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Abstract

Infliximab versus alpha interferon in the treatment of Behçet's disease: the BIO BEHÇET'S RCT

Robert J Moots,[1,2*] Farida Fortune,[3] Richard Jackson,[4] Tony Thornburn,[5] Ann W Morgan,[6] Dan Carr,[7] Philip Ian Murray,[8] Graham Robert Wallace[8] and Deva Situnayake[9]

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Background: While biologic therapy, typically with infliximab or Roferon, was used for Behçet syndrome after first-line immunosuppressants, no high-quality randomised trials or predictive biomarkers were available.

Objective: To undertake a randomised controlled clinical trial of infliximab versus Roferon in Behçet syndrome and identify potential biomarkers for response.

Design: Pragmatic, standard of care, single-masked, randomised, two-arm, parallel head-to-head trial, with exploratory study on potential role of interferon lambda 3 and interferon lambda 4 single nucleotide polymorphisms and urinary metabolomics biomarkers.

Setting: Three national UK Behçet syndrome centres and allied clinics.

Participants: Patients with active Behçet syndrome, fulfilling International Study Group 1990 criteria, with inadequate response to or intolerance of first-line treatment.

Intervention: Randomisation to infliximab (5 mg/kg intravenous infusion) or Roferon (subcutaneous injection), utilising the UK Behçet syndrome drug pathway protocol.

Outcomes: Primary outcome: modified Behçet's disease activity index at 12 weeks of therapy. Secondary outcomes: (1) modified Behçet's disease activity index score at 24 weeks and (2) significant improvement at 12 and 24 weeks from baseline in vitreous haze and best corrected visual acuity change, oral ulcer severity score, number of genital ulcers, arthritis pain, adverse events, reduction in dose of glucocorticoid, quality-of-life scores and Physician's Global Assessment of disease activity.

Sample size: Utilising a Bayesian analysis of covariance model (80% credible interval), initial sample size was 45/arm (Bayesian power 90%). With an anticipated 10% dropout rate, 100 patients were to be recruited. Following recommendations to reduce the overall length of the trial, this was revised down to 80 patients (36 in each arm, allowing for 10% dropout): 80% equi-tailed credibility interval, Bayesian power 88%. In total, 79 patients were eventually recruited for the study.

Methods: Patients with refractory active Behçet syndrome underwent stratified block randomisation, based on randomly permuted blocks with random block sizes of two and four, allocating treatment to either infliximab or Roferon. Follow up with symptom-directed examination at weeks 12 and 24 according to standard of care. Analysis of the primary end point was undertaken using a Bayesian analysis of covariance approach. Informative priors for the anticipated treatment effect were derived from a cohort of six international experts prior to the start of the study.

Results: In this first prospective head-to-head randomised controlled clinical trial of two biologic drugs in Behçet syndrome, both infliximab and Roferon were equally effective [mean difference (80% credibility interval) = 0.13 (–0.19 to 0.46)], with a trend for minor benefit in favour of infliximab in terms of tolerability and treatment persistence. Genetic data suggested a potential association between patient outcome and carriage of either rs4803221 or rs7248668 variants in the interferon lambda 3 (interleukin 28B) gene locus in the Roferon-treated arm. However, with the relatively small sample size, statistical significance of the association was lost when correcting for multiple tests. Metabolomic analysis identified potential markers of a metabolic response to treatment with infliximab.

Limitations: Single-masked design. Slow recruitment with fewer patients recruited in total, limiting the strength of analysis for secondary outcomes and mechanistic studies.

Conclusion: We report clinical efficacy in both infliximab and Roferon in refractory active Behçet syndrome, together with the potential for a novel metabolomic biomarker identifying response to infliximab.

Future work: Further work will characterise the appropriate metabolite(s) from existing samples to inform future prospective trials to study this in more detail clinically. The efficacy of Roferon in Behçet syndrome may support future manufacture of this drug.

Trial registration: This trial is registered as EudraCT Number: 2014-005390-36; ISRCTN49793874.

Funding: This award was funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme (NIHR award ref: 12/205/46) and is published in full in *Efficacy and Mechanism Evaluation*; Vol. 11, No. 17. See the NIHR Funding and Awards website for further award information.

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List of abbreviations

AE	adverse event
ANCOVA	analysis of covariance
AUC	area under the curve
BCVA	best corrected visual acuity
BDCAF	Behçet's Disease Current Activity Form
BD-QoL	Behçet's disease-specific quality of life
BS	Behçet syndrome
CV	curriculum vitae
eCRF	electronic case report form
EQ-5D-5L	EuroQol-5 Dimensions, five-level version
GCP	good clinical practice
ICH	International Conference for Harmonisation
IFNL3	interferon lambda 3
IFNL4	interferon lambda 4
IL-28B	interleukin 28B
IMP	investigational medicinal product
ISG	International Study Group
ITT	intention to treat
LCTC	Liverpool Clinical Trials Consortium
mBDAI	modified Behçet's disease activity index
MDT	multidisciplinary team
NMR	nuclear magnetic resonance
PCR	polymerase chain reaction
PI	principal investigator
QC	quality control
QoL	quality of life
RCT	randomised controlled trial
SAE	serious adverse event
SNP	single nucleotide polymorphism
TNF	tumour necrosis factor
TSC	Trial Steering Committee
WOCBP	women of childbearing potential

Plain language summary

Behçet syndrome, a very rare disease in the UK, causes major illness. Yet without high-quality, randomised, controlled trials, choosing treatment is somewhat hit and miss. We set up a randomised controlled clinical trial to study the two most widely used biologic drugs in Behçet syndrome, infliximab and Roferon, head-to-head and searched for potential blood and urinary markers for response.

Patients with active Behçet syndrome, in the United Kingdom national Behçet syndrome centres and allied clinics, who had not responded to or could not tolerate first-line treatment were randomised to either infliximab infusions or Roferon injections. The primary outcome was modified Behçet's disease activity index at 12 weeks of therapy. Secondary outcomes included modified Behçet's disease activity index at 24 weeks and significant improvements in individual organs, quality of life and Physician's Global Assessments of activity at 12 and 24 weeks. Initial assessment suggested 100 patients were required for a statistically meaningful outcome but was revised down to 80 following recommendations to shorten the trial.

In this first prospective head-to-head randomised controlled clinical trial of two biologics in Behçet syndrome, both drugs worked equally well. There was a non-significant trend for minor benefits of infliximab in terms of tolerability and treatment persistence. Genetic data suggested a potential association between patient outcome and carriage of either rs4803221 or rs7248668 variants in the interferon lambda 3 (interleukin 28B) gene locus in the Roferon arm, but statistical significance was lost with the relatively small sample size. Metabolomics analysis identified potential markers of a metabolic response to infliximab.

The limitations of the study included the single-masked design: patients (but not clinicians) were aware of their treatment, and fewer patients were studied than planned. This limited the strength of analysis for secondary outcomes and mechanistic studies. We now plan to characterise the metabolite(s) from existing samples to design future trials to study if there can be effective targeting of treatment in Behçet syndrome.

Scientific summary

Background

Behçet syndrome (BS), a multisystem inflammatory vasculitis, is infrequent in the UK, but it has the potential to cause significant morbidity and mortality. The evidence base supporting biologic treatment, which is used for active disease after failure of standard immunosuppression or when prognosis is poor, is largely based on uncontrolled studies. At the time of the trial, the UK National guideline for therapy of Behçet's indicated that either the tumour necrosis factor alpha inhibitor infliximab or the interferon alpha drug Roferon could be employed as treatment for patients following failure of first-line treatment with standard immunomodulators. The Bio Behçet's trial was conceived to exploit the opportunity of the three UK National Centres of Excellence for Behçet's and associated satellite centres to undertake the first randomised clinical trial to compare infliximab and Roferon as treatment for BS, together with an exploratory analysis of potential genomic and metabolomic biomarkers of therapeutic response.

Methods

The Bio Behçet's trial is a pragmatic, standard of care, single-masked, randomised, two-arm, parallel trial comparing the efficacies of infliximab and Roferon employed after failure of first-line therapy in BS. Patients with BS, diagnosed according to the International Study Group 1990 criteria, with active disease who had failed to respond to, or were intolerant of, first-line treatment of BS with topical steroids or small-molecule immunosuppressants were randomised to treatment with either infliximab (Remicade) 5 mg/kg intravenous infusions or Roferon subcutaneous injection (in variable dose), utilising the treatment protocol for each of these drugs in normal clinical care as detailed in the BS drugs pathway for England.

The trial utilised a Bayesian design utilising priors informed by a small survey of international experts in BS. Utilising a Bayesian analysis of covariance model, with an 80% credible interval, a sample size of 45 patients per arm was deemed appropriate and gave a Bayesian power of 90%. Allowing for an anticipated 10% dropout rate, 100 patients were planned to be recruited but reduced to 80 following recommendations to reduce the overall length of the trial. Allowing for a 10% dropout rate, estimates of study power based on 72 evaluable patients (36 on each arm) and an 80% credible interval a Bayesian power of 88% was obtained.

Between June 2016 and February 2020, 161 patients were screened, and 79 patients were randomised. The intention-to-treat analysis was restricted to 37 subjects allocated to infliximab and 37 to Roferon.

Based on previous work with hepatitis C infection and response to interferon therapy and given the role of the innate immune system in the pathogenesis of BS, we examined interferon lambda 3 (IFNL3) and interferon lambda 4 (IFNL4) single nucleotide polymorphisms (SNPs) as biomarkers of response to treatment with alpha interferon and/or infliximab in BS. We also examined the potential for urine metabolomics to act as biomarkers for drug response.

The primary outcome was a modified version of the Behçet's disease activity index (mBDAI) after 3 months of therapy. Secondary outcomes comprised mBDAI score after 6 months of therapy and significant improvement in organ systems after 3 and 6 months (week 12 and week 24 visits) assessed by: reduction in vitreous haze using the SUN consensus group grading scale and best corrected visual acuity change [using the logarithm of the minimal angle of resolution (LogMAR) chart at 4 m] from baseline; change in oral ulcer severity score; change in number of genital ulcers; arthritis pain (10 cm Likert scale); adverse events (AEs) in each group; reduction in dose of prednisolone (or equivalent glucocorticoid) at 3 months

(week 12 visit); reduction in dose of prednisolone (or equivalent glucocorticoid) at 6 months (week 24 visit); quality-of-life (QoL) scores at 3 and 6 months (week 12 and week 24 visits) and Physician's Global Assessment of disease activity at 3 and 6 months (week 12 and week 24 visits).

Results

Baseline characteristics of the two treatment arms did not differ significantly by sex, ethnic profile, baseline disease characteristics and steroid use.

For the primary outcome measure, change in mBDAI between baseline and 3 months (and as a secondary outcome between baseline and 6 months) did not differ significantly between the two treatments [mean difference (80% CrI) = 0.13 (−0.19 to 0.46)].

A significantly higher proportion of patients randomised to Roferon swapped away from their randomised treatment compared to those randomised to infliximab treatment (Roferon 11 of 37, infliximab 3 of 37; $p = 0.0104$).

The clinician's overall perception of disease activity indicated a reduction in disease activity for most patients between baseline and 3 months and between baseline and 6 months, with a median reduction of −2.0 (infliximab) and −1.0 (Roferon) at 3 months and −3.0 (infliximab) and −2.0 (alpha interferon) at 6 months. There was a small but significant difference in favour of infliximab compared to Roferon at both 3 and 6 months ($p < 0.05$).

There were no significant differences between the two treatments at 3 or 6 months for secondary outcome measures, including oral ulcer activity score, genital ulcer activity score and Likert pain score, though, for each of these secondary outcome measures, there were important clinically significant within-group reductions over time at 3 and 6 months. There were no important differences between the two treatments for QoL measures. A modest steroid-sparing effect was observed for each treatment.

In total, 46 patients reported 270 Aes. There were a greater number of AEs observed on Roferon ($p < 0.001$). Eight serious adverse events (SAEs) from five patients were reported across the study. One patient on the infliximab arm reported four SAEs [hypertension (×2), bacterial urinary tract infection and blood pressure inadequately controlled]. In total, three patients (six events) were reported on the infliximab arm, and two patients (two events) were reported on the Roferon arm. There were no suspected drug interactions and no suspected unexpected serious adverse reactions (SUSARS) reported in the study.

The genetic data suggest the possibility of an association between response to treatment and carriage of either rs4803221 or rs7248668 variants in the IFNL3 (interleukin 28B) gene locus only for the alpha interferon-treated arm, in line with association between these two SNPs and Roferon treatment outcome in hepatitis C. These results must be treated with caution due to small numbers in responder subgroups.

There were no baseline differences in metabolomic analysis between the patients before randomisation, indicating no major confounding factors that may have influenced response to a particular treatment. Comparison of 24-week urine samples from responders and non-responders to the same drug using principal component analysis revealed, for infliximab, one specific bin of metabolites that remained significantly different comparing responders to non-responders. This effect was weaker for Roferon, but further study will be required to identify individual metabolites and the associated metabolic pathways responsible for these results.

Conclusion

Using a Bayesian trial design, in this first randomised controlled study comparing infliximab with Roferon when used after primary treatment failure, both were found to be effective and largely equivalent, with minor benefits favouring infliximab in terms of efficacy and tolerability. Mechanistic studies utilising genomics and metabolomics to identify predictors of response to treatment revealed opportunities for further study based on our initial findings. The UK National Behçet's Centres of Excellence and associated satellite centres can be an effective resource to support clinical trials in the management of BS.

Trial registration

This trial is registered as EudraCT Number: 2014-005390-36; ISRCTN49793874.

Funding

This award was funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme (NIHR award ref: 12/205/46) and is published in full in *Efficacy and Mechanism Evaluation*; Vol. 11, No. 17. See the NIHR Funding and Awards website for further award information.

Chapter 1 Introduction

Background

Behçet syndrome (BS; also called Behçet's disease or, simply, Behçet's) is a systemic inflammatory vasculitis of unknown aetiology, characterised by recurrent episodes of acute inflammation in a variety of organs, typically including mucus ulceration in the mouth and genitally, but also manifestations in other organs from the skin to the eyes, where it can cause blindness.[1,2] These clinical features, the absence of specific autoantibodies and its spontaneously relapsing and remitting nature have led to its characterisation as a polygenic autoinflammatory disorder. It is considered to be a very rare disease in the UK. The early estimates of prevalence in the UK ranged from 0.27 to 0.64 per 100,000, but a recent unvalidated population-based estimate utilising a large primary care database suggested a higher range of between 12 and 14/100,000, with the rider that this may be an overestimate as a proportion of such cases will not have been validated by an expert multidisciplinary team (MDT). Behçet's disease is up to 10-fold more prevalent in the Middle East and Far East along the 'silk route' to Japan and increases towards Southern Europe.

There is considerable variation in its clinical presentation between and within individuals, and there appear to be differences in disease manifestations and response to therapy in patients in the UK compared to those in southern European or Far East countries. While little is known about the underlying pathophysiological processes, recent years have witnessed the successful application of biologic therapies, with good outcomes in patients who had not previously responded to standard therapy with steroids and/or immunosuppressants such as azathioprine.[3–5] Much of the evidence base for biologic therapy has arisen from case series or other uncontrolled trials performed in countries outside the UK – where the phenotype appears to differ. For example, more severe ocular disease is reported in patients in Turkey and Japan compared to the UK and Western populations, where mucocutaneous and gastrointestinal (GI) manifestations are more prevalent. Accordingly, most trials have reported effects on ocular disease. Very few data are available to inform useful systematic reviews, and most guidelines therefore stem from expert-based consensus.[6]

The establishment of three National Centres of Excellence for Behçet's disease in England[7] led to the creation of a national cohort of patients with agreed pathways for assessment and treatment by MDTs, comprising the specialist and support staff needed to cover the wide spectrum of organ involvement and the ability to fund biologic therapy when indicated. At the time of the trial, it was generally considered that only two biologics, infliximab and alpha interferon (Roferon), had sufficient evidence to support their use as first-line biologics in refractory disease. Other biologics, such as alemtuzumab, with a less favourable adverse event (AE) profile, were reserved for patients who were refractory to or intolerant of infliximab and alpha interferon.

Infliximab is a mouse/human chimeric monoclonal antibody originally developed for use in rheumatoid arthritis that works by neutralising tumour necrosis factor (TNF) alpha. Its long-term safety record is well established in rheumatoid arthritis,[8] and its utility in Behçet's disease reported, typically in uncontrolled studies and in countries outside the UK.[3] In 2011, Arida *et al*. conducted an extensive PubMed/MEDLINE search on the published experience of 375 patients treated with a TNF-inhibitor for Behçet's disease[9] and with a variety of organ systems involved. Of these, the vast majority (325) were treated with infliximab, and all had been inadequately controlled with, or were intolerant to, other immunosuppressive regimens such as glucocorticoids, azathioprine and ciclosporin. Sustained organ-specific clinical responses were evident in 90%, 89%, 100% and 91% of patients with resistant mucocutaneous, ocular, GI and central nervous system involvement, respectively. None of those trials were randomised or placebo controlled.

At the time of the trial, Roferon was used in several inflammatory and rheumatic disorders, including Behçet's disease, and was well summarised by Kotter *et al*. in 2010.[10] Much of the reported experience of alpha interferon in Behçet's disease originated from uncontrolled studies in patients with ocular disease. Kotter *et al*. reported in 2003 an open, non-randomised, uncontrolled prospective study using Roferon in 50 patients with inflammatory eye disease due to Behçet's disease.[11] An overall ocular response rate of 92% was reported. A rapid response: with the posterior uveitis score of the affected eye falling by 46% per week and full remission achieved by week 24. A retrospective single-centre uncontrolled series reported by Bodaghi *et al*.[12] of ocular Behçet's disease also reported a high response rate of 82.6%, with other groups reporting similar findings. Reports of controlled trials of alpha interferon and its efficacy in extraocular disease are limited. Alpsoy *et al*.[13] published a randomised placebo-controlled study of 50 patients with mucocutaneous Behçet's disease randomised to alpha interferon or placebo, reporting that alpha interferon was effective in the management of mucocutaneous lesions, with a trend towards improvement of joint symptoms. The formulation of and dosing regimens for alpha interferon have varied between these studies, most utilising the preparation Roferon, with short half-life and more frequent administration. The subsequent development of pegylated alpha interferon (Viraferon Peg)[14] allowed dosing once a week. However, a UK prospective trial evaluating Viraferon Peg in Behçet's disease reported only modest benefit, with responses far less than that for Roferon.[15]

Assessment of disease activity: As a multisystem disease with lack of laboratory biomarkers to determine activity and limited disease-specific outcome measures, devising and undertaking a clinical trial of active medicinal products in Behçet's disease is challenging, and international standards for this have not yet been adopted. Mumcu *et al*.[16] summarised the various methods used to measure overall clinical activity. The International Scientific Committee on Behçet's disease produced the 'Behçet's Disease Current Activity Form' (BDCAF), with investigators from five countries participating.[17] Thirty dichotomous questions were reduced to a Behçet's disease activity index (BDAI) lying between 0 and 12 but then transformed to a 0–20 scale for international comparisons. In Iran, the Iranian Behçet's Disease Dynamic Measure is used.[18] The Behçet's Syndrome Activity Scale was developed as a patient-reported outcome measure, which correlates with the BDCAF.[19] A Behçet's Disease-specific quality-of-life measure (BD-QoL) was derived by the Psychometric Group in the Leeds Institute of Rheumatic and Musculoskeletal Medicine, Leeds University; it consists of 30 easily answered dichotomised questions.[20] Other disease activity measurements have been proposed for specific organ symptoms. We chose to use a slightly modified form of the BDAI as the primary outcome for the proposed clinical trial, as this is a verified measure of the overall severity of the disease and is routinely used in the three UK Centres of Excellence.

As in other complex chronic diseases, there is a need in BS to not only better understand the phenotype of patients in clinical trials but also to explore the potential to identify biomarkers that may help target therapies and minimise AEs. No such biomarkers are currently available. We, therefore, built into the study the exploratory analysis of two potential genetic and metabolomic biomarkers that focused on potential responses to alpha interferon and infliximab, respectively. Three genome-wide association studies in patients with hepatitis C virus genotype 1 infection implicated single nucleotide polymorphisms (SNPs) in the vicinity of the interferon lambda 3 (IFNL3) [interleukin 28B (*IL28B*)] gene on chromosome 19q13.13 with response to alpha interferon therapy.[21–23] Patients with the CC genotype at rs12979860 had higher response rates to alpha interferon.[24] A recent parallel sequencing study[25] was able to show that rs4803221 and rs7248668 predicted failure to respond better than rs12979860. IFNL3 encodes a lambda type of infliximab, while the SNP at rs12979860 affects interferon-stimulated gene production as part of the innate immune response, but the actual mechanism is unclear.[26] Despite this, treatment algorithms incorporating IFNL3 genotyping are now used in many clinics for the treatment of hepatitis C.[27] A recent study[28] showed that rs12979860 is in linkage disequilibrium with a frameshift variant, ss469415590[ΔG], which also creates a new gene, interferon lambda 4 (*IFNL4*),

reduced expression of which may be associated with reduced responsiveness of cells to alpha interferon 8. Whether the same SNPs affect response to alpha interferon in other diseases is unclear, but given the role of the innate immune system in the pathogenesis of BS,[29] it was biologically plausible that a similar effect to that seen in hepatitis C with alpha interferon may be operating in BS. We intended to test this hypothesis in this trial.

To our knowledge, no convincing genetic predictors had been identified through genome-wide association studies as determinants of response to infliximab. We therefore chose initially to pursue a metabolomic route to explore this. Nuclear magnetic resonance (NMR)-based metabolomics allows the examination of the changes in hundreds or thousands of low-molecular-weight metabolites in an intact tissue or biofluid and offers several distinct advantages in a clinical setting since it can be carried out on standard preparations of blood cells, serum, plasma or urine. Pattern recognition techniques are applied to the NMR spectra of samples taken from individuals. Metabolomic analysis was able to distinguish between patients with rheumatoid arthritis who responded to anti-TNF therapy compared to those who did not, with a sensitivity of 88.7% and a specificity of 85.9%.[30] We have previously shown that metabolomic analysis of vitreous humour could separate with high sensitivity and specificity samples from patients with two inflammatory conditions, lens-induced uveitis and idiopathic chronic uveitis with urea and oxaloacetate levels associated with the different conditions.[31] There is therefore a sound rationale for exploring similar methodology in the current study of patients with BS.

Assessing biomarkers as part of this trial also promised the potential to not only help in identifying determinants of response but also provide insights into the potential mechanisms of action of these biologics in BS patients.

Rationale

Behçet syndrome is associated with significant morbidity and mortality in the UK and abroad. In the UK, it can take up to 12 years to diagnose, leads to blindness and stroke, often does not respond to simple immunosuppressive therapy and, as we have previously reported, has a major impact on quality of life (QoL).[32] Although the biologic drugs infliximab and alpha interferon have been reported to be effective in refractory BS, they are expensive [at the time of starting the trial, the cost of infliximab was < £20,000/year and alpha interferon (Roferon) was £4000/year] and have not been subjected to rigorously undertaken randomised controlled trials (RCTs) compared directly against each other for efficacy and safety. Evidence for their efficacy arose from uncontrolled studies in other countries, where the disease phenotype may exhibit subtle differences from that in the UK.[6] Funding for biologic drugs for BS in England is held by the three National Centres of Excellence, allocated from highly specialised NHS commissioning.[33] Anecdotal experiences from the three centres of excellence also indicated that response rates to the individual biologics used in this study might differ when used within a UK population compared to results reported from other international cohorts. While these biologic drugs were generally considered effective for patients with BS in the UK, the national centres anecdotally observed efficacy rates to differ from those reported in other countries, with variable and unpredictable responses, and therefore there was a poor evidence base on which to inform clinical decisions. The identification of novel biomarker(s) predicting response to biologic therapy was deemed necessary to allow a more precision-based approach to treatment. A polymorphism in the IFNL3 (IL28B) was predictive of reduction in viral load in response to alpha interferon in hepatitis B or C infections.[21,34,35] As similar alpha interferon-mediated pathways of innate immunity are involved in BS, the potential effect of IFNL3 SNP on response to therapy with alpha interferon may well be relevant in BS. Similarly, metabolic analyses of urine samples from patients afforded the potential to provide biomarkers for treatment response to infliximab and/or alpha interferon.

Objectives

The aim of the study was to create the evidence base to underpin clinically effective prescribing of the biological drugs infliximab and alpha interferon for BS.

The objectives of the study were to:

1. Undertake a RCT to compare infliximab versus alpha interferon in patients with BS who were unresponsive to standard oral therapy.
2. Examine whether IFNL3 and IFNL4 SNPs can predict responses to alpha interferon and/or infliximab in BS.
3. Examine the potential for urine metabolomics to act as biomarkers for drug responses to infliximab and/or alpha interferon in BS.

Chapter 2 Research methods

Trial design

Bio Behçet's was a randomised, two-arm, parallel design comparing the efficacies of infliximab versus alpha interferon. The population was patients with refractory disease eligible for the first biologic drug. Patients were recruited from the National Behçet's Centres and supporting clinics in England and randomised to the two arms of the trial with stratification by centre. A total of 80 patients were to be randomised on a 1 : 1 ratio for arms. Recruitment was scheduled to take approximately 36 months. Assessments were made at baseline, 12, 24 and 36 weeks. The end of study was when the final patient completed their 6-month follow-up assessment.

Study setting and study population

Study setting

The study was carried out in the three Behçet's Centres of Excellence in England, with additional recruiting clinics. The additional recruiting centres were chosen on the basis of:

1. having at least one lead clinician with a specific interest in, and responsibility for, supervising and managing patients with BS
2. showing significant enthusiasm to participate in the study
3. ensuring that sufficient time, staff and adequate facilities were available for the trial
4. providing information to all supporting staff members involved with the trial or with other elements of the patient's management
5. acknowledging and agreeing to conform to the administrative and ethical requirements and responsibilities of the study, including signing up to good clinical practice (GCP) and other regulatory documentation.

Each recruiting centre then met the following inclusion criteria:

1. positive Site-specific Assessment by local NHS research and development offices
2. local Health Research Authority approval
3. signed Research Site Agreement
4. receipt of evidence of completion of (1) and (2) by Liverpool Clinical Trials Consortium (LCTC)
5. completion and return of 'Signature and Delegation Log' to LCTC
6. curriculum vitae (CV), including a record of International Conference for Harmonisation (ICH) of GCP training – principal investigator (PI)
7. curriculum vitae, including a record of ICH GCP training – other personnel on the delegation log
8. Clinical Study Protocol Receipt Form:
   a. Investigator Brochure's Receipt Form
9. local laboratory accreditation/Quality Check
10. local laboratory reference ranges
11. patient information sheet, consent form and general practitioner letter on trust-headed paper
12. local pharmacy practice form.

Centres that did not meet the above criteria were excluded from the trial.

Study population

The population studied was drawn from patients attending the three Behçet's Centres of Excellence in England, with additional recruiting sites working in collaboration. There were an estimated 800 patients

with BS in England. The Behçet's Centres, established by UK National Specialist Commissioning in 2012, are funded to provide a comprehensive service for diagnosis and management of BS, including full funding for biologic drugs in patients with refractory disease who are intolerant of or inadequate responders to therapy with corticosteroids and/or immunosuppressants. Each centre runs at least weekly multidisciplinary clinics for patients with Behçet's disease, attended by consultants in oral medicine, ophthalmology, neurology, dermatology, genito urinary medicine medicine or gynaecology and rheumatology and supported by a specialist nurse, clinical psychologist and support worker.

Eligibility criteria

Inclusion criteria:

1. Diagnosed to have BS by International Study Group (ISG) criteria or International Criteria for Behçet's Disease.
2. Had refractory disease as defined by the UK Centres of Excellence criteria (failure to respond to steroid and/or immunosuppressive therapy with significant or major organ-threatening disease) and therefore qualify for biologic therapy with either infliximab or alpha interferon. Patients who had failed to respond to, or been intolerant of, azathioprine at a dose of > 2 mg/kg (or comparable drug) and/or prednisolone at a dose of > 40 mg/day typically for more than 3 months, or with evidence of either organ-threatening disease or unacceptable AEs from immunosuppressive medication.
3. Able to give informed consent.
4. Have not previously received a biologic agent.
5. Aged over 18 years.

Exclusion criteria:

1. Contraindication to either infliximab or alpha interferon (e.g. active infection, severe liver disease, neutropenia, previous malignancy).
2. Unlikely to comply (e.g. cannot attend for assessments because of excessive travel requirements).
3. A strong preference for one of the two potential therapies.
4. Severe heart failure that would contraindicate the use of infliximab.
5. Diagnosed with multiple sclerosis.
6. Evidence of infection with human immunodeficiency virus (HIV):
    a. Women of childbearing potential (WOCBP) who were unwilling or unable to use an acceptable method to avoid pregnancy for the study duration plus 6 months.
    b. Women who were pregnant or breastfeeding.
    c. Sexually active, fertile men, not using effective birth control if their partners are WOCBP.
7. Active tuberculosis.

Trial interventions

Arm A infliximab intravenous infusion (Remicade®)

Remicade (and not biosimilar infliximab) was used in this study and was supplied from local stock. No additional labelling by site pharmacy was required.

The cost of the investigational medicinal product (IMP) was covered by existing funding arrangements with the National Behçet's Centres.

Patients continued with concomitant immunosuppressants such as methotrexate or azathioprine unless otherwise clinically indicated.

Patients in Arm A received Remicade at a standard dose of 5 mg/kg at weeks 0, 2 and 6 as loading, then every 8 weeks for the remaining length of the trial (unless there was primary ocular, neurological or vascular disease when infusions were repeated at 6 weekly intervals after loading).

Remicade was administered according to the standard preparation and infusion procedures of each investigational centre.

Arm B alpha interferon (Roferon-A®) prefilled syringes

Roferon was used in this study and was supplied from local stock and handled according to the instructions within the corresponding summary of product characteristics. No additional labelling by site pharmacy was required.

The cost of the IMP was covered by existing funding arrangements with the National Behçet's Centres.

A decreasing dose of Roferon-A was given to patients randomised to Arm B. All doses were given subcutaneously. See *Table 1*.

Immunosuppressants were discontinued in Arm B by rapid tapering after commencement of therapy. In the absence of an AE, tapering down of Roferon occurred at 4-weekly intervals. The development of an AE [such as leucopenia, persistent fever, raised liver function tests (alanine aminotransferase or aspartate aminotransferase > 3 times the upper limit of normal), persistent unacceptable fatigue, flu-like symptoms or severe depression] prompted a reduction in dose and/or frequency according to the above schedule.

Roferon-A prefilled syringes were dispensed from hospital stocks using the usual prescribing and dispensing practices.

Schedule of trial procedures

The schedule of trial procedures are listed in *Table 2*.

Data capture

Trial data were captured using electronic Case Report Forms (eCRFs) and transcribed to a MACRO database. This database was designed and maintained by the LCTC, which was also responsible for the randomisation. The eCRF was the primary data collection instrument for the study.

All eCRFs were entered directly into a MACRO database and accessed via a secure web page by research site staff and the clinical trial co-ordinator at LCTC. The client application was secured with

TABLE 1 Dosing schedule for Roferon

Dose	Frequency	Duration
3 million units	Daily	3 days
6 million units[a]	Daily	2–4 weeks
4.5 million units	Daily	2–4 weeks
3 million units	Daily	2–4 weeks
3 million units	3 times a week	2–4 weeks
3 million units	Twice weekly	To trial end

a The 6 million units dose was only to be administered to males weighing over 80 kg with major organ-threatening disease (e.g. severe eye involvement). Males < 80 kg and females started with 4.5 million units once daily to minimise the development of side effects.

TABLE 2 Schedule of trial procedures

Time (weeks)	Screening	0 Randomisation/ baseline[b]	12 weeks	24 weeks End of trial	36 weeks[a] End of trial
Informed written consent	x				
Assessment of eligibility criteria	x				
Review of medical history	x				
Review of concomitant medications	x	x	x	x	x
Pregnancy test	x	x	x	x	x
Randomisation		x			
Compliance with study intervention			x	x	x
Height	x				
Weight	x	x	x	x	x
Heart rate, respiratory rate, blood pressure	x	x	x	x	x
EQ-5D-5L Health Questionnaire		x	x	x	x
BD-QoL questionnaire		x	x	x	x
BDAI		x	x	x	x
Collection of 9 ml blood for translational research		x			
Collection of (3 × 1 ml) urine for translational research		x	x	x	x
Laboratory assessments (FBC)	x	x	x	x	x
Laboratory assessments – biochemical profile (liver, bone and renal)	x	x	x	x	x
Laboratory assessments (ESR)	x		X	X	X
Laboratory assessments (CRP)	x		X	X	X
Laboratory assessments (hepatitis B and C serology)	x[c]				
HIV screening	x[c]				
TB screening	x				
Assessment of AEs	x		x	x	x
Routine clinical assessment [eyes, ulcers (mouth/genital), musculoskeletal, skin and systemic problems] as clinically indicated[b]	x	x	x	x	x
Steroid use	x	x	x	x	x
Visual acuity (using LogMAR chart)[b]	x	x	x	x	x
Intraocular inflammation (SUN grading)[b]	x	x	x	x	x
Burden of skin rash[b]	x	x	x	x	x
Musculoskeletal Likert pain score[b]	x	x	x	x	x

TABLE 2 Schedule of trial procedures (*continued*)

Time (weeks)	Screening	0 Randomisation/ baseline[b]	12 weeks	24 weeks End of trial	36 weeks[a] End of trial
Genital Ulcer Severity Score[b]	x	x	x	x	x
Oral Ulcer Severity Score[b]	x	x	x	x	x
PHQ-9 Questionnaire	x	x	x	x	x

CRP, C-reactive protein; EQ-5D-5L, EuroQol-5 Dimensions, five-level version; ESR, erythrocyte sedimentation rate; FBC, full blood count; PHQ-9, Patient Health Questionnaire-9 items; TB, tuberculosis.
a Week 36 – for patients that swap treatment at week 12.
b Performed at baseline, then a symptom-directed approach for follow-up visits (weeks 12, 24 and 36).
c Not to be completed if already carried out up to 6 months earlier.
Study intervention to be administered as per protocol.

a unique username/password combination allocated to each delegated member of the research team. When data were entered into an eCRF, it was electronically stamped with the date, time and the person who entered it. If data were changed on an eCRF, it was electronically stamped with the change and would be accompanied with the date, time, person and a reason for making the change or correction. The previous value was recorded in an audit trail for each data item.

Each eCRF contained specific validation checks on the data being entered. If any values were outside what was expected, or data missing, this was flagged up and raised as a discrepancy on the main database system. Regular reports were generated to identify discrepancies in the data and allow for follow-up. Comprehensive guidelines for eCRF data entry were provided to all staff who have been delegated the responsibility for data collection. Where the site was unable to upload data using the eCRF, a backup paper CRF was available to use and accessed from the LCTC portal. In such cases, the site research staff would enter the data onto the trial MACRO database following the assessment.

Electronic and paper screening logs were kept in clinics to record the number of patients declining participation and, when volunteered, the reason given. All data were kept in a secure, locked location on NHS premises. All routine eCRFs were to be completed within 14 days of the study visit occurring.

Paper versions of the CRFs were available for download from the LCTC website, www.LCTC.org.uk and used as an aid to research staff. Quality control (QC) processes, including onsite source data verification for primary and secondary end points, were put in place in line with the eCRF platform.

Biological samples

Blood samples/genotyping

A 9 ml blood sample was collected at baseline and then transported using Royal Mail Safeboxes to the Wolfson Centre for Personalised Medicine, University of Liverpool. Following this, DNA was extracted using an automated Chemagic platform (Perkin Elmer), and four SNPs were genotyped including rs12979860, rs4803221, rs7248668 and rs368334815 using 'off the shelf' validated allelic discrimination assays (Applied Biosystems). This was carried out by a trained technician with Real-Time polymerase chain reaction (PCR) utilising a QuantStudio six Fast Real-Time PCR System (Applied Biosystems). Strict QC measures were followed to ensure systematic validation of the genotype results.

The genotypic analysis was an exploratory analysis to determine whether any of the SNPs show an effect of efficacy of alpha interferon based on primary and secondary outcomes.

Urine metabolite

Urine samples from the patients were analysed by NMR spectroscopy and principal component analysis. Urine samples (3 × 1 ml) were collected at baseline, week 12, week 24 and week 36, snap frozen and stored at −80 °C and then transported to the Centre for Translational Medicine, The University of Birmingham. After thawing, urine samples were centrifuged at 13,000 *g*, prepared using a standard protocol and loaded into a standard 5-mm NMR tube for spectroscopy. For sample preparation, 450 µl of urine was mixed with 150 µl of 400 mM phosphate buffer at pH 7.0. 1D-NOESY presaturation ^{1}H NMR spectra were acquired on a Bruker 600 MHz IvDR NMR system equipped with a *z*-axis gradient 5 mm TXI probe. Sixteen steady-state scans and a total of 128 transients were acquired per spectrum. All samples were shimmed to achieve a TMSP linewidth below 1 Hz prior to data acquisition. The spectral width was set to 20 ppm, the interscan relaxation delay was set to 10 seconds and a total of 32,768 complex data points were acquired. All spectra were processed using the MetaboLabPy software, including manual phase correction and data pre-processing. Data pre-processing included excluding regions > 9.8497 ppm, between 6.4522 and 5.6194 ppm and < 0.3168 ppm; segmental alignment of 71 spectral regions; noise filtering; bucketing of 32 data points (0.005 ppm); spectral normalisation using probabilistic quotient normalisation; variance stabilisation using Pareto scaling and finally export into an Excel spreadsheet for statistical data analysis.

Lists of metabolites providing the greatest discrimination between groups were identified using multivariate analyses and metabolites identified using an NMR database (Human Metabolome Database version 2.5) and Chenomx NMR suite. Strict QC measures were adhered to, ensuring proper validation of genotype results.

Outcome measures

Primary outcome

Modified Behçet's disease activity index (mBDAI) after 3 months of treatment (week 12 visit), with 20% change in means being defined as the zone of equivalence of treatment.

Secondary outcomes

Modified Behçet's disease activity index after 6 months of treatment (week 24 visit).

- Original BDAI after 3 and 6 months of treatment (week 12 and week 26 visits).
- Significant improvement in organ systems after 3 and 6 months (week 12 and week 24 visits) assessed by:
  - Ocular: reduction in vitreous haze using the SUN consensus group grading scale and best corrected visual acuity (BCVA) change (using the LogMAR chart at 4 m) from baseline. A reduction of 2 or more in vitreous haze and a difference of 15 letters or more in BCVA are considered to be clinically significant.
  - Oral ulcer activity: change in ulcer severity score. An improvement of 20% is considered to be clinically meaningful.
  - Change in number of genital ulcers: a reduction of 20% is considered to be clinically significant.
  - Musculoskeletal: Likert pain score assessed by arthritis pain (10 cm) Likert scale on Rheumatology and Flare Data Collection Form (an improvement of 20% is considered to be clinically meaningful).
- Adverse events in each group.
- Reduction in dose of prednisolone (or equivalent glucocorticoid) at 3 months (week 12 visit): a clinically meaningful reduction is considered to be 50% of baseline or dose of < 15 mg/day prednisolone.

- Reduction in dose of prednisolone (or equivalent glucocorticoid) at 6 months (week 24 visit): a clinically meaningful reduction is considered to be 50% of baseline or dose of < 7.5 mg/day prednisolone.
- Quality-of-life scores at 3 and 6 months (week 12 and week 24 visits) compared to baseline. The QoL instruments used will be EuroQol-5 Dimensions and BD-QoL: a reduction of 20% would be of clinical importance.
- Physician's Global Assessment of disease activity [a 7-point Likert scale completed as part of (but assessed independently of) the BDAI] at 3 and 6 months (week 12 and week 24 visits) (a change of 2 points is considered to be clinically meaningful).

Chapter 3 Statistical methods

Sample size

The primary outcome was a modified version of the BDAI after 3 months of therapy, which will range from 0 to 30 for a patient.

If a traditional frequentist equivalence design were to be used, then based on equivalence being defined as the difference in means being < 20% (i.e. 20% of mean BDAI of 10 = 2), then for significance level, 0.20 and power 90%, a sample size of 176 (88 per arm) is required. (Here we have assumed standard deviation of 4 for BDAI at 3 months, a difference in means of 0.5, in accordance with the opinions of the international experts recruited for the Bayesian design. Also, baseline measurements have not been taken into account which would be expected to reduce the sample size to some extent.) As the recruitment of this number of patients is not feasible, the Bayesian design is adopted.

Bayesian design: Analysis of the data obtained from the small survey of experts both from the UK and regions of high prevalence of BS internationally, described in *Chapter 4* (Research Design), gives a prior distribution of the difference in mean values of BDAI as *N* (0.52, 1.062) and < 24% difference in means to define equivalence. The mode for the latter was 20%, and this value is used in the sample size calculation as it fits better with FDA guidelines.

If the difference in means, D, of BDAI at 3 months is considered without the use of baseline BDAI, then (assuming the above) prior for D and a normal distribution, *N* (10, 42) for the distribution of the 3-month BDAI scores. For a sample size of 45 per arm, the Bayesian power based on an equi-tailed 80% credible interval for testing for equivalence is 0.71.

To be more accurate, a simulation exercise was carried out using R and WinBUGS to establish the sample size for the analysis of covariance (ANCOVA) model. For one arm, random baseline and 3-month BDAI scores were generated from a bivariate normal distribution with mean vector (12, 10), variances 4.0 for both and correlation *r*. For the other arm, random baseline and 3-month BDAI scores were generated from a bivariate normal distribution with mean vector (12, 10+ m), variances 4.0 for both and correlation *r*. For each simulation, *r* was randomly chosen from a uniform distribution (0.05, 0.5) and *m* from a *N* (0.52, 1.062) distribution. For a sample size of 45 per arm, testing for equivalence using an 80% equi-tailed credible interval calculated from the posterior distribution, Bayesian power of 91% was obtained. When a 90% credible interval was used, the Bayesian power dropped to 73%.

Using this design with the 80% credible interval, a sample size of 45 patients per arm was deemed suitable, which, allowing for 10% dropout, requires 100 patients to be recruited.

Following recommendations to reduce the overall length of the study, the study was amended to recruit a total of 80 patients. Including a 10% dropout rate, estimates of study power are based on 72 evaluable patients (36 on each arm). Here, when an 80% equi-tailed credibility interval is used, a power of 88% is obtained; when a 90% credible interval is used, the power drops to 71%.

Randomisation

Stratified block randomisation, based on randomly permuted blocks with random block sizes of 2 and 4, was employed. The randomisation code list was generated by the LCTC trial statistician with the software package Stata® using the 'ralloc' statement. The trial was open-labelled. The stratification factor included in the design is Centre. Data from the eCRFs will be entered onto a MACRO4 database with

extensive data validation checks alerting all missing data to be queried. Missing data were monitored, and strategies were developed to minimise its occurrence. Central statistical data monitoring will summarise missing or inconsistent data periodically.

Analysis

Primary outcome

Alpha interferon. A Bayesian ANCOVA model will be used:

$y = \beta 0 + \beta 1 \times x + \alpha \times tr + \varepsilon,$

where y = BDAI at 3 months, x = baseline BDAI, tr = 0 if a patient is in the infliximab group and tr = 1 if a patient is in the alpha interferon group. β0, β1 and α are the parameters to be estimated, and ε is the error term with variance σ^2 (to be estimated). The parameter of particular interest is α, as it measures the difference between the two treatment groups. Prior distributions will be placed on β0, β1, α and σ^2 WinBUGS will be used to fit the model.

Prior information

Vague priors for β0 and β1 were set as following a normal distribution with mean zero and a larger variance [i.e. ~ *N* (0.0, 100,000)]. The prior distribution for σ was set as a uniform distribution with limits of 0 and 3, respectively [i.e. ~ U (0, 3)].

The prior distribution for alpha is based on data obtained from a small group of international BS experts using the question:

On the assumption that the average BDAI score for patients treated with infliximab is 10, share out 100 points on the following scale on how better or worse alpha interferon is compared to infliximab at relieving/controlling BS symptoms.

Alpha interferon better	Alpha interferon worse
Scale: 21% + 16–20% 11–15% 6–10% 0–5% |	0–5% 6–10% 11–15% 16–20% 21%+
For example 0 0 10 20 3 20 10 10 0 0	

The experts' answers revealed a mean of 0.053 and a variance equal to 0.0126. The results are also shown in *Table 3* and *Figure 1*.

A second question assessed where a point of equivalence between the two drugs was reached. The question posed was:

> *Suppose you are generally prescribing just one of the two drugs (infliximab or alpha interferon) at the moment. If you were told that the efficacies of infliximab and alpha interferon are exactly the same, then presumably you would not change to prescribing the other drug. However, if you were told that the other drug is 40% more efficacious than the one you are currently prescribing, then you would presumably change. Somewhere between 0% and 40% you would probably change from prescribing the current drug to the other. What % would this be? (Ignore any other factors such as cost of drug. Percentages are based on a mean of 10 for the BDAI.)*

The responses to the second question informed the boundaries as to where infliximab and alpha interferon can be considered to be equivalent and also where one is superior to the other.

TABLE 3 Results from experts' survey

Difference	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Expert 6
−25	0	0	0	0	0	0
−18	0	0	5	0	0	0
−13	10	0	10	5	5	0
−8	10	0	15	20	10	5
−3	15	0	20	20	10	10
3	30	5	20	5	10	20
8	15	15	15	25	10	30
13	15	20	10	25	10	20
18	5	30	5	0	10	10
25	0	30	0	0	35	5

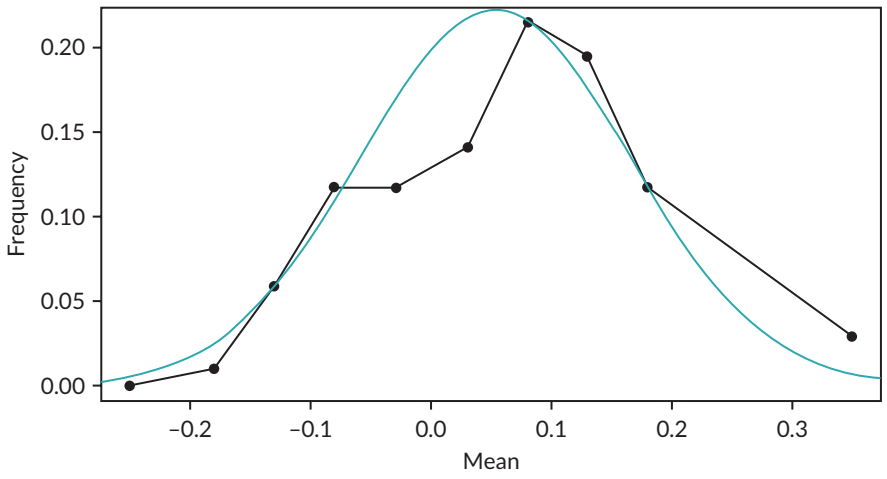


FIGURE 1 Prior distribution.

Let the equivalence boundary be given by γ. Then the equi-tailed, 80% Bayesian credible region (α_L, α_U) obtained from the posterior distribution for α will be used to describe the difference in efficacy for the two treatments, guided by the following:

- if (αL, αU) lies between (−γ, γ), then equivalent. if αU < −γ, then infliximab is superior
- if α_U < −γ and α_U < γ, then infliximab could be equivalent or superior. if α_U < −and α_U > γ, then equipoise
- if α_U > −γ and α_U > γ, then alpha interferon could be equivalent or superior. if α_L > γ, then alpha interferon is superior.

Secondary outcomes

As this trial is for a very rare disease, clinical decisions and recommendations were based on the analyses of both primary and all secondary outcomes, weighing up the evidence in the true spirit of statistics but keeping in mind the problems of multiple testing and overinterpretation.

The intention-to-treat (ITT) principle will be used for the primary analysis. Secondary sensitivity analyses will be carried out on (1) all patients including the data for both arms for patients who switched

treatment (the ANCOVA model can cope with this); (2) those patients who responded to treatment, whether it was their original treatment or the one to which they may have switched; and (3) all patients who remained on their original treatment and complied with the protocol. Data on the number of patients who switch treatments and their reasons for doing so will be recorded and analysed. Another sensitivity analysis will be carried out to investigate the effect of the prior distributions in the Bayesian analysis, especially on the parameter of prime interest (the difference in means between treatments), where results using a vague prior will be compared to those using the prior based on expert opinion.

The Independent Safety and Data Monitoring Committee (ISDMC) reviewed safety and the data after 12 patients had their 3-month follow-up visit and again when 45 patients had their 3-month follow-up visit. In addition, the ISDMC met before the trial commenced and at least yearly during the trial. No specific stopping rules were to be applied, but the ISDMC was able to recommend continuation or stopping of the trial based on safety data and efficacy data based on the primary and secondary outcomes. The recommendation to stop the trial would only have been made if the reasons for stopping would convince clinical experts in BS.

A single statistical analysis plan was produced during the trial. This document detailed how the final analysis and interim analysis shall be carried out, as well as including all relevant information for inspection by the IDSMC. This document was approved by the Trial Steering Committee (TSC) and the IDSMC prior to any analysis being carried out.

Separate protocols and statistical analysis plans were produced for the second two objectives of the study (genotyping and metabolomics), which are discussed briefly below.

Genotyping for IFNL3 and IFNL4 SNPs: DNA was extracted from all blood samples after recruitment and transported to the Wolfson Centre for Personalised Medicine using Royal Mail Safe Boxes. Genotyping for four SNPs was undertaken (rs12979860, rs4803221 and rs7248668 and ss469415590[ΔG]) using Real-Time PCR utilising a 7900HT Fast Real-Time PCR System (Applied Biosystems). Genotyping was performed by a trained technician. Test-specific standard operating procedures were written prior to the start of genotyping, and strict QC measures were adhered to ensure proper validation of genotype results. This was an exploratory analysis to determine whether any of the SNPs showed an effect with respect to the efficacy of alpha interferon based on the primary and secondary outcomes. If a strong effect was found for a SNP based on one of the primary and secondary outcomes, or as a 'trend' over several of the outcomes, the SNP with the highest predictive value was to be tested in approximately 200 other patients (based on power calculations) where DNA is available from our collaborators or in future studies.

Metabolomic analysis: (3 × 1 ml) urine samples were taken from patients at each trial visit, snap frozen and stored at −800 °C before transporting the Birmingham in batches. After thawing, urine samples were centrifuged at 13,000 *g* and prepared using a standard protocol and loaded into a standard 5-mm NMR tube for spectroscopy. One-dimensional (1-D) 1H spectra were acquired at 300 °K using a standard spin-echo pulse sequence with water suppression using excitation sculpting on a Bruker DRX 500 MHz NMR spectrometer equipped with a cryoprobe. Glutamine levels were measured in the urine samples using high-performance ion exchange chromatography. Xanthurenic acid levels were measured using a fluorometric method.

Lists of metabolites providing the greatest discrimination between groups will be identified using multivariate analyses, and metabolites will be identified using a NMR database (Human Metabolome Database version 2.5) and Chenomx NMR suite.

Rationale for mechanistic studies

The mechanistic studies were designed to (1) lead to important developments in the elucidation of the as-yet-unknown pathophysiological processes underlying BS; (2) clarify the role of two inflammatory

pathways involved in a variety of manifestations of the disease and responses (or not) to two distinct biologic drugs that target different inflammatory processes; and (3) identify the potential usefulness of two promising novel biomarkers to facilitate cost-effective targeting of therapy, derived from the greater mechanistic understanding of disease processes that (1) and (2) will provide. Assuming the frequency of the CC genotype is 55%, then the power is approximately 75% for detecting a difference in response of 20% (CC genotype 95% vs. non-CC genotype 75%, giving an overall response rate of approximately 85%) using a one-sided test and significance level 0.2. This high significance level is inevitable for a sample size of 45. However, if the overall response rate is 80%, then a difference in response rate of 35% (CC genotype 95% vs. non-CC genotype 60%) could be detected with 75% power with a two-sided test and significance level 0.05. To further strengthen the power of the analyses, patient responses will also be classified on an ordinal scale of 'no response', 'poor response' and 'good response' according to BDAI score. Results from techniques such as ordinal logistic regression might then be more conclusive. It should be noted that analysis of the mechanistic study is limited to observable differences within treatment groups as opposed to measuring the effect of differences between groups. This is primarily due to the study sample size, but it does limit the utility of any conclusions that may be drawn.

Chapter 4 Results

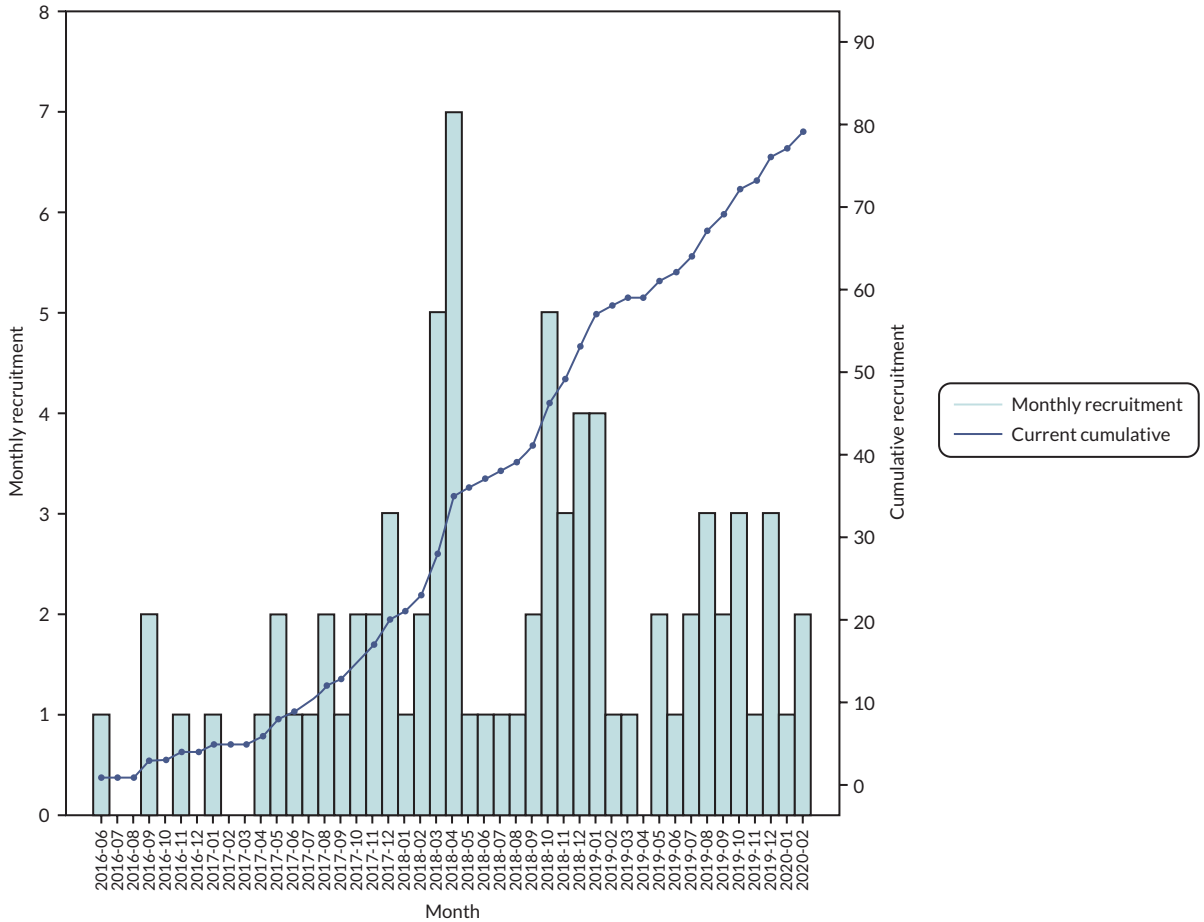
Trial recruitment and disposition

Patients were recruited between June 2016 and February 2020. Recruitment by centre is summarised in *Table 23* (see *Appendix 1*) and occurred at a linear rate that was slower than anticipated (*Figure 2*), resulting in the premature termination of the trial and fewer participants than originally planned. One hundred and sixty-one patients were screened, and seventy-nine patients randomised. The subsequent disposition of the randomised patients is summarised in *Figure 3*.

For the reasons outlined, the ITT analysis was restricted to 37 subjects allocated to infliximab and 37 to Roferon.

Assessment of data quality

Withdrawals from the study protocol within each treatment arm are summarised, together with their reasons and losses to follow-up, in *Table 4*.



(Created using Recruitment.xlsx found on S:\Statistical Documents\Trials\Bio-Behçet's\Closed\3. Final Analysis\3.1 Data and Programs\Data on 17SEP2021)
Population: All randomised patients

FIGURE 2 Trial recruitment over time.

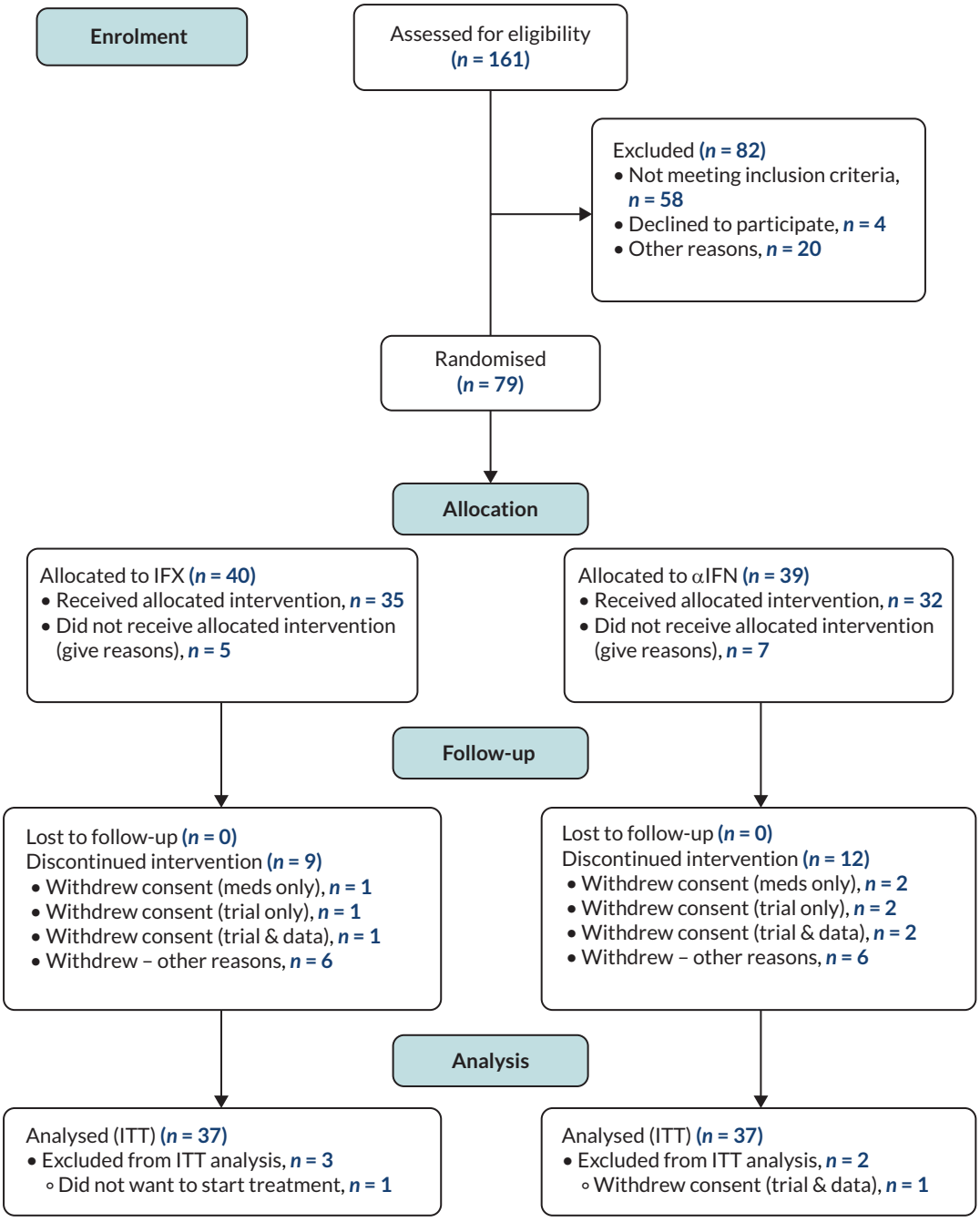


FIGURE 3 Patient disposition. αIFN, alpha interferon; IFX, infliximab.

A summary of study protocol deviations is provided in *Appendix 1* of *Table 24*: Summary of study protocol deviations. Patients with a major protocol deviation were removed from analyses performed on the per-protocol data set.

Description of baseline subject characteristics

Baseline characteristics of the study population are summarised in *Table 4* for the 74 trial participants. Mean age [interquartile range (IQR)] was 39.1(31.6–47.2) and did not differ significantly between the two treatment arms. There were 50 (68%) female and 24 male (32%) participants with similar proportions in each treatment arm by sex. Ethnic profile and baseline disease characteristics did not differ between treatment arms. Steroid use was also similar in each treatment arm (IFX 49%, alpha interferon 51%). These are detailed in *Table 5*.

TABLE 4 Withdrawals and losses to follow-up

Reason	Infliximab (*N* = 37)	Roferon (*N* = 37)	Total (*N* = 74)
Total discontinued protocol treatment, *n* (%)			
Clinician decision (not AE)	1 (3)	2 (5)	3 (4)
Inadequate response	1 (3)	1 (3)	2 (3)
Other	3 (8)	2 (5)	5 (7)
Unacceptable AE	1 (3)	2 (5)	3 (4)
Reason missing	0 (0)	1 (3)	1 (1)
Days from randomisation to withdrawal from protocol treatment			
Median (IQR)	169.0 (144.0–205.0)	164.5 (58.0–186.5)	169.0 (85.0–191.0)
Range	30.0–455.0	28.0–269.0	28.0–455.0
Total withdrawn from trial, *n* (%)			
Lost to follow-up	1 (2.7)	0 (0)	1 (1.4)
Other	3 (8.1)	6 (16.2)	9 (12.2)
Reason missing	1 (2.7)	0 (0)	1 (1.4)
Days from randomisation to withdrawal from trial			
Median (IQR)	8.0 (0.0–91.0)	68.5 (39.5–126.0)	51.0 (8.0–105.0)
Range	0.0–169.0	20.0–153.0	0.0–169.0

IQR, interquartile range.

Note
Created using SAS (Version 9.4) on 6 May 2021 16:23:01.
Created using data set randomisation, EOS and EOT data sets (Stats Server) by EH.
Population: ITT.

TABLE 5 Baseline characteristics

Characteristic	Infliximab (*N* = 37)	Roferon (*N* = 37)	Total (*N* = 74)
Age in years			
Median (IQR)	38.9 (31.8–48.7)	39.3 (31.6–46.5)	39.1 (31.6–47.2)
Sex, *n* (%)			
Female	24 (65)	26 (70)	50 (68)
Male	13 (35)	11 (30)	24 (32)
Ethnicity, *n* (%)			
White – British	34 (92)	30 (81)	64 (86)
Caribbean	0 (0)	1 (3)	1 (1)
Black – other	1 (3)	0 (0)	1 (1)
Other	0 (0)	1 (3)	1 (1)
White – European	1 (3)	1 (3)	2 (3)
White – other	0 (0)	2 (5)	2 (3)

continued

TABLE 5 Baseline characteristics (*continued*)

Characteristic	Infliximab (*N* = 37)	Roferon (*N* = 37)	Total (*N* = 74)
White and black Caribbean	0 (0)	1 (3)	1 (1)
Pakistani	1 (3)	1 (3)	2 (3)
Smoking status, *n* (%)			
Missing	0 (0)	1 (3)	1 (1)
Current smoker	8 (22)	6 (16)	14 (19)
Ex-smoker	17 (46)	9 (24)	26 (35)
Never smoked	12 (32)	21 (57)	33 (45)
Alcohol status, *n* (%)			
Missing	0 (0)	1 (3)	1 (1)
None	14 (38)	11 (30)	25 (34)
Sporadic	18 (49)	18 (49)	36 (49)
Regular	5 (14)	7 (19)	12 (16)
Steroid use, *n* (%)			
Missing	1 (3)	0 (0)	1 (1)
No	18 (49)	18 (49)	36 (49)
Yes	18 (49)	19 (51)	37 (50)
Ocular, *n* (%)			
Missing	11 (30)	8 (22)	19 (26)
Primary	10 (27)	7 (19)	17 (23)
Other	16 (43)	22 (59)	38 (51)
Oral, *n* (%)			
Missing	12 (32)	7 (19)	19 (26)
Primary	12 (32)	15 (41)	27 (36)
Other	13 (35)	15 (41)	28 (38)
Genital, *n* (%)			
Missing	12 (32)	7 (19)	19 (26)
Primary	11 (30)	14 (38)	25 (34)
Other	14 (38)	16 (43)	30 (41)
Musculoskeletal, *n* (%)			
Missing	16 (43)	13 (35)	29 (39)
Primary	10 (27)	10 (27)	20 (27)
Other	11 (30)	14 (38)	25 (34)
Previous septic arthritis in the last 12 months, *n* (%)			
No	37 (100)	37 (100)	74 (100)
Previous septic arthritis in prosthetic joints ever, *n* (%)			
No	37 (100)	37 (100)	74 (100)

TABLE 5 Baseline characteristics (*continued*)

Characteristic	Infliximab (*N* = 37)	Roferon (*N* = 37)	Total (*N* = 74)
Malignancy, *n* (%)			
No	36 (97)	37 (100)	73 (99)
Yes	1 (3)	0 (0)	1 (1)
Urine catheter, *n* (%)			
No	37 (100)	37 (100)	74 (100)
Heart failure, *n* (%)			
No	37 (100)	37 (100)	74 (100)
Skin rash, *n* (%)			
No	23 (62)	23 (62)	46 (62)
Yes	14 (38)	14 (38)	28 (38)

Notes
Created using SAS (Version 9.4) on 11 May 2021 16:15:45.
Created using data set randomisation, Baseline, Demographic, Medical History data sets (Stats Server) by EH.
Population: ITT.

Exposure to treatment and compliance

Summary information is provided in *Appendix 1*: describing patients' exposure to treatment with infliximab (mean dose per patient and the percentage of patients continuing to receive treatment over time) and interferon (dose changes over time and mean number of missed doses recorded at weeks 12, 24 and 36 reviews).

Analysis of primary outcome measures

The primary outcome for the trial was defined as the change in mBDAI between baseline and 3 months (with 6 months as a secondary outcome). The statistical analysis examined the difference in mean mBDAI scores between the two treatment arms at 3 and 6 months, with clinically significant response defined as a difference in 20% or more between the two treatment arms (assessed using Bayesian ANCOVA for change in mean from baseline adjusted for baseline score). Analysis based on planned ITT therefore included 37 patients allocated to infliximab and 37 to Roferon.

Table 6 presents the results from the Bayesian linear regression model to estimate the impact of treatment group on mBDAI. The linear model includes as an adjusting covariate the baseline mBDAI, and so there are three parameters presented:

- β0: model intercept
- β1: covariate associated with baseline mBDAI
- α (alpha interferon vs. infliximab): impact of treatment group.

Results are presented in terms of model estimates [standard error (SE)] and the 80% credibility interval, which is consistent with the study design.

The results show that 3-month BDAI is reliant on the baseline BDAI, which is demonstrated by an 80% credibility interval which does not include zero for the β1 parameter. The impact of treatment is not

statistically significant based on the 80% credibility interval [Est (SE) = 0.13 (0.25), 80% CI = (−0.19 to 0.46)]. So the two treatment arms did not differ significantly with respect to change in BDAI at 3 or 6 months. These results show that, after adjustment for baseline BDAI, patients who were treated with alpha interferon had a BDAI score of 0.13 points higher than those on infliximab. The study set a margin of equivalence of 20% change between the two treatment arms. Here, for example, a patient with a baseline BDAI score of 8 points could expect a follow-up BDAI score of approximately 4.45 on infliximab and 4.58 on alpha interferon, representing an approximate 3% change between the two treatment groups.

Figure 4 demonstrates this graphically, as the probability density for the prior does not differ significantly from the posterior.

However, *Figure 5* also shows that both treatments appear to be associated with significant improvements in BDAI at 3 and 6 months. Defining response categories as 20%, 50% and 70% improvement with respect to baseline revealed that for infliximab, 17, 13 and 8 patients met the 20%, 50% and 70% response definitions. For Roferon, figures were 22, 12 and 7, respectively.

The improvement in mBDAI within both treatment arms is illustrated further in *Figures 6* and *7* which show the BDAI score measured at baseline and 6 months for infliximab and Roferon.

TABLE 6 Results for primary outcome

Parameter	Estimate	SE	80% credibility interval
β0	0.13	0.35	(−0.31 to 0.59)
β1	0.54	0.19	(0.29 to 0.78)
α (Roferon vs. infliximab)	0.13	0.25	(−0.19 to 0.46)

SE, standard error.

Note
Created by richj23 using R version 3.5.1 (2 July 2018) on 17 September 2021.

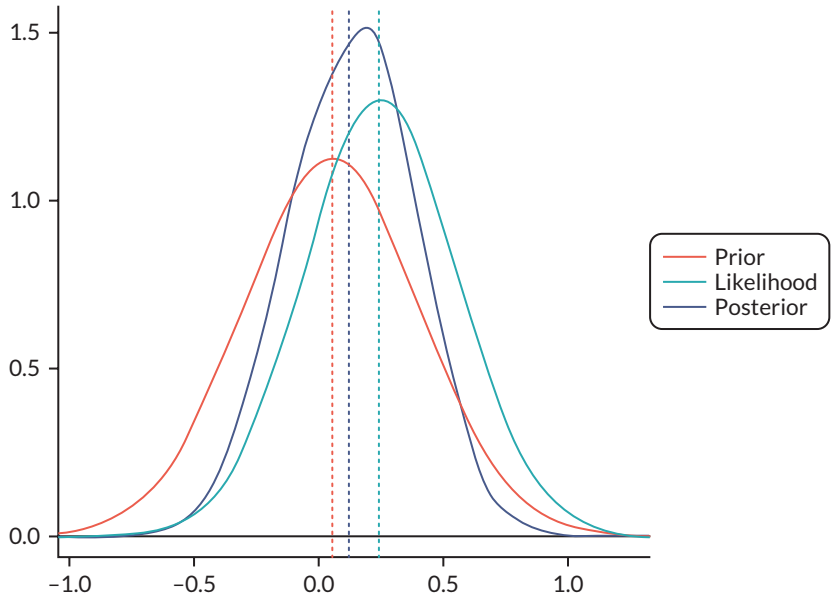


FIGURE 4 Density of prior distribution, likelihood and posterior distribution for mBDAI.

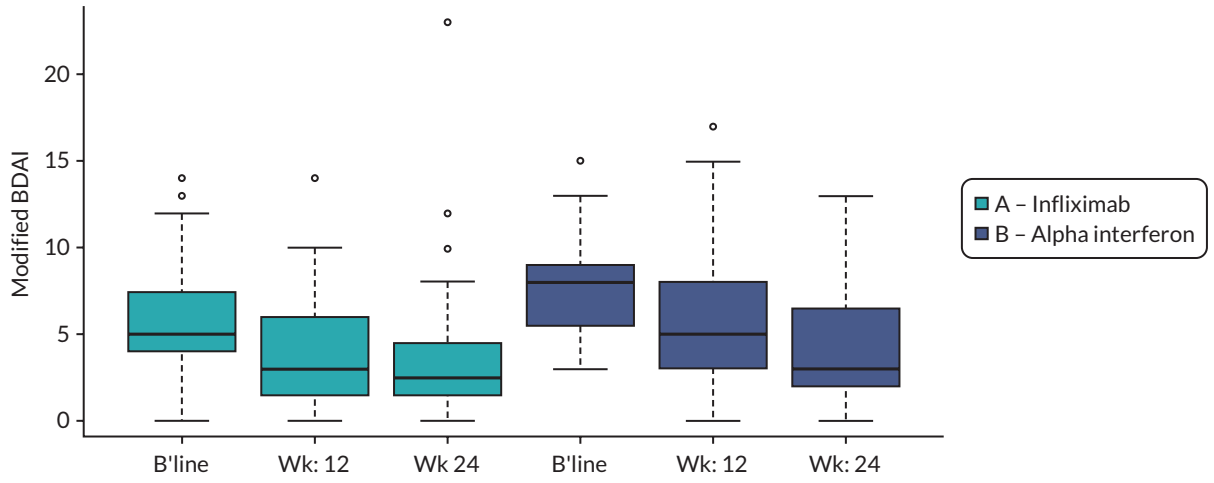


FIGURE 5 Boxplot of mBDAI at baseline, 3 and 6 months across treatment groups.

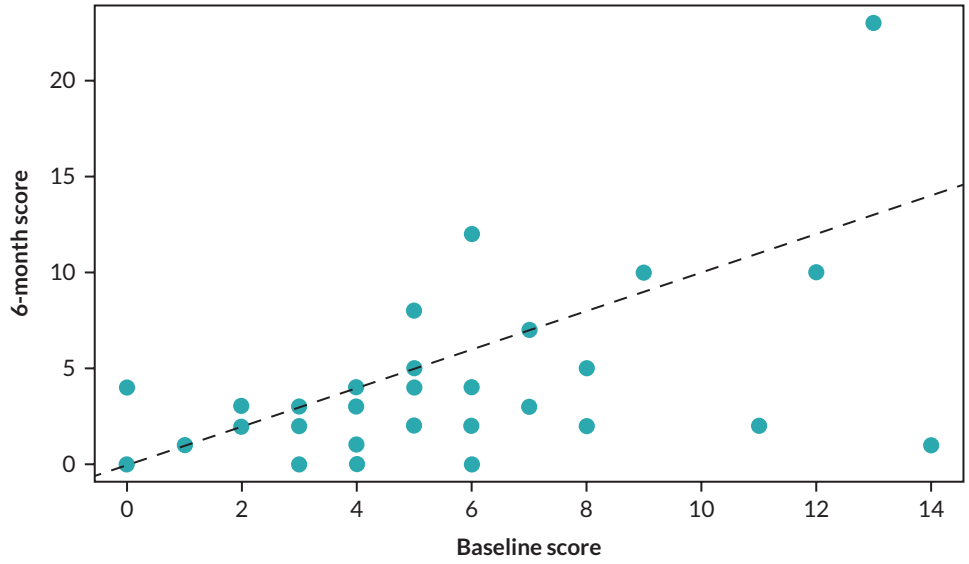


FIGURE 6 Change in BDAI score – infliximab.

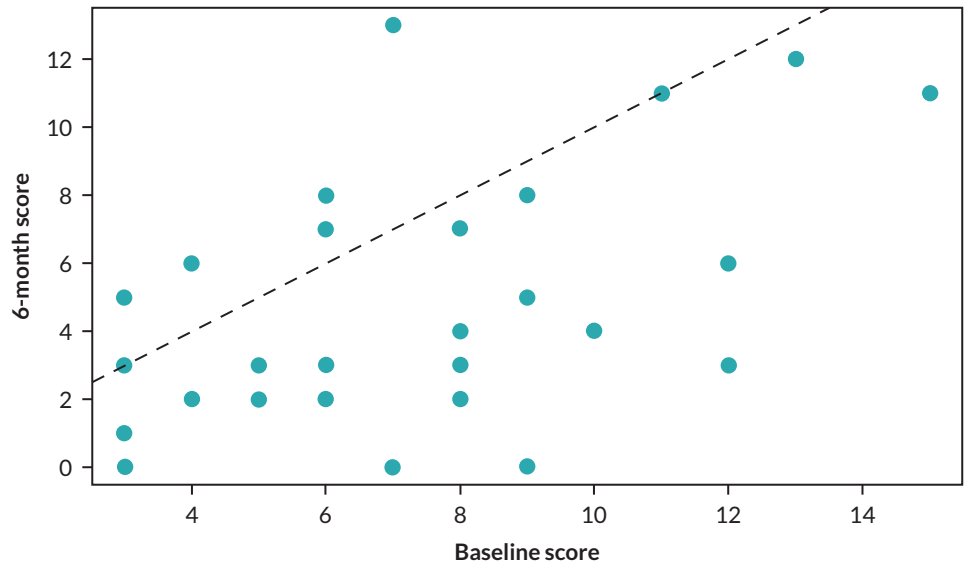


FIGURE 7 Change in BDAI score – Roferon.

If there were no impact of treatment, we would expect each point to lie close to the diagonal red dashed line. However, most points lie above the line, showing a higher BDAI at baseline than at 6 months. This illustrates the reduction in BDAI associated with both treatments. The median (IQR) change for infliximab is −1.5 (−4 to 0) BDAI, and regressing the 6-month BDAI data against the baseline BDAI reveals a significant difference and estimated that on average, the 6-month BDAI score is 70% [0.7 (0.109); $p < 0.001$] that of the baseline score (i.e. a 30% reduction). The median (IQR) change for Roferon is −3 (−5.25 to −1) BDAI and regressing the 6-month BDAI data against the baseline BDAI also indicates a significant difference and estimates that, on average, the 6-month BDAI score is 61% [0.61 (0.069); $p < 0.001$] that of the baseline score (i.e. a 39% reduction).

Results of the sensitivity analyses demonstrate that the protocol variations and switches did not influence the conclusion that the two treatments did not differ overall in relation to response and provide confidence that the estimate of the difference between the two treatments is reliable.

A significantly higher proportion of patients randomised to alpha interferon swapped away from their randomised treatment compared to those randomised to infliximab treatment (Roferon 11 of 37, infliximab 3 of 37; *Table 7*, $p = 0.0104$) (*Table 6*).

Reasons for switching are summarised in *Table 8*. It should be noted that for four of the patients switching away from alpha interferon, the reason for the switch was not recorded.

Analyses of secondary outcome measures

Improvements in organ systems after 3 and 6 months compared to baseline were evaluated as secondary outcome measures.

TABLE 7 Oral ulcer activity – baseline vs. 6 months

	Infliximab (*N* = 37)	αIFN (*N* = 37)	*p*-value
Baseline			
n	19	25	
Median (IQR)	26.0 (22.0–42.0)	25.0 (16.0–34.0)	
6 months			
n	19	25	
Median (IQR)	14.0 (7.0–27.0)	14.0 (5.0–23.0)	
Difference between baseline and 6 months			
Absolute difference – median (IQR)	−8.0 (−26.0 to 12.0)	−11.0 (−20.0 to 0.0)	
Percentage difference – median (IQR)	−53.8 (−69.0 to −10.0)	−53.8 (−66.7 to −24.0)	
Proportion with at least 20% reduction	11 (58%)	16 (64%)	0.7600

αIFN, alpha interferon.

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, mouth ulcer data sets (Stats Server).
Population: ITT.

TABLE 8 Reasons for switching treatment

Reason	Infliximab (*N* = 37)	Roferon (*N* = 37)
Reason switched treatments, *n* (%)		
Clinician decision (not AE)	2 (6)	0 (0)
Inadequate response	1 (3)	3 (9)
Unacceptable AE	0 (0)	4 (12)
Missing reason	0 (0)	4 (12)

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, Bioassess data sets (Stats Server).
Population: ITT.
Notes for *Table 8*:
Alpha interferon – There were four patients who had a reported treatment swap away from their randomised treatment of alpha interferon, but the reason for the treatment swap was missing:
- *site 1024, personid 6.*
- *site 1024, personid 12.*
- *site 1024, personid 16.*
- *site 690, personid 19.*

Original Behçet's disease activity index

The box plot results for the original BDAI at baseline and after 3 and 6 months by treatment group are presented in *Figure 5* and *Table 27* (see *Appendix 1*) and, as expected, did not differ from those presented for the mBDAI. There were no statistically significant differences demonstrated between groups for the Bayesian linear model comparing the original BDAI at baseline and 3 months and between baseline and 6 months. Statistics for within-group changes over time are not presented, but they show that both treatments appear to be associated with significant improvements in BDAI at 3 and 6 months.

Ophthalmological assessments included assessment for intraocular inflammation (vitreous haze) and visual function (BCVA; numbers of letters read) at baseline, 3 months and 6 months. For vitreous haze at baseline, 11 patients (infliximab) and 16 patients (Roferon) were evaluated. For BCVA, 18 patients underwent baseline assessments. Not all patients had follow-up assessments. As ocular examination was symptom-directed, the number of patients with these measurements was small. *Tables 28–31* (see *Appendix 1*) summarise the results for vitreous haze and BCVA, respectively, comparing measurements at baseline with results at 3 and 6 months for each eye. For each of the outcome measures, there were no notable differences between treatment groups.

Oral ulcer activity score

Most patients experienced a reduction in their oral ulcer activity score between baseline and 3 months (*Table 9*) and between baseline and 6 months (*Table 7*), with a median reduction (for both treatment arms) of 50% at 3 months and 53.8% at 6 months.

Using caution, due to small sample sizes, a slightly higher proportion of patients randomised to alpha interferon experienced a clinically significant (at least 20%) reduction compared to patients randomised to infliximab (64% and 58%, respectively) at both 3 and 6 months; however, this small difference was not statistically significant at the 5% level (Fisher's exact test $p = 0.7600$).

Genital ulcer activity

A similar pattern emerged for genital ulceration. Most patients experienced a reduction in their genital ulcer activity between baseline and 3 months (*Table 10*) and between baseline and 6 months (*Table 11*), with a median percentage reduction (for both treatment arms) of 100% at 3 months and 100% at 6 months.

Noting the small sample sizes, there was no statistically significant difference (at the 5% level) in the proportion of patients in each treatment arm who experienced a clinically significant (at least 20%) reduction in their genital ulcer activity at 3 months (Fisher's exact test $p = 1.0000$) and 6 months (Fisher's exact test $p = 0.7600$).

TABLE 9 Oral ulcer activity – baseline vs. 3 months

	Infliximab (*N* = 37)	Roferon (*N* = 37)	*p*-value
Baseline			
n	19	25	
Median (IQR)	26.0 (22.0–42.0)	25.0 (16.0–34.0)	
3 months			
n	19	25	
Median (IQR)	8.0 (0.0–22.0)	18.0 (8.0–20.0)	
Difference between baseline and 3 months			
Absolute difference – median (IQR)	−13.0 (−25.0 to 0.0)	−7.0 (−21.0 to 0.0)	
Percentage difference – median (IQR)	−50.0 (−100 to −8.3)	−50.0 (−68.0 to −20.0)	
Proportion with at least 20% reduction	11 (58%)	16 (64%)	0.7600

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data sets randomisation, mouth ulcer data sets (Stats Server).
Population: ITT.

TABLE 10 Genital ulcer activity – baseline vs. 3 months

	Infliximab (*N* = 37)	Roferon (*N* = 37)	*p*-value
Baseline			
N	11	10	
Median (IQR)	17.0 (0.0–32.0)	22.5 (0.0–34.0)	
3 months			
N	11	10	
Median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–14.0)	
Difference between baseline and 3 months			
Absolute difference – median (IQR)	−17.0 (−24.0 to 0.0)	−11.0 (−29.0 to 0.0)	
Percentage difference – median (IQR)	−100.0 (−100 to −75.0)	−100.0 (−100 to −32.4)	
Proportion with at least 20% reduction	7 (64%)	7 (70%)	1.0000

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, genital ulcer data sets (Stats Server).
Population: ITT.

Likert pain score

Though sample sizes were small, there was no statistically significant difference (at 5% level) in the proportion of patients in each treatment arm who experienced a clinically significant (at least 20%) improvement in their Likert pain score at 3 months (Fisher's exact test $p = 1.0000$) and 6 months (Fisher's exact test $p = 0.1142$). Summary data (see *Appendix Tables 32* and *33*) indicate modest early improvements for both infliximab and Roferon which were lost by 6 months for the infliximab group.

Prednisolone usage

Prednisolone use was reported as a binary (yes/no) variable longitudinally throughout the study. *Table 12* details the number (percentage) of patients receiving prednisolone (or another steroid). The two groups were well matched a baseline with similar proportions receiving steroids [20/39 (51.3%)] and there appeared a modest steroid-sparing effect observed in each group. For infliximab at baseline, 15 patients were using steroids, reducing to 12 at 24-week follow-up. This results in 20% of patients on

TABLE 11 Genital ulcer activity – baseline vs. 6 months

	Infliximab (*N* = 37)	Roferon (*N* = 37)	*p*-value
Baseline			
N	11	10	
Median (IQR)	17.0 (0.0–32.0)	22.5 (0.0–34.0)	
6 months			
n	11	10	
Median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–16.0)	
Difference between baseline and 6 months			
Absolute difference – median (IQR)	−17.0 (−32.0 to 0.0)	−13.5 (−29.0 to 0.0)	
Percentage difference – median (IQR)	−100.0 (−100 to −100)	−100.0 (−100 to −18.9)	
Proportion with at least 20% reduction	7 (64%)	5 (50%)	0.7600

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, genital ulcer data sets (Stats Server).
Population: ITT.

TABLE 12 Prednisolone (steroid) usage

Timepoint	Infliximab (*n* = 39) (%)	Roferon (*n* = 29) (%)
Baseline	20/39 (51.3)	20/39 (51.3)
Week 12 review	16/34 (47.1)	18/33 (54.5)
Week 24 review	12/29 (41.4)	11/32 (34.4)
Week 36 review	1/2 (50)	5/9 (55.6)

Note
Created by richj23 using R version 3.5.1 (2 July 2018) on 17 September 2021.

TABLE 13 Logistic model for prednisolone (steroid) usage

Model term	Est (SE)	OR (95% CI)	*p*-value
Intercept	0.05 (0.32)	1.05 (0.562 to 1.972)	0.873
Treatment (αIFN vs. infliximab)	0 (0.453)	1 (0.411 to 2.43)	1
Baseline	−0.17 (0.47)	0.84 (0.336 to 2.121)	0.719
Week 12	−0.4 (0.495)	0.67 (0.254 to 1.768)	0.419
Week 24	−0.05 (1.45)	0.95 (0.055 to 16.294)	0.972
Baseline: treatment (Roferon vs. infliximab)	0.3 (0.667)	1.35 (0.365 to 4.995)	0.653
Week 12: treatment (Roferon vs. infliximab)	−0.3 (0.697)	0.74 (0.189 to 2.91)	0.669
Week 24: treatment (Roferon vs. infliximab)	0.22 (1.629)	1.25 (0.051 to 30.477)	0.891

αIFN, alpha interferon; OR, odds ratio.

Note
Created by richj23 using R version 3.5.1 (2 July 2018) on 17 September 2021.

steroids ceasing their use. The overall rate decreased from 15/29 (52%) to 12/29 (41%). For Roferon at baseline, 16 patients were using steroids which reduced to 9 at 24-week follow-up. This results in 44% of patients on steroids ceasing their use. Two patients on Roferon began taking steroids; therefore, the overall rate decreased from 16/32 (50%) to 11/32 (34%).

The results of a logistic regression model are presented in *Table 13* to explore the impacts of time points and treatment on the use of steroids. Treatment and time are included as an interaction to detect either a consistent overall difference due to treatment or a difference between treatments that appears over the course of the study. No significant differences are observed, showing that there is no evidence of any difference in prednisolone (or other steroid) dose reduction between the treatment groups. Further analysis using actual steroid doses will be explored.

Clinician's overall perception (Physician's Global Assessment disease activity)

The physician's overall perception of disease activity (a 7-point Likert scale) was completed as part of (but assessed independently of) the BDAI at baseline, 3 and 6 months. A change of 2 points in the score was considered a clinically meaningful change. The clinician's overall perception of disease activity indicated a reduction in disease activity for most patients between baseline and 3 months and between baseline and 6 months, with a median reduction of −2.0 (infliximab) and −1.0 (Roferon) at 3 months and −3.0 (infliximab) and −2.0 (Roferon) at 6 months. There was a statistically significant difference (at the 5% level) between treatment arms in the change in clinician's overall perception of disease activity at 3 months from baseline (Wilcoxon test $p = 0.0421$) and at 6 months from baseline (Wilcoxon test $p = 0.0420$) in favour of infliximab providing a greater reduction in the clinician's overall perception of disease activity.

At 3 months, physician's overall perception of disease activity was higher for the alpha interferon arm compared to the IFX arm at the 5% level ($p = 0.002$) and remained significantly higher at 6 months at the 5% level ($p = 0.001$).

These results are summarised in *Tables 14* and *15*.

TABLE 14 Clinician's overall perception of disease activity – baseline vs. 3 months

	Infliximab (*N* = 37)	Roferon (*N* = 37)	*p*-value[a]	*p*-value[b]
Baseline				
n	31	29		
Mean (SD)	4.8 (1.28)	4.9 (1.22)		
Median (IQR)	5.0 (4.0–6.0)	5.0 (4.0–6.0)		
3 months				
n	31	29		
Mean (SD)	2.5 (1.29)	3.8 (1.57)		
Median (IQR)	2.0 (1.0–3.0)	4.0 (2.0–5.0)		
Difference between baseline and 3 months				
Mean (SD)	−2.3 (1.81)	−1.1 (1.99)	0.0187	
Median (IQR)	−2.0 (−4.0 to −1.0)	−1.0 (−3.0 to 0.0)		0.0421

SD, standard deviation.
a *p*-value calculated using independent sample *t*-test.
b *p*-value calculated using Wilcoxon test.

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, disease activity data sets (Stats Server).
Population: ITT.

TABLE 15 Clinician's overall perception of disease activity – baseline vs. 6 months

	Infliximab (*N* = 37)	Roferon (*N* = 37)	*p*-value[a]	*p*-value[b]
Baseline				
N	26	29		
Mean (SD)	4.9 (1.26)	4.9 (1.14)		
Median (IQR)	5.0 (4.0–6.0)	5.0 (4.0–6.0)		
6 months				
N	26	29		
Mean (SD)	2.3 (1.46)	3.2 (1.28)		
Median (IQR)	2.0 (1.0–3.0)	3.0 (2.0–4.0)		
Difference between baseline and 6 months				
Mean (SD)	−2.6 (1.86)	−1.7 (1.51)	0.0550	
Median (IQR)	−3.0 (−4.0 to −2.0)	−2.0 (−3.0 to −1.0)		0.0420

SD, standard deviation.
a *p*-value calculated using independent sample *t*-test.
b *p*-value calculated using Wilcoxon test.

Notes
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, disease activity data sets (Stats Server).
Population: ITT.

Quality-of-life measures

Quality-of-life measures included the number of patients with reported problems (levels 2, 3, 4, 5) in the EuroQol-5 Dimensions, five-level version (EQ-5D-5L) domains of Mobility, Self-Care, Usual Activities, Pain/Discomfort and Anxiety/Depression at baseline, 3 and 6 months for infliximab and Roferon. There were no differences between groups for these important subdimensions of the EQ-5D-5L score evaluating the number of patients who reported problems.

There were also no significant differences between the two treatment groups for EQ-visual analogue score assessment comparing the difference between baseline versus 3 months and baseline versus 6 months comparing infliximab with alpha interferon (3 months Wilcoxon test, $p = 0.8318$; 6 months Wilcoxon test, $p = 0.8600$).

For BD-QoL, only minor differences emerged in favour of infliximab compared to Roferon when comparing differences in scores for QoL at 3 months versus baseline between the two treatment groups (Wilcoxon test, $p = 0.0274$), but this was no longer present at 6 months (Wilcoxon test, $p = 0.3029$).

Quality-of-life measures results are summarised in *Tables 35–39* (see *Appendix 1*).

Analysis of safety and tolerability

Table 40 evaluates the difference in AE severity across the two treatment arms for all reported AEs. In total, 46 patients reported 270 events. A proportion test shows that there were a greater number of AEs observed on alpha interferon ($p < 0.001$). A Fisher's test to evaluate any differences in the distribution of AEs across treatment arms was not significant ($p = 0.224$). The overview of AEs by severity can be found in *Table 16*.

In total, eight serious adverse events (SAEs) from five patients were reported across the study. One patient on the infliximab arm reported four SAEs [hypertension (×2), bacterial urinary tract infection and blood pressure inadequately controlled]. In total, three patients (six events) were reported on the infliximab arm and two patients (two events) were reported on the alpha interferon arm. There were no suspected drug interactions and no suspected unexpected serious adverse reactions (SUSARS) reported in the study.

Summaries of all study-related SAEs together with an aggregated summary of AEs are listed in *Table 40* (see *Appendix 1*).

Genetics analysis

Four SNPs within the IFNL4 gene locus were selected owing to a priori knowledge of effects on gene/protein function or clinical association.

TABLE 16 Overview of AEs by severity: number of events

Patient group	Mild (%)	Moderate (%)	Severe (%)	Total
A – Infliximab	51 (50)	47 (47)	3 (3)	101
B – Roferon	80 (47)	88 (52)	1 (1)	169
Total	131 (49)	135 (50)	4 (1)	270

Note
Created by richj23 using R version 3.5.1 (2 July 2018) at 09:51:32 on 17 September 2021.

Genotyping was undertaken, and a summary of genotype counts and minor allele frequencies can be found in *Table 17*. Hardy–Weinberg *p*-values were within the tolerable threshold (> 0.0001), indicating that genotype distributions are not significantly different to those that might be expected, and therefore there were no quality control issues associated with the genotyping assays.

Genotypes were obtained for a total of 62 individuals (30 in Arm A and 32 in Arm B) for all SNPs except for rs7248668 where a genotype for one individual (Arm B) could not be obtained despite repeated attempts.

The data suggest that there is high linkage disequilibrium between rs12979860 and rs368234815, and rs4803221 and rs7248668, summarised in *Table 18*.

Genotype association with a binary response outcome based on either 20%, 50% or 70% response was undertaken using a Pearson's chi-squared test for all SNPs in all individuals plus stratifying for Arm A or Arm B only (*Table 19*).

These analyses suggest the only statistically significant associations are for the rs4803221 and rs7248668 SNPs in Arm B and only when applying the 70% response binary phenotype ($p = 0.021$ and 0.025, respectively). However, after correction for multiple testing [false discovery rate (FDR)], these associations are no longer significant ($p > 0.05$).

Subsequent analysis determined genetic association with four continuous variable outcome measures: BDAI at baseline, 3 months, 6 months and a baseline-adjusted BDAI area under the curve (AUC). This used an ANOVA with Bonferroni correction (*Table 20*).

TABLE 17 Single nucleotide polymorphisms within *IFNL4* gene locus

rs number	Chr (position) (GRCh38.p13)	Locus position	Effect
rs12979860	chr19:39248147	g.5710	Intronic variant
rs368234815	chr19:39248514-39248515	n.343del	Non-coding transcript variant
rs4803221	chr19:39248489	n.368G > C	Non-coding transcript variant
rs7248668	chr19: 39253181	g.676C > T	Promoter variant

TABLE 18 Summary statistics for the genotyping data

	A_1	A_2		A_1/A_1	A_1/A_2	A_2/A_2	MAF	HW *p*-value
rs12979860	C	T	Arm A (*n* = 30)	17	11	2	0.25	0.90
			Arm B (*n* = 32)	17	11	4	0.30	0.32
			Total (*n* = 62)	34	22	6	0.27	0.39
rs368234815	TT	G	Arm A (*n* = 30)	17	11	2	0.25	0.90
			Arm B (*n* = 32)	16	12	4	0.31	0.47
			Total (*n* = 62)	33	23	6	0.28	0.51
rs4803221	C	G	Arm A (*n* = 30)	21	8	1	0.17	0.83
			Arm B (*n* = 32)	22	8	2	0.19	0.31
			Total (*n* = 62)	43	16	3	0.18	0.36
rs7248668	G	A	Arm A (*n* = 30)	21	8	1	0.17	0.83
			Arm B (*n* = 31)	22	7	2	0.18	0.21
			Total (*n* = 61)	43	15	3	0.17	0.28

TABLE 19 Interferon lambda 4 SNP association with binary response phenotype (20%, 50% or 70% response)

		Arm A (*n* = 30)						Arm B (*n* = 32)						Overall (*n* = 62)					
rs12979860		**C/C**	**C/T**	**T/T**	**Total**	**MAF**	***p*-value**	**C/C**	**C/T**	**T/T**	**Total**	**MAF**	***p*-value**	**C/C**	**C/T**	**T/T**	**Total**	**MAF**	***p*-value**
20%	Non-responder	7	4	2	13	0.31		4	5	1	10	0.35		11	9	3	23	0.33	
	Responder	10	7	0	17	0.21	0.239	13	6	3	22	0.27	0.454	23	13	3	39	0.24	0.640
50%	Non-responder	8	7	2	17	0.32		12	7	1	20	0.23		20	14	3	37	0.27	
	Responder	9	4	0	13	0.15	0.303	5	4	3	12	0.42	0.237	14	8	3	25	0.28	0.824
70%	Non-responder	13	7	2	22	0.25		14	9	2	25	0.26		27	16	4	47	0.26	
	Responder	4	4	0	8	0.25	0.511	3	2	2	7	0.43	0.347	7	6	2	15	0.33	0.731
rs368234815		**TT/TT**	**TT/G**	**G/G**	**Total**	**MAF**	***p*-value**	**TT/TT**	**TT/G**	**G/G**	**Total**	**MAF**	***p*-value**	**TT/TT**	**TT/G**	**G/G**	**Total**	**MAF**	***p*-value**
20%	Non-responder	7	4	2	13	0.31		4	5	1	10	0.35		11	9	3	23	0.33	
	Responder	10	7	0	17	0.21	0.239	12	7	3	22	0.30	0.616	22	14	3	39	0.26	0.716
50%	Non-responder	8	7	2	17	0.32		11	8	1	20	0.25		19	15	3	37	0.28	
	Responder	9	4	0	13	0.15	0.303	5	4	3	12	0.42	0.252	14	8	3	25	0.28	0.745
70%	Non-responder	13	7	2	22	0.25		13	10	2	25	0.28		26	17	4	47	0.27	
	Responder	4	4	0	8	0.25	0.511	3	2	2	7	0.43	0.344	7	6	2	15	0.33	0.787
rs4803221		**C/C**	**C/G**	**G/G**	**Total**	**MAF**	***p*-value**	**C/C**	**C/G**	**G/G**	**Total**	**MAF**	***p*-value**	**C/C**	**C/G**	**G/G**	**Total**	**MAF**	***p*-value**
20%	Non-responder	9	3	1	13	0.19		6	4	0	10	0.20		15	7	1	23	0.20	
	Responder	12	5	0	17	0.15	0.492	16	4	2	22	0.18	0.304	28	9	2	39	0.17	0.814
50%	Non-responder	11	5	1	17	0.21		14	6	0	20	0.15		25	11	1	37	0.18	
	Responder	10	3	0	13	0.12	0.597	8	2	2	12	0.25	0.144	18	5	2	25	0.18	0.483
70%	Non-responder	16	5	1	22	0.16		18	7	0	25	0.14		34	12	1	47	0.15	
	Responder	5	3	0	8	0.19	0.628	4	1	2	7	0.36	**0.021**	9	4	2	15	0.27	0.201

TABLE 19 Interferon lambda 4 SNP association with binary response phenotype (20%, 50% or 70% response) (*continued*)

		Arm A (*n* = 30)						Arm B (*n* = 32)						Overall (*n* = 62)					
rs7248668		**G/G**	**G/A**	**A/A**	**Total**	**MAF**	***p*-value**	**G/G**	**G/A**	**A/A**	**Total**	**MAF**	***p*-value**	**G/G**	**G/A**	**A/A**	**Total**	**MAF**	***p*-value**
20%	Non-responder	9	3	1	13	0.19		6	3	0	9	0.17		15	6	1	22	0.18	
	Responder	12	5	0	17	0.15	0.492	16	4	2	22	0.18	0.472	28	9	2	39	0.17	0.934
50%	Non-responder	11	5	1	17	0.21		14	5	0	19	0.13		25	10	1	36	0.17	
	Responder	10	3	0	13	0.12	0.597	8	2	2	12	0.25	0.172	43	15	3	61	0.17	0.550
70%	Non-responder	16	5	1	22	0.16		18	6	0	24	0.13		34	11	1	46	0.14	
	Responder	5	3	0	8	0.19	0.628	4	1	2	7	0.36	**0.025**	9	4	2	15	0.27	0.201

Note
Data represent genotype frequencies in Arm A, Arm B and the full cohort. Statistical significance determined by Pearson's chi-squared test ($p < 0.05$ in bold).

TABLE 20 Interferon lambda 4 SNP associations with BDAI (baseline, 3 and 6 months) and baseline-adjusted BDAI AUC for Arm A, Arm B and the full cohort

	Arm A (mean ± SD)				Arm B (mean ± SD)				Overall (mean ± SD)			
rs12979860	**C/C**	**C/T**	**T/T**	**ANOVA *p*-value**	**C/C**	**C/T**	**T/T**	**ANOVA *p*-value**	**C/C**	**C/T**	**T/T**	**ANOVA *p*-value**
BDAI (baseline)	6.15 ± 2.94	6.67 ± 3.48	7.00	0.877	6.89 ± 2.85	8.07 ± 3.08	8.80 ± 4.71	0.390	6.51 ± 2.88	7.34 ± 3.31	8.50 ± 4.28	0.278
BDAI (3 months)	4.50 ± 3.12	4.58 ± 3.96	7.00 ± 1.41	0.625	6.47 ± 4.00	6.58 ± 4.93	3.67 ± 2.52	0.555	5.52 ± 3.68	5.58 ± 4.49	5.00 ± 2.65	0.956
BDAI (6 months)	4.67 ± 5.92	2.81 ± 1.72	5.50 ± 2.12	0.547	3.94 ± 2.86	5.45 ± 3.78	4.25 ± 5.97	0.559	4.13 ± 4.64	4.13 ± 3.17	4.67 ± 4.76	0.961
Baseline-adjusted BDAI AUC	−1.21 ± 1.81	−2.00 ± 2.50	2.25 ± 3.89	0.055	−1.31 ± 2.82	−1.27 ± 2.94	−2.25 ± 3.68	0.866	−1.26 ± 2.32	−1.64 ± 2.69	−0.45 ± 4.08	0.639
rs368234815	**TT/TT**	**TT/G**	**G/G**	**ANOVA *p*-value**	**TT/TT**	**TT/G**	**G/G**	**ANOVA *p*-value**	**TT/TT**	**TT/G**	**G/G**	**ANOVA *p*-value**
BDAI (baseline)	6.15 ± 2.94	6.67 ± 3.48	7.00	0.877	6.83 ± 2.92	8.07 ± 2.96	8.80 ± 4.71	0.367	6.47 ± 2.91	7.37 ± 3.25	8.50 ± 4.28	0.254
BDAI (3 months)	4.50 ± 3.12	4.58 ± 3.96	7.00 ± 1.41	0.625	5.93 ± 3.45	7.23 ± 5.26	3.667 ± 2.52	0.401	5.22 ± 3.32	5.96 ± 4.78	5.00 ± 2.65	0.748
BDAI (6 months)	4.67 ± 5.92	2.81 ± 1.72	5.50 ± 2.12	0.547	3.94 ± 2.95	5.33 ± 3.63	4.25 ± 5.97	0.601	4.32 ± 4.71	4.13 ± 3.09	4.67 ± 4.76	0.959
Baseline-adjusted BDAI AUC	−1.21 ± 1.81	−2.00 ± 2.50	2.25 ± 3.89	0.055	−1.55 ± 2.72	−0.96 ± 3.01	−2.25 ± 3.68	0.754	−1.37 ± 2.26	−1.46 ± 2.76	−0.45 ± 4.08	0.732
rs4803221	**C/C**	**C/G**	**G/G**	**ANOVA *p*-value**	**C/C**	**C/G**	**G/G**	**ANOVA *p*-value**	**C/C**	**C/G**	**G/G**	**ANOVA *p*-value**
BDAI (baseline)	6.12 ± 2.81	7.11 ± 4.08	7.00	0.706	7.19 ± 3.22	8.20 ± 3.16	9.50 ± 3.54	0.489	6.65 ± 3.04	7.68 ± 3.56	8.67 ± 2.89	0.319
BDAI (3 months)	4.15 ± 2.89	5.78 ± 4.41	6.00	0.468	6.27 ± 4.67	6.44 ± 3.43	4.00	0.868	5.26 ± 4.02	6.11 ± 3.85	5.00 ± 1.41	0.736
BDAI (6 months)	4.45 ± 5.39	2.63 ± 1.69	7.00	0.537	4.05 ± 3.20	6.50 ± 4.17	1.50 ± 2.12	0.117	4.25 ± 4.38	4.56 ± 3.67	3.33 ± 3.51	0.892
Baseline-adjusted BDAI AUC	−1.26 ± 1.66	−1.38 ± 3.88	−0.50	0.943	−1.25 ± 2.75	−1.16 ± 2.92	−6.25	0.226	−1.26 ± 2.24	−1.27 ± 3.32	−3.38 ± 4.07	0.530

TABLE 20 Interferon lambda 4 SNP associations with BDAI (baseline, 3 and 6 months) and baseline-adjusted BDAI AUC for Arm A, Arm B and the full cohort (*continued*)

	Arm A (mean ± SD)				Arm B (mean ± SD)				Overall (mean ± SD)			
rs7248668	G/G	G/A	A/A	ANOVA *p*-value	G/G	G/A	A/A	ANOVA *p*-value	G/G	G/A	A/A	ANOVA *p*-value
BDAI (baseline)	6.12 ± 2.81	7.25 ± 4.33	7.00	0.672	7.19 ± 3.23	8.33 ± 3.32	9.50 ± 3.54	0.470	6.65 ± 3.04	7.82 ± 3.75	8.67 ± 2.89	0.290
BDAI (3 months)	4.15 ± 2.89	5.78 ± 4.41	6.00	0.468	6.27 ± 4.67	6.50 ± 3.66	4.00	0.869	5.26 ± 4.02	6.12 ± 3.97	5.00 ± 1.41	0.745
BDAI (6 months)	4.45 ± 5.39	2.63 ± 1.69	7.00	0.537	4.05 ± 3.20	5.57 ± 3.51	1.50 ± 2.12	0.276	4.25 ± 4.38	4.00 ± 3.00	3.33 ± 3.51	0.920
Baseline-adjusted BDAI AUC	−1.26 ± 1.66	−1.38 ± 3.88	−0.50	0.943	−1.25 ± 2.75	−1.46 ± 3.02	−6.25	0.238	−1.26 ± 2.44	−1.42 ± 3.38	−3.38 ± 4.07	0.523

SD, standard deviation.

Note
Data represent mean ± standard deviation. Statistical significance determined by one-way ANOVA.

A notionally statistically significant association was observed for baseline-adjusted BDAI AUC for both rs12979860 and rs368234815 in Arm A only (infliximab) (p = 0.055). However, this was no longer significant after correction for multiple testing (FDR) (p > 0.05) (*Table 20*).

Metabolomics

Urine samples from the patients with BS were analysed by NMR spectroscopy and principal component analysis. Initial results showed no significant metabolite differences at baseline between patients allocated to drug A (Infliximab) or drug B (Roferon) (*Figure 8*). NMR data were analysed as bins which contain multiple metabolites. Three bins showed significant differences between patient groups A and B (*Figure 9*). However, when corrected for multiple comparisons of all bins, none remained significant (*Figure 10*).

To test whether drug treatments altered metabolite profiles, baseline samples from patients on infliximab (see *Figures 8–10*) or Roferon (*Figures 11–13*) were compared to samples from the same patient taken at week 24. PCA analysis, *Figures 8* and *11*, showed no major difference in metabolite profiles between the two samples. Specific bins, *Figures 9* and *12*, did show significance, though this was lost when correction for multiple comparisons was made (see *Figures 10* and *13*).

To determine whether metabolite profiles were associated with responses to the individual drugs, urine samples at week 24 from responders and non-responders were compared. Samples from patients on infliximab showed similar clustering by PCA analysis (*Figure 14*). Specific bins once again showed significant differences (*Figure 15*), with one bin remaining significant after correction for multiple comparisons (*Figure 16*).

For responders versus non-responders to Roferon, there was weak separation between the groups by PCA analysis, particularly clustering of responder samples (*Figure 17*). This pattern may have been due to specific bins which were significantly different between the groups (*Figure 18*), although such significance was lost when corrected for multiple comparisons (*Figure 19*).

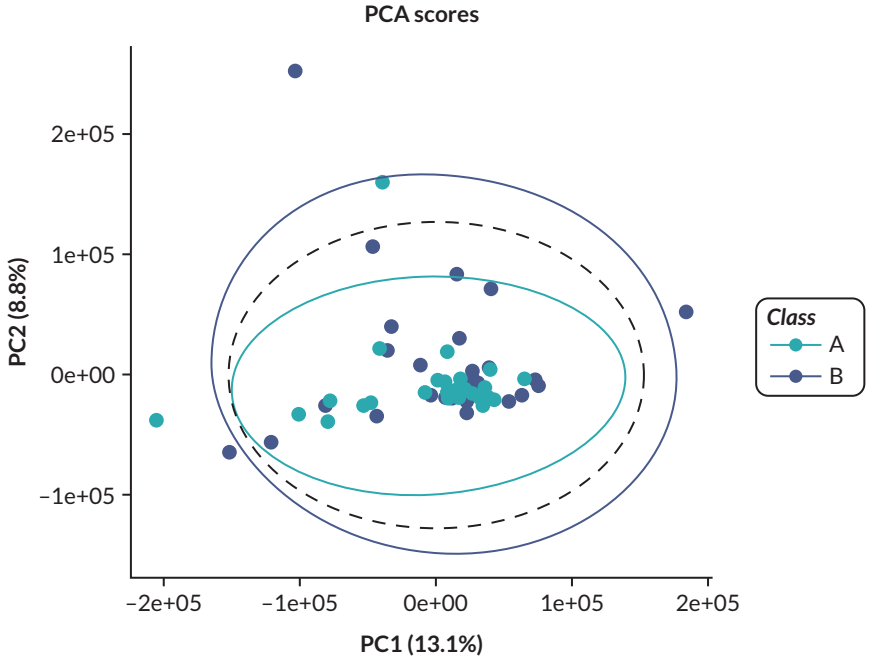


FIGURE 8 PCA analysis of baseline urine samples prior to randomised drug treatment. PCA, principle component analysis.

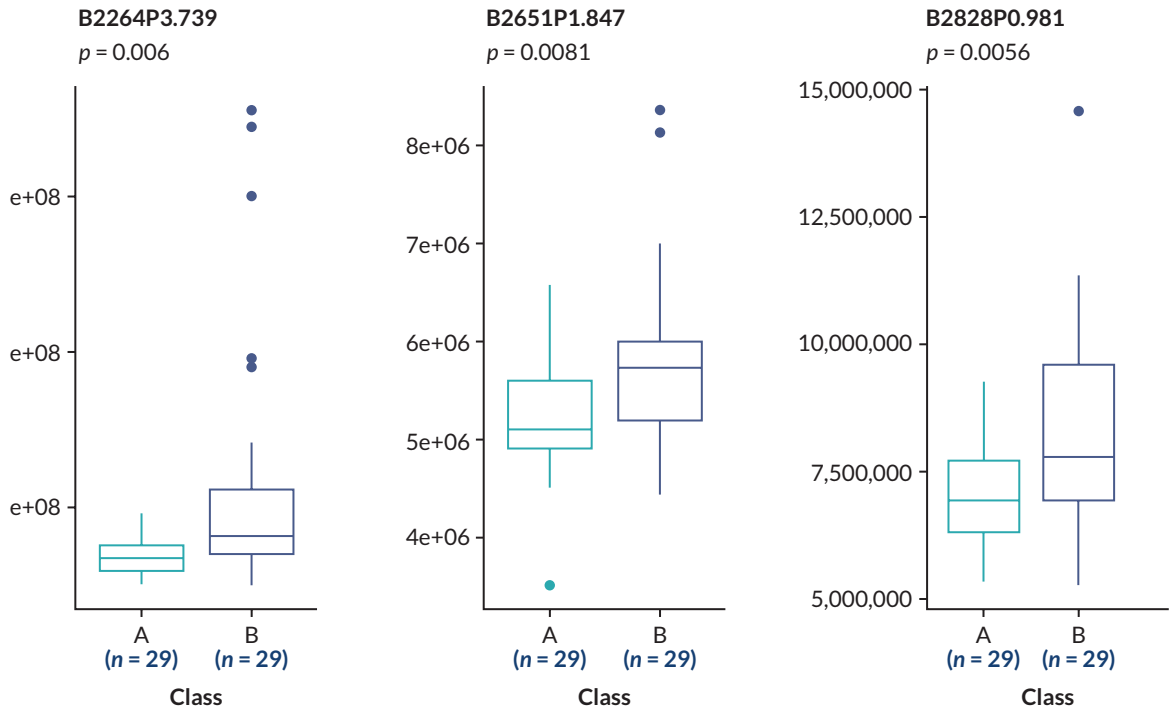


FIGURE 9 PCA analysis of baseline urine samples showing specific bins significantly different between groups.

2-group unpaired *t*-test (unequal variance)

	p.value ($p \leq 0.05$)	q.value (FDR ≤ 0.05)
n.significant	41	0

FIGURE 10 PCA analysis of baseline urine samples prior to randomised drug treatment; unpaired *t*-test for multiple comparisons.

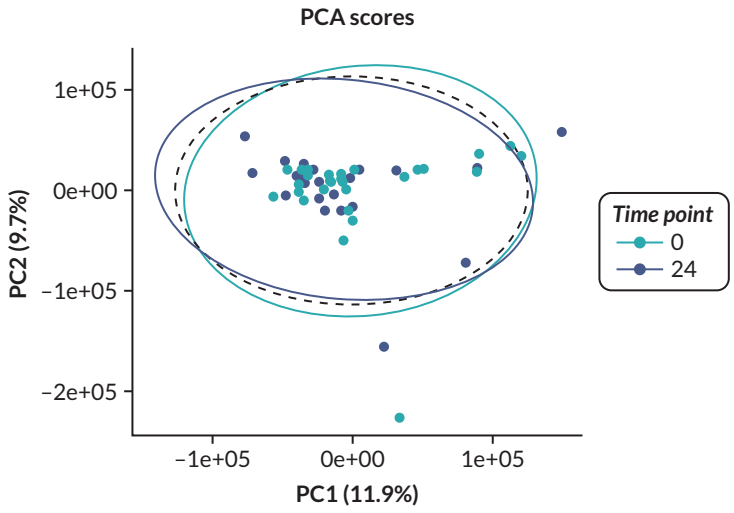


FIGURE 11 Analysis of NMR analysis of urine samples from patients on infliximab at baseline vs. 24 weeks. PCA analysis.

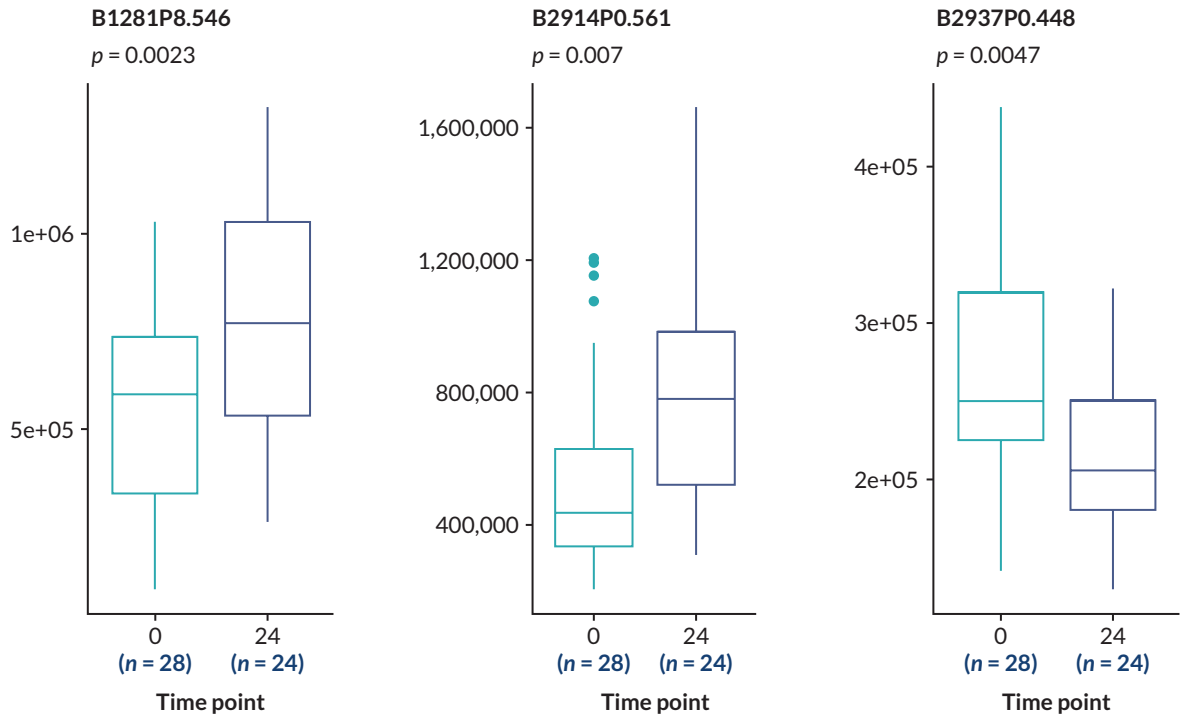


FIGURE 12 Analysis of NMR analysis of urine samples from patients on infliximab at baseline vs. 24-week significant bins.

2-group unpaired *t*-test (unequal variance)

	p.value ($p \leq 0.05$)	q.value (FDR ≤ 0.05)
n.significant	39	0

FIGURE 13 Unpaired *t*-test for multiple comparisons.

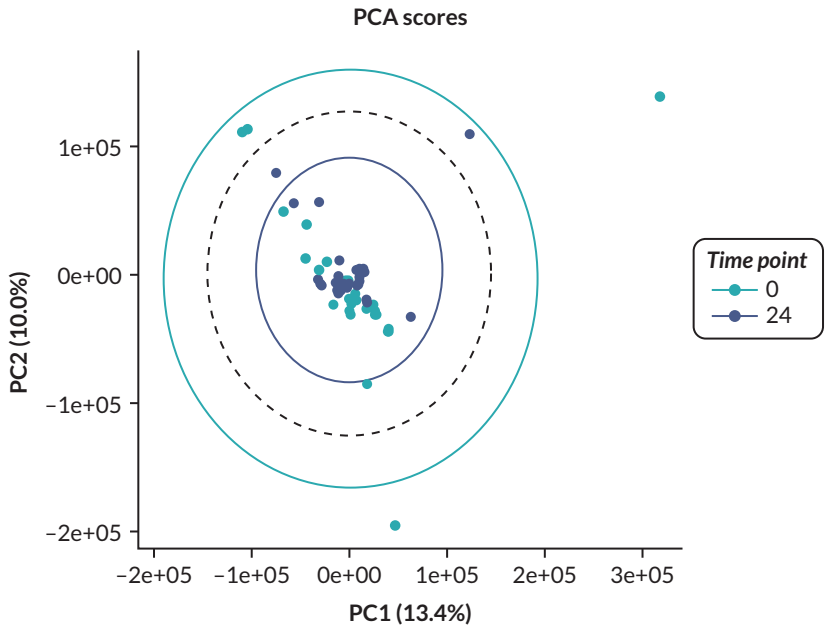


FIGURE 14 Analysis of NMR analysis of urine samples from patients on Roferon at baseline vs. 24 weeks. PCA analysis.

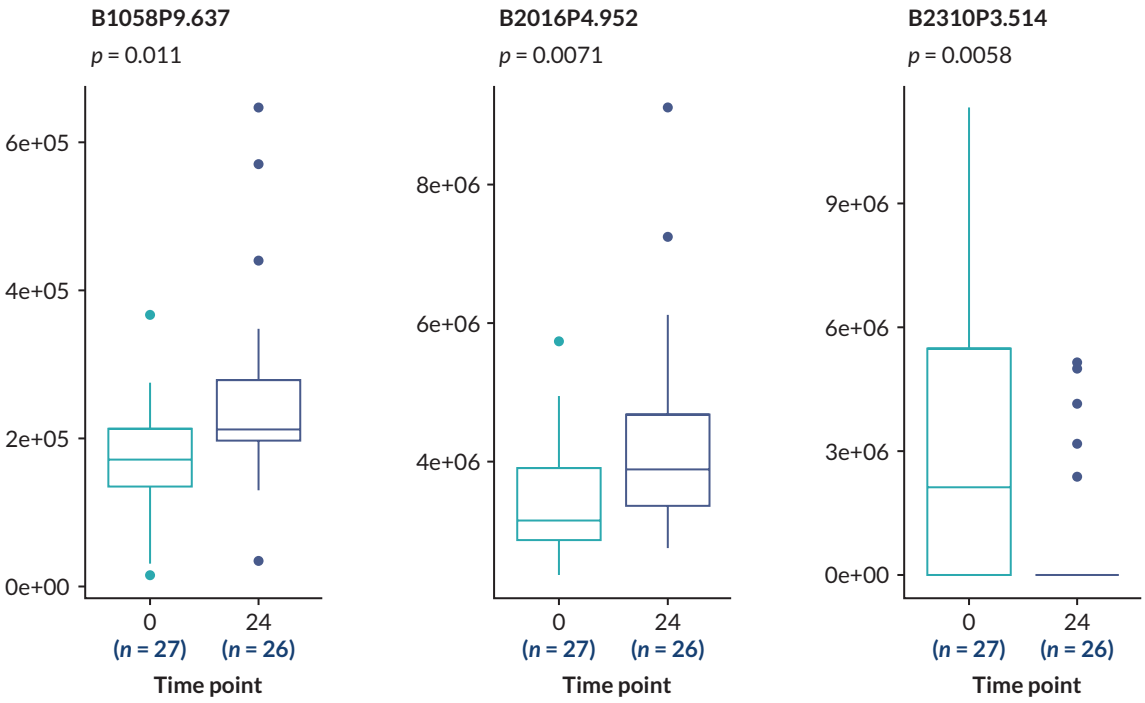


FIGURE 15 Analysis of NMR analysis of urine samples from patients on Roferon at baseline vs. 24 weeks. PCA analysis – significant bins.

2-group unpaired *t*-test (unequal variance)

	p.value ($p \leq 0.05$)	q.value (FDR ≤ 0.05)
n.significant	30	0

FIGURE 16 Analysis of NMR analysis of urine samples from patients on Roferon at baseline vs. 24 weeks. PCA analysis – unpaired *t*-test for multiple comparisons.

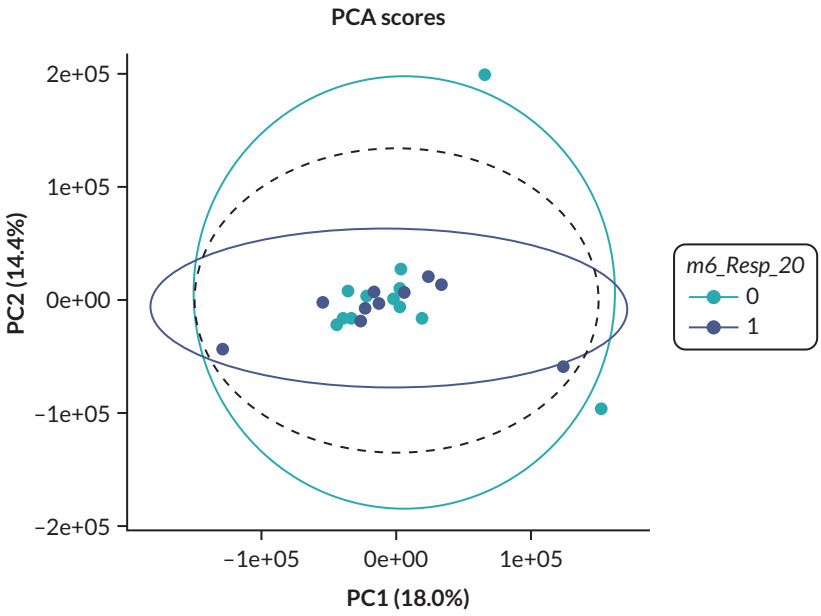


FIGURE 17 Nuclear magnetic resonance metabolite analysis of urine samples from responders to infliximab compared to non-responders. PCA analysis.

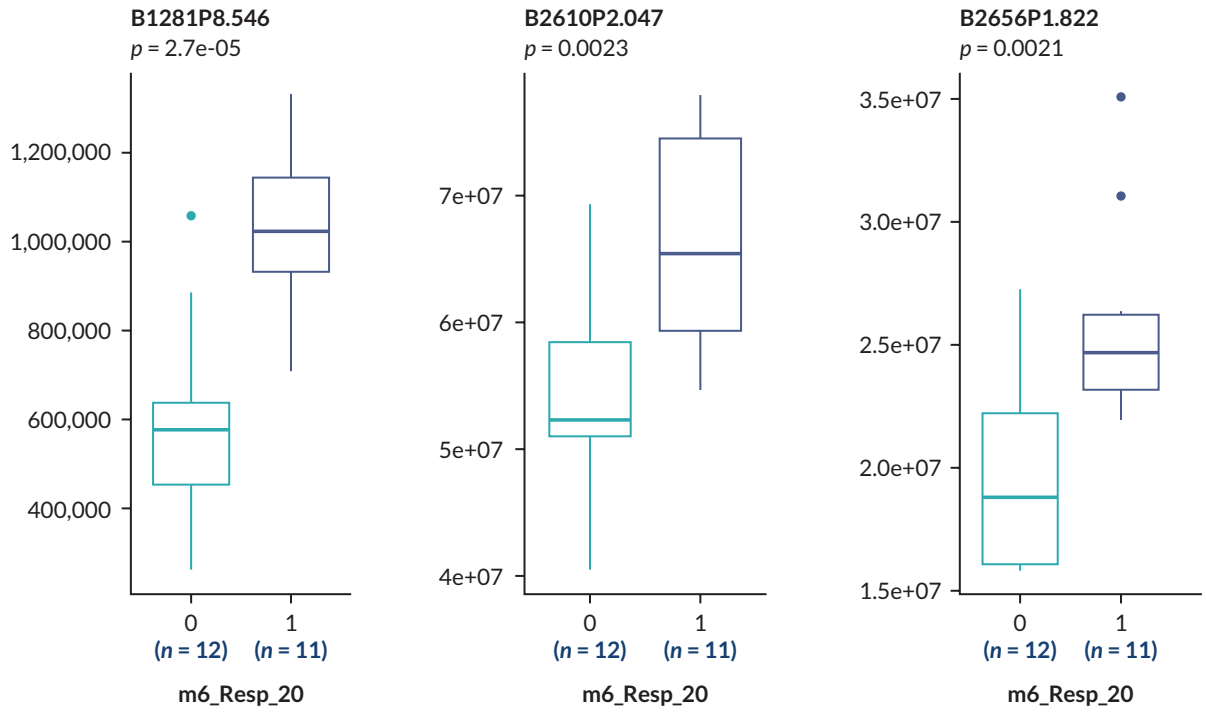


FIGURE 18 Nuclear magnetic resonance metabolite analysis of urine samples from responders to infliximab compared to non-responders. PCA analysis showing significantly different specific bins.

2-group unpaired *t*-test (unequal variance)

	p.value ($p \leq 0.05$)	q.value (FDR ≤ 0.05)
n.significant	63	1

FIGURE 19 Nuclear magnetic resonance metabolite analysis of urine samples from responders to infliximab compared to non-responders. PCA analysis – unpaired *t*-tests for multiple comparisons.

Metabolomic analysis showed no significant differences between the patients at baseline before randomised allocation to either of the study drugs (*Figure 9*). This confirms that there were no major confounding factors that may have influenced response to a particular treatment. Analysis between individual patient's urine metabolite profile at baseline compared to 24 weeks indicated no major differences, suggesting that the drugs were not inducing wide-ranging changes to the patients' metabolic processes, but rather that any effects would be due to specific changes to each drug's target pathways. This was supported by comparison of 24-week urine samples from responders and non-responders to the same drug. For infliximab, one bin remained significant after multiple corrections (*Figure 18*), and PCA clustering was weaker for patients on Roferon.

While of interest, it should be noted that comparisons between responders and non-responders split each group, leading to a smaller number of samples for analysis. However, the results for infliximab seem to indicate that within one of the bins we have detected potential marker(s) of a metabolic response to treatment which is worthy of further study to identify the individual metabolites and associated metabolic pathways responsible. The signal was weaker for Roferon and may not necessarily be due to the same metabolites and pathways (*Figures 20–23*). The specific bins that vary between each comparison will therefore be further analysed to determine the specific metabolites responsible for this difference, which may identify the particular pathways being influenced by each treatment.

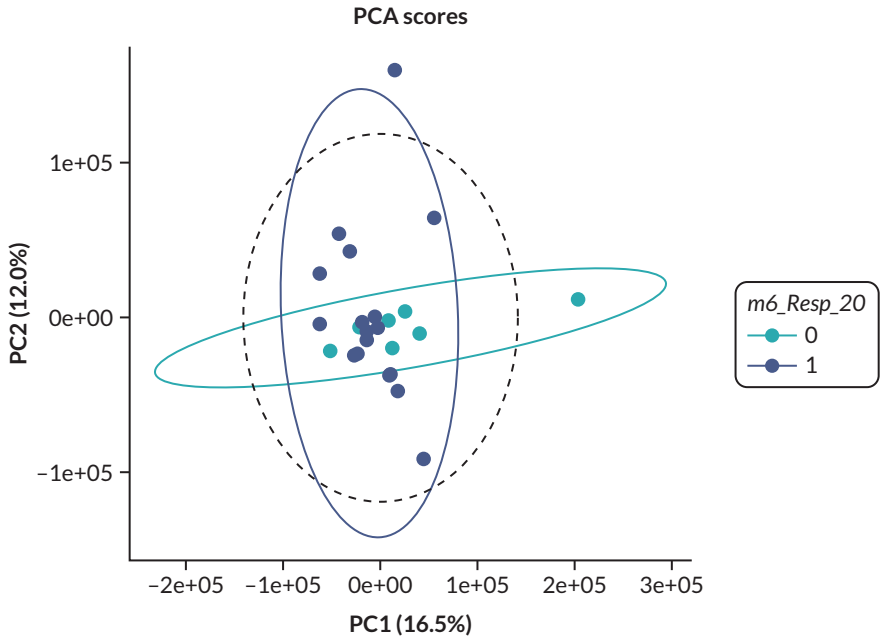


FIGURE 20 Nuclear magnetic resonance metabolite analysis of urine samples from responders to Roferon compared to non-responders. PCA analysis.

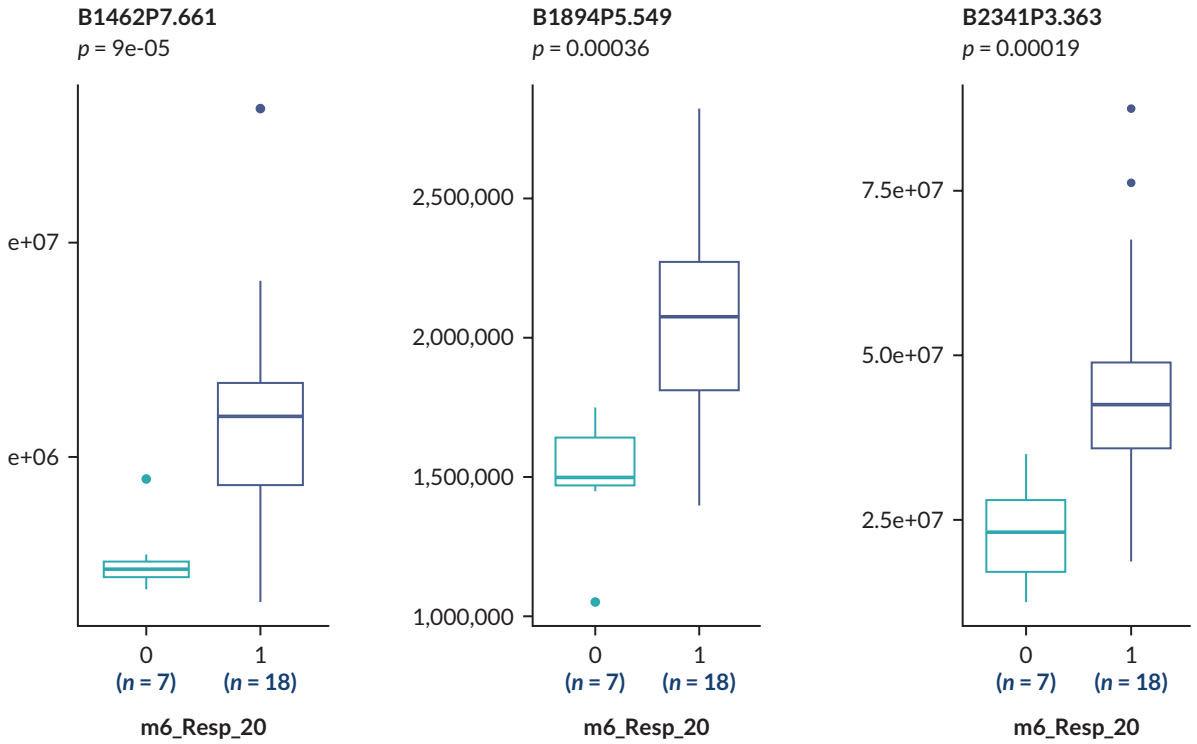


FIGURE 21 Nuclear magnetic resonance metabolite analysis of urine samples from responders to Roferon compared to non-responders. PCA analysis, showing significantly different specific bins.

2-group unpaired *t*-test (unequal variance)

	p.value ($p \leq 0.05$)	q.value (FDR ≤ 0.05)
n.significant	139	0

FIGURE 22 Nuclear magnetic resonance metabolite analysis of urine samples from responders to Roferon compared to non-responders. PCA analysis results using unpaired *t*-tests for multiple comparisons.

Chapter 5 Discussion

We report the first prospective RCT comparing infliximab with Roferon for subjects with refractory active BS, including all aspects of this multisystem disease and not restricted to single organ involvement. The study demonstrated that both infliximab and Roferon are effective in the treatment of BS and equivalent in terms of efficacy, with a numerical trend for minor benefit in favour of infliximab in terms of tolerability and treatment persistence.

This trial leveraged the benefits from the provision of expert multidisciplinary care provided by UK National Centres of Excellence (CoE) and linked satellite clinics. This enabled the first time the evaluation of a Western cohort of BS confirmed by an expert MDT for a head-to-head comparison of the two most widely used biologics for refractory disease at the time of the trial. BS is considerably less prevalent in the UK compared to Silk Road countries, and with the potential for subtle differences in the UK phenotype compared to those reported elsewhere, the study was designed as a pragmatic, standard-of-care trial, utilising the diagnostic and treatment pathways developed by and employed at the UK Centres. The 1990 ISG classification criteria were employed for diagnosis; inclusion criteria comprised severe disease that was refractory to or intolerant of therapy with steroid and/or immunosuppression with azathioprine or organ-threatening involvement where early use of biologic agents was deemed clinically important.

To address the challenges in designing and especially in powering a trial for a rare multisystem disease, a Bayesian approach was employed, leveraging the opinions of leading international experts to establish a prior distribution for the difference in mean values of the internationally validated clinical composite disease activity instrument, the BDAI, at 3 months between the two treatments. Change in BDAI was chosen as primary outcome, as this encompassed disease activity in individual organ systems. The secondary outcomes focused on significant clinical changes within affected organs. Ethnically diverse subjects with BSs were recruited from a wide geographic area within England.

Omeract recommended a mandatory set of domains to be used in all trials of BS and for separate subsets of domain-specific measures to be used for each type of organ or system involvement for trials testing organ-specific or subphenotype-based outcomes.[36] As our study was not focused on a particular organ or clinical subphenotype, we utilised some but not all of the Omeract measures for non-organ-specific trials in BS. This included overall disease (BDAI) and, as components of BDAI, Physician Global Assessment, Patient Global assessment, vascular, GI, central nervous system, together with mucocutaneous (oral ulcer activity score, genital ulcer activity score), ocular activity and damage (vitreous haze, BCVA), musculoskeletal (Likert pain score, but not tender or swollen joints) and quality of life (EQ-5D-5L, BD-QoL).

These measures comprised the routine data collected by the three CoEs and were therefore more likely to be evaluated reliably. Together, they provided a comprehensive assessment of treatment on disease activity, impact and function. However, for these reasons, the study design lacked the power to detect important treatment signals for clinical subphenotypes, particularly for those with ocular disease.

This trial was undertaken with a single-masked design. While assessors were masked to treatment arm, it was considered impractical for patients to be masked to study drugs, given the different routes, frequency of administration and major differences in adverse effect profiles of the two drugs.

Analysis of the impact of treatment on mBDAI revealed that, within each treatment arm, clinically significant improvements of mBDAI were observed over time (*Figures 6* and *7*), representing a 30% [0.7 (0.109); $p < 0.001$] reduction from baseline for infliximab [median, IQR change; −1.5 (−4 to 0)] and a 39% reduction [0.61 (0.069); $p < 0.001$] for Roferon [median, IQR change; −3 (−5.25 to −1)]. For infliximab,

17, 13 and 8 patients of 37 met 20%, 50% and 70% response criteria, respectively, and for Roferon, 22, 12 and 7 patients.

There was no statistically significant difference in response between the two treatment arms (*Figures 6* and *7*), demonstrating equivalence of infliximab and Roferon at both the 3- and 6-month end points. Sensitivity analysis, taking account of patients switching treatments, or of those who had major protocol deviations, did not influence the conclusions (*Table 21*). However, a significantly higher proportion of patients randomised to alpha interferon swapped to the other study drug, infliximab (*Table 22*; $p = 0.0104$), largely due to inadequate response or unacceptable side effects, as would be expected from the literature. A total of 46 patients reported 270 Aes, significantly more in those taking alpha interferon (see *Tables 39* and *40*; $p < 0.001$).

The Physicians Global Assessment of Disease activity (a component of BDAI and therefore correlated with it) improved significantly in both groups, with small differences at the 5% level in favour of infliximab at both 3 and 6 months (*Tables 14* and *15*). The subgroup of patients included with ophthalmological involvement was small, reflecting the lower proportion of ocular BS observed in the UK, compared to Silk Road countries, and consequently, no significant differences between the two treatment arms were detected. Multiple previous, largely open and uncontrolled studies of both Roferon and infliximab in subjects with ocular disease have demonstrated evidence of rapid improvements for each drug individually.

A recent narrative systematic review of anti-TNF therapy[37] confirmed the pivotal role of TNF alpha in the immunopathogenesis of BS. Eleven comparative studies reported a beneficial effect of treatment. Four were prospective trials, and seven were retrospective. Anti-TNF therapy often appeared to show early clinical benefit for all subphenotypes, including ocular disease. However, no previous studies have prospectively compared anti-TNF therapy with an alternate biologic agent.

The literature for Roferon is largely from uncontrolled observational studies, many of which report significant, early and sustained ocular responses with improvements in BCVA. Ocular response is rapid, with remission achievable by 24 weeks and response rates which vary between 80% and 90%. One recent Turkish retrospective study, employing identical treatment regimens to our trial, compared treatments over 12 months for patients with refractory BS uveoretinitis taking infliximab (20 patients) with alpha interferon (33 patients).[38] The same study reported significant improvements in BCVA in both treatment groups, with a reduction in laser flare photometry and vitreous haze at 6 months (infliximab 0.97 ± 0.2 to 0.5 ± 0.14) and (IFN 1.2 ± 0.2 to 0.5 ± 0.1). After 1 year, there was an 85% response rate for alpha interferon versus 80% for infliximab.

TABLE 21 Results of sensitivity analyses for specific groups (after 6 months of treatment)

	Estimate	SE	80% credibility interval
Cohort 1			
Roferon vs. infliximab	0.13	0.25	(−0.19 to 0.46)
Cohort 2			
Roferon vs. infliximab	0.1	0.27	(−0.25 to 0.43)
Cohort 3			
Roferon vs. infliximab	0.13	0.26	(−0.21 to 0.47)

Notes
Created by richj23 using R version 3.5.1 (2 July 2018) on 17 September 2021.
- *Cohort 1: all patients including the data from both arms for patients who switched treatment.*
- *Cohort 2: all patients who remained on their original treatment and complied with the protocol.*
- *Cohort 2: all patients aside from those who had a major protocol deviation.*

TABLE 22 Number of patients switching treatment

	Infliximab (*N* = 37)	Roferon (*N* = 37)	*p*-value
Switched treatments, *n* (%)			
Switched	3 (9)	11 (33)	0.0104
Not switched	31 (91)	21 (64)	
Missing response	0 (0)	1 (3)	

Notes
p-value calculated using Fisher's exact test.
Created using SAS (Version 9.4) on 17 September 2021.
Created using data set randomisation, Bioassess data sets (Stats Server).
Population: ITT.
Notes for *Table 22*:
Infliximab – *The number of patients totals 34 rather than 37, as 3 patients did not complete a treatment swap form for the following reasons:*
- *1 patient could not be contacted after the screening visit.*
- *1 patient received mental health treatment and was not fit to continue on the trial beyond baseline.*
- *1 patient did not provide further information.*

Roferon– *The number of patients totals 33 rather than 37, as 4 patients did not complete a treatment swap form for the following reasons:*
- *2 patients stopped medication after baseline.*
- *1 patient had a treatment swap prior to week 12 follow-up. Did not complete treatment swap form. PI wants to keep patient on follow-up within the study.*
- *1 patient did not provide further information.*

Genetic variability, acquired or innate antibodies, receptor dysregulation or antibodies stimulated by treatment may modulate clinical responsiveness to IFN-α2a. Increased frequencies of anti-IFN-α and various autoantibodies associated with IFN-α2a treatment have been suggested to be associated with a better clinical response.[38] These aspects were not examined in this study.

One potential advantage of Roferon compared to infliximab may be its ability to induce lasting and prolonged drug-free remission of disease, ranging from 20% after a mean of 33 months of treatment, remaining relapse-free for a mean of 37 months[39] to 68% relapse-free for a mean of 43 months after a mean of 32 months' treatment.[40] More prolonged therapy may result in even more durable remission of ocular disease.[41] Many of these studies, while primarily demonstrating ocular efficacy, also demonstrated benefits for mucocutaneous and articular disease, as evidenced in our study. Unfortunately, due to supply constraints and the subsequent withdrawal of production of alpha interferon, long-term open-label follow-up was not possible: all patients completing in our trial who were randomised to Roferon and who noted both efficacy and tolerability have stopped treatment with this drug and, if exhibiting a flare, were mostly switched to anti-TNF therapy.

Our study also included a mechanistic component to address the potential to predict the response to either infliximab or Roferon treatment which both possess distinct modes of action, utilising genomics and urine metabolomics in order to further improve cost-effectiveness and precision. Genotyping for four SNPs in the region of IFNL3 (IL28B) was undertaken based on the literature supporting their role in predicting viral clearance for hepatitis C virus infection and NMR-based urine metabolomics which has shown promise to predict response in RA.

Genetic data are suggestive of an association between patient outcome and carriage of either rs4803221 or rs7248668 variants in the *IFNL3 (IL-28B)* gene locus, but only in the alpha interferon-treated arm (*Table 11*). This observation is in line with previous findings, which demonstrate significant association between these SNPs and Roferon treatment outcomes in hepatitis C.[23] This may be indicative of the potential to predict patient outcomes for alpha interferon according to genotype in this patient population. However, given the relatively small sample size of Arm B and the fact that the

statistical significance of the association is negated by correction for multiple testing, the results must be treated with caution. Larger, adequately statistically powered genetic studies are required to verify this finding in the context of BS.

This is the first prospective study to utilise metabolomics to examine the potential for differential effects of two biologic agents when used head-to-head in the treatment of BS.

Metabolomic analysis showed no significant differences between the patients at baseline before randomisation, confirming that there were no major confounding factors that may have influenced response to a particular treatment. Analysis between individual patient's urine metabolite profile at baseline compared to 24 weeks indicated no major differences, suggesting that the drugs were not inducing wide-ranging systemic changes to the patients' metabolic processes, rather suggesting that any effects would be due to specific metabolic changes to each drug's target pathway. This was supported by a comparison of 24-week urine samples from responders and non-responders to the same drug. For infliximab, one bin remained significant after multiple corrections (*Figure 9*), though PCA clustering was weaker for patients on Roferon. The results for infliximab seem to indicate that within one of the bins we have detected potential marker(s) of a metabolic response to treatment which is worthy of further study to identify the individual metabolites and associated metabolic pathways responsible. The signal was weaker for Roferon and may not necessarily be due to the same metabolites and pathways.

It should be noted that comparisons between responders and non-responders split each group, leading to a smaller number of samples for analysis. The specific bins that vary between each comparison will therefore be further analysed to determine the specific metabolites responsible for this difference, which may indicate the selective pathways being influenced by each treatment. The metabolomic analysis in this study supports the process used for patient randomisation and, for the first time, directs future research towards identifying the direct effect of each drug when investigating a patient's response to treatment.

A limitation of this study was the use of only urine and NMR analysis of metabolites. Changes in serum lipid markers have been described previously in serum from patients with BS and healthy controls using mass spectrometry analysis.[42,43] Analysis of urine samples from patients with BS and healthy controls using mass spectroscopy identified a biomarker panel composed of 10 metabolites which, when selected as biomarker panel of BS showed clear discrimination between the groups.[44] Amino acids, including glutamate and valine, were identified potential biomarkers in synovial fluid from patients with BS with arthritis compared to patients with seronegative arthritis.[45]

The current study advances these reports by comparing two drug therapies between patients with BD. It also supports the randomisation process of patients and helps direct our future research towards the direct effect of each drug-limiting process rather than altering it in a patient's response to treatment.

Costs of therapy are also relevant when evaluating benefits and risks of treatment options, though costs of biologics and in particular anti-TNF therapy have fallen significantly since the introduction of biosimilars. The significant difference in annual treatment costs identified at the design stage for this study comparing infliximab with interferon is no longer relevant as the annual NHS cost for infliximab has fallen significantly to the order of £2512 at the time of writing and Roferon is no longer commercially available.

Overall, this trial had several significant strengths. It is the first prospective RCT evaluating two biologic drugs head-to-head in BS. Given the potential adverse effect profiles of both infliximab and Roferon, and especially with the significant risk of secondary inefficacy with infliximab (due to the development of anti-drug antibodies), it was important to create a sound evidence base from which to inform optimal usage and targeting of these drugs. The trial demonstrated comparable clinical effectiveness of

both infliximab and Roferon, with infliximab associated with slightly lower adverse effects and better tolerability. Furthermore, at the time of trial design and funding approval, there was a major difference in acquisition cost, with Roferon significantly less expensive than infliximab (Remicade). However, with the widespread availability of biosimilar infliximab, the price differential reduced considerably to near parity. Based on our results for a UK-based cohort, the trial outcomes do not appear to differ from those in the published international literature for both infliximab and Roferon.

There are also several weaknesses. Recruitment to the study was slower than anticipated, with fewer participants recruited than was expected. Furthermore, the duration of follow-up was limited. While this still allowed appropriate statistical evaluation of the primary end point, the numbers were too few to allow a detailed evaluation of all secondary outcomes. Concerns about the potential adverse effect profile for Roferon, especially the (very rare) risk of mental health problems, prevented many patients from participating in the trial – it was encouraging to note that no serious effects on mental health were recorded. We observed a more favourable side effect profile for Roferon compared to that feared in practice. Unfortunately, towards the end of the trial, the manufacturers of Roferon ceased production of this drug for commercial reasons. While sufficient study drugs were made available to continue the trial, the expiry date of the final batch meant that long-term open-label follow-up was not possible, and those patients requiring Roferon had to swap to an anti-TNF drug (typically infliximab) when flaring after Roferon was discontinued.

The loss of availability of Roferon is of significant concern in the management of BS. Not all patients with severe disease respond to infliximab, with overall response rates estimated at 80%. In those who initially respond, there is a significant risk of secondary inefficacy after prolonged use or development of infliximab antibodies. Infliximab would also be contraindicated in patients with comorbid diseases with an increased risk of infection. This is particularly relevant in areas where the prevalence of TB is high. There remains a pressing need to identify other targeted therapies, with different mechanisms of action to infliximab, for severe refractory BS either for all clinical aspects or for specific BS subphenotypes after primary or secondary alpha tumour necrosis factor failure. Recent Phase II and III trials of the PGE4-inhibitor, Apremilast, have led to its licence for BS – but this drug appears to be more useful for mucosal ulceration than ocular or vascular involvement. Other biologic drugs, such as IL-17 inhibitors, for example secukinumab,[46] ustekinumab[47,48] and IL-6 inhibitors such as tocilizumab,[49–51] have been suggested anecdotally to be effective in BS but currently lack robust clinical trial evaluation in this setting.

Patient and public involvement

This has been embedded into the Bio Behçet's study from its inception. This trial was planned with involvement of patients with Behçet's. The National UK patient charity (Behçet's UK: Chair Mr Tony Thornburn) was a co-applicant/coinvestigator on the grant. The patient charity helped provide awareness of the study and will be fully involved in dissemination of results. Patients have been involved in data analysis, and a representative from Behçet's UK was part of the TSC.

Equality, diversity and inclusion

Our aim has been to be inclusive and reflective of the diverse population of patients with Behçet's in the UK in the recruitment and running of this study. We have recruited through the National Centres for Behçet's in the UK – to understand response to drugs in the UK Behçet's population, as set out in the grant application. As part of this, we have recruited patients from a broad spectrum of ethnicities found in UK Behçet's patients, but we are aware that the spectrum of ethnicities in the UK may differ from that in regions of greater preference for Behçet's, such as in Silk Route countries.

Additional information

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Robert J Moots (https://orcid.org/0000-0001-7019-6211) (Professor of Rheumatology) led the design of the study, recruited patients, contributed to analysis of results and preparation of the manuscript.

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Richard Jackson (https://orcid.org/0000-0002-7814-5088) (Lecturer in Statistics) contributed to analysis of results and preparation of the manuscript.

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Philip Ian Murray (https://orcid.org/0000-0001-8491-3795) (Professor of Ophthalmology) contributed to study design, analysis of results and preparation of the manuscript.

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Deva Situnayake (https://orcid.org/0000-0002-0643-0760) (Consultant Rheumatologist) contributed to study design, recruitment of patients, analysis of results and preparation of the manuscript.

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Data-sharing statement

All available data can be obtained from the corresponding author via the Liverpool Clinical Trials Consortium.

Ethics statement

This study was assessed and approved by the NRES Committee North West – Liverpool Central. Date of approval: 17 March 2015. Reference number: 15/NW/0008.

Information governance statement

The University of Liverpool is committed to handling all personal information in line with the UK Data Protection Act (2018) and the General Data Protection Regulation (EU GDPR) 2016/679. Under Data Protection legislation the University of Liverpool is the Data Processor and the Data Controller, and we process personal data in accordance with their instructions. You can find out more about how we handle personal data, including how to exercise your individual rights and the contact details for the University of Liverpool Data Protection Officer https://www.liverpool.ac.uk/legal/data_protection/.

Disclosure of interests

Full disclosure of interests: Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page at https://doi.org/10.3310/HTFC6304.

Primary conflicts of interest: Ann W Morgan reports Investigator Initiator grant from Roche for GCA; consultancy and honoraria from Roche and Chugai; consultancy from AstraZeneca, Sanofi, Regeneron and Vifor; travel subsidence from Roche and MRC DPFS panel membership. Philip Ian Murray reports payments from Oxford University Press and payments made to the author's institution from Scope Eyecare. Graham Robert Wallace received travel subsidence from Cerrahpasa University Hospital to attend the Cerrahpasa Behçet's Disease Symposium. All other authors have no interests to disclose.

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Appendix 1 Supporting figures and tables

TABLE 23 Trial recruitment by centre

Site name	Date of greenlight	Date of first randomisation	Date of last randomisation	Rand to IFX (*N* = 40)	Rand to αIFN (*N* = 39)	Total randomised (*N* = 79)
Behçet's Syndrome Centre of Excellence (London)	15 February 2017	11 April 2017	7 February 2020	11	13	24
Aintree University Hospital	30 March 2016	21 June 2016	18 February 2020	12	11	23
Sandwell Hospital (Birmingham)	13 January 2017	16 June 2017	15 July 2019	8	7	15
Chapel Allerton Hospital	19 October 2016	25 November 2016	21 October 2019	5	5	10
Freeman Hospital (Newcastle Upon Tyne)	9 May 2017	19 May 2017	23 September 2019	2	2	4
Addenbrooke's Hospital (Cambridge)	22 December 2017	5 February 2018	19 December 2018	2	1	3
University College Hospital (London)	15 March 2017	.	.	0	0	0
Manchester Royal Infirmary	4 October 2017	.	.	0	0	0

αIFN, alpha interferon.

TABLE 24 Summary of study protocol deviations

Type	Description of deviation	IFX	αIFN	Total
Major	17: Major: patient management/assessment – patient examination/Test	2	3	5
	3: Major: administration of wrong treatment or incorrect dose, etc.	1	1	2
	5: Major: major protocol deviation in patient management and/or assessment	2	6	8
	6: Major: other major protocol deviation	0	1	1
Minor	19: Minor: source data	0	1	1
	23: Minor: IMP issue	0	2	2
	24: Minor: blood result	29	42	81
	25: Minor: translational	18	17	35
	29: Minor: visit time point	2	4	6
	30: Minor: patient examination/test	20	29	49
	7: Minor: other protocol deviations (not expected to have an impact on defined end points of the trial)	4	4	8

αIFN, alpha interferon.

TABLE 25 Details on treatment and compliance – infliximab

Measure	IFX
Patients received treatment at week 0, *n* (%)	14 (78%)
Patients received treatment at week 2, *n* (%)	14 (78%)
Patients received treatment at week 6, *n* (%)	13 (72%)
Patients received treatment at week 14, *n* (%)	12 (67%)
Patients received treatment at week 22, *n* (%)	11 (61%)
Patients received treatment at week 30, *n* (%)	5 (28%)
Patients with at least one dose delay, *n* (%)	388.3 (18.06)
Patients with at least one dose reduction, *n* (%)	5 (2, 6)

Notes
Please note that details of treatment received were obtained for 18 patients which provides the denominator for summaries in *Table 25*. There were no patients with dose delays/reductions, although five patients prematurely ended all study treatment while on infliximab.

TABLE 26 Details on treatment and compliance – Roferon

Measure	αIFN
Patient taken full dose of injections up to week 12 review, *n* (%)	21 (56%)
Patient taken full dose of injections up to week 24 review, *n* (%)	18 (49%)
Patient taken full dose of injections up to week 36 review, *n* (%)	13 (35%)
Patients with treatment dosage change between baseline and week 12 review, *n* (%)	11 (30%)
Patients with treatment dosage change between week 12 review and week 24 review, *n* (%)	27 (73%)
Patients with treatment dosage change between week 24 review and week 36 review, *n* (%)	5 (14%)
Mean number of doses missed per patient recorded at week 12 review	0.93
Mean number of doses missed per patient recorded at week 24 review	4.47
Mean number of doses missed per patient recorded at week 36 review	1.11

αIFN, alpha interferon

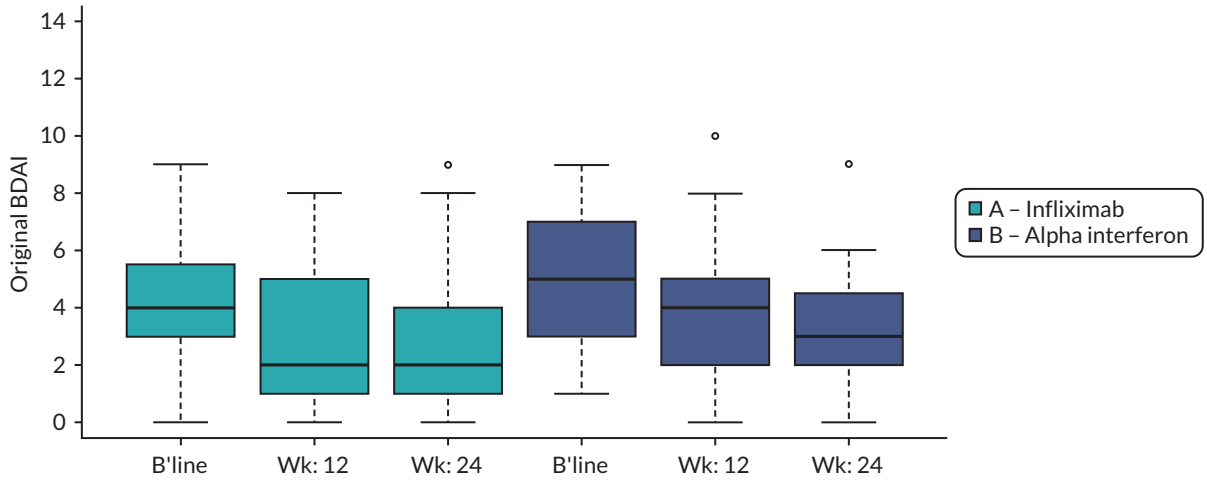

FIGURE 23 Original BDAI after 3 and 6 months by treatment group.

TABLE 27 Results for original BDAI

	Estimate	SE	80% credibility interval
αIFN vs. IFX (3 months)	0.12	0.25	(−0.2 to 0.44)
αIFN vs. IFX (6 months)	0.06	0.26	(−0.27 to 0.4)

αIFN, alpha interferon.

TABLE 28 Vitreous haze – baseline vs. 3 months

	IFX (*N* = 37)	αIFN (*N* = 37)	IFX (*N* = 37)	αIFN (*N* = 37)
	Right eye	Right eye	Left eye	Left eye
Baseline, *n* (%)				
0	8 (100)	9 (75)	6 (75)	10 (83)
0.5+	0 (0)	2 (17)	2 (25)	1 (8)
1+	0 (0)	1 (8)	0 (0)	1 (8)
3 months, *n* (%)				
0	8 (100)	10 (83)	7 (88)	9 (75)
0.5 +	0 (0)	1 (8)	1 (13)	2 (17)
2 +	0 (0)	1 (8)	0 (0)	1 (8)
Difference between baseline and 3 months, *n* (%)				
−0.5	0 (0)	1 (8)	1 (13)	1 (8)
0	8 (100)	10 (83)	7 (88)	10 (83)
2	0 (0)	1 (8)	0 (0)	1 (8)

αIFN, alpha interferon.

TABLE 29 Vitreous haze – baseline vs. 6 months

	IFX (*N* = 37)	αIFN (*N* = 37)	IFX (*N* = 37)	αIFN (*N* = 37)
	Right eye	Right eye	Left eye	Left eye
Baseline, *n* (%)				
0	6 (86)	10 (83)	4 (57)	11 (92)
0.5 +	1 (14)	1 (8)	3 (43)	0 (0)
1 +	0 (0)	1 (8)	0 (0)	1 (8)
6 months, *n* (%)				
0	6 (86)	10 (83)	6 (86)	10 (83)
0.5 +	1 (14)	2 (17)	1 (14)	2 (17)
Difference between baseline and 6 months, *n* (%)				
−0.5	0 (0)	1 (8)	2 (29)	1 (8)
0	7 (100)	10 (83)	5 (71)	10 (83)
0.5	0 (0)	1 (8)	0 (0)	1 (8)

αIFN, alpha interferon.

TABLE 30 Best corrected visual acuity (number of letters read) – baseline vs. 3 months

	IFX (*N* = 37)	αIFN (*N* = 37)	IFX (*N* = 37)	αIFN (*N* = 37)
	Right eye	Right eye	Left eye	Left eye
Baseline				
n	18	18	18	18
Median (IQR)	55.0 (50.0–60.0)	55.0 (50.0–60.0)	55.0 (50.0–60.0)	54.5 (50.0–60.0)
3 months				
n	14	16	14	16
Median (IQR)	55.0 (49.0–63.0)	59.5 (50.5–65.0)	55.0 (52.0–65.0)	58.0 (49.0–64.5)
Difference between baseline and 3 months				
N	13	14	13	14
Median (IQR)	0.0 (–5.0 to 3.0)	3.5 (0.0–9.0)	3.0 (–1.0 to 9.0)	1.5 (–1.0 to 7.0)

αIFN, alpha interferon.

TABLE 31 Best corrected visual acuity (number of letters read) – baseline vs. 6 months

	IFX (*N* = 37)	αIFN (*N* = 37)	IFX (*N* = 37)	αIFN (*N* = 37)
	Right eye	Right eye	Left eye	Left eye
Baseline				
N	18	18	18	18
Median (IQR)	55.0 (50.0–60.0)	55.0 (50.0–60.0)	55.0 (50.0–60.0)	54.5 (50.0–60.0)
6 months				
N	13	15	13	15
Median (IQR)	60.0 (46.0–60.0)	60.0 (56.0–65.0)	60.0 (50.0–65.0)	60.0 (55.0–65.0)
Difference between baseline and 6 months				
N	12	13	12	13
Median (IQR)	–0.5 (–3.0 to 5.0)	5.0 (0.0–6.0)	0.5 (0.0–9.0)	1.0 (0.0–2.0)

αIFN, alpha interferon.

TABLE 32 Likert pain score – baseline vs. 3 months

	IFX (*N* = 37)	αIFN (*N* = 37)	*p*-value
Baseline			
N	13	16	
Median (IQR)	5.0 (4.0–8.0)	6.0 (5.0–7.5)	
3 months			
N	13	16	
Median (IQR)	6.0 (4.0–7.0)	5.5 (2.0–7.5)	
Difference between baseline and 3 months			
Absolute difference – median (IQR)	–1.0 (–2.0 to 0.0)	–0.5 (–2.5 to 1.0)	
Percentage difference – median (IQR)	–12.7 (–25.0 to 10.0)	–7.1 (–50.0 to 16.3)	
Proportion with at least 20% improvement	5 (38%)	7 (44%)	1.0000

αIFN, alpha interferon.

TABLE 33 Likert pain score – baseline vs. 6 months

	IFX (*N* = 37)	αIFN (*N* = 37)	*p*-value
Baseline			
N	13	16	
Median (IQR)	5.0 (4.0–8.0)	6.0 (5.0–7.5)	
6 months			
N	13	16	
Median (IQR)	6.0 (5.0–8.0)	5.0 (1.5–7.0)	
Difference between baseline and 6 months			
Absolute difference – median (IQR)	0.0 (−1.0 to 1.0)	−1.5 (−4.0 to 0.5)	
Percentage difference – median (IQR)	6.3 (−11.1 to 26.8)	−17.4 (−70.0 to 7.1)	
Proportion with at least 20% improvement	2 (15%)	8 (50%)	0.1142

αIFN, alpha interferon.

TABLE 34 Quality of life – patients from EQ-5D-5L with reported problems (i.e. levels 2, 3, 4, 5)

Time point	IFX (*N* = 37) (%)	αIFN (*N* = 37) (%)	*p*-value
Mobility			
Baseline	19 (52.8)	25 (69.4)	0.2265
3 months	19 (57.6)	20 (62.5)	0.8014
6 months	19 (61.3)	18 (56.3)	0.7994
Self-care			
Baseline	13 (36.1)	13 (36.1)	1.0000
3 months	9 (27.3)	13 (40.6)	0.3015
6 months	8 (25.8)	13 (40.6)	0.2869
Usual activities			
Baseline	24 (66.7)	28 (77.8)	0.4304
3 months	20 (60.6)	23 (71.9)	0.4339
6 months	17 (54.8)	17 (53.1)	1.0000
Pain/discomfort			
Baseline	32 (88.9)	32 (88.9)	1.0000
3 months	25 (75.8)	28 (87.5)	0.3389
6 months	23 (74.2)	23 (71.9)	1.0000
Anxiety/depression			
Baseline	22 (61.1)	22 (61.1)	1.0000
3 months	18 (54.5)	19 (59.4)	0.8036
6 months	16 (51.6)	20 (62.5)	0.4500

αIFN, alpha interferon.

TABLE 35 Quality of life – difference from baseline in EQ-VAS – baseline vs. 3 months

	IFX (*N* = 37)	αIFN (*N* = 37)	*p*-value	*p*-value
Baseline				
N	33	29		
Mean (SD)	60.1 (19.48)	51.2 (21.03)		
Median (IQR)	60.0 (50.0–74.0)	50.0 (30.0–65.0)		
3 months				
N	33	29		
Mean (SD)	71.6 (20.91)	59.7 (19.73)		
Median (IQR)	75.0 (65.0–85.0)	65.0 (50.0–75.0)		
Difference between baseline and 3 months				
Mean (SD)	11.5 (24.81)	8.4 (24.93)	0.6315	
Median (IQR)	10.0 (0.0–20.0)	10.0 (–5.0 to 20.0)		0.8318

αIFN, alpha interferon; SD, standard deviation.

TABLE 36 Quality of life – difference from baseline in EQ-VAS – baseline vs. 6 months

	IFX (*N* = 37)	αIFN (*N* = 37)	*p*-value	*p*-value
Baseline				
n	31	31		
Mean (SD)	59.5 (19.98)	53.1 (21.48)		
Median (IQR)	60.0 (45.0–74.0)	50.0 (30.0–70.0)		
6 months				
n	31	31		
Mean (SD)	70.5 (20.54)	64.9 (19.06)		
Median (IQR)	75.0 (55.0–90.0)	70.0 (50.0–80.0)		
Difference between baseline and 6 months				
Mean (SD)	11.1 (24.79)	11.8 (24.41)	0.9100	
Median (IQR)	10.0 (–5.0 to 25.0)	8.0 (–5.0 to 30.0)		0.8600

αIFN, alpha interferon; SD, standard deviation.

TABLE 37 Quality of life – difference from baseline in BD-QoL– baseline vs. 3 months

	IFX (*N* = 37)	αIFN (*N* = 37)	*p*-value	*p*-value
Baseline				
n	32	31		
Mean (SD)	13.5 (8.70)	17.1 (7.67)		
Median (IQR)	12.5 (7.0–20.5)	18.0 (12.0–24.0)		
3 months				
N	32	31		
Mean (SD)	10.0 (8.47)	16.3 (7.58)		
Median (IQR)	8.5 (2.0–17.5)	18.0 (12.0–22.0)		
Difference between baseline and 3 months				
Mean (SD)	−3.5 (5.04)	−0.8 (4.57)	0.0282	
Median (IQR)	−3.0 (−6.0 to 0.0)	0.0 (−3.0 to 2.0)		0.0274

αIFN, alpha interferon; SD, standard deviation.

TABLE 38 Quality of life – difference from baseline in BD-QoL– baseline vs. 6 months

	IFX (*N* = 37)	αIFN (*N* = 37)	*p*-value	*p*-value
Baseline				
n	31	31		
Mean (SD)	14.0 (8.57)	17.6 (8.01)		
Median (IQR)	13.0 (7.0–21.0)	19.0 (12.0–25.0)		
6 months				
n	31	31		
Mean (SD)	10.9 (8.88)	15.4 (9.09)		
Median (IQR)	10.0 (3.0–18.0)	15.0 (7.0–23.0)		
Difference between baseline and 6 months				
Mean (SD)	−3.2 (5.18)	−2.2 (6.26)	0.5100	
Median (IQR)	−3.0 (−5.0 to 0.0)	−2.0 (−5.0 to 3.0)		0.3029

αIFN, alpha interferon; SD, standard deviation.

TABLE 39 Line listing of study SAEs

ID	Age	Gender	Trial arm	Onset date	Offset date	Outcome	Overall diagnosis	MedDra def	System organ class	Drug interact.	SAE grade	SUSAR
2	18	Female	A – Infliximab	9 January 2020	14 January 2020	Resolved	Hypertension	Hypertension	Vascular disorders		Moderate	0
3	44	Male	B – Alpha interferon	8 March 2020	12 March 2020	Resolved	Admitted to hospital with abdominal pain on 8 March 2020 (right iliac fossa) Initially queried appendicitis, normal appendix seen on CT report CT report conclusion: Distal small bowel obstruction due to Meckel's diverticulum. Diagnostic laparoscopy division of bond adhesives on 10 March 2020. Discharged on 12 March 2020. Awaiting MRI scan and results follow-up in 8/52	Ileus	GI disorders		Severe	0
4	41	Female	A – Infliximab	14 March 2018	15 March 2018	Resolved with sequelae	Diffuse abdominal pain			No	Moderate	
5	48	Female	A – Infliximab	9 October 2017	10 October 2017	Resolved with sequelae	Emergency cholecystectomy	Cholecystectomy	Surgical and medical procedures	No	Moderate	
6	18	Female	A – Infliximab	6 August 2019	9 August 2019	Resolved with sequelae	Patient admitted to hospital due to vomiting and high temperature. Diagnosed with UTI and given IV antibiotics	Urinary tract infection bacterial	Infections and infestations		Severe	
7	18	Female	A – Infliximab	6 October 2019	8 October 2019	Resolved	• Admitted with high blood pressure • Athralgias and arthritis flare • facial erythema acne type reaction • periorbital involvement • initially febrile (temp 38.8)	Blood pressure inadequately controlled	Vascular disorders		Severe	

TABLE 39 Line listing of study SAEs (*continued*)

ID	Age	Gender	Trial arm	Onset date	Offset date	Outcome	Overall diagnosis	MedDra def	System organ class	Drug interact.	SAE grade	SUSAR
8	18	Female	A – Infliximab	17 December 2019	23 December 2019	Resolved	Hypertensive crisis	Hypertension	Vascular disorders		Moderate	
9	39	Female	B – Alpha interferon	1 February 2019	11 February 2019	Resolved	Neutropenia – Grade 3	Neutropenia	Blood and lymphatic system disorders	No	Severe	0

CT, computerised tomography; IV, intravenous.

TABLE 40 Aggregated AEs: number of patients (number of events)

CTC category	Short name	Arm	Mild	Moderate	Severe
Blood and lymphatic system disorders	Anaemia	A – Infliximab	0 (0)	1 (1)	0 (0)
	Anaemia	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Neutropenia	A – Infliximab	2 (3)	0 (0)	0 (0)
	Neutropenia	B – Alpha interferon	1 (5)	9 (9)	0 (0)
	Neutropenia aggravated	A – Infliximab	1 (1)	0 (0)	0 (0)
	Neutropenia aggravated	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Cardiac disorders	Chest ache	A – Infliximab	0 (0)	0 (0)	1 (1)
	Chest ache	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Chest pain	A – Infliximab	0 (0)	1 (1)	0 (0)
	Chest pain	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Dizziness	A – Infliximab	0 (0)	1 (1)	0 (0)
	Dizziness	B – Alpha Interferon	0 (0)	0 (0)	0 (0)
	Dizzy	A – Infliximab	0 (0)	0 (0)	0 (0)
	Dizzy	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Palpitations	A – Infliximab	0 (0)	0 (0)	0 (0)
	Palpitations	B – Alpha interferon	1 (1)	0 (0)	0 (0)
Ear and labyrinth disorders	Ear ache	A – Infliximab	0 (0)	0 (0)	0 (0)
	Ear ache	B – Alpha Interferon	1 (1)	0 (0)	0 (0)
	Ear buzzing	A – Infliximab	0 (0)	0 (0)	0 (0)
	Ear buzzing	B – Alpha interferon	1 (1)	0 (0)	0 (0)
Eye disorders	Blurred vision	A – Infliximab	0 (0)	0 (0)	0 (0)
	Blurred vision	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Dry eyes	A – Infliximab	0 (0)	1 (1)	0 (0)
	Dry eyes	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Eye irritation	A – Infliximab	0 (0)	0 (0)	0 (0)
	Eye irritation	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Stye	A – Infliximab	0 (0)	0 (0)	0 (0)
	Stye	B – Alpha interferon	0 (0)	1 (1)	0 (0)

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Uveitis	A – Infliximab	0 (0)	0 (0)	0 (0)
	Uveitis	B – Alpha interferon	1 (1)	0 (0)	0 (0)
GI disorders	Abdominal pain	A – Infliximab	0 (0)	0 (0)	0 (0)
	Abdominal pain	B – Alpha interferon	1 (1)	1 (1)	0 (0)
	Bloody stool	A – Infliximab	1 (1)	0 (0)	0 (0)
	Bloody stool	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Diarrhoea	A – Infliximab	0 (0)	0 (0)	0 (0)
	Diarrhoea	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Diarrhoea	A – Infliximab	0 (0)	2 (2)	0 (0)
	Diarrhoea	B – Alpha interferon	2 (2)	4 (4)	0 (0)
	Dry mouth	A – Infliximab	0 (0)	0 (0)	0 (0)
	Dry mouth	B – Alpha interferon	1 (1)	1 (1)	0 (0)
	Loose stools	A – Infliximab	1 (1)	0 (0)	0 (0)
	Loose stools	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Mouth paraesthesia	A – Infliximab	0 (0)	0 (0)	0 (0)
	Mouth paraesthesia	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Nausea	A – Infliximab	1 (2)	1 (1)	0 (0)
	Nausea	B – Alpha interferon	0 (1)	3 (3)	0 (0)
	Oral ulceration	A – Infliximab	1 (1)	0 (0)	0 (0)
	Oral ulceration	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Oral viral infection	A – Infliximab	0 (0)	0 (0)	0 (0)
	Oral viral infection	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Pericoronitis	A – Infliximab	0 (0)	1 (1)	0 (0)
	Pericoronitis	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Sore throat	A – Infliximab	0 (0)	0 (0)	0 (0)
	Sore throat	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Sore throat NOS	A – Infliximab	0 (0)	0 (0)	0 (0)
	Sore throat NOS	B – Alpha interferon	1 (1)	0 (0)	0 (0)

continued

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Stomach cramps	A – Infliximab	0 (0)	0 (0)	0 (0)
	Stomach cramps	B – Alpha interferon	3 (3)	0 (0)	0 (0)
	Stools loose	A – Infliximab	0 (0)	0 (0)	0 (0)
	Stools loose	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Throat infection	A – Infliximab	0 (0)	2 (2)	0 (0)
	Throat infection	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Ulcers aphthous oral	A – Infliximab	0 (0)	0 (0)	0 (0)
	Ulcers aphthous oral	B – Alpha interferon	0 (2)	1 (1)	0 (0)
	Vomiting	A – Infliximab	1 (1)	0 (0)	1 (1)
	Vomiting	B – Alpha Interferon	1 (1)	2 (2)	0 (0)
General disorders and administration site conditions	Application site paraesthesia	A – Infliximab	0 (0)	0 (0)	0 (0)
	Application site paraesthesia	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Chills and fever	A – Infliximab	1 (1)	0 (0)	0 (0)
	Chills and fever	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Exhaustion	A – Infliximab	0 (0)	0 (0)	0 (0)
	Exhaustion	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Fatigability	A – Infliximab	0 (0)	0 (0)	0 (0)
	Fatigability	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Fatigue	A – Infliximab	1 (2)	1 (1)	0 (0)
	Fatigue	B – Alpha interferon	1 (2)	2 (3)	1 (1)
	Fatigue extreme	A – Infliximab	0 (0)	0 (0)	0 (0)
	Fatigue extreme	B – Alpha interferon	0 (0)	2 (2)	0 (0)
	Flu-like symptoms	A – Infliximab	0 (1)	2 (2)	0 (0)
	Flu-like symptoms	B – Alpha interferon	3 (3)	2 (2)	0 (0)
	Generalised aching	A – Infliximab	1 (1)	0 (0)	0 (0)
	Generalised aching	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Generally unwell	A – Infliximab	0 (0)	0 (0)	0 (0)
	Generally unwell	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Inflammatory swelling	A – Infliximab	0 (0)	0 (0)	0 (0)

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Inflammatory swelling	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Injection site bruising	A – Infliximab	0 (0)	0 (0)	0 (0)
	Injection site bruising	B – Alpha Interferon	1 (1)	0 (0)	0 (0)
	Injection site irritation	A – Infliximab	0 (0)	0 (0)	0 (0)
	Injection site irritation	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Irritability	A – Infliximab	0 (0)	0 (0)	0 (0)
	Irritability	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Lethargy	A – Infliximab	1 (1)	0 (0)	0 (0)
	Lethargy	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Pain in face	A – Infliximab	0 (0)	0 (0)	0 (0)
	Pain in face	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Syringe issue	A – Infliximab	0 (0)	0 (0)	0 (0)
	Syringe issue	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Weakness	A – Infliximab	0 (0)	1 (1)	0 (0)
	Weakness	B – Alpha interferon	0 (0)	1 (1)	0 (0)
Hepatobiliary disorders	Unspecified disorder of gallbladder	A – Infliximab	0 (0)	1 (1)	0 (0)
	Unspecified disorder of gallbladder	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Immune system disorders	Angioedema aggravated	A – Infliximab	0 (0)	2 (2)	0 (0)
	Angioedema aggravated	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Autoimmune neutropenia	A – Infliximab	0 (0)	0 (0)	0 (0)
	Autoimmune neutropenia	B – Alpha interferon	0 (0)	2 (2)	0 (0)
	Erythema nodosum	A – Infliximab	0 (0)	0 (0)	0 (0)
	Erythema nodosum	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Skin sensitisation	A – Infliximab	0 (0)	0 (0)	0 (0)
	Skin sensitisation	B – Alpha interferon	1 (1)	0 (0)	0 (0)
Infections and infestations	Chest infection	A – Infliximab	0 (1)	3 (3)	0 (0)
	Chest infection	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Common cold	A – Infliximab	0 (0)	0 (0)	0 (0)

continued

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Common cold	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Ear infection	A – Infliximab	0 (0)	0 (0)	0 (0)
	Ear infection	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Flu symptoms	A – Infliximab	0 (0)	0 (0)	0 (0)
	Flu symptoms	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Fungal infection	A – Infliximab	0 (0)	0 (0)	0 (0)
	Fungal infection	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Genital abscess	A – Infliximab	0 (0)	0 (0)	0 (0)
	Genital abscess	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Genital herpes	A – Infliximab	0 (0)	1 (1)	0 (0)
	Genital herpes	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Gummata and ulcers due to yaws	A – Infliximab	1 (1)	0 (0)	0 (0)
	Gummata and ulcers due to yaws	B – Alpha interferon	0 (0)	2 (2)	0 (0)
	Infected finger	A – Infliximab	0 (0)	0 (0)	0 (0)
	Infected finger	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Papulopustular rash	A – Infliximab	0 (0)	0 (0)	0 (0)
	Papulopustular rash	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Pleurisy viral	A – Infliximab	1 (1)	0 (0)	0 (0)
	Pleurisy viral	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Tonsillitis	A – Infliximab	0 (0)	1 (1)	0 (0)
	Tonsillitis	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Tonsillitis bacterial	A – Infliximab	1 (1)	1 (1)	0 (0)
	Tonsillitis bacterial	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Urinary tract infection bacterial	A – Infliximab	3 (3)	0 (0)	0 (0)
	Urinary tract infection bacterial	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Injury, poisoning and procedural complications	Extensive limb swelling	A – Infliximab	0 (0)	0 (0)	0 (0)
	Extensive limb swelling	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Falling down	A – Infliximab	1 (1)	0 (0)	0 (0)

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Falling down	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Wrist injury	A – Infliximab	0 (0)	1 (1)	0 (0)
	Wrist injury	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Investigations	ALT decreased	A – Infliximab	0 (0)	0 (0)	0 (0)
	ALT decreased	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	ALT increased	A – Infliximab	0 (1)	1 (1)	0 (0)
	ALT increased	B – Alpha interferon	0 (5)	3 (3)	0 (0)
	Blood in urine	A – Infliximab	0 (0)	0 (0)	0 (0)
	Blood in urine	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Blood neutrophils	A – Infliximab	1 (1)	0 (0)	0 (0)
	Blood neutrophils	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Blood neutrophils abnormal	A – Infliximab	0 (0)	0 (0)	0 (0)
	Blood neutrophils abnormal	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Body temperature increased	A – Infliximab	0 (0)	0 (0)	0 (0)
	Body temperature increased	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Increased blood pressure	A – Infliximab	1 (1)	0 (0)	0 (0)
	Increased blood pressure	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Low platelets	A – Infliximab	1 (1)	0 (0)	0 (0)
	Low platelets	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Neutrophils reduced	A – Infliximab	0 (1)	1 (1)	0 (0)
	Neutrophils reduced	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Plasma neutrophils abnormal	A – Infliximab	0 (0)	0 (0)	0 (0)
	Plasma neutrophils abnormal	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Vitamin D decreased	A – Infliximab	0 (0)	1 (1)	0 (0)
	Vitamin D decreased	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Metabolism and nutrition disorders	Appetite absent	A – Infliximab	0 (0)	0 (0)	0 (0)
	Appetite absent	B – Alpha interferon	1 (1)	0 (0)	0 (0)

continued

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Decreased appetite	A – Infliximab	0 (0)	0 (0)	0 (0)
	Decreased appetite	B – Alpha interferon	1 (1)	1 (1)	0 (0)
	Hepatic steatosis	A – Infliximab	1 (1)	0 (0)	0 (0)
	Hepatic steatosis	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Oedema	A – Infliximab	0 (0)	1 (1)	0 (0)
	Oedema	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Thyrotoxicosis	A – Infliximab	0 (0)	0 (0)	0 (0)
	Thyrotoxicosis	B – Alpha interferon	0 (0)	1 (1)	0 (0)
Musculoskeletal and connective tissue disorders	Arthralgia	A – Infliximab	1 (1)	0 (0)	0 (0)
	Arthralgia	B – Alpha interferon	1 (1)	1 (1)	0 (0)
	Arthralgia aggravated	A – Infliximab	0 (0)	0 (0)	0 (0)
	Arthralgia aggravated	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Back pain	A – Infliximab	0 (0)	0 (0)	0 (0)
	Back pain	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Generalised joint pains	A – Infliximab	1 (1)	0 (0)	0 (0)
	Generalised joint pains	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Generalised muscle aches	A – Infliximab	0 (0)	0 (0)	0 (0)
	Generalised muscle aches	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Generalised joint pain	A – Infliximab	0 (0)	0 (0)	0 (0)
	Generalised joint pain	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Joint pain	A – Infliximab	0 (0)	1 (1)	0 (0)
	Joint pain	B – Alpha interferon	2 (2)	1 (1)	0 (0)
	Joint stiffness	A – Infliximab	0 (0)	0 (0)	0 (0)
	Joint stiffness	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Knee pain	A – Infliximab	0 (0)	1 (1)	0 (0)
	Knee pain	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Myalgia	A – Infliximab	0 (0)	0 (0)	0 (0)
	Myalgia	B – Alpha interferon	1 (1)	0 (0)	0 (0)

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Neck tightness	A – Infliximab	1 (1)	0 (0)	0 (0)
	Neck tightness	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Nonallopathic lesions of abdomen and other sites, not elsewhere classified	A – Infliximab	0 (0)	1 (1)	0 (0)
	Nonallopathic lesions of abdomen and other sites, not elsewhere classified	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Pain in (l) knee	A – Infliximab	1 (1)	0 (0)	0 (0)
	Pain in (l) knee	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Pain in (r) hip	A – Infliximab	0 (0)	0 (0)	1 (1)
	Pain in (r) hip	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Painful joints	A – Infliximab	0 (0)	1 (1)	0 (0)
	Painful joints	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Tennis elbow	A – Infliximab	1 (1)	0 (0)	0 (0)
	Tennis elbow	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Skin tags	A – Infliximab	0 (0)	0 (0)	0 (0)
	Skin tags	B – Alpha interferon	0 (0)	1 (1)	0 (0)
Nervous system disorders	Blurred vision	A – Infliximab	1 (1)	1 (1)	0 (0)
	Blurred vision	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Confusion	A – Infliximab	1 (1)	0 (0)	0 (0)
	Confusion	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Dizzy spells	A – Infliximab	0 (0)	0 (0)	0 (0)
	Dizzy spells	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Forgetfulness	A – Infliximab	0 (0)	0 (0)	0 (0)
	Forgetfulness	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Frequent headaches	A – Infliximab	1 (1)	0 (0)	0 (0)
	Frequent headaches	B – Alpha interferon	0 (0)	3 (3)	0 (0)
	Headache	A – Infliximab	0 (0)	3 (3)	0 (0)
	Headache	B – Alpha interferon	1 (1)	3 (3)	0 (0)

continued

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Headache discomfort	A – Infliximab	0 (0)	0 (0)	0 (0)
	Headache discomfort	B – Alpha interferon	0 (1)	1 (1)	0 (0)
	Headache NOS	A – Infliximab	0 (0)	0 (0)	0 (0)
	Headache NOS	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Insomnia	A – Infliximab	0 (0)	0 (0)	0 (0)
	Insomnia	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Mental concentration decreased	A – Infliximab	0 (0)	0 (0)	0 (0)
	Mental concentration decreased	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Numbness	A – Infliximab	0 (0)	1 (1)	0 (0)
	Numbness	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Numbness in hands, forearms, elbows	A – Infliximab	0 (0)	1 (1)	0 (0)
	Numbness in hands, forearms, elbows	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Numbness of upper arm	A – Infliximab	1 (1)	0 (0)	0 (0)
	Numbness of upper arm	B – Alpha Interferon	0 (0)	0 (0)	0 (0)
	Poor sleep	A – infliximab	0 (0)	0 (0)	0 (0)
	Poor sleep	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Tingling of extremity	A – Infliximab	1 (1)	0 (0)	0 (0)
	Tingling of extremity	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Psychiatric disorders	Anxiety	A – Infliximab	0 (0)	1 (1)	0 (0)
	Anxiety	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Anxiety reaction	A – Infliximab	0 (0)	0 (0)	0 (0)
	Anxiety reaction	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Distress	A – Infliximab	1 (1)	0 (0)	0 (0)
	Distress	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Feeling down	A – Infliximab	0 (0)	0 (0)	0 (0)
	Feeling down	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Lack of motivation	A – Infliximab	0 (0)	0 (0)	0 (0)

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Lack of motivation	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Low mood	A – Infliximab	0 (0)	0 (0)	0 (0)
	Low mood	B – Alpha interferon	1 (1)	2 (2)	0 (0)
	Panic attacks	A – Infliximab	0 (0)	1 (1)	0 (0)
	Panic attacks	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Worry	A – Infliximab	0 (0)	1 (1)	0 (0)
	Worry	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Reproductive system and breast disorders	Genital discharge	A – Infliximab	0 (0)	0 (0)	0 (0)
	Genital discharge	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Genital ulceration	A – Infliximab	1 (1)	0 (0)	0 (0)
	Genital ulceration	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Heavy periods	A – Infliximab	0 (0)	0 (0)	0 (0)
	Heavy periods	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Hot flushes NOS	A – Infliximab	1 (1)	0 (0)	0 (0)
	Hot flushes NOS	B – Alpha interferon	0 (0)	0 (0)	0 (0)
Respiratory, thoracic and mediastinal disorders	Acute tonsillitis (excl. proven streptococcal)	A – Infliximab	1 (1)	0 (0)	0 (0)
	Acute tonsillitis (excl. proven streptococcal)	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Chest cold	A – Infliximab	0 (0)	0 (0)	0 (0)
	Chest cold	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Chest infection	A – Infliximab	0 (0)	2 (2)	0 (0)
	Chest infection	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Chest tightness	A – Infliximab	0 (0)	0 (0)	0 (0)
	Chest tightness	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Cough nonproductive	A – Infliximab	0 (0)	0 (0)	0 (0)
	Cough nonproductive	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Generalised chest pains	A – Infliximab	1 (1)	0 (0)	0 (0)

continued

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Generalised chest pains	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Intercostal pain	A – Infliximab	0 (0)	0 (0)	0 (0)
	Intercostal pain	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Laryngotracheobronchitis	A – Infliximab	0 (0)	1 (1)	0 (0)
	Laryngotracheobronchitis	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Nasal ulcer	A – Infliximab	1 (1)	0 (0)	0 (0)
	Nasal ulcer	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Nose bleeds	A – Infliximab	0 (0)	0 (0)	0 (0)
	Nose bleeds	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Oropharyngeal pain	A – Infliximab	1 (1)	0 (0)	0 (0)
	Oropharyngeal pain	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Persistent nonproductive cough	A – Infliximab	0 (0)	0 (0)	0 (0)
	Persistent nonproductive cough	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Sinus infection	A – Infliximab	0 (0)	0 (0)	0 (0)
	Sinus infection	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Sore throat	A – Infliximab	0 (0)	0 (0)	0 (0)
	Sore throat	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Throat infection	A – Infliximab	0 (0)	0 (0)	0 (0)
	Throat infection	B – Alpha interferon	0 (0)	1 (1)	0 (0)
Skin and subcutaneous tissue disorders	Abscess on buttock	A – Infliximab	0 (0)	1 (1)	0 (0)
	Abscess on buttock	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Cellulitis	A – Infliximab	0 (1)	1 (1)	0 (0)
	Cellulitis	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Dermatitis photosensitive	A – Infliximab	0 (0)	0 (0)	0 (0)
	Dermatitis photosensitive	B – Alpha interferon	3 (3)	0 (0)	0 (0)
	Facial rash	A – Infliximab	1 (2)	1 (1)	0 (0)
	Facial rash	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Hair loss	A – Infliximab	0 (0)	0 (0)	0 (0)

TABLE 40 Aggregated AEs: number of patients (number of events) (*continued*)

CTC category	Short name	Arm	Mild	Moderate	Severe
	Hair loss	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Other early skin lesions of yaws	A – Infliximab	0 (0)	0 (0)	0 (0)
	Other early skin lesions of yaws	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Psoriasis	A – Infliximab	0 (0)	0 (0)	0 (0)
	Psoriasis	B – Alpha interferon	0 (1)	1 (1)	0 (0)
	Psoriasis of scalp	A – Infliximab	1 (1)	0 (0)	0 (0)
	Psoriasis of scalp	B – Alpha interferon	0 (0)	0 (0)	0 (0)
	Rash face	A – Infliximab	0 (0)	0 (0)	0 (0)
	Rash face	B – Alpha interferon	1 (1)	0 (0)	0 (0)
	Skin inflammation	A – Infliximab	0 (0)	0 (0)	0 (0)
	Skin inflammation	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Skin lesion	A – Infliximab	0 (0)	0 (0)	0 (0)
	Skin lesion	B – Alpha interferon	0 (0)	1 (1)	0 (0)
Vascular disorders	Migraine	A – Infliximab	0 (0)	0 (0)	0 (0)
	Migraine	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Migraine headache	A – Infliximab	0 (0)	0 (0)	0 (0)
	Migraine headache	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Nose bleeds	A – Infliximab	0 (0)	0 (0)	0 (0)
	Nose bleeds	B – Alpha interferon	0 (0)	1 (1)	0 (0)
	Nose bleeds	A – Infliximab	0 (0)	0 (0)	0 (0)
	Nose bleeds	B – Alpha interferon	1 (1)	0 (0)	0 (0)

ALT, alanine aminotransferase; NOS, no other symptoms.

Notes
The number of patients (number of events) for each AE are categorised using MedDra definitions. For each patient the worst grade of each event is retained. The vast majority of events are sporadic. There is evidence of neutropenia associated with Roferon, but aside from this there are no other notable differences between treatment arms.
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