished the study early after their 9-month follow-up visit.

Numbers analysed

Primary outcome analysis was performed on 29 patients in the rituximab group and 28 patients in the placebo group, with all participants remaining in their original randomisation groups. Some of the secondary outcome and additional analyses (in particular physical activity and fatigue diary, and muscle bioenergetics, respectively), were performed on smaller numbers because of issues with usability of the data (MR-based bioenergetics and physical activity monitoring) and patient compliance of a sufficient level to meet assessment duration requirements (fatigue diaries and physical activity monitoring).

Data were analysed using a pragmatic ITT approach throughout. A sensitivity analysis for a compliant ITT (± 1 week at visit 16 at 3 months) for the primary outcome was additionally specified. No participants completed their PBC-40 questionnaire, which included the primary fatigue domain outcome, within 83–97 days from the date of randomisation. When the more appropriate time between first infusion at visit 2 to completion of the PBC-40 questionnaire at 3 months was used, only 17 out of 55 participants had completed primary outcome questionnaire data within a week of the primary outcome collection target date. However, departures from this were generally quite small, with an overall median of 9 days in the combined arms [interquartile range (IQR) 7–15 days, range 0–29 days] from the due date, and similar between arms. The compliant ITT subgroup analysis was not performed because of the small numbers of those who were compliant (17/55 participants), even using the less stringent infusion time point rather than randomisation date.

Outcomes and estimation

Baseline data

The baseline clinical data are outlined in *Tables 1* and *2*. The study population was predominantly female, in keeping with the established demographic profile of PBC.¹² The distribution of UK-PBC risk score data at baseline suggested a low overall risk, in keeping with the established observation that fatigue severity in PBC is unrelated to the severity of underlying disease. Participants were predominantly of white ethnicity and most were not current smokers. More than two-thirds of participants had been managed at Newcastle Clinics for Research and Service in Themed Assessment centre for at least 1 year. The median age was ≈ 54 years and typical body mass index scores were relatively high, indicating that there were some potentially overweight participants in each arm. Alcohol consumption was modest in both arms and well below the recommended

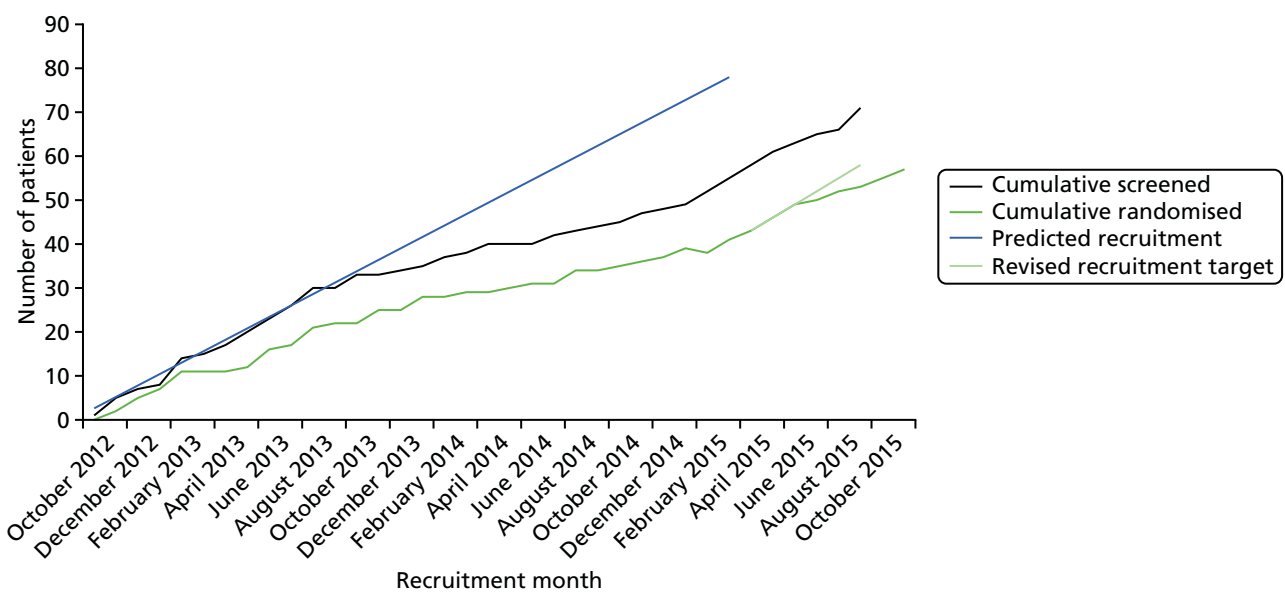


FIGURE 3 B-cell-depleting therapy (RiTuximab) as a treatment for fatigue in Primary Biliary Cirrhosis trial recruitment tracker.

TABLE 1 Baseline categorical characteristics by trial arm

Categorical variable	Trial arm, <i>n</i> (%)	
	Rituximab (<i>N</i> = 29)	Placebo (<i>N</i> = 28)
Sex: female	28 (96.5)	27 (96.5)
Ethnicity		
White	28 (96.5)	28 (100)
Non-white	1 (3.5)	0 (0)
Smoking status		
Never	16 (55)	12 (43)
Past	7 (24)	8 (28.5)
Current	6 (21)	8 (28.5)
Managed by the Newcastle Clinics for Research and Service in Themed Assessment centre for at least 1 year		
Yes	20 (74)	19 (68)
UDCA use: yes	24 (89)	27 (96.5)
If yes: responder	19 (79)	16 (59)

TABLE 2 Baseline continuous characteristics by trial arm

Continuous variable	Trial arm					
	Rituximab (<i>N</i> = 29)			Placebo (<i>N</i> = 28)		
	<i>n</i>	Median (IQR)	Range	<i>n</i>	Median (IQR)	Range
Age (years)	28	55.9 (48.8–60.0)	34.0–66.7	27	53.3 (49.9–58.8)	39.2–72.1
Alcohol consumption (units per week: drinkers)	17	4 (2–8)	1–12	9	4 (2–12)	1–14
Alcohol consumption (units per week: all)	29	1 (0–4)	0–12	28	0 (0–1.5)	0–14
Body mass index (kg/m ²)	29	28.7 (24.5–30.5)	19.8–40.5	28	26.7 (22.9–30.7)	18.7–43.5
UK-PBC risk score at 10 years	25	1.26 (0.94–1.74)	0.21–3.55	27	1.75 (1.12–3.04)	0.20–12.90

weekly intake. The distributions were well balanced between the intervention and control arms, with the exception of the proportion of UDCA responders and UK-PBC risk score at 10 years prediction.

Primary outcome

Data for the primary outcome, fatigue severity assessed at 3 months using the PBC-40 fatigue domain, are summarised in *Table 3*. The primary comparison was at 3 months, in which the mean PBC-40 fatigue scores were 36.2 in the rituximab arm and 38.1 in the placebo arm. This represents a decrease in score from baseline of around 5 units in each arm. The unadjusted mean PBC-40 fatigue score was 1.9 units lower in the rituximab arm at 3 months, but when the distribution of covariates was taken into account, the adjusted mean difference was –0.9 (95% CI –4.6 to 3.1). Although the 95% CIs are wide, they do not extend beyond the clinically important difference of 5.

There was little difference in mean fatigue scores between trial arms at any of the time points. The time course of the comparison between the intervention and control arms over the 12-month follow-up period

TABLE 3 The PBC-40 fatigue domain score at different time points by trial arm

Time point	Trial arm						Difference in PBC-40 (scale range 11–55) fatigue domain score at 3 months [adjusted difference in means ^b (rituximab – placebo) (95% CI)]
	Rituximab			Placebo			
	<i>n</i>	Mean PBC-40 (scale range 11–55) fatigue domain score ^a (SD)	Range in PBC-40 (scale range 11–55) fatigue domain score	<i>n</i>	Mean PBC-40 (scale range 11–55) fatigue domain score ^a (SD)	Range in PBC-40 (scale range 11–55) fatigue domain score	
Baseline	29	41.2 (5.5)	32–54	28	43.0 (5.9)	31–54	–0.9 (–4.6 to 3.1)
3 months	27	36.2 (8.4)	14–52	28	38.1 (8.7)	17–51	
6 months	27	36.6 (7.6)	19–51	28	39.9 (7.5)	18–54	
9 months	25	38.1 (8.3)	20–53	28	39.6 (8.6)	17–55	
12 months	23	39.5 (8.2)	26–54	26	39.5 (6.5)	30–53	
Repeated measures ANOVA up to 12 months between:							
Trial arms: <i>F</i> -test (<i>p</i> -value)			1.81 (0.18)				
Arms × time points: <i>F</i> -test (<i>p</i> -value)			0.41 (0.80)				
a Unadjusted comparison of means.							
b 95% CI for adjusted mean difference with adjustment for baseline measurements.							

was assessed for the primary outcome (PBC-40 fatigue domain) using repeated measures ANOVA at the time points of baseline and 3, 6, 9 and 12 months. There was no significant difference between the two trial arms ($F = 1.81$; $p = 0.18$) or in the interaction between arms over time ($F = 0.41$; $p = 0.80$).

Secondary outcomes

The prespecified secondary outcome data are presented in the following sections by category.

Immunological

Immunological analyses suggested that rituximab was effective in mediating depletion of B-cells, its primary proposed mode of action. Virtually complete B-cell depletion was seen in all rituximab-treated patients by the 3-month assessment point (*Table 4*). Assessment was through CD19, a surface marker distinct from the molecular target for the drug (CD20), excluding treatment-related surface marker change following drug action as an explanation. No depletion was seen in the placebo arm. Complete depletion was maintained at 6 months, with gradual repopulation to 50% of the baseline level by 12 months. These kinetics for cell numbers are in keeping with previous reports of rituximab therapy.^{54,55} The kinetics of fatigue reduction in the rituximab group mirrored the kinetics of B-cell depletion and recovery. However, in the placebo group the improvement in fatigue score was not mirrored with similar changes in kinetics of B-cell depletion and recovery.

Distribution of AMA titre outcome is given in *Table 5*. Reduction in the levels of anti-PDC antibody (the characteristic autoantibody of PBC) was also seen in the rituximab (but not the placebo) arm (*Tables 5* and *6*). Although reduction was seen at 3 months, peak reduction was seen at 6 months and sustained at 9 months. This correlates with the known mechanistic effects of rituximab.

A reduction in IgM fraction was seen at 3 months in the rituximab arm (*Table 7*). There was a small reduction in total Ig and IgG levels in the rituximab-treated group and to a lesser extent in the placebo arm. The incomplete nature of antibody depletion in contrast to the complete depletion of B-cells is in keeping with previous reports^{54,55} of rituximab therapy. The known cellular targeting (CD20) expression is seen at a much lower level in plasma cells than in earlier lineage B-cells, leading to more effective depletion of the latter.

TABLE 4 B-cell outcome up to 12 months by trial arm

Trial arm		Time point													
		Baseline		3 months		6 months		9 months		12 months					
		<i>n</i>	Median (IQR) in CD19 B-cell depletion (%) ^a	Range in CD19 B-cell depletion (%) ^a	<i>n</i>	Median (IQR) in CD19 B-cell depletion (%) ^a	Range in CD19 B-cell depletion (%) ^a	<i>n</i>	Median (IQR) in CD19 B-cell depletion (%) ^a	Range in CD19 B-cell depletion (%) ^a	<i>n</i>	Median (IQR) in CD19 B-cell depletion (%) ^a	Range in CD19 B-cell depletion (%) ^a		
Rituximab	27	11.9 (8.4–14.8)	3.75–27.9	26	0.1 (0.02–0.07)	0–0.49	25	0.1 (0.05–2.9)	0.02–3.95	24	2.3 (0.6–4.9)	0.2–12.2	24	5.3 (1.9–8.4)	1.2–16.3
Placebo	28	9.9 (6.7–13.9)	3.1–29.0	28	10.9 (6.88–14.1)	3.09–25.3	28	10.4 (7.3–13.8)	3.2–27.3	28	10.5 (8.2–14.6)	3.5–27.5	26	9.3 (7.7–14.7)	2.6–29.4

^a The value given is the number of CD19-positive cells (i.e. B-cells) as a percentage of the CD45-positive cells (i.e. total lymphocyte population). When effects of rituximab (anti-CD20) wore off, B-cells should have increased and the percentage should have gone up after depletion.

TABLE 5 Anti-mitochondrial antibody titre categories at 3 months by trial arm

Time point, AMA titre category	Trial arm, <i>n</i> (%)	
	Rituximab	Placebo
Baseline		
40	1 (5)	1 (5)
80	1 (5)	1 (5)
160	2 (9)	1 (5)
320	3 (14)	7 (33)
> 640	15 (68)	11 (52)
3 months		
40	2 (11)	1 (6)
80	0 (0)	2 (12)
160	4 (21)	0 (0)
320	5 (27)	4 (24)
> 640	8 (42)	10 (59)

TABLE 6 Anti-PDC antibody levels up to 12 months by trial arm

Time point	Trial arm					
	Rituximab			Placebo		
	<i>n</i>	Mean % from baseline (SD)	Range in % from baseline	<i>n</i>	Mean % from baseline (SD)	Range in % from baseline
3 months	27	70.4 (18.0)	29.5–101.5	28	92.6 (12.5)	60.9–110.3
6 months	26	54.4 (21.6)	12.9–93.2	28	96.7 (15.2)	45.5–124.8
9 months	25	59.9 (24.1)	14.9–121.4	28	99.5 (15.3)	61.8–140.9
12 months	24	78.5 (29.0)	22.2–144.4	26	102.2 (21.6)	77.6–186.3

Additional patient-reported outcome measures

Additional PROMs were used to assess other aspects of the patient experience (the five non-fatigue domains of the PBC-40 addressing cognitive, emotional, social and mixed symptoms along with itch), the ESS (daytime somnolence), OGS (autonomic dysfunction), COGFAIL (cognitive symptoms), HADS (depression and anxiety) and fatigue diary scores (*Table 8*). The unadjusted questionnaire score means showed little difference between trial arms at 3 months and, when the distribution of covariates was taken into account, there was still little or no difference between mean questionnaire scores. The 95% CIs were generally wide but there was no suggestion that the results were consistent with any clinically important differences.

Two observations are, however, worthy of note. In contrast to fatigue, in which some improvement over time was seen in both the rituximab and placebo arms, no improvement in the other measures was seen in either group. Any placebo effect was therefore restricted to fatigue, being absent even from the closely linked cognitive symptom domain. The second observation is that the mean ESS levels (12.3 and 13.3 at baseline in the two groups) were very high, showing a high level of sleep disturbance suggestive of central fatigue.

TABLE 7 Immunoglobulin-level outcomes up to 12 months by trial arm

Ig fraction	Trial arm								Difference in mean serum Ig levels at 3 months [adjusted difference in means (rituximab – placebo) (95% CI) ^b]
	Rituximab				Placebo				
	<i>n</i>	Mean serum Ig levels ^a (SD)	Median serum Ig levels ^a (IQR)	Range in serum Ig levels ^a	<i>n</i>	Mean serum Ig levels ^a (SD)	Median serum Ig levels ^a (IQR)	Range in serum Ig levels ^a	
Total IgG									
Baseline	22	12.5 (3.4)	11.6 (10.3–13.8)	7.5–22.2	22	11.8 (2.55)	11.7 (10.4–12.8)	7.6–17.9	0.98 (0.50 to 1.50)
3 months	20	11.0 (2.8)	10.8 (9.4–12.1)	6.1–18.6	19	11.2 (2.4)	10.9 (9.4–12)	7.7–17.1	
Total IgM									
Baseline	22	3.7 (2.1)	3.2 (2.3–5)	0.9–8.7	22	3.2 (2.1)	3.0 (1.8–4.3)	0.5–10.3	1.24 (0.76 to 1.88)
3 months	20	2.0 (1.0)	1.7 (1.3–2.7)	0.5–3.8	19	3.0 (2.2)	2.8 (1.5–3.8)	0.5–9.9	
Total Ig level									
Baseline	22	18.6 (5.0)	17.4 (15.4–19.7)	11.4–32	22	17.1 (4.4)	17 (14.4–19.1)	9.8–28.3	
3 months	20	15.16 (3.6)	14.2 (13.8–15.7)	8.6–26.3	19	16.2 (4.1)	15.3 (13.8–17.9)	10.2–27.3	
^a Unadjusted comparison of means.									
^b 95% CI for adjusted mean difference with adjustment for baseline measurement and predicted UK-PBC risk score at 10 years.									

TABLE 8 Questionnaire scores up to 12 months by trial arm

Questionnaire domain	Time point or period																
	Baseline			3 months				6 months		9 months		12 months		Up to 12 months; repeated measures ANOVA between:			
	n	Mean score (SD)	Range in score	n	Mean score ^a (SD)	Range in score	Adjusted difference in means ^b (rituximab – placebo) (95% CI)	n	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	Trial arms		Arms × time points	
														F-test	p-value	F-test	p-value
PBC-40 domain																	
Itch (0–15)																	
Rituximab	29	4.7 (2.7)	0–9	27	4.5 (2.8)	0–10	–0.8 (–0.3 to 1.8)	27	4.3 (2.7)	25	4.4 (3.1)	23	4.7 (3.8)	5.45	0.023	1.13	0.343
Placebo	28	6.5 (3.4)	0–15	28	5.5 (3.5)	0–15		28	6.4 (3.6)	28	6.4 (3.4)	26	6.2 (3.0)				
Cognitive (6–30)																	
Rituximab	28	20.0 (4.2)	12–30	27	19.3 (4.0)	9–27	0.8 (–1.5 to 3.3)	27	19.4 (3.2)	25	19.2 (4.5)	22	19.6 (3.0)	0.16	0.690	0.22	0.928
Placebo	27	19.8 (5.3)	7–28	28	18.3 (5.7)	6–30		28	19.3 (5.8)	28	19.0 (4.8)	26	20.0 (4.7)				
Social (8–50)																	
Rituximab	28	33.0 (7.6)	21–50	27	32.6 (7.1)	16–47	1.5 (–1.6 to 5.0)	27	31.9 (8.3)	25	32.2 (7.6)	28	32.5 (7.6)	0.00	0.945	1.07	0.374
Placebo	27	33.3 (6.4)	23–43	28	30.8 (7.4)	15–45		28	32.9 (8.1)	28	31.3 (8.1)	26	30.3 (7.5)				
Emotional (3–15)																	
Rituximab	28	9.4 (3.3)	4–15	27	9.3 (3.3)	4–15	0.6 (–0.9 to 2.0)	27	9.3 (3.5)	25	9.1 (3.5)	22	9.0 (2.9)	0.26	0.609	0.67	0.617
Placebo	26	9.8 (2.9)	3–15	28	9.0 (3.2)	3–15		28	9.6 (3.6)	28	9.6 (3.4)	25	9.1 (3.4)				
Other symptoms (6–35)																	
Rituximab	29	17.6 (4.4)	7–27	27	16.9 (3.7)	8–23	0.2 (–1.2 to 1.5)	27	18.2 (4.9)	25	17.8 (5.1)	23	18.5 (4.7)	0.01	0.908	0.59	0.671
Placebo	28	18.5 (3.4)	10–26	28	17.6 (3.3)	10–23		28	18.2 (3.5)	28	18.1 (4.0)	26	18.9 (4.7)				
ESS (0–24)																	
Rituximab	28	12.3 (5.5)	0–24	26	10.9 (6.1)	1–24	–0.7 (–3.0 to 1.6)	27	11.4 (5.4)	25	10.8 (5.9)	24	11.2 (5.8)	1.51	0.224	0.80	0.523
Placebo	27	13.3 (5.0)	4–24	28	11.9 (5.1)	3–24		27	12.6 (5.9)	28	13.3 (5.2)	26	11.8 (4.5)				
continued																	

continued

TABLE 8 Questionnaire scores up to 12 months by trial arm (*continued*)

Questionnaire domain	Time point or period																
	Baseline			3 months				6 months		9 months		12 months		Up to 12 months; repeated measures ANOVA between:			
	n	Mean score (SD)	Range in score	n	Mean score ^a (SD)	Range in score	Adjusted difference in means ^b (rituximab – placebo) (95% CI)	n	Mean score (SD)	n	Mean score (SD)	n	Mean score (SD)	Trial arms		Arms × time points	
														F-test	p-value	F-test	p-value
OGS (0–20)																	
Rituximab	28	4.1 (3.1)	0–13	26	4.7 (4.0)	0–15	0.4 (–1.0 to 1.9)	25	5.5 (4.1)	24	5.7 (4.0)	23	4.7 (4.1)	0.28	0.601	0.91	0.459
Placebo	26	4.9 (3.3)	0–12	28	4.8 (4.1)	0–15		26	5.7 (4.3)	28	5.4 (3.8)	25	5.8 (4.1)				
PROMIS HAQ (0–100)																	
Rituximab	29	9.4 (8.7)	0–40	26	13.7 (14.0)	0–56.3	1.4 (–3.3 to 7.3)	27	11.2 (11.2)	25	12.4 (10.6)	24	12.6 (11.1)	1.65	0.205	1.25	0.290
Placebo	28	12.0 (11.2)	0–50	28	14.0 (13.1)	0–50		27	16.5 (14.5)	28	17.0 (17.1)	26	16.5 (15.2)				
COGFAIL (0–100)																	
Rituximab	27	57.3 (13.6)	31–81	25	59.7 (14.7)	32–82	1.7 (–5.7 to 8.6)	27	57.6 (15.7)	24	57.1 (16.2)	22	57.8 (14.7)	0.96	0.331	0.50	0.739
Placebo	26	52.2 (16.2)	17–77	28	52.9 (17.2)	22–93		26	54.2 (19.2)	28	54.4 (20.1)	26	55.7 (17.3)				
HADS (0–42)																	
Rituximab	28	13.5 (6.7)	5–29	22	12.4 (6.5)	3–25	–1.1 (–3.8 to 1.2)	25	13.8 (7.9)	24	13.7 (8.6)	24	13.0 (6.6)	0.03	0.872	0.52	0.723
Placebo	27	12.1 (7.4)	4–30	28	12.3 (6.7)	2–31		25	14.0 (7.9)	26	14.2 (7.5)	25	13.0 (7.6)				
Fatigue diary (1–6)																	
Rituximab	18	3.79 (0.80)	1.4–4.9	18	3.51 (1.04)	1–5.14	0.1 (–0.4 to 0.7)	17	3.34 (1.07)	18	3.48 (1.15)	16	3.80 (1.09)	0.58	0.453	0.28	0.923
Placebo	20	3.95 (0.49)	2.86–5	20	3.59 (1.11)	1.14–5		20	3.79 (1.10)	20	3.61 (1.07)	18	3.77 (0.97)				
^a Unadjusted comparison of means.																	
^b 95% CI for adjusted mean difference with adjustment for baseline questionnaire score. PBC-40 itch domain was additionally adjusted for UK-PBC risk score at a 10-year prediction.																	

Physical activity

Physical activity in the study group was assessed using wrist accelerometry, with values derived over a minimum of 5 days for the overall mean activity and the best 5-hour period (*Table 9*). There were considerable missing data in each arm [there were 16/57 (28%) and 23/55 (42%) participants with missing data at baseline and 3 months, respectively]. Missing data were typically a result of patients not wearing the activity monitor for sufficient time during the assessment period. ENMO outcomes differed little between arms at 3 months, but the exercise parameters were slightly higher in the rituximab arm at baseline. After adjustment for baseline values, the mean exercise parameters were slightly lower in the rituximab arm, but there was little indication of a large and meaningful difference.

Bioenergetics

We used a combination of MR spectroscopy of muscle and anaerobic threshold assessment using CPET to calculate changes in pH and recovery time post exercise (*Table 10*).

In keeping with previous reports,⁴⁷ anaerobic threshold at baseline was low in the PBC patient group. The mean values rose in the rituximab arm from baseline to 3-month follow-up, with no change in the placebo arm. The anaerobic threshold score at 3 months was higher in the rituximab arm than in the placebo arm, which was also the case after adjustment for baseline values (adjusted difference 1.41, 95% CI 0.03 to 2.80).

In keeping with our previous reported findings, although muscle pH values at rest were within the normal range, the minimum pH following the specific exercise task was highly variable among the PBC patients, with substantial acidosis seen in some patients. Minimum pH in muscle seen following exercise was slightly higher at 3 months than at baseline, but this was seen in both trial arms. No reduction in the time taken to recover to baseline pH after exercise and no reduction in the AUC for pH (a factor combining the degree of acidosis and the length of time taken to recover to the baseline level and an estimate therefore of degree of muscle intracellular acid exposure) were seen in either trial arm. Comparison of bioenergetics outcomes between trial arms shows that the adjusted differences in means at 3 months were all small with relatively narrow 95% CIs for the pH parameters; however, the 95% CI was wide for the comparison of AUC.

TABLE 9 Euclidian Norm Minus One outcomes at baseline and 3 months by trial arm

Time point	Trial arm						Difference at 3 months [adjusted difference in means (rituximab – placebo) (95% CI) ^b]
	Rituximab			Placebo			
	<i>n</i>	Mean (SD) ENMO outcome ^a	Range in ENMO outcome	<i>n</i>	Mean (SD) ENMO outcome ^a	Range in ENMO outcome	
Baseline (pre)							
Average ENMO	18	26.7 (4.8)	19.6–36.7	23	24.5 (7.6)	14.0–46.7	N/A
ENMO best 5 hours	18	51.6 (11.6)	34.6–70.6	23	46.6 (16.9)	24.9–95.3	N/A
3 months (post)							
Average ENMO	16	25.3 (4.9)	17.2–33.8	16	25.3 (7.7)	12.4–43.2	–1.19 (–3.44 to 1.23)
ENMO best 5 hours	16	47.8 (10.8)	34.3–73.5	16	46.0 (15.3)	18.0–80.2	–1.33 (–6.04 to 3.09)
N/A, not applicable.							
a Unadjusted comparison of means.							
b 95% CI for adjusted mean difference with adjustment for baseline ENMO measurements.							

TABLE 10 Bioenergetics outcomes at baseline and 3 months by trial arm

Bioenergetics outcome, time point	Trial arm								Difference at 3 months [adjusted difference in means (rituximab – placebo) (95% CI) ^b]
	Rituximab				Placebo				
	<i>n</i>	Mean ^a (SD)	Median (IQR)	Range	<i>n</i>	Mean ^a (SD)	Median (IQR)	Range	
pH resting									
Baseline	18	7.024 (0.023)	7.01 (7.009–7.040)	6.991–7.074	23	7.015 (0.022)	7.01 (6.996–7.032)	6.977–7.053	N/A
3 months	19	7.023 (0.019)	7.022 (7.012–7.037)	6.993–7.063	24	7.017 (0.021)	7.017 (7.007–7.029)	6.970–7.058	
Minimum pH post exercise									
Baseline	18	6.75 (0.40)	6.93 (6.67–6.99)	5.73–7.04	23	6.84 (0.36)	6.98 (6.80–7.00)	5.28–7.01	–0.1 (–0.29 to 0.04)
3 months	19	6.81 (0.32)	6.97 (6.64–7.01)	5.82–7.04	24	6.90 (0.11)	6.95 (6.81–6.98)	6.61–7.07	
Recovery time (seconds)									
Baseline	18	24.6 (32.0)	11.5 (0–40)	0–120	23	60.8 (96.9)	20 (0–70)	0–310	13.5 (–16.9 to 35.0)
3 months	19	63.7 (78.6)	50 (0–80)	0–290	24	52.1 (86.3)	20 (0–50)	0–320	
pH fall with exercise									
Baseline	18	0.28 (0.39)	0.09 (0.03–0.40)	0.01–1.27	24	0.1 (0.36)	0.04 (0.02–0.21)	0–1.76	0.10 (–0.04 to 0.30)
3 months	19	0.21 (0.31)	0.05 (0.02–0.38)	0.004–1.19	24	0.12 (0.11)	0.09 (0.02–0.20)	0.01–0.40	
AUC for pH									
Baseline	18	59.60 (82.7)	19.96 (5.08–84.31)	2.41–261.7	24	41.76 (84.2)	8.95 (4.17–48.7)	0–405.8	26.9 (–11.6 to 75.74)
3 months	19	56.45 (84.47)	11.76 (3.42–112.7)	0.66–308.99	24	31.51 (38.03)	20.64 (3.39–43.06)	0.93–153.4	
Anaerobic threshold									
Baseline	28	11.32 (2.70)	11.5 (9.5–13)	7–18	28	10.89 (2.20)	11 (9–13)	7–15	1.41 (0.03 to 2.80)
3 months	23	12.70 (2.88)	13 (10–15)	8–19	22	10.86 (2.49)	10 (9–13)	7–17	

N/A, not applicable.

a Unadjusted comparison of means.

b 95% CI for adjusted mean difference with adjustment for baseline measurement. AUC and recovery time analyses were additionally adjusted for age in years at randomisation and recovery time also included predicted UK-PBC risk score at 10 years in final model.

Plots of the changes in bioenergetics outcomes against changes in fatigue scores are shown in *Figure 4* and the correlations are given in *Table 11*. The changes were frequently close to zero, with the exception of the anaerobic threshold: there were more non-zero changes in the rituximab arm, but these were not consistently in one direction. The correlation coefficients were all close to zero. There was little evidence that improved fatigue scores are associated with better bioenergetics outcomes. However, sample sizes in each arm were small and correlations of combined arms are based on just 41–45 participants, depending on outcome measure.

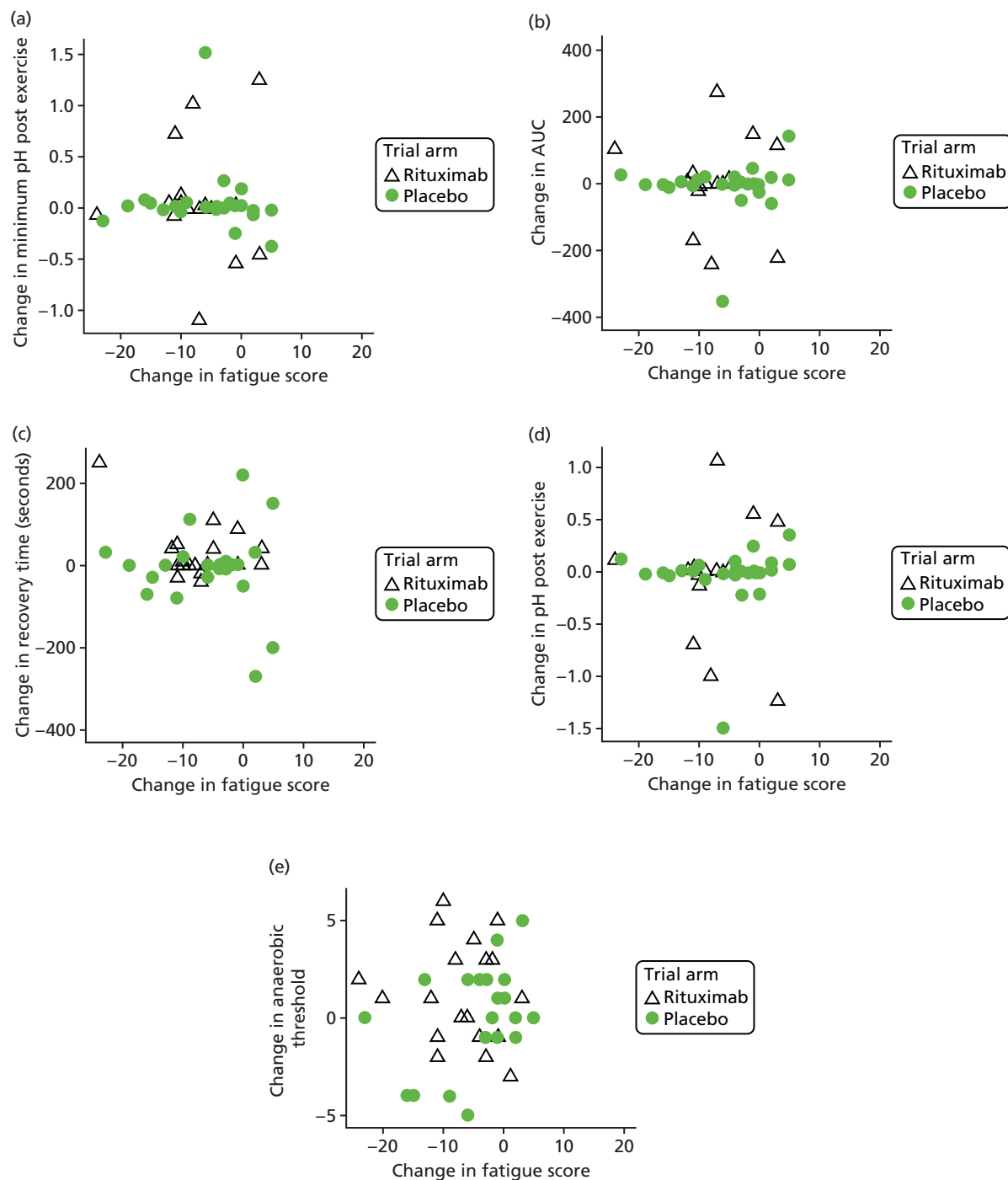


FIGURE 4 Change in muscle bioenergetics outcomes compared with change in fatigue score. (a) Change in minimum pH post exercise; (b) change in AUC; (c) change in recovery time; (d) change in pH post exercise; and (e) change in anaerobic threshold.

TABLE 11 Correlations (Pearson's *r*) between changes in PBC-40 fatigue domain score and changes in bioenergetics outcomes by trial arm

Change in PBC-40 fatigue score, correlation with:	Trial arm								
	Rituximab			Placebo			Overall		
	<i>n</i>	Correlation	95% CI	<i>n</i>	Correlation	95% CI	<i>n</i>	Correlation	95% CI
Minimum pH post exercise	18	0.034	−0.440 to 0.493	23	−0.062	−0.463 to 0.359	41	−0.013	−0.319 to 0.296
pH fall with exercise	18	−0.044	−0.501 to 0.432	24	0.065	−0.347 to 0.457	42	0.011	−0.294 to 0.314
Recovery time (seconds)	18	−0.443	−0.754 to 0.030	23	−0.059	−0.460 to 0.362	41	−0.194	−0.474 to 0.121
AUC for pH	18	−0.082	−0.529 to 0.400	24	0.075	−0.338 to 0.465	42	−0.007	−0.310 to 0.297
Anaerobic threshold	23	−0.119	−0.507 to 0.308	22	0.416	−0.006 to 0.713	45	0.117	−0.183 to 0.396

Ancillary analyses

Impact on biochemical disease parameters

The B-cell-depleting therapy (Rituximab) as a treatment for fatigue in Primary Biliary Cirrhosis (RITPBC) trial was not designed or powered to explore the impact of the drug on liver injury. The cohort of treated patients, however, exceeds the total world experience of PBC patients treated with biological agents to date. Furthermore, because the primary target was fatigue, a non-stage-associated symptom, treated patients were typically early in the disease course. This contrasts with other trials of biological agents, which have typically been performed in patients with advanced/aggressive disease. The RITPBC trial therefore presents a unique insight into the impact of rituximab therapy in early disease, which could inform future trials of disease-modifying therapy. It is for this reason that the biochemical results are reported in *Tables 12–15*.

Baseline biochemical parameters were typical of early-stage PBC, with median ALP values of 136 U/l and 131 U/l in the rituximab and placebo groups, respectively. Subsequent to the design of the trial, definitions have been developed for the risk status for PBC patients. These include dichotomous scoring systems such as the Toronto Criteria and continuous variable scoring systems such as the UK-PBC risk score.^{4,62} Over the 12-month period of assessment in the placebo group, a typical PBC natural history pattern was seen, with small increases in the ALT and bilirubin levels (the latter of which were within the normal range). There were no significant differences between trial arms or in the interaction between arms over time in repeated measure ANOVA up to 12 months for bilirubin, but there were significant differences between trial arms ($F = 6.17$; $p = 0.016$) and in the interaction between arms over time ($F = 2.91$; $p = 0.023$) for ALP outcome (see *Tables 12 and 13*).

TABLE 12 Full blood count and liver function test laboratory parameters at baseline and 3 months by trial arm

Laboratory parameter	Trial arm															
	Rituximab								Placebo							
	Baseline				3 months				Baseline				3 months			
	n	Mean (SD)	Median (IQR)	Range	n	Mean (SD)	Median (IQR)	Range	n	Mean (SD)	Median (IQR)	Range	n	Mean (SD)	Median (IQR)	Range
Full blood count																
Haemoglobin (g/l)	29	127.8 (10.9)	126 (122–134)	104–152	27	127.9 (10.7)	128 (122–134)	96–153	28	131.0 (8.2)	132 (126–136)	110–151	28	131.8 (9.0)	132 (124.5–137.5)	116–148
WBC (10 ⁹ /l)	29	5.8 (1.5)	5.5 (4.3–7.1)	3.7–8.6	27	5.7 (1.9)	5.3 (4.5–6.5)	3.4–11.8	28	6.3 (1.4)	6.3 (5.3–7.2)	3.6–8.8	28	6.3 (1.6)	6.2 (5.3–7.1)	2.9–9.9
Platelet count (10 ⁹ /l)	29	274.6 (59.1)	272 (240–316)	132–397	27	277.7 (64.1)	260 (230–322)	162–444	28	276.9 (91.8)	255 (206–343.5)	105–444	28	282.0 (100.7)	262 (212.5–334.5)	89–507
Liver function test																
PT (seconds)	28	10.9 (0.6)	11 (10.5–11)	10–12	19	10.6 (0.7)	11 (10–11)	9–12	27	11.0 (0.7)	11 (11–11)	10–13	18	10.7 (0.8)	11 (10–11)	10–12
Bilirubin (μmol/l)	29	7.3 (3.9)	7 (6–8)	3–22	27	6.9 (2.9)	6 (5–8)	3–15	28	7.4 (3.7)	6 (5–9)	3–21	28	7.6 (3.3)	7 (6.5–8)	3–21
ALP (U/l)	29	156.6 (72.4)	136 (102–197)	52–339	27	120.1 (65.0)	106 (72–145)	44–307	28	217.1 (166.8)	131 (101–302.5)	45–642	28	223.9 (175.9)	137 (100–335.5)	44–682
ALT (U/l)	29	48.4 (33.0)	34 (28–60)	10–150	27	39.0 (34.8)	29.0 (17–47)	7–178	28	50.2 (29.2)	42 (30.5–63)	11–135	28	52.0 (43.7)	37.8 (27–63.3)	13–231
AST (U/l)	28	45.0 (25.8)	36 (30.5–51)	19–128	27	38.1 (27.6)	27.4 (23–36)	17–125	24	46.6 (24.9)	38 (30–58.5)	14–105	27	41.8 (25.0)	33 (24–53)	16–104.2
Albumin (g/l)	29	44.7 (2.1)	44 (44–46)	40–49	27	45.1 (1.9)	45 (44–46)	41–48	28	43.1 (2.2)	43 (42–44.5)	37–47	28	43.6 (2.8)	43.5 (41.5–45)	39–51
GGT (U/l)	28	140.8 (157.7)	85 (35–172)	22–559	26	123.0 (164.0)	44 (22–120)	13–688	28	197.4 (224.5)	121.5 (46.5–215.5)	12–853	25	182.4 (206.0)	87 (36–255)	11–733
APTT (seconds)	28	34.0 (5.4)	33 (31–35.5)	26–52	19	33.7 (4.3)	33 (31–35)	28–45	27	32.8 (3.2)	32 (30–35)	28–43	18	32.1 (3.7)	32 (31–33)	25–41
CRP (mg/l)	27	6.9 (5.5)	5 (56)	5–33	23	6.2 (4.0)	5 (5–5)	5–23	27	7.2 (4.8)	5 (5–6)	5–21	26	6.0 (2.7)	5 (5–5)	5–16
APTT, activated partial thromboplastin time; PT, prothrombin time; U, unit; WBC, white blood cell.																

TABLE 13 Full blood count and liver function test laboratory parameters at 6, 9 and 12 months by trial arm

Laboratory parameter	Trial arm														
	Rituximab									Placebo					
	6 months			9 months			12 months			6 months			9 months		
	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]
Haemoglobin (g/l)	25	127.1 (14.5)	128 (122–135) [84–148]	25	128.8 (14.1)	129 (121–142) [86–147]	24	128.8 (12.8)	131.5 (125–136) [83–148]	28	132.7 (8.4)	127.5 (127.5–137) [119–157]	28	133.7 (7.4)	134.5 (127–139.5) [121–146]
WBCs (10 ⁹ /l)	25	5.9 (2.6)	5.3 (4.4–6.8) [2.4–13.3]	25	5.8 (1.8)	5.8 (4.7–6.4) [2.9–11.9]	24	6.0 (2.3)	5.8 (4.5–6.8) [3.2–12.7]	28	6.5 (1.8)	6.3 (5.6–7.3) [3.6–12.5]	28	6.5 (1.7)	6.1 (5.2–7.8) [4.1–11.6]
Platelet count (10 ⁹ /l)	25	274.0 (70.8)	263.0 (228–300) [167–457]	25	274.4 (63.4)	263 (232–291) [168–446]	24	278.5 (76.5)	283 (235–309) [100–482]	28	274.4 (92.9)	252 (219.5–317) [90–485]	28	274 (96.8)	252.5 (212–321) [100–518]
Bilirubin ^a (μmol/l)	27	7.5 (3.5)	7 (6–8) [3–21]	25	6.6 (2.5)	6 (5–7) [4–15]	24	7.2 (3.5)	7 (5–8) [3–19]	28	7.8 (3.4)	7 (5–9) [4–17]	28	8.1 (3.5)	7.5 (5–10) [3–18]
ALP ^b (U/l)	27	128.5 (71.0)	107 (82–164) [44–328]	25	137.9 (72.0)	108 (93–173) [46–330]	24	148.8 (86.2)	129.5 (89–181.5) [42–407]	28	225.7 (170.5)	157 (105–336) [47–665]	28	244.4 (195.6)	167 (106–367.5) [28–758]
ALT (U/l)	27	38.1 (30.5)	26 (18–50) [13–136]	25	48.0 (39.5)	31.4 (24.2–68) [14–195.7]	24	52.8 (46.8)	36.2 (26.5–64) [11–236.5]	28	53.2 (37.9)	46.2 (26.1–59.5) [15–181]	28	62.0 (54.4)	47.1 (29.5–64.2) [13–237.7]
AST (U/l)	23	36.6 (23.8)	25 (20–48) [18–106]	25	41.7 (34.0)	29 (22–49) [11.2–182]	21	49.8 (59.3)	29.4 (25–49) [20.3–295.2]	25	44.4 (27.4)	3 (28.4–47) [18–129]	26	47.6 (30.4)	35.2 (25–56) [16.3–110]
Albumin (g/l)	27	45.1 (2.1)	45 (44–46) [39–48]	25	45 (2.3)	44 (44–46) [41–51]	24	45 (1.9)	45 (44–46.5) [41–48]	28	43.3 (2.6)	43.5 (42–45) [38–48]	28	43.6 (2.7)	44 (43–45.5) [36–47]
GGT (U/l)	26	137.0 (184.4)	49.5 (25–109) [16–790]	22	147.2 (187.5)	45 (28–221) [16–721]	21	126.8 (150.0)	872 (2–167) [18–557]	25	167.6 (200.2)	123 (33–184) [10–907]	24	184.4 (221.9)	109 (39.5–240) [13–997]
CRP (mg/l)	24	6.6 (3.6)	5 (5–5) [5–18]	23	7.1 (4.9)	5 (5–6) [5–25]	22	9.5 (12.9)	5 (5–8) [5–65]	28	6.8 (3.7)	5 (5–7) [5–19]	25	8.8 (7.2)	5 (5–9) [5–32]

U, unit; WBC, white blood cell.

a There were no significant differences between trial arms or in the interaction between arms over time in repeated measure ANOVA up to 12 months.

b There were significant differences between trial arms ($F=6.17$; $p=0.016$) and in the interaction between arms over time ($F=2.91$; $p=0.023$) in repeated measure ANOVA up to 12 months.

TABLE 14 Lipid profile and U&E laboratory parameters at baseline and 3 months by trial arm

Laboratory parameter	Trial arm															
	Rituximab								Placebo							
	Baseline				3 months				Baseline			3 months				
	<i>n</i>	Mean (SD)	Median (IQR)	Range	<i>n</i>	Mean (SD)	Median (IQR)	Range	<i>n</i>	Mean (SD)	Median (IQR)	Range	<i>n</i>	Mean (SD)	Median (IQR)	Range
Lipid profile																
LDL (mmol/l)	14	3.9 (0.9)	4.0 (3.1–4.3)	2.7–5.6	14	4.1 (1.1)	4.0 (3.5–4.4)	2.8–7	7	3.4 (0.8)	3.4 (2.9–4.2)	2.3–4.3	7	3.3 (0.9)	3.5 (2.4–4)	2.2–4.5
HDL (mmol/l)	26	1.8 (0.7)	1.8 (1.2–2.1)	0.9–3.3	23	1.8 (0.5)	1.8 (1.3–2.2)	1–2.9	21	1.6 (0.6)	1.5 (1.2–1.7)	0.9–2.9)	23	1.7 (0.6)	1.6 (1.3–2)	1.1–2.9
Triglyceride (mmol/l)	27	1.7 (0.7)	1.4 (1.1–2.3)	0.6–3	23	1.6 (0.8)	1.5 (1–2.1)	0.7–4.1	25	1.6 (1.1)	1.4 (0.8–2)	0.6–5.3	23	1.6 (0.8)	1.6 (1–1.9)	0.6–4.4
U&E																
Urea (mmol/l)	24	4.9 (1.0)	5 (4.3–5.7)	2.7–7.2	24	5.1 (0.9)	5.1 (4.3–5.7)	3.8–7	24	5.6 (2.5)	5.1 (4.3–6.3)	2.1–12.4	24	5.3 (1.6)	5.4 (4.7–6)	2.2–9
Creatinine (mmol/l)	24	67.8 (10)	66 (61–78.5)	50–86	24	69.1 (9.2)	69 (60–75)	56–93	24	70.0 (17.2)	69.5 (57–74)	42–124	24	65.8 (14.8)	64.5 (53–75)	44–102
Sodium (mmol/l)	24	140.3 (1.8)	140 (139.5–141)	136–144	24	140.4 (2.3)	140 (138.5–142.5)	137–145	24	140.0 (2.5)	139.5 (138.5–141)	135–145	24	140.0 (2.2)	140 (138.5–142)	136–143
Potassium (mmol/l)	24	4.3 (0.3)	4.3 (4.1–4.6)	3.7–4.9	24	4.3 (0.3)	4.3 (4–4.5)	3.9–5.2	24	4.4 (0.5)	4.5 (4.1–4.6)	3.7–6.2	24	4.4 (0.3)	4.4 (4.1–4.5)	3.9–5.3

TABLE 15 Lipid profile and U&E laboratory parameters at 6, 9 and 12 months by trial arm

Laboratory parameter	Trial arm																	
	Rituximab									Placebo								
	6 months			9 months			12 months			6 months			9 months			12 months		
	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]	<i>n</i>	Mean (SD)	Median (IQR) [range]
Lipid profile																		
LDL (mmol/l)	12	4.2 (0.9)	4.1 (3.8–4.7) [2.8–6]	9	4.0 (1.6)	4.4 (3.3–4.5) [0.9–6.8]	9	4.4 (1.6)	4.1 (3.4–4.3) [2.4–7.5]	12	3.6 (1.2)	3.4 (3–4) [1.8–6.3]	9	3.2 (1.1)	3.1 (2.8–3.6) [1.3–5.4]	9	3.6 (1.9)	3 (2.5–3.9) [1.6–7.9]
HDL (mmol/l)	22	1.8 (0.6)	1.8 (1.3–2.2) [0.9–3]	19	1.9 (0.8)	1.8 (1.2–2.2) [0.9–4.4]	20	1.7 (0.5)	1.6 (1.3–2.1) [0.9–2.8]	23	1.7 (0.6)	1.6 (1.2–1.9) [0.9–3.2]	22	1.9 (0.7)	1.9 (1.4–2.1) [1.1–3.2]	21	1.8 (0.6)	1.7 (1.4–2.2) [1–3.3]
Triglyceride (mmol/l)	23	1.5 (0.6)	1.2 (1.1–1.7) [0.9–3]	20	1.7 (1.0)	1.5 (1.2–1.9) [0.9–5.6]	20	1.4 (0.5)	1.2 (1.1–1.8) [0.6–2.2]	23	1.7 (1.1)	1.4 (0.9–2.5) [0.5–4.3]	22	1.6 (1.0)	1.4 (1.0–1.9) [0.6–4.6]	22	1.7 (1.1)	1.5 (1–2) [0.5–4.9]
U&E																		
Urea (mmol/l)	27	4.9 (1.0)	4.8 (4.4–5.4) [2.6–8.3]	23	5.2 (1.1)	4.9 (4.5–5.9) [3.8–7.8]	24	5.1 (1.5)	4.7 (4.1–5.8) [3.3–9.3]	27	5.3 (1.8)	5 (4.1–6.3) [2.7–12]	27	5.7 (2.2)	5 (4.2–6.5) [2.7–11.8]	25	5.8 (2.1)	5.6 (4.3–7.2) [2.4–12.2]
Creatinine (mmol/l)	27	66.8 (8.3)	66 (62–71) [51–89]	23	68.1 (7.8)	66 (64–73) [54–84]	24	69.4 (9.8)	68.5 (62.5–75.5) [48–91]	27	69.6 (15.8)	69 (56–77) [40–111]	27	68.4 (13.9)	70 (56–78) [49–114]	25	70.5 (17.4)	70 (59–72) [44–127]
Sodium (mmol/l)	27	140.1 (1.9)	140 (139–141) [136–144]	23	140.4 (1.9)	140 (140–142) [136–144]	24	140.8 (2.2)	141 (139–142.5) [135–145]	27	139.6 (2.4)	140 (138–142) [135–144]	27	139.5 (2.3)	139 (138–141) [135–144]	25	140.6 (2.5)	141 (139–142) [135–145]
Potassium (mmol/l)	27	4.3 (0.3)	4.3 (4.1–4.5) [3.9–4.9]	23	4.3 (0.3)	4.3 (4.1–4.4) [3.9–4.9]	24	4.4 (0.3)	4.3 (4.1–4.6) [3.8–5.1]	27	4.5 (0.3)	4.4 (4.3–4.7) [3.8–5.1]	27	4.4 (0.4)	4.4 (4–4.6) [3.7–5.6]	25	4.4 (0.4)	4.4 (4.1–4.6) [3.9–5.2]

Median ALP level fell from 136 U/l to 106 U/l over 3 months in the rituximab arm, whereas the median ALP level rose from 131 U/l to 137 U/l in the placebo arm. Ninety-three per cent of patients in the rituximab arm had a normal ALP level at 3 months, compared with 65% in the placebo arm (*Table 16*). All parameters progressively returned to baseline levels by 12 months of follow-up.

There were no notable differences between the rituximab and placebo arms for any lipid parameters, creatinine concentration or serum electrolyte levels or any change from baseline measurements up to 12 months (see *Tables 14* and *15*).

Safety

There were four SAEs during the course of the trial, all of which were unrelated to treatment (*Table 17*). There was one death from lobar pneumonia and chronic obstructive pulmonary disease, which occurred after consent but prior to baseline visit. This participant did not receive any intervention. One participant in the placebo arm was hospitalised for right optic neuritis and, owing to concerns that this might be treatment related, emergency unblinding was carried out. This same participant was hospitalised for left optic neuritis at a later date, but made a full recovery on both occasions. One participant in the placebo group with a background of chronic abdominal pain was hospitalised for abdominal pain, but subsequently made a full recovery after being treated with opiates.

Severe adverse events

For SAEs, we collected information on the number of patients in each trial arm who experienced an event and the number of patients assessed (see *Table 17*).

TABLE 16 Proportion of patients with clinically significant ALP level and those in the normal range by trial arm

ALP	Trial arm, n (%)	
	Rituximab	Placebo
Clinically significant 15% drop in ALP from baseline		
3 months (N = 55)	18 (67)	4 (14)
12 months (N = 49)	8 (33)	2 (8)
Patients with ALP in normal range ^a		
Baseline (N = 57)	21 (78)	17 (61)
3 months (N = 55)	25 (92.5)	18 (64.5)
12 months (N = 49)	20 (77)	17 (65.5)
Patients with ALP in normal range or with 15% drop from baseline		
3 months (N = 55)	25 (92.5)	19 (68)
12 months (N = 49)	20 (83.5)	17 (68)
^a A Normal range for ALP is between 30 U/l and 130 U/l; applying Toronto Criteria for response [$\leq 1.67 \times$ upper limit of normal (130 U/l) giving a value of 217], the range in these fatigued patients is 40–217 or 15% drop at 3 months from baseline level.		

TABLE 17 Serious adverse events up to 12 months by trial arm

Subject ID	SAE in medical terms	Case description	Onset of first sign/symptom	Seriousness	Outcome of SAE	Study infusion start date	Study infusion last date	Causality to study intervention	Trial arm
09LP	Right optic neuritis	2-week history of headache and reduced vision in right eye. Reviewed by ophthalmology and diagnosis of right optic neuritis made. Admitted to neurology ward at the Royal Victoria Infirmary for further investigations	3 May 2013	Life-threatening (excluding pre-planned admissions)	Completely recovered	4 March 2013	19 March 2013	No (unblinded)	Placebo
17SO	Death	When reviewing medical notes on 23 August 2013 at 14.00, a notification of death was found in notes. Patient died at home on 25 May 2013. Post-mortem: (1a) lobar pneumonia, and (1b) COPD		Subject died	Death			No	Died before randomisation
09LP	Left optic neuritis	Patient started with headache on Tuesday 3 September. Noticed decreased vision 5 September 2013 and went to eye casualty on 5 September 2013. Admitted, treated with six doses of methylprednisolone	3 September 2013	Life-threatening (excluding pre-planned admissions)	Completely recovered	4 March 2013	19 March 2013	No	Placebo
62ET	Abdominal pain	Complained of abdominal pain on the background of known chronic abdominal pain. Saw GP who referred to hospital. Was admitted from 18 to 21 January 2016. Had ultrasound and radiography – both normal. Treated with opiates and discharged on 21 January 2016 with pain team follow-up	18 January 2016	Other significant medical event	Completely recovered	20 July 2015	3 August 2015	No	Placebo

COPD, chronic obstructive pulmonary disease; GP, general practitioner; ID, identification.

Chapter 4 Discussion

Summary of main findings

Fifty-seven eligible participants aged ≥ 18 years with PBC and moderate or severe fatigue (PBC-40 fatigue domain score of > 33) were randomised to rituximab or placebo, and trial attrition was very low. Rituximab was found to be safe in this patient group, with no disease-attributable SAEs.

Immunological analyses showed that rituximab was effective in mediating depletion of B-cells, as expected. However, there was no statistically significant difference in the primary outcome (mean PBC-40 fatigue score at 3 months) between the rituximab and placebo arms (adjusted mean difference -0.9 , 95% CI -4.6 to 3.1); improvement in mean PB-40 fatigue score was observed at 3 months in both arms.

There were no significant differences in secondary outcomes at 3 months, covering patient-reported, immunological, exercise and bioenergetics outcomes, except for in anaerobic threshold and immunoglobulin serum levels (IgG and IgM), which improved with rituximab but not placebo. Overall, there was no evidence to support the use of rituximab as therapy for the treatment of fatigue in this group of patients.

Strengths

Levels of patient interest were high, as was retention within the study, both indicating a high degree of patient acceptability. This experience mirrors that of recent trials targeting itch as a symptom and supports the view that trials of therapy targeting symptoms in PBC are deliverable and highly acceptable to patients.⁶⁴

Over a period of 1 year, rituximab was found to be safe. No SAEs were observed in the study participants who received the active drug (compared with three in the placebo group). In particular, no worsening of liver function was observed. This is in keeping with other reports of the agent in PBC and in contrast to the observation of worsening of liver function in murine models of PBC treated with anti-CD20.^{54,55,65} This divergence of effects is likely to reflect the limitations of current murine models of PBC and challenges their relevance to therapeutics development. We believe that the observation of a benign safety profile (within the constraints of a 1-year follow-up study) will be informative both for the use of rituximab in other related liver disease (most obviously immunoglobulin G4 disease) and in the development of future biological therapy strategies in PBC.

Limitations

Recruitment fell short of the original target. Although levels of patient interest were high, and numbers of patients with potential eligibility-matched pre-trial estimates, a significant number of people were lost at the pre-screening point. This was largely due to exclusion criteria related to potential chronic infection and malignancy, which were frequently met in a chronic disease population typically aged in their mid-50s. Although patients affected did not typically exhibit overt chronic infection malignancy, 'soft' findings meant that such processes could not be excluded to a sufficient degree to allow randomisation. We had estimated a likely exclusion rate on the basis of 'actual' criteria rates and had not anticipated the scale of 'potential' exclusion criteria. This issue has important implications for the design of future trials of biological therapies in PBC. A further issue was visit burden, which is an emerging challenge in PBC trials. This is particularly an issue with younger patients who are either working or have young families. Emerging data from the UK-PBC programme suggest that both symptom severity and risk of non-response to primary therapy are increased in younger patients, making them an important target population for clinical

trials. This will be an important issue to address when designing future trials in PBC. We do not feel that the shortfall in recruitment materially altered the outcome of the trial.

Issues were encountered with a number of the secondary outcome measures (in particular MR-based muscle bioenergetics, physical activity monitoring and fatigue diaries). Data sets were incomplete for these measures because of either issues of compliance (fatigue diaries and physical activity monitoring for which, despite instructions emphasising the importance of the time span for data capture, insufficient data were retrieved for some patients to allow inclusion in the analysis) or analysis (MR-based muscle bioenergetics and physical activity monitoring where extraction of reliable, usable data issues of not possible for some completed assessments). The reliability of data capture limits their utility in trials of fatigue and its mechanisms. Further work is needed on these measures before they can be reliably used as trial outcome measures as opposed to experimental study methodologies.

Interpretation

This was the first trial of any liver disease in which the primary treatment target was fatigue and its impact on quality of life. No difference was found in the primary outcome between arms, so there is no evidence to support the use of rituximab as a treatment for fatigue in otherwise unselected PBC patients with moderate to severe fatigue. B-cell depletion was complete in all the rituximab-treated patients, excluding a technical drug failure as an explanation for this finding, with progressive reconstitution over the year of follow-up (although the baseline level was not reached by the 12-month final assessment point).

A mean reduction of 5 units in the fatigue domain of the PBC-40 (the predefined level for a clinically significant fall) was observed in both the active and placebo drug groups. This remained present at 6 months, with a gradual increase, almost back to baseline levels, by 12 months. Within the trial arms, 16 out of the 27 (59%) rituximab-treated and 10 out of the 28 (36%) placebo-treated participants showed a 5-point or greater fall in fatigue scores. Potential explanations for the apparent reduction in fatigue severity in the placebo arm include a significant placebo effect and regression to the mean. The lack of beneficial effect on physical activity level, an objective measure that has relevance to the subjective domain of fatigue and which correlates in PBC with fatigue severity, would provide support for the view that there is no significant fatigue improvement with rituximab in PBC. Interestingly, the degree of response seen in the placebo group (36%) mirrors that seen in the placebo arm in recent trials of novel antipruritic therapy in PBC, suggesting that this may represent a natural placebo response rate in this symptomatic condition.⁶⁴ This observation will help with the design of future studies of symptomatic therapy in the condition. It is striking that any change in levels of patient-reported outcomes was restricted to the primary outcome parameter, namely fatigue. No change over time was seen in the closely linked cognitive domain of the PBC-40, or even in the itch domain (a parameter previously demonstrated to be susceptible to placebo effect in trials of antipruritic therapy). It is also interesting to observe that the restriction of a placebo effect to the primary outcome symptom seen in this trial is mirrored in recent trials of antipruritic therapy, in which a placebo effect was seen for pruritus severity but not fatigue.⁶⁴ One approach to mitigating the placebo effect in future trials in PBC might therefore be to make the target more generic ('symptom burden' rather than a specific symptom). The very specific nature of the placebo effect does also raise the question of whether or not psychological interventions may have a value in at least mitigating the impact of symptoms in individual patients.

The variability in the apparent response to rituximab was significant, with some recipients exhibiting almost complete resolution in their fatigue and others showing no change. Although this could represent a further manifestation of a placebo effect, it also raises the possibility that fatigue is heterogeneous in nature and that only a subgroup of patients, with a particular form of the disease, are responders to rituximab. Since the design of the trial, it has become clear that a stratified approach to therapeutics in PBC is highly effective (the advent of targeted second-line therapy has led to recent progress in prognostic therapy in PBC), something that had previously been thought to be a major challenge when using

unselected patient approaches.¹⁴ The RITPBC trial used an unselected approach that may be a limitation. Again, subsequent to the design of the trial, it has become increasingly accepted that central and peripheral fatigue have quite distinct characteristics in PBC.²⁴ The underpinning data and study hypothesis in the RITPBC trial were based on peripheral muscle abnormality (and peripheral fatigue). The study population showed, at baseline, significant levels of sleep disturbance [40/53 trial participants (76%) had baseline ESS scores of ≥ 10] and cognitive impairment, which are features strongly associated with central fatigue.^{66,67} There is a need to determine rituximab response in the different fatigue variants in PBC in order to further understand the biology of this important symptom in different endotype groups in order to inform future trials of fatigue-modifying therapy in PBC. We do not believe that there is, at present, any rationale for further trials of rituximab.

Significant bioenergetic abnormality was seen at baseline in the participants, mirroring the findings in the original studies that informed the trial.^{39,40} Although resting muscle pH was normal, the minimum pH seen following exercise was again dramatically (but variably) lowered. The time for recovery to the baseline level was again prolonged. Rituximab therapy was associated with a small rise in post-exercise muscle pH (and a small fall in the pH drop with exercise); however, this effect was not significant. Recovery time was not affected (in keeping with a model in which it is autonomic regulation of muscle blood flow and proton efflux from acidotic muscle cells, neither of which would be predicted to change with rituximab therapy). Similarly, the AUC for total muscle acid exposure, which is driven to a significant degree by pH recovery time, was not improved by rituximab therapy. In contrast to muscle bioenergetic function on MR, anaerobic threshold, which was again found to be low in the PBC group, mirroring earlier observations, improved significantly with rituximab (but not placebo), with a degree of change that was biologically significant. Serum anti-PDC antibody levels were significantly reduced with rituximab, although the antibody remained present at lowered levels.⁴⁷ This is in keeping with previous reports of the use of rituximab in PBC and in other disease states, and with the cellular distribution of CD20 (it is present on B-cells but not plasma cells). Peak depletion of anti-PDC antibody was seen at 6 months rather than the 3-month primary end point. In terms of the bioenergetic model for fatigue in PBC, a number of conclusions can be drawn. The first is that the previously described bioenergetic abnormality is confirmed in this study in a cohort of highly fatigued patients. The second is that anaerobic threshold impairment is reduced in patients who exhibit depletion of anti-PDC antibodies. Further analysis will be required to determine whether or not this link is causal; however, the finding would be at least in keeping with the hypothesis that anti-PDC antibodies have a direct metabolic role in increasing anaerobic metabolism. If this is indeed the case, it may be that the peak effect has been missed given that the peak in anti-PDC antibody depletion was at 6 months, 3 months after the last anaerobic threshold assessment. The third conclusion is that the link between anti-PDC antibodies and anaerobic threshold, and between muscle bioenergetic abnormality and perceived fatigue, is limited. Change in post-exercise muscle pH with rituximab was limited, as was any change in fatigue. The apparent disconnect between anaerobic threshold, on the one hand, and muscle bioenergetics abnormality on MR spectroscopy, on the other, would suggest that factors other than the balance between aerobic and anaerobic metabolism play the predominant role in regulating muscle pH. The obvious candidate would be the capacity of muscle to handle protons and lactate: capacity that may be modified by addressing autonomic dysfunction (highly prevalent in PBC and postulated to impact on both transport of protons and lactate out of muscle cells and vascular outflow from muscle tissue) or through exercise therapy (which is known to increase proton/lactate transporter levels and to increase vascular outflow). There is evidence, albeit very limited, to suggest the efficacy of both approaches in PBC.^{40,68}

This study was not designed or powered to explore the impact on rituximab on liver disease severity in PBC. In the course of the study, however, it became clear that the information that could be derived would be useful in assisting in the design of future studies of disease-modifying drugs. There has been recent advance in the treatment of PBC, with second-line therapy being approved for use in patients showing inadequate response to first-line therapy with UDCA.¹⁴ The successful agent, obeticholic acid, is, however, a modified bile acid and thus represents a more effective version of the UDCA approach. Trials of the obvious alternative approach, immunotherapy to modify the immune injury to bile ducts that leads to the

cholestasis in the first place, have, in marked contrast, been unsuccessful.⁶⁹ One potential explanation for this lack of efficacy would be that the biological agents may have been used at the wrong point in the disease pathway, following proven failure of treatment with UDCA (if immune injury is an early phenomenon, and cholestasis a late one, then failure to respond to UDCA would imply the presence of 'downstream' aggressive cholestasis, meaning that only then moving to treatment of 'upstream' immune activity would be counterintuitive). Because the treatment target in the RITPBC trial was fatigue, which is unrelated to disease severity and UDCA therapy, this trial provides a unique data set relating to use of a biological agent in patients who have not previously had UDCA therapy.^{19,70} As far as we are aware, this is the only such data set available in the world. Our data strongly suggest that in a cohort of PBC patients not characterised by UDCA treatment failure, rituximab, in addition to being safe, has a measurable effect on both individual parameters of disease severity (ALP and ALT levels) and the recently derived UK-PBC, which is a fully validated predictive model for the disease. Ninety-three per cent of participants in the rituximab arm had normal liver function at 3 months, compared with 65% of participants in the placebo arm. These findings might support the concept that rituximab has a beneficial effect on disease activity when given early in the disease course.

Chapter 5 Conclusions

The following conclusions can be drawn from this study:

- There is no evidence to support the use of rituximab for the treatment of fatigue in otherwise unselected populations of PBC patients with moderate to severe fatigue. On average, there was a 5-point reduction in PBC-40 fatigue severity (measured on the PBC-40 fatigue domain) over 3 months (which was of clinical significance), but this was seen in both trial arms.
- Trials of fatigue-modulating therapy are deliverable in PBC and acceptable to patients (retention was 96.5%). However, there is a significant issue with placebo effects that are similar in scale to those seen in recent trials of anti-itch therapy.
- Rituximab when used in PBC was safe, with no attributable SAEs.
- The bioenergetics abnormality reported previously in fatigued PBC patients and that underpinned this trial concept was also seen in this trial.
- Anaerobic threshold was significantly improved by rituximab. Muscle bioenergetics abnormality on MR spectroscopy was not, however, significantly improved. The capacity of muscle to handle protons and lactate may therefore play a more important role in fatigue expression than PBC autoantibody. Future trials of therapy for fatigue might address these aspects (exercise therapy and targeting autonomic dysfunction).
- Although the trial was not designed or powered to explore impact on liver biochemistry, our findings point to a positive impact of rituximab on liver function when used in patients not defined by UDCA failure.

Acknowledgements

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Contributions of authors

Amardeep Khanna (Clinical Research Associate) was involved in the clinical co-ordination of the trial.

Laura Jopson (Clinical Research Associate) was involved in the clinical co-ordination of the trial.

Denise Howel (Senior Lecturer in Statistics) contributed to the design of the study, contributed to the statistical analysis plan and supervised the final analyses.

Andrew Bryant (Trial Statistician) contributed to the statistical analysis plan and conducted the final analyses.

Andrew Blamire (Professor of Magnetic Resonance Physics) conducted the MR imaging studies.

Julia L Newton (Clinical Professor of Ageing and Medicine) contributed to the design and development of the trial protocol.

Jennifer Wilkinson (Senior Trial Manager) developed the trial protocol and oversaw the management of the trial.

Alison J Steel (Trial Manager) managed the trial.

Jennifer Bainbridge (Research Nurse) conducted trial visits.

Renaë Stefanetti (Research Associate) contributed to data analysis.

Sophie Cassidy (Research Associate) contributed to performing exercise testing.

David Houghton (Research Associate) contributed to performing exercise testing.

David E Jones (Professor of Liver Immunology) was chief investigator, and designed and developed the trial protocol.

Data sharing statement

Anonymised data from this study may be available to the scientific community subject to regulatory and ethics approval. Requests for data should be directed to the corresponding author.

Patient data

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: <https://understandingpatientdata.org.uk/data-citation>.

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Appendix 1 Research and development approval

The Newcastle upon Tyne Hospitals

JP/SS

14th September 2012

Professor of Liver Immunology,
Newcastle University,

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

Dear Professor Jones,

Trust R&D Project:	5997
Title of Project:	<i>Rituximab for the Treatment of Fatigue in Primary Biliary Cirrhosis (RITPBC)</i>
Principal Investigator:	Professor David Jones
Number of patients:	78
Funder {proposed}:	NIHREfficacy and Mechanism Evaluation Programme & Department of Health
Sponsor {proposed}:	The Newcastle upon Tyne Hospitals NHS Foundation Trust
REC number:	12/NE/0095
EudraCT number:	2012-000145-12
CLRN ID:	90909

NHS Foundation Trust

Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP

Tel: [REDACTED]

Fax: [REDACTED]

www.newcastle-hospitals.nhs.uk

Having carried out the necessary risk and site assessment for the above research project, Newcastle upon Tyne Hospitals NHS Foundation Trust grants NHS Permission for this research to take place at this Trust dependent upon:

- (i) you, as Principal Investigator, agreeing to comply with the Department of Health's Research Governance Framework for Health and Social Care, and confirming your understanding of the responsibilities and duties of Principal Investigators by signing the Investigator Responsibilities Document. A copy of this document will be kept on file within the Joint Research Office.
- (ii) you, as Principal Investigator, ensuring compliance of the project with all other legislation and guidelines including Caldicott Guardian approvals and compliance with the Data Protection Act 1998, Health and Safety at Work Act 1974, any requirements of the MHRA (eg CTA, EudraCT registration), and any other relevant UK/European guidelines or legislation (eg reporting of suspected adverse incidents).
- (iii) where applicable, you, as Principal Investigator, should also adhere to the GMC supplementary guidance *Good practice in research and Consent to research* which sets out the good practice principles that doctors are expected to understand and follow if they are involved in research – see http://www.gmc-uk.org/guidance/ethical_guidance/5991.asp

Sponsorship

The Newcastle upon Tyne Hospitals NHS Foundation Trust w/11 act as Sponsor for this project, under the Department of Health's guidelines for research in health and social care.

In addition, the Trust has a Research Governance Implementation Plan, agreed with the Department of Health, in order to fully comply with Research Governance and fulfil the responsibility of a Sponsor.

As the Trust is acting as Sponsor for the research and where some of the research is taking place outside of Newcastle upon Tyne, then all costs must be met for research governance audit visits to those sites. It is the responsibility of the PI to provide confirmation to the Trust of who will pay these costs. Audit is required under the Research Governance Framework for Health and Social Care. (Please note that the Trust randomly audits 10% of approved research projects annually.)

NHS Permission applies to the research described in the protocol and related documentation as listed on the favourable ethical opinion(s) from Newcastle & North Tyneside 1 Research Ethics Committee, dated 16 May 2012, 17 May 2012, 29 June 2012 and 28 August 2012. Specifically, the following versions of the key documents are approved:

Document	Version	Date
Protocol	4.0	07 August 2012
Summary of Product Characteristics	Rituximab	10 January 2012
Participant Information Sheet	3.0	15 May 2012
Participant Consent Form	2.0	23 April 2012
GP Letter	1.0	20 February 2012
Fatigue Diary	1.0	20 February 2012
Validated Questionnaire: PBC40		
Validated Questionnaire: PROMIS HAQ		
Validated Questionnaire: COGFAIL		
Validated Questionnaire: ESS		
Validated Questionnaire: OGS		
Validated Questionnaire: HADS		

Any changes to these documents, or any other amendments to the study must be submitted to the Research Ethics Committee and MHRA (if relevant) for review (see <http://www.nres.npsa.nhs.uk/applications/after-ethical-review/amendments/> for guidance). All amendments must be submitted to the R&D office for review in parallel with ethical and regulatory review so that implications of the amendment can be assessed. You must send a copy of all amendment documents to the R&D office and if the changes or amendments to the study have implications for costs or use of resources, you must also submit details of these changes.

It is the Principal Investigator's responsibility to ensure that all staff involved in the research have Honorary Research Contracts or the necessary Letters of Access. These must be issued prior to commencing the research.

In addition, unless otherwise agreed with the Trust, the research will be covered for negligence under the CNST (Clinical Negligence Scheme for Trusts), however cover for no-fault harm is the responsibility of the Principal Investigator to arrange if required.

Please also note that for any NHS employee who generates Intellectual Property *in the normal course of their duties*, it is recognised that the Intellectual Property Rights remain with the employer and not the employee.

Yours sincerely

Research Management & Governance CRM&GI Manager

Appendix 2 Ethics/Research Ethics Committee approval



Health Research Authority

NRES Committee North East - Newcastle & North Tyneside 1

TEDCO Business Centre
Room 002
Rolling Mill Road

16 May 2012

Institute of Cellular Medicine
4th Floor, William Leech Building Newcastle University
Framlington Place
Newcastle upon Tyne NE2 4HH

Dear Professor

Study title: Rituximab for the Treatment of Fatigue in Primary Biliary Cirrhosis

REC reference: 12/NE/0095

Protocol number: 5997

EudraCT number: 2012-000145-12

Thank you for your letter of 23 April 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites listed in the application, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Covering Letter	Professor David Jones	06 March 2012
GP/Consultant Information Sheets	1.0	20 February 2012
Investigator CV	Professor David Jones	02 February 2012
Investigator CV	Professor David Jones	23 April 2012
Other: Summary of Product Characteristics	Rituximab	10 January 2012
Other: GCP Certificate	Professor David Jones	10 March 2009
Other: Newcastle University Indemnity Confirmation Letter	Kelly Lovelock	01 February 2012
Other: EME Funding Award Confirmation Letter	Lucy Knight, Program Manager	24 January 2012
Other: Department of Health Subvention Award Confirmation Letter	Trudi Simmons, Senior Manager	08 December 2011
Other: Newcastle upon Tyne Hospitals Treatment Costs Confirmation Email	Gary Ford	19 December 2011
Other: Newcastle upon Tyne Hospitals NHS Foundation Trust SOP 13	2.0	01 August 2009
Participant Consent Form	1.0	20 February 2012
Participant Consent Form	2.0 (Clean and Tracked Changes)	23 April 2012
Participant Information Sheet	1.0	20 February 2012
Participant Information Sheet	2.0 (Clean and Tracked Changes)	23 April 2012
Protocol	1.0	20 February 2012
Protocol	2.0 (Clean and Tracked Changes)	23 April 2012
Questionnaire: Validated Questionnaire: PBC40		

Questionnaire: Validated Questionnaire: PROMIS HAQ		
Questionnaire: Validated Questionnaire: COGFAIL		
Questionnaire: Validated Questionnaire: ESS		
Questionnaire: Validated Questionnaire: OGS		
Questionnaire: Validated Questionnaire: HADS		
REC application	IRAS Version 3.4, 90909/300787/1/993	07 March 2012
Response to Request for Further Information	Professor David Jones	23 April 2012
Sample Diary/Patient Card	Fatigue Diary, Version 1.0	20 February 2012

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “*After ethical review – guidance for researchers*” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/NE/0095

Please quote this number on all correspondence

With the Committee's best wishes for the success of this

project Yours sincerely

Vice Chair

Email:

Enclosures: "After ethical review – guidance for researchers"

Appendix 3 Medicines and Healthcare products Regulatory Agency clinical trial authorisation approval

Safeguarding public health

NEWCASTLE-UPON-TYNE HOSPITALS NHS FOUNDATION TRUST

15/08/2012

Dear

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I.2004/1031

Our Reference: 17136/0261/001-0001
 Eudract Number: 2012-000145-12
 Product: MabThera
 Protocol number: 5997

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 09/08/2012.

Authorisation of your clinical trial is subject to the following condition(s):
 *The normal saline used as the placebo comparator is a UK marketed product.
 If these conditions are met, the trial is authorised and you do not need to respond to this letter. If your trial does not meet these conditions, your trial does not have authorisation and therefore you can not proceed with the trial. You must inform the MHRA immediately if the trial does not meet the above conditions. All changes to the terms and conditions of this trial must be made as a request for a substantial amendment to this clinical trial authorisation.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

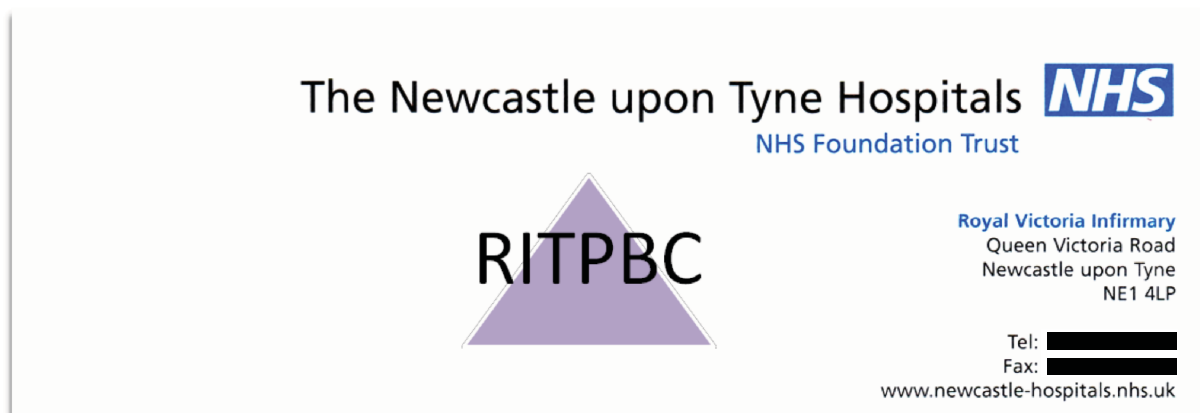
Clinical Trials Unit
MHRA



Medicines and Healthcare products Regulatory Agency
 151 Buckingham Palace Road London SW1W 9SZ
 T 0203 080 6000 www.mhra.gov.uk

An executive agency of the Department of Health

Appendix 4 Participant information sheet



PARTICIPANT INFORMATION SHEET

Title of Project: Rituximab for the Treatment of Fatigue in Primary Biliary Cirrhosis (RITPBC Study)

Chief Investigator: Professor David Jones

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. One of our team will go through the information sheet with you and answer any questions you have. Please ask the study doctor or nurse if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this leaflet.

Please start by reading the study summary. If you think you might be interested in taking part, then please go on to read the remainder of this information sheet.

STUDY SUMMARY

Primary Biliary Cirrhosis (PBC) is a liver disease that predominantly affects females, can present for the first time at any age and which develops over many years. It is caused by the immune system attacking the body's own tissues. People with PBC frequently experience profound fatigue or tiredness which they liken to their "batteries running down" and although people still want to undertake normal activities they often lack the energy to be able to do them. This reduces quality of life, makes it difficult for people to work and can end up with them becoming isolated in the community. At present we have no treatment for fatigue in PBC. Finding a treatment for fatigue in PBC is one of the highest research priorities identified by patient groups.

The aim of this study is to undertake a clinical trial to examine the effects of a treatment ("Rituximab") on severe fatigue in PBC to help us understand whether this will be a potentially useful treatment. The information that this will give us about how energy generation changes in patients with PBC with and without the treatment will also help us to develop new treatments for fatigue in other diseases. The study has the potential to improve the quality of life of many patients with PBC, for whom there is currently no hope of improvement.

We will perform a randomised controlled study of Rituximab therapy in PBC compared to placebo.

A randomised controlled trial (RCT) is an experimental design used for testing the effectiveness of a new medication. Individuals are assigned randomly to a treatment group (experimental therapy) and a control group (placebo or standard therapy) and the outcomes are compared. This trial is strengthened by being a double-blind study.

Randomisation increases the likelihood that the two groups being treated will be similar. Each participant will be allocated a unique randomisation number, which will be concealed until after each eligible patient has been accepted for the trial. These precautions mean that people who decide whether a patient is eligible to participate in the trial cannot influence which treatment a patient is allocated to receive. This protects the study from conscious or unconscious bias, which would make the test unreliable.

Many trials are set up so that no one knows who has been allocated to receive which treatment. This is known as blinding and helps reduce the effects of bias when comparing the outcomes of the treatments. As this study is a double-blind trial, both the medical staff organising the treatment and those taking part in the trial do not know who is receiving which treatment.

For this study there is a 50% chance that you will receive the study drug Rituximab and a 50% chance that you will receive the placebo.

You can find more information on randomisation and blinding on the following website:

<http://www.nhs.uk/Conditions/Clinical-trials/Pages/Fairtests.aspx>

The study will be performed in a specialised clinical research environment at Clinical Research Facility Royal Victoria Infirmary. We have, for many years, worked closely with PBC patient groups to focus on the problems that are important to our patients. This study is fully supported by Liver North, a liver disease charity and patient support group.

The study will take place over approximately one year and involve between 9 and 20 visits depending on the planning of some investigations. We will ask you to provide some extra blood (up to 6 teaspoonfuls) at the start of the study and after three, six, nine and twelve months. In addition to this we will ask you to complete some quality of life questionnaires, you will have your activity levels monitored for a week using a small device (the size of a wrist watch), exercising twice on an exercise bike to measure your oxygen used, and have two MRI scans.

You are invited to participate in the study as your doctor feels you are eligible to take part.

Any information collected about you during this study will be kept strictly confidential.

Taking part in this study is entirely voluntary. If you do agree to participate, you are free to withdraw at any time and without having to provide a reason. You will be asked to sign a consent form to confirm that you are willing to take part.

If you are interested in taking part in the study please continue to read the rest of this information sheet.

Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about how the study will be carried out.

INFORMATION SHEET PART 1

What is the purpose of this study?

You have been invited to participate in this study because you have Primary Biliary Cirrhosis (PBC) and have fatigue. Rituximab is a relatively new drug that has been used in patients with Rheumatoid Arthritis and found to improve fatigue in this patient group. Rituximab treatment results in depletion of B lymphocytes; these are immune cells that are thought to play an important role in PBC. Rituximab is administered via intravenous infusion over several hours. One treatment course comprises two infusions two weeks apart. The main purpose of this study is to investigate whether Rituximab improves fatigue in PBC and if it does, how this happens. To this end we will measure markers of muscle function, activity and quality of life before and up to one year after the first infusion of Rituximab. We hope to find whether Rituximab affects fatigue and how this happens. Participation in the study will last approximately one year.

Who can take part?

To take part in this study you must:

- have moderate or severe fatigue assessed as having a fatigue domain score of >33
- be aged 18 years or older
- willing and able to provide written informed consent
- available for the duration of the study
- willing and able to comply with the procedures required as described in this information leaflet and as directed by the study doctor or nurse

You cannot take part in this study if:

- the above does not apply to you
- you are currently taking part in another clinical study
- you have any condition which, in the opinion of the investigator, might interfere with evaluating the study objectives
- there is any reason you might not be able to have an MRI scan
- you have had major surgery within 4 weeks of study entry
- you have had vaccination within 4 weeks of study entry (patients requiring seasonal Flu or travel vaccines will be required to wait 4 weeks post vaccination to enrol in the study)
- you are pregnant or planning to become pregnant for the duration of study

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

Which treatments are being used in the study?

If you agree to take part in the study you may receive an infusion through a drip that either contains Rituximab or a salt solution (placebo). Neither the medical staff organising the treatment, nor you, will know which treatment you will receive.

Rituximab/placebo therapy

B-cells are involved in fighting infection and causing inflammation. We also believe that they play an important role in causing fatigue in PBC. The number of B-cells in the blood can be greatly reduced by using Rituximab. Rituximab/placebo is given by drip and involves being in the Clinical Research Facility for several hours on the day of your treatment. A course of Rituximab/placebo consists of two drips given a fortnight apart. Before each Rituximab/placebo treatment all participants will receive Paracetamol and an antihistamine orally and a steroid by a drip. These help to minimise side effects that sometimes occur when Rituximab/placebo is given. The paracetamol and steroid may temporarily improve the symptoms of your PBC but the purpose of the study is to look at the effects of the treatment over a year. All participants will remain double-blinded throughout the study.

What will happen to me if I take part?

If you decide to participate in this study, you will be allocated at random to receive either Rituximab treatment or a salt solution (placebo). The study will take place over approximately one year and involve between 9 and 20 visits depending on the planning of some investigations. All patients will be asked to provide some extra blood (up to 6 teaspoonfuls) at the start of the study and after three, six, nine and twelve months for analysis of the cells and proteins in your blood and will collect normal clinical information about the severity of your liver disease. In addition you will have your activity levels monitored for a week using two different activity monitors. They are small devices,, one worn on the wrist and one worn on the upper arm. You will also have an exercise test on an exercise bike t and a MRI scan before and after the treatment. Full details of what will happen to you are in Part 2 of this information sheet.

Reimbursement

You will be reimbursed travel expenses for study related visits.

What do I have to do?

To take part in the study you must make yourself available for all study visits and comply with the instructions given to you. You also need to report any possible side effects and new health problems. It is advised patients are vaccinated against Flu and receive Pneumovax before treatment.

What are the side-effects of any treatment received when taking part?

Infusion of Rituximab leads to infusion reactions in about 30% of patients during the first infusion, but the chance is lower with subsequent infusions. Usually these are mild and self-limited and include local skin irritation, light-headedness, dizziness and nausea. Severe allergic reactions requiring emergency

treatment are very rare. Rituximab leads to a modest increase of infections, notably upper respiratory and urinary tract infections.

What are the possible disadvantages and risks of taking part?

Blood sampling can sometimes cause bruising and soreness of the arms, or very rarely a blockage of the vein or a small nerve injury which can cause numbness and pain. Normally these problems resolve with time. Some people may faint while blood is being drawn.

Measurement of physical activity is a routine clinical investigation which involves wearing two small devices, one is like an armband to be worn on the back of the upper right arm and the other is worn on the wrist for a week.

Two MR scans of your muscles will take place whilst you perform gentle exercises by repeated flexion of the foot against a weight and the MRI scanner measures acid accumulation. MR scanning can be very noisy, and sometimes people tell us they feel more tired than normal after they have exercised in the scanner.

Detection of unsuspected abnormalities on subjects undergoing research MR scans at the Newcastle MR Centre

Research MR scans undertaken at the Newcastle MR Centre are for research, not clinical purposes. As such they will not be routinely examined or reported by a radiologist. However, if a previously unsuspected abnormality is detected by one of the MR Centre radiographers or other staff, then the scan will be referred to a qualified radiologist for a radiological opinion. The study doctor will then be informed by the radiographer and may then discuss the case with the radiologist and/or other specialists as appropriate. The decision as to what further action is needed, including how to communicate such findings to you and your GP, will be the responsibility of the study doctor.

Should I take contraception during the study?

If appropriate we will advise you about contraception before you decide whether to take part in the study. Male patients should continue to use a reliable method of contraception for 12 months after the last Rituximab/placebo treatment.

Can I become pregnant or breast feed during the study?

You must not take part if you are breastfeeding, pregnant, planning to become pregnant or are not using a reliable method of contraception. A pregnancy test will be performed in women of child-bearing potential before starting treatment. If you are treated with Rituximab/placebo then you should not fall pregnant or breastfeed for 12 months after your last Rituximab/placebo treatment course.

What are the possible benefits of taking part?

You may not gain any direct medical benefit from participating in this study, but we hope to find that Rituximab has a positive effect on fatigue in PBC. If, during the course of the study, we find that you have a medical condition of which you are unaware, we will inform you of our findings and refer you to your GP or an appropriate doctor.

What happens when the research study stops?

At the end of the study, information will be held securely for a maximum of 15 years. You will be provided with contact details of the research team in case you want to discuss any aspect of taking part in the study at a later date.

The study drug (Rituximab) will not be provided after the study has finished.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practices and all information about you will be handled in confidence. The details are included in Part 2.

This completes Part 1 of the information sheet. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

INFORMATION SHEET PART 2**What will happen to me if I take part?**

If you decide to participate in this study, you will be allocated at random (by chance, like the toss of a coin) to receive either Rituximab treatment or a salt solution (placebo). You are free to decline any drug treatment that is offered to you. All of your other drug therapy will remain unchanged, unless your doctor tells you otherwise.

The study will take place over approximately one year and involve between 9-20 visits depending on the planning of some investigations. If you take part you will be asked to complete quality of life questionnaires and have your activity levels monitored for a week using two small devices to be worn on the wrist and on the upper arm. During two of the visits you will exercise on an exercise bike and at two separate visits your muscle function will be assessed during an MR scan. All study visits will take place at the Newcastle Clinical Research Facility (NCRF) at the Royal Victoria Infirmary (RVI) apart from the MR scans which will take place at the Newcastle Magnetic Resonance Centre (NMRC) which is based at the Campus for Ageing and Vitality. Please see the Table of Events Flow Diagram at the end of this information sheet.

We will ask you to provide some extra blood (up to 6 tea spoon-full) at the start of the study and after three, six, nine and twelve months for analysis of the cells and proteins in your blood. In the future, we hope that these samples will help us predict which patients will respond best to treatment.

Visit 0 (screening)

Your first visit is to take place up to 2 weeks before visit 1. At this screening visit you will have the opportunity to ask any questions and discuss the study in full. If you decide you want to take part in the study you will then:

- sign the Informed Consent Form
- be asked questions about your general health
- be asked permission for us to inform your GP that you are taking part in a clinical research study
- be given a full physical examination
- have blood samples taken for a number of routine laboratory tests to check it is safe for you to start Rituximab (unless this has been done in the past month)

If any of the tests or examinations detects an infection or another problem, there may be a need to stop your participation in the study. This is in order to ensure your safety. If we detect any problems we will discuss these with you and refer you to the appropriate clinician for treatment or care. It may be possible to be screened again for entry into the study after treatment of some infections or problems. This will depend on the nature of the condition and will be at the discretion of the study doctor.

We will be able to discuss most results with you on the day of your visit but some test results can take up to 10 working days to be processed. Once we have received your results we will contact you to talk about the next step.

Visit 1 (baseline investigations and randomisation)

This visit will take place prior to your first Rituximab/placebo infusion and will involve:

- completion of quality of life questionnaires (PBC-40, PROMIS HAQ, COGFAL, OGS, ESS and HADS)
- blood samples (approximately 30 ml equivalent to 6 teaspoons)
- an assessment of muscle function using MR (a scan of your muscles whilst you take gentle exercise)
- an assessment of your exercise capacity (measuring how much oxygen you use whilst cycling on an exercise bike)
- assessment of physical activity using two monitors that you wear over a week (the monitors are the size of an armband to be worn on the back of the upper right arm and on the wrist, touching the skin.)
- pregnancy test (for female patients with childbearing potential)
- issuing you with a fatigue diary. You will be asked to complete it for a period of one week during the first week of each month for visits at baseline, 1, 3, 6, 9 and 12 months
- randomisation to receive either Rituximab or placebo infusion

Visit 2 (1st Infusion)

This visit will take place within 4 weeks of the baseline investigations. At this visit you will receive your infusion of Rituximab or placebo. You will receive information about the procedures involved as part of

your baseline visit. The infusion will last about 5 hours and you will be asked to stay for another hour after the infusion for observation. Lunch and refreshments will be provided during this visit. You will be asked to:

- undergo a physical examination (including vital signs, height and weight)
- repeat blood samples
- you will be asked if you have had any adverse events or if there have been any changes to other medication you were taking since your last visit (visit 1)

Visit 3 (safety visit)

At this visit you will be asked to:

- undergo a physical examination (including vital signs, height and weight)
- repeat blood samples
- you will be asked if you have had any adverse events or if there have been any changes to other medication you were taking since your last visit (visit 2).

Visit 4 (treatment 2)

This visit will take place two weeks after visit 2. At this visit you will receive your second infusion of Rituximab or placebo. The infusion will last about 4 hours and you will be asked to stay for another hour after infusion for observation. Lunch and refreshments will be provided during this visit. You will be asked to:

- undergo a physical examination (including vital signs, height and weight)
- repeat blood samples
- you will be asked if you have had any adverse events or if there have been any changes to other medication you were taking since your last visit (visit 3)

Visits 5-15 (follow-up visits)

These visits will take place weekly for 12 weeks following your 2nd infusion with telephone calls by a Research Nurse. At these visits you will be asked by the Research Nurse if you have had any adverse events or if there have been any changes to other medication you were taking at baseline.

Visit 16 (follow up)

This study visit takes place in the Clinical Research Facility at the Royal Victoria Infirmary 12 weeks after your second infusion. At this visit you will involve:

- an assessment of muscle function using MR (a scan of your muscles whilst you take gentle exercise)
- an assessment of your exercise capacity (measuring how much oxygen you use whilst cycling on an exercise bike)
- undergoing a physical examination (including vital signs, height and weight)
- repeat blood samples as per baseline visit
- completing quality of life questionnaires (PBC-40, PROMIS HAQ, COGFAIL, OGS, ESS and HADS)

- assessment of physical activity using two monitors that you wear over a week (you will be reminded by the Research Nurse to return the monitor either by post in a self addressed envelope or prearranged taxi at the end of the assessment period.)
- you will be reminded to complete your fatigue diary for one week at the beginning of the month for this 3 month visit
- you will be asked if you have had any adverse events or if there have been any changes to other medication you were taking since your last visit (visit 15).

Visits 17-18 (follow up)

These visits will take place at 6 and 9 months respectively after your second infusion. At these visits you will be asked to:

- undergo a physical examination (including viral signs, height and weight)
- complete quality of life questionnaire (PBC-40, PROMIS HAQ, COGFAIL, OGS, ESS and HADS)
- repeat blood samples as per baseline visit
- you will be reminded to complete your fatigue diary for one week at the beginning of the month for the 6, 9 and 12 month visits
- you will be asked if you have had any adverse events or if there have been any changes to other medication you were taking since your last visit

Visit 19 (final assessments)

This final study visit takes place at 12 months after your second infusion. At this visit you will be asked to:

- undergo a physical examination (including vital signs, height and weight)
- complete quality of life questionnaires (PBC-40, PROMIS HAQ, COGFAIL, OGS, ESS and HADS)
- repeat blood samples as per baseline visit
- return your completed fatigue diary
- you will be asked if you have had any adverse events or if there have been any changes to other medication you were taking since your last visit (visit 18)

Travel expenses

You can claim back any travel expenses associated with attending the hospital for the research.

Can I find out the results of the research?

If you would like to know the final results of the study then a copy of the journal article will be sent to you on request. We will also present the findings of the study at LiverNorth meetings which are open to the public and which will be publicised widely.

Who can I speak to if I want further information?

If you would like to speak to someone in the Research team or have any problems or queries, contact Kathryn Houghton on [REDACTED] or email [REDACTED], Dr Laura Jopson on [REDACTED] or email [REDACTED]. If you would like to speak to another health professional who is not directly involved in the study, Dr Jopson can arrange this for you.

What if new information becomes available?

Sometimes during the course of a study, new information becomes available about the drug being tested. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, the study doctor will make arrangements for your usual routine care to continue. On receiving new information, the study doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your usual routine care to continue.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time, without giving a reason, and without your medical care or legal rights being affected. If you choose to stop treatment, you will be asked to continue to attend for follow up visits until the end of the study, but you may choose not to do so. If you withdraw from the study completely the blood samples taken as part of the study so far will be stored and analysed, and the data you have provided in the study will be kept and stored.

What if there is a problem?

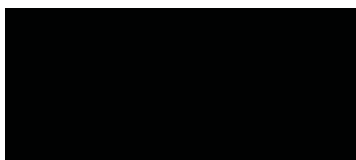
If you have a concern about any aspect of this study, you should ask to speak to the Chief Investigator, Professor David Jones, who will do his best to answer your questions. Contact details are: Professor David Jones, telephone: [REDACTED], email: [REDACTED]. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure by speaking to a member of the PALS (Patient Advice and Liaison Service) team directly during Monday to Friday from 9:00am until 5:00pm. Outside of these hours you can leave a message on an answer-machine and you will be contacted the next working day. The service can be contacted on:

Freephone: [REDACTED]

Text: [REDACTED]

Email: [REDACTED]

or by writing to them at their Freepost address.



Alternatively, you can find more information on taking part in clinical trials on the following websites:

<http://www.nhs.uk/Conditions/Clinical-trials/Pages/Introduction.aspx>

<http://www.mrc.ac.uk/Achievementsimpact/Clinicaltrials/TakingPartInATrial/index.htm>

In the event that something goes wrong and you are harmed during this research due to someone's negligence, then you may have grounds for a legal action for compensation against Newcastle upon Tyne Hospitals NHS Foundation Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). NHS Indemnity does not offer no-fault compensation (i.e. for harm that is not anyone's fault). Neither the sponsor (The Newcastle upon Tyne Hospitals NHS Foundation Trust) who has undertaken to manage the study, nor

the management of the hospital/research centre you are attending for your routine treatment, is able to agree in advance to pay compensation for non-negligent harm.

Private medical insurance

Anyone who has private medical insurance is advised to contact their provider to ensure that participating in this study does not affect their cover.

Will my taking part in this study be kept confidential?

Yes. Any personal information pertaining to your participation in the study will remain confidential. Access to this information is strictly controlled by authorised staff. All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital or clinic will have your name and address removed so that you cannot be recognised. You will be assigned a unique study number which will be used to identify your information and biological samples that leave the hospital. We will also enter your information onto a computer database. We will only use your unique study number, your initials and date of birth in this database (your name and address will not be included).

The information we collect from you during the course of the study will be processed for the purpose of the study and for ensuring compliance with medical, ethical, and pharmaceutical laws and regulations. Your information may be made available to regulatory authorities for the purpose of inspecting and validating our work and it may be disclosed on a strict 'need to know' basis in case of medical emergencies.

Members of the research team, other authorised staff from your hospital, the legal sponsor (The Newcastle upon Tyne Hospitals NHS Foundation Trust), or regulatory authorities may need access to your study documents and medical records. By signing the consent form you will permit authorised staff participating in the research as collaborators or acting on behalf of regulatory authorities to review and use your study documents and/or medical records. Your personal information may be reviewed and copied (your identifying personal data will not be copied) by such people during and after the study to verify clinical and scientific research procedures and/or data to the extent permitted by applicable laws and regulations and without breaching the confidentiality of the records. Even if you withdraw your consent, your personal data may still be processed so that we can verify our work.

Information we collect as part of this study will be held by the research team in paper and electronic format for future reference. Personal data will be held for a maximum of 12 months after the end of the study, and research data for a maximum of 15 years.

Contacting your GP

We will ask you for your permission to contact your GP who will be informed that you are participating in a clinical study.

What will happen to my samples taken as part of the study?

The samples being taken will be treated as a 'gift', and you will not benefit financially if this research leads to the development of a new treatment or medical test. Samples will be sent to Newcastle University for processing and storage. Your name and address will be removed so that you cannot be recognised. After the samples have been analysed, they will be destroyed.

What will happen to the results of the research study?

The results of the study will be published in a peer-reviewed scientific journal. Results may also be presented at clinical conferences. You will not be identified in any publications or reports.

Who is organising and funding the research?

This study was awarded by the Efficacy and Mechanism Evaluation (EME) Programme, and is funded by the Medical Research Council (MRC) and managed by the National Institute for Health Research (NIHR) on behalf of the MRC-NIHR partnership. The Newcastle Clinical Trials Unit is managing the study. The Newcastle upon Tyne Hospitals NHS Foundation Trust is the legal sponsor of the study. This means that they are responsible for the conduct and management of the study.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by NRES Committee North East - Newcastle and North Tyneside 1 Research Ethics Committee.

What should I do now?

If you have any questions about the study, or you think you would like to take part, please contact:

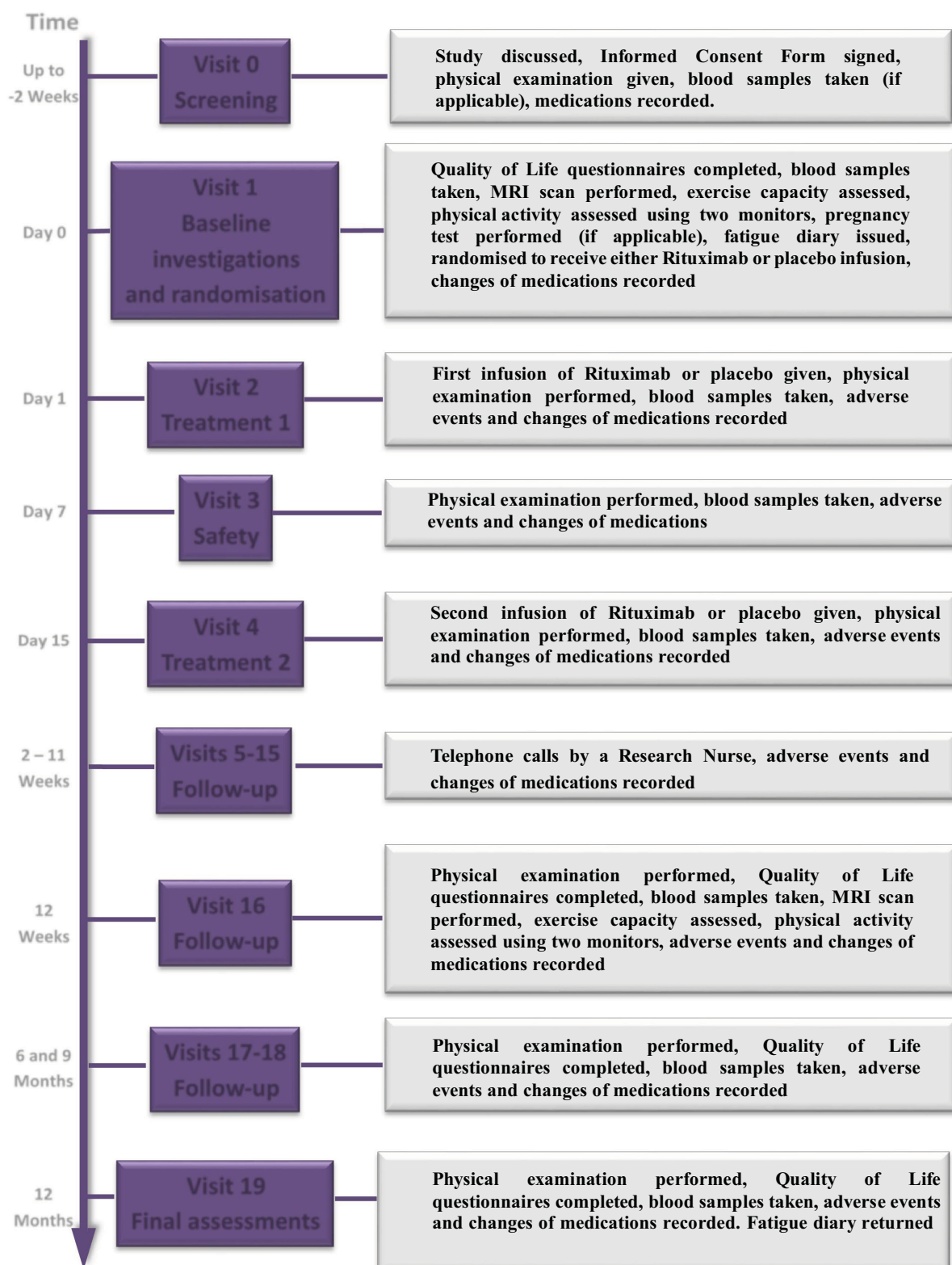
Research Nurses:

Kathryn Houghton, Tel: [REDACTED] or email [REDACTED]

Jenny Bainbridge, Tel: [REDACTED]

Dr Laura Jopson, Tel: [REDACTED], email: [REDACTED]

Table of Events Flow Diagram



Appendix 5 Standard operating procedure for sample handling

Standard operating procedure (SOP) for reception and handling of RITPBC samples (12/11/2012 to 02/11/2016)

Blood samples for each of visits 1, 16, 17, 18 & 19 were physically collected from the Clinical research facility (CRF) (RVI Level 6) as soon as contacted (Jennifer Bainbridge and team).

A single SST II Advance tube (5ml whole blood) with Gold Hemogard Closure (BD 367954) and two K2EDTA tubes (10ml whole blood) with Lavender Hemogard Closure (BD 367525) were handed over and transported (in a covered polystyrene box) 300 metres to the laboratory (M3063 3rd Floor William Leech Building, The Medical School) where the samples were immediately processed.

At this point the subject identifier (eg 01JC), date of collection, NHS number, MRN Number and subject's date of birth were recorded on an Excel spreadsheet database.

Serum preparation:

The Gold top tube containing 5ml of whole blood was centrifuged @ 1300xg for 10min at RT°C and the serum lying above the gel plug was drawn off to a 7ml bijou tube. This serum was mixed and aliquoted into 1.5ml eppendorf tubes (4 x 500ul). The tubes were then immediately stored at -80°C.

Peripheral Blood Mononuclear Cell (PBMC) purification:

The remaining whole blood from the K2EDTA tubes was pooled (~16mls) into a 50ml Flacon tube and diluted (1:1) with Phosphate Buffered Saline (PBS).

15ml Lymphoprep (AXIS-SHIELD, Oslo, Norway) was dispensed on top of the frit of a 50ml Leucosep tube (227290 Greiner Bio-one) and centrifuged for 5min @400xg at RT°C.

The diluted blood was then poured on top of the frit and the tube centrifuged for 25min @ 800xg with no braking at RT°C.

The "buffy (PBMC containing) layer" was then carefully aspirated (avoiding taking a significant amount of lymphoprep) using a sterile Pasteur pipette to a fresh 50ml Falcon tube containing PBS. This tube was topped up to 50ml with PBS and centrifuged for 5mins @400g at RT°C.

The supernatant was carefully decanted and the pellet resuspended in PBS and topped up to 50ml and re-centrifuged as above.

Following this wash spin the supernatant was decanted and the pellet resuspended in 3ml of freezing medium (10% DMSO + 90% FCS). The PBMCs were aliquoted into Cryovials (3 x 1ml) and placed in a "Mr Frosty" freezing vessel and placed at -80°C overnight. The following day the tubes were taken out and stored long term in liquid nitrogen.

Flow cytometry:

Flow Cytometry Buffer was made (PBS + 2% (v/v) FBS (10mls in 500mls) + 1mM EDTA (1ml of 0.5M)). This buffer acts as antibody diluent, wash buffer and Flow Cytometry running buffer.

Whole blood (100ul) (collected in K2EDTA tubes) was dispensed into clear polystyrene FACS tubes.

Add 5ul Human TruStain FcX™ (Fc Receptor Blocking Solution) (Biolegend 422302). Gently mix and incubate 10-15min RT°C.

Add specific antibodies (see panel below) and incubate 30min RT°C away from direct light. Add 2mls 1xFACSLyse Solution (Dilute from 10x - Becton Dickinson Cat No. 349202)

Gently mix and incubate 10min RT°C.

Centrifuge 400xg for 5min and discard supernatant.

Resuspend cells with 2ml Flow Buffer and centrifuge 400xg for 5min. Repeat for 3x washes

Resuspend cells in 150ul Flow Buffer and run immediately or store 4°C for no longer than 12hours.

Samples were analysed in the Flow Cytometry Core Facility (William Leech Building (2nd floor Room M2.099) using the FACSCanto II (machine 3) analyser throughout the period of study).

Data was securely stored and analysed using FlowJo 10.

Antibody Pa

Unstained	No antibodies	
Tube 1	CD19-FITC/CD69-PE/CD45-PerCP	10ul
Tube 2	CD20-PE	10ul
	CD27-APC	10ul
	CD38-PerCP-Cy5.5	40ul of 1:50 in Flow Buffer (<2wks)
	IgD-FITC	20ul
	CD19-APC-Cy7	5ul
Tube 3	CD19-PerCP-Cy5.5	3.5ul
	CD80-PE	10ul
	CD86-APC	10ul
	CD268-FITC	40ul of 1:50 in Flow Buffer (<2wks)
Isotype Controls:		
Tube 5	IgG2b-PE	10ul
	IgG1-PerCP-Cy5.5	40ul of 1:50 in Flow Buffer (<2wks)
Tube 6	IgG1-FITC	10ul
	IgG1-PE	10ul
	IgG1-APC	10ul
	IgG1-PerCP	10ul

A decorative graphic consisting of numerous thin, parallel green lines that curve from the left side of the page towards the right, creating a sense of movement and depth.

**EME
HS&DR
HTA
PGfAR
PHR**

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