Autologous Stem cell Transplantation In refractory Crohn's disease – Low Intensity Therapy Evaluation (ASTIClite)

ASTIClite

A multicentre, randomised controlled trial to evaluate the efficacy of autologous haematopoietic stem cell transplantation with low dose cyclophosphamide mobilisation and reduced intensity conditioning.

Sponsor:	Barts Health NHS Trust
Representative of the Sponsor:	**Mays Jawad** Director of Research Services JRMO QM Innovation Building 5 Walden Street London E1 2EF Phone: 020 7882 7275 Email:Research.Governance@qmul.ac.uk

RESEARCH PROTOCOL
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Chief Investigator Agreement Page

The clinical study as detailed within this research protocol (Version 7.1, dated 12 July 2019), or any subsequent amendments, involves the use of an investigational medicinal product and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trial regulations.

Chief Investigator Name: Prof James Lindsay

Chief Investigator Site: Barts Health NHS Trust

Signature and Date: 12th July 2019

Print name: James Lindsay

Statistician Agreement Page

The clinical study as detailed within this research protocol (Version 7.1, dated 12 July 2019), or any subsequent amendments, involves the use of an investigational medicinal product and will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996), Principles of ICH-GCP, and the current regulatory requirements, as detailed in the Medicines for Human Use (Clinical Trials) Regulations 2004 (UK S.I. 2004/1031) and any subsequent amendments of the clinical trials regulations.

Statistician Name: Mike Bradburn

Statistician Job Title: Senior Statistician

Organisation: Sheffield Clinical Trials Research Unit

Signature and Date:  15th July 2019

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Autologous **S**tem cell **T**ransplantation **I**n refractory **C**rohn's disease – **L**ow **I**ntensity **T**herapy **E**valuation (ASTIClite)

This document describes a clinical study, and provides information about procedures for entering participants. The protocol is not intended for use as a guide to the treatment of other patients. Amendments may be necessary; and where required, these will be circulated to known participants in the trial.

Contents

Abbreviations

ADWP	Autoimmune Disease Working Party
AE	Adverse Event
AR	Adverse Reaction
ATG	Anti-thymocyte globulin
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CI	Chief Investigator
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of an Investigational Medicinal Product
CTRU	Clinical Trials Research Unit
DMEC	Data Monitoring and Ethics Committee
DMP	Data Management Plan
DPA	Data Protection Act
EBMT	European Society for Blood and Marrow Transplantation
EME	Efficacy and Mechanism Evaluation
EQ-5D	EuroQol Five Dimensions Questionnaire
EudraCT	European Union Drug Regulatory Agency Clinical Trial
GCP	Good Clinical Practice
G-CSF	Granulocyte-colony stimulating factor
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
HSCT	Haematopoietic stem cell transplantation
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire
IMP	Investigational Medicinal Product
ITT	Intention to Treat
JACIE	Joint Accreditation Committee-ISCT & EBMT
MaRIA	Magnetic resonance index of activity
MDT	Multidisciplinary Team
MHRA	Medicines and Healthcare products Regulatory Agency
MRI	Magnetic resonance imaging
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NHSE	NHS England
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NIMP	Non-Investigational Medicinal Product
PBMC	Peripheral blood mononuclear cell
PI	Principal Investigator
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SES CD	Simple Endoscopic Score for Crohn's Disease
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TNF	Tumour Necrosis Factor
TSC	Trial Steering Committee
WPAI	Work Productivity and Activity Impairment

1. Project Details

1.1 Investigator Details

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1.3 Sponsor Details

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1.4 Committees

Trial Steering Committee:

Dr John Mansfield (Chair)	Consultant Gastroenterologist
Dr Kim Orchard	Consultant Haematologist
Prof Ailsa Hart	Consultant Gastroenterologist
Dr Victoria Cornelius	Statistician
Dr Elena Ricart	Consultant Gastroenterologist
Ms Helen Bartlett	Patient representative
Ms Charlotte Howe	Patient representative

Data Monitoring and Ethics Committee:

Dr Tariq Iqbal (Chair)	Consultant Gastroenterologist
Prof David Marks	Consultant Haematologist
Prof Matthieu Allez	Consultant Gastroenterologist
Prof Dominique Farge-Bancel	Professor of Medicine
Mrs Siobhan Creanor	Statistician

1.5 Participating Centres

Site	Principal Investigator	Lead Haematologist
Cambridge University Hospitals NHS Foundation Trust	Dr Miles Parkes	Dr Ben Uttenthal
Edinburgh Western General Hospital, NHS Lothian	Dr Shahida Din	Dr Peter Johnson
Oxford University Hospitals NHS Foundation Trust	Prof Simon Travis	Dr Andy Peniket
Sheffield Teaching Hospitals NHS Foundation Trust	Prof Alan Lobo	Prof John Snowden
Nottingham University Hospitals NHS Trust	Dr Gordon Moran	Dr Jenny Byrne
Barts Health NHS Trust	Dr James Lindsay	Prof John Gribben
Guy's & St Thomas' NHS Foundation Trust *(non-treatment site)*	Dr Peter Irving	Dr Majid Kazmi

Royal Liverpool and Broadgreen University Hospital NHS Trust *(Transplant Centre: Clatterbridge Cancer Centre NHS Foundation Trust)*	Dr Sreedhar Subramanian	Dr Amit Patel
King's College Hospital NHS Foundation Trust *(non-recruiting site)*	Dr Majid Kazmi	Dr Majid Kazmi

1.5.1 Participant Identification Centres (PIC)

Site	Address
Circle Nottingham (Nottingham Treatment Centre)	Circle Nottingham Nottingham NHS Treatment Centre Lister Road Nottingham NG7 2FT

1.6 Role of the Funder

The funder has reviewed the research protocol but will have no role in data collection, analysis, data interpretation, report writing or in the decision to submit the report for publication. The funder has approved the selection of members for oversight committees.

1.7 Protocol amendments

Protocol version	Changes made
V3.0	Original approved version
V4.0	Clarity added around procedures relating to mechanistic studies; corrections/typos where required; increased window for some screening investigations prior to randomisation, and timing of CDAI in relation to colonoscopy; inclusion of the use of the IBD BioResource to identify potential participants; addition of HBI between mobilisation and conditioning; CDAI and HBI at both screening and baseline; added stoma version of IBDQ for participants where this is required
V5.0	Changes to PI at Edinburgh and Nottingham sites; secondary outcome added for MRI and MaRIA score; additional mechanistic serum sample added at week 40 visit; addition of potential storage of stem cell samples for use in future research; added Karnofsky performance status at screening and week 48 for all participants
V5.1	Addition of Circle Nottingham as PIC site

V5.2	Addition of King's College Hospital as treatment site for patients recruited at Guy's & St Thomas'
V6.0	Administrative changes; increase the window for screening investigations from within 4 weeks to 8 weeks of randomisation; provided clarity on how the primary outcome will be assessed for patients who have had ileo and/or colonic resection
V6.1	Corrected an error in consistency across the protocol, that either MUGA scan or Echo is permitted as part of screening
V7.0	Refined statistical analysis section following approval of v1.0 SAP; administrative changes due to staff changes; flowchart updated to be consistent with earlier protocol amendments; addition of protocol changes section, ability to test for monogenic disease as part of screening is appropriate, option of lower doses or no cyclophosphamide if a second attempt at mobilisation is required.
V7.1	Increased dose of methylprednisolone (NIMP) in response to two SAEs.

1.8 Study Summary

Study Title:	Autologous Stem cell Transplantation In refractory Crohn's disease – Low Intensity Therapy Evaluation (ASTIClite)
EudraCT no:	2017-002545-30
Sponsor:	Bart's Health NHS Trust
Funder:	NIHR EME (project number 15/178/09)
ISRCTN no:	17160440
Project start date:	1st August 2017
Project end date:	31st March 2022
Study Design:	Open label, multicentre, parallel group, randomised controlled trial
Participants:	99 participants with refractory Crohn's Disease
Setting:	Participants will be recruited from 8 secondary care centres, and HSCT will be carried out within centres JACIE accredited for allogenic HSCT, or for autologous HSCT if they have previous experience of autologous HSCT for CD.
Inclusion/exclusion criteria (see section 6.2 & 6.3)	• Patients aged 18-60 with a diagnosis of CD for at least six months, refractory to at least two classes of biologic therapy and where surgery is not appropriate, with impaired quality of life. They must have endoscopic ulceration in at least one segment of bowel as assessed using the ulcer size subscore of the SES CD scoring system. • Patients must be well nourished, able to give full informed consent, and have active CD activity, confirmed by endoscopy at screening (SES CD). • Patients must have a satisfactory EBMT Autoimmune Disease Working Party recommended screening assessment prior to HSCT, and must be willing to discontinue all current immunosuppressant medication.

	• Patients must be deemed medically fit enough for HSCT based on clinical tests, and the opinion of the investigator • Patients are ineligible if they have a diagnosis of ulcerative colitis or indeterminate colitis, have strictures preventing endoscopic assessment of affected bowel, have undrained perianal fistulae, perianal or intra-abdominal sepsis, evidence of enteric or systemic infection, mycobacterial infection, currently pregnant or unwilling to use adequate contraception during the trial, or there is a contraindication to the use of any of the IMPs.
Intervention Treatment Summary:	Reduced intensity mobilisation: • Cyclophosphamide 1g/m^2 on day 1 • G-CSF (filgrastim) 5µg/kg daily, starting from day 5 • Monitoring of full blood count and CD34+ counts from day 8 until CD34+ exceeds 10x10^6/L (expected to be onwards from day 10) • Stem cell harvest (leukapheresis) until a minimum of 2.0 x10^6/kg CD34+ are collected for cryopreservation • Mesna in line with local clinical practice Conditioning (after stem cell harvest): • Fludarabine 25mg/m^2 IV on days -6, -5, -4, -3 and -2. • Cyclophosphamide 60mg/kg/day on days -3 and -2. • Standard hydration and diuretics throughout administration of cyclophosphamide, with mesna. • Rabbit ATG (Thymoglobulin; Sanofi-Genzyme) 2.5mg/kg on days -3, -2 and -1. • Methylprednisolone 2mg/kg per day on days -3, -2 and -1. • Stem cell reinfusion on day 0 • G-CSF 5µg/kg/day started on day +5 and continued until the absolute neutrophil counts reach >1.0x10^9/L for 2 consecutive days. At week 24, those participants with evidence of disease activity on endoscopy / colonoscopy or MRI will be started on maintenance anti-TNF therapy. As routine standard of care, participants will receive the necessary vaccinations, including an annual influenza vaccine.
Usual Care	The control group will continue current gold standard medical care. Usual care can include steroid therapy, immunomodulators, any licensed/approved biologic therapy and enteral or intravenous nutrition. After the week 48 assessment of the primary outcome they will be offered ongoing standard care which may include HSCT if permitted by NHSE specialist commissioning at that time.
Randomisation:	Participants will be randomised to either the intervention arm, or control arm, in the ratio 2:1.
Anticipated recruitment period	Three years
Duration of follow-up:	Participants will be followed up for 48 weeks from day 0. For the intervention arm, day 0 is the date of stem cell re-infusion.

	For the control arm, day 0 is 49 days after the date of randomisation.
Hypothesis:	HSCTlite will induce and maintain regression of intestinal ulceration in patients with refractory CD, reduce clinical disease activity and enhance quality of life compared to standard care. HSCTlite will have an acceptable safety profile.
Primary Objective:	To assess the efficacy of HSCTlite compared to standard care at inducing regression of intestinal ulceration in patients with refractory Crohn's disease at week 48.
Secondary Objectives:	1. To assess whether low dose cyclophosphamide and G-CSF is a safe and effective mobilisation regimen for patients with refractory CD. 2. To assess the impact of HSCTlite on clinical disease activity, quality of life and adverse events compared to standard care. 3. To assess the safety and efficacy of anti-TNF therapy in patients who demonstrate endoscopic disease recurrence at week 24 after HSCTlite. Mechanistic objectives: 1. Intestinal MRI will be performed to determine the early impact of HSCTlite on mucosal disease. 2. Immune profiling of peripheral blood and mucosal biopsies will: a. Characterise immune re-constitution after HSCT, and assess impact of HSCT on disease activity b. Assess immunological events that precede disease recurrence post HSCT c. Assess the mechanism of restoration of responsiveness to anti-TNF therapies d. Serum will be stored for future analysis of responses to vaccination post HSCT
Definition of end of trial	The end of the trial is defined as the date of the last recruited participant's week 48 follow up visit. Sites will be closed once data cleaning is completed and the regulatory authority and ethics committee will be informed.
Other related research	**ASTIClite EBMT Registry Follow up** (IRAS number: 228818) Participants taking part in the ASTIClite study will be invited to consent to long term follow up through the EBMT registry. This takes place in routine care following HSCT, and this additional consent from participants will allow this registry data to be collected, and used as part of the research.

2. Introduction

2.1 Background

Intestinal inflammation in Crohn's disease (CD) is caused by mucosal immune system reactivity to luminal antigen and results in debilitating symptoms, reduced

quality of life, impaired work productivity and significant health care costs (1). CD accounts for 27,000 hospital admissions/year and costs £6695 (7835 euro) per patient per year (2). Acquisition of biological medications accounts for the largest section of patient costs both in secondary and tertiary care (3). This has increased from 64% to 72% over the last 2 years (2).

Many patients respond to conventional and biologic therapies; however, the on-going NIHR portfolio cohort trial PANTS (UKCRN 14175 & 17747) of 1500 CD patients commencing anti- TNF therapy, reports primary non-response in between 16.9% and 23.7% and secondary loss of response in a further 29% over 2 years (4). Second line therapy with Vedolizumab is approved by NICE for refractory CD. However, it did not achieve its induction primary endpoint in a phase III trial in anti TNF exposed patients (5) and real world data report steroid free remission in only 30% (6). Therefore, patients with treatment refractory CD face chronic symptoms related to on-going disease activity in addition to the morbidity associated with chronic steroid exposure. Surgery may be an option but will often result in a permanent stoma and may be declined. Case reports suggest exceptional benefit after haematopoietic stem cell transplant (HSCT) for patients with refractory CD (7,8).

The ASTIC trial

A previously conducted randomised controlled trial (ASTIC) was designed to answer (i) does HSCT 'cure' CD (ii) is any observed benefit derived from the cyclophosphamide or the stem cell transplant (9). Eligible patients underwent high dose (4g/m^2) cyclophosphamide / G-CSF mobilisation and were then randomised to immediate HSCT or conventional care for one year. Few patients in either group achieved the ambitious primary endpoint of clinical disease remission for three months, off all medication, with no evidence of active disease on imaging and endoscopy. In retrospect, this primary endpoint was more ambitious than that used in any other trial published in this disease area. In addition, it may not be in a patient's interest to have a protocolled withdrawal of therapy to meet the primary endpoint (off all therapy), given that it is known patients can relapse after HSCT and respond to therapy. In ASTIC, there were patients who failed to achieve clinical remission (CDAI<150), but had achieved no evidence of active disease on any assessment. In these patients, it is likely that previous intestinal tract damage was driving non-inflammatory type symptoms. Finally, there were a high number of adverse and serious adverse events that were not clinically acceptable, or acceptable to patients in this population, and one patient death. In view of this, HSCT is rarely used in the UK for patients with refractory Crohn's disease.

However, ASTIC did demonstrate significant benefits in endpoints more traditional for therapeutic trials in this area, such as steroid free clinical remission, mucosal healing and quality of life (9) These endpoints are still of significant clinical importance, and importance to patients. After the primary endpoint, patients in the control arm underwent HSCT. Subsequent analysis of the impact of HSCT in all patients in the ASTIC program reported a significant reduction in clinical and endoscopic disease activity with 50% patients showing regression of all endoscopic ulceration at one year. In addition, clinical factors that predict treatment success or adverse events were identified (10).

Risks and Benefits

The incidence of Crohn's disease (CD) is increasing, particularly in young adults who may live with their disease for up to 6 decades (1). Patients with refractory CD suffer impaired quality of life and disease or treatment related morbidity. Surgery may be inappropriate, is not a cure, and is often refused. In addition, refractory CD is associated with a heavy burden of direct health care costs including disease assessment, outpatient care, inpatient care, intravenous nutrition, surgery and the acquisition costs of medication (2,3). Biological therapies (both licensed and in phase III trials) have reduced efficacy in treatment-experienced patients and high acquisition costs. The PANTS cohort study will identify approximately 570 CD patients with primary or secondary loss of response to anti-TNF therapy over the next 2 years. In the absence of an effective alternative, these patients are likely to be exposed to a sequence of expensive therapies with diminishing potential for benefit and increasing risk of harm.

The ASTIC trial (9) reported a high burden of adverse events and one death. Subsequent expert review has suggested that the high dose cyclophosphamide used at both mobilisation and conditioning may have been a factor for many of the mobilisation related infectious adverse events (11,12). Research has also highlighted the importance of supportive care in reducing the incidence of SAEs (13). Analysis of the entire ASTIC cohort using more traditional endpoints demonstrates a significant benefit of this therapy at one year. Importantly, this analysis and previous studies have suggested that HSCT appears to restore responsiveness to anti-TNF therapies to which patients were previously refractory (8,10). Furthermore, a recent single centre cohort study with long term clinical and endoscopic follow up showed benefit extending to five years. Although many patients did require re-introduction of therapy, they derived prolonged benefit from therapies to which they were previously refractory (14). Finally, reduced intensity mobilisation and conditioning regimens are associated with lower morbidity in malignant and auto-immune disease (13,15–17).

ASTIClite

The ASTIClite study is a multicentre, parallel group, randomised controlled trial to evaluate the efficacy of HSCTlite compared with standard care at inducing regression of intestinal ulceration in patients with refractory CD. Participants will be recruited from eight sites that have tertiary referral IBD clinics and HSCT will be carried out in centres that are either JACIE accredited for allogenic HSCT, or for autologous HSCT if they have previous experience of autologous HSCT for CD. Patients with clinical and endoscopic CD activity who are refractory to at least two classes of biologic therapy, and in whom surgery is inappropriate or has been declined, will be recruited.

Our study attempts to answer the top priorities identified by The James Lind Alliance Priority Setting Partnership between the UK CD patient charity, the British Society of Gastroenterology (BSG), the NIHR gastroenterology specialist group and research charities: selecting the optimal therapy for patients with refractory disease. A formal qualitative research programme (DECIDES) recruiting patients with refractory CD who would be eligible to participate in this trial confirms that ASTIClite is focused on issues that matter to patients.

Eligible patients will be randomised to receive either autologous stem cell transplantation using the HSCTlite regimen, or standard care, in the ratio 2:1, and the primary endpoint will be assessed at week 48.

An internal pilot will be incorporated to confirm whether the HSCTlite mobilisation regimen delivers effective stem cell harvest without a flare up of CD activity. Ability to

recruit to target will be assessed at month 10 of recruitment with STOP/GO criteria set at 60% of the anticipated recruitment at that time.

The study will be conducted in accordance with the protocol, GCP, and the Medicines for Human Use (Clinical Trials) Regulations 2004.

2.2 Rationale for the Study

The ASTIC trial of autologous haematopoietic stem cell transplantation in CD (9) did not achieve its primary endpoint and was associated with significant toxicity. However, many experts feel it would be inappropriate to interpret this complex clinical trial with the simple message that HSCT is not effective for refractory CD for the following reasons (11,12):

a. The primary endpoint was more ambitious than in any other CD trial & the low frequency of patients that achieved it suggests that the trial was underpowered
b. Both control and intervention group received 4g/m^2 cyclophosphamide to mobilise stem cells which had significant short term beneficial impact on disease activity
c. No maintenance therapy was used after HSCT in patients with recurrent disease
d. HSCT achieved statistical significance over control for secondary endpoints including clinical remission and endoscopic disease activity.
e. Combined uncontrolled data of all transplanted patients shows striking reduction in clinical and endoscopic disease activity at one year (10)
f. The high dose of cyclophosphamide used increased adverse events (11,12)
g. Reduced intensity HSCT regimens and enhanced supportive care have reduced HSCT morbidity (13,17)

The ASTIClite study will assess the clinical efficacy and long-term impact of low dose cyclophosphamide/GCSF mobilization with reduced intensity conditioning in patients with active CD refractory to biologic therapies (HSCTlite). Embedded mechanistic studies will assess the timeline of response, immune reconstitution after HSCT and the mechanism by which HSCT restores anti TNF responsiveness in this previously refractory group.

2.3 Justification of the Study Design

The ASTIClite study is a multicentre, open-label randomised controlled trial.

This trial design will allow direct comparisons between the HSCTlite regimen and standard care, using endpoints, and timing of the primary outcome that are traditional for clinical trials. An additional assessment has been included at week 24 to assess disease recurrence, and if required, maintenance anti-TNF therapy can be introduced in patients who have undergone HSCTlite (see section 8.1.4 for details).

Over the last two decades in HSCT practice, conditioning regimens have been made less intensive by reducing the high doses of alkylating chemotherapeutic agents (such as cyclophosphamide), and introducing purine analogue drugs, which maintain immunosuppression whilst reducing cytotoxic side effects on tissues and organs. Such ‘reduced intensity conditioning’ regimens are associated with fewer acute regimen-related toxicities in other settings, including infections and gut mucosal damage (18).

Given the concerns about cyclophosphamide toxicity in both the ASTIC trial (9,19), and other studies(13,14), the conditioning regimen in the ASTIClite study uses a lower dose of cyclophosphamide (1g/m^2) for mobilisation and a reduced intensity regimen consisting of a combination of a reduced dose of cyclophosphamide (120mg/kg), rabbit ATG and fludarabine, a purine analogue chemotherapy agent commonly used for immunosuppression in transplantation and allogeneic HSCT. We believe that the lower dose mobilisation regimen can be more safely delivered in an outpatient setting than the current EBMT recommended regimen (of 2-4g/m^2). Likewise, we will investigate the safety and efficacy of autologous HSCT with the 'Flu/Cy/ATG' reduced intensity conditioning regimen. This regimen is recommended for paediatrics in the EBMT Guidelines (15), but we have extended its use to adults in ASTIClite as it is likely to be better tolerated by patients with CD, whilst providing intense and durable immunosuppression for disease control. It is anticipated that the lower doses of chemotherapy will be less toxic to vital organs, the gut mucosa and reproductive/endocrine function, whilst providing high levels of immunosuppression against the CD. The final doses chosen were decided upon through consensus agreement between Haematologists at participating centres, and the ASTIClite trial hopes to test the efficacy of the chosen treatment regimen. Furthermore, the efficacy of the mobilisation and conditioning regimen will be assessed by the Data Monitoring and Ethics Committee (DMEC), after the first 10 patients have received treatment. Updates will be made to the protocol, including changes to IMP doses, if required, based on recommendations from this DMEC assessment.

3. Aims and objectives

The main aims of this study are to assess whether stem cell mobilisation with low dose cyclophosphamide 1g/m^2 and G-CSF followed by autologous transplantation with a reduced intensity ('HSCTlite') conditioning regimen (fludarabine 125mg/m^2, cyclophosphamide 120mg/kg and rabbit-ATG 7.5mg/kg) is safe and effective in inducing regression of intestinal ulceration, in patients with refractory CD, when compared with standard care.

This multi-centre study has been designed to have direct clinical applicability in the management of refractory Crohn's Disease in the UK following completion.

3.1 Efficacy

The clinical objectives of this study are:

3.1.1 Primary objective

1. To assess the efficacy of HSCTlite compared to standard care at inducing regression of Intestinal ulceration in patients with refractory CD at week 48.

3.1.2 Secondary objectives

2. To assess the impact of HSCTlite on clinical disease activity and quality of life compared to standard care.

3.2 Safety objectives

The safety objectives in this study will initially be assessed as part of the embedded pilot study. The Data Monitoring and Ethics Committee (DMEC) will assess whether low dose cyclophosphamide and G-CSF is a safe and effective mobilisation regimen

for patients with refractory CD. Ongoing monitoring of toxicity of chemotherapy using NCI CTCAE criteria will continue throughout the trial.

Adverse Event (AE) and Serious Adverse Event (SAE) data will be collected from all participants throughout their participation in the trial. Further detail on AE and SAE recording and reporting is in section 10.

3.3 Exploratory objectives

In addition to the above objectives, the following will also be assessed:

1. The safety and efficacy of anti-TNF therapy in patients who demonstrate endoscopic disease recurrence at week 24 after HSCTlite

3.4 Mechanistic objectives

HSCT is thought to induce regression of autoimmune diseases by altering the diversity of the T cell receptor repertoire and generating functional renewal of regulatory T cells (20,21). Neither the mechanism of action nor the time-course of response is known in CD.

The mechanistic objectives in this study are:

1. Intestinal MRI will be performed to determine the early impact on mucosal disease at week 4.
2. Immune profiling of peripheral blood and mucosal biopsies will:
    a. Characterise immune re-constitution after HSCT, and assess impact of HSCT on disease activity
    b. Assess immunological events that precede disease recurrence post HSCT
    c. Assess the mechanism of restoration of responsiveness to anti-TNF therapies
    d. Serum will be stored for future assessment of response to vaccination post HSCT

4. Study Design

This study is a parallel-group, controlled trial that will randomise eligible patients in the ratio 2:1 to low dose cyclophosphamide and G-CSF mobilisation and reduced intensity conditioning HSCT (HSCTlite) versus conventional care. Patients with clinical and endoscopic CD activity who are refractory to at least two classes of biologic therapy and in whom surgery is inappropriate or has been declined will be recruited.

Patients in the intervention arm with endoscopic evidence of disease recurrence at week 24 after HSCTlite will receive induction and maintenance anti-TNF therapy.

The primary endpoint of treatment success will be assessed at week 48. All patients undergoing HSCTlite, either as part of the trial, or control participants who subsequently receive HSCTlite, will be entered onto the EBMT registry for four years' annual follow up of safety, efficacy, quality of life and health care resource utilisation (see section 15).

Mechanistic studies are integral to the study, with assessment of the timeline of response to HSCTlite and interrogation of the systemic and mucosal immune compartments. Intestinal MRI will be performed at week 4 and 24 to assess early

response. Serum, whole blood, and mucosal biopsies will be collected at multiple time points. In addition, stool, serum and whole blood will be collected and stored for future research grant applications.

At each follow up visit, participants will complete questionnaires on quality of life and health care resource utilisation, and information will be gathered on adverse events. Where participant recall is limited, the hospital medical notes will be used to identify information relating to adverse events and medication. Participants will give permission for the research team to access their medical notes as part of the consent process. Entry into the trial will be documented in the medical notes, with copies of study documents for clinicians' reference. Consent will be reconfirmed at each study visit, and this will be documented in the medical notes.

All potentially eligible participants will be discussed on a case-by-case basis by a panel comprising of the clinical members of the TMG, for adjudication on eligibility, and will be asked to sign a separate consent form to allow this discussion to take place. This will be done at one of the scheduled monthly meetings, or via email if the timing of referral does not coincide with meeting dates. At least two members of the panel must be in agreement on inclusion of the potential participant. If the TMG members deem a potential participant ineligible, they will not be consented to the study, unless specific actions are requested, such as further screening investigations. In this case, patients will come back to the TMG for further discussion on eligibility, once required actions are complete.

All referrals to the panel of potentially eligible patients should include sufficient supporting clinical information, as documented on the referral form. Further information may be requested by the panel if this is required to make the decision on eligibility. All discussions around eligibility will be documented in either the meeting minutes, or the email discussion, and these will be retained with the patient file.

Participants who are found to be ineligible after randomisation will be excluded from trial participation, and this will be reported to the Sponsor as a protocol non-compliance.

The safety of the HSCTlite treatment regimen will be assessed by the Data Monitoring and Ethics Committee after the first ten participants and at each DMEC meeting after this (see section 2 and 4.1). The DMEC can recommend early stopping of the trial, if this is in the interests of the safety of participants. The study also incorporates an internal pilot phase in which the feasibility of the recruitment target is considered. No formal interim analyses of efficacy are planned.

4.1 Feasibility Outcomes

An embedded pilot study has been designed to ensure that HSCTlite achieves adequate mobilisation without causing a flare up of CD activity prior to conditioning (reported in other conditions) (17). CD activity during mobilisation will be monitored by assessment of disease activity indices (Harvey Bradshaw Index) and biochemical markers of inflammation (CRP). The DMEC will assess efficacy and safety of the HSCTlite mobilisation regimen after 10 patients and subsequently at each DMEC meeting. Should the protocol fail to mobilise 2 x 10^6/kg CD34+ cells (haematopoietic stem and progenitor cells) in more than 10% patients, or if greater than 10% patients experience a disease flare up (increase in Harvey Bradshaw Index of >30% from

baseline associated with a rise in CRP) during mobilisation, a protocol amendment will be submitted to modify the mobilisation regimen for subsequent patients.

Trial feasibility will be assessed by the TSC and DMEC at month 10 to ensure that >60% predicted patients have been recruited. DMEC will continue to monitor safety throughout the study period.

4.2 Primary Endpoint

Treatment success at week 48 defined as mucosal healing (no endoscopic ulceration (SES CD ulcer size sub score = 0, assessed by adjudication panel blind to allocation and time of assessment)) without surgery or death. Patients who do not complete the week 48 endoscopic assessment will be categorised as treatment failures.

Endoscopic primary endpoints are now the gold standard for clinical trials in CD as several pivotal studies have reported poor correlation between mucosal healing and clinical disease activity indices (22). In addition, patients who are eligible for inclusion to ASTIClite are likely to have sustained significant digestive disease damage from prior inflammation. This is known to result in a burden of non-inflammatory symptoms that may not be alleviated by transplant, even in the setting of a complete resolution of mucosal ulceration. This was seen in the ASTIC trial, where patients with regression of all disease activity on oesophago-gastro-duodenoscopy (OGD), small intestinal MRI and colonoscopy reported persistent symptoms relating to fixed intestinal damage (9).

4.3 Secondary Endpoints

Clinical endpoints

1. Clinical remission (CDAI <150)
2. Steroid free clinical remission (CDAI <150)
3. Clinical remission (Harvey Bradshaw Index ≤4)
4. Clinical remission (PRO2 – mean scores – abdominal pain ≤1, stool frequency ≤1.5)
5. Absolute CDAI at week 48
6. Absolute SES CD at week 48
7. Change in CDAI and SES CD between baseline and week 48
8. Proportion of patients in complete endoscopic remission (SES CD score of 0)
9. Absolute MaRIA score at week 48

Safety endpoints

1. Toxicity of chemotherapy using NCI CTCAE criteria version 4.03
2. Adverse events (AEs) and Serious Adverse Events (SAEs), including mortality

Patient-reported endpoints

1. Disease specific quality of life using the IBDQ
2. Disease specific quality of life using the IBD Control
3. Quality of life using the EQ-5D-5L
4. Health care resource utilisation questionnaire

Exploratory secondary endpoints

1. Efficacy of re-introduction of anti-TNF therapy in patients with disease recurrence post-HSCT (change in CDAI at 6 weeks and change in SES CD at 22 weeks after initiation)
2. Safety of re-introduction of anti-TNF therapy in patients with disease recurrence post-HSCT
3. Presence of any of the late side effects of HSCT, documented through adverse events.

***Table 1**: Suggested long term screening for late effects of HSCT, assessed as part of standard care following a stem cell transplant*

Recommended timing of assessment	**3 months**	**6 months**	**1 year**
Corresponding study visit	**Week 14**	**Week 24**	**Week 48**
General			
Weight	1	1	1
Blood pressure	1	1	1
Performance status (Karnofsky/Lansky)	1	1	1
Haematology			
FBC	1	1	1
Renal			
Renal function	1	1	1
Urine protein (dipstick)	1	1	1
Liver			
Liver function	1	1	1
Iron studies		1	1
Endocrine			
Thyroid function TSH, Free T4	1	1	1
Gonadal function FSH, LH, oestradiol, Progesterone (women <=50 years) FSH, LH, Testosterone (men)	1	1	1
Sexual function assessment (as per patient report)		1	1
Bone			
Bone profile	1	1	1
Bone density scan Women and men with evidence of hypogonadism Patients on prolonged corticosteroids or calcineurin inhibitors			1
Respiratory			
Clinical assessment	1	1	1
Pulmonary function test			1
Chest radiograph		*	*
Counselling re: smoking cessation	1	1	1
Nervous system			
Neurological assessment			1
Vascular			

Cardiovascular risk factors			1
Echocardiogram			1
HbA1c		1	1
Lipid profile and abdominal girth		1	1
Immune System			
CD4 subsets	1	1	1
Immunoglobulin levels	1	1	1
Antimicrobial prophylaxis as per local protocol	1	1	1
Immunisation and antibody levels as per local protocol			1
Oral complications			
Dental assessment		1	1
Ocular			
Cataracts assessment	1	1	1
Second cancers			
Mammograms (Women >40 years)			1
Vigilance and self-examination		1	1
Second autoimmune diseases			
Second autoimmune diseases		1	1
Psychosocial			
Psychosocial/psychosexual issues, by standard holistic needs assessment	1	1	1

1 = recommended for all transplant patients

* = reassessment recommended if previously abnormal

Mechanistic endpoints

1. Timeline of response to HSCTlite using MRI at week 4 and 24
2. Nature of immune re-constitution after HSCT
3. Immunological events that precede the onset of disease recurrence post HSCT
4. Mechanism of restoration of responsiveness to anti-TNF therapies if appropriate

4.4 Blinding

In view of the nature of HSCT, neither patients nor their treating physicians will be blinded to the treatment allocation. However, an adjudication panel blind to both the timing of procedure and treatment allocation will assess videos of all endoscopic procedures used to determine the primary endpoint. Likewise, expert physicians unaware of the timing of investigation or prior treatment will perform central MRI review for a percentage of completed scans, and calculate the MaRIA score using anonymised electronic copies of the appropriate images. All completed MRI scans will be reported by the local research team.

The trial statistician(s) will remain blinded throughout the study, but will be unblinded at database freeze, for analysis. The Senior Statistician will be unblinded to the treatment allocation throughout the trial, but will review and approve the statistical analysis plan version 1 before seeing any outcome data.

5. Ancillary sub-studies

Mechanistic studies are planned alongside the main ASTIClite trial. Intestinal MRI will be performed at week 4 and 24 to assess early response.

Serum, whole blood and peripheral blood mononuclear cells (isolated from whole blood) will be collected at baseline, week 8, week 14, week 24, week 32, week 40 (serum only) and week 48. Mucosal biopsies will also be collected at baseline, week 24 and week 48.

Stool samples, in addition to samples of serum, whole blood and mucosal biopsies, will be collected and stored for future studies, the funding for which will be sought via research grant applications.

The objectives of the mechanistic sub-study are:

1. To determine the early impact on mucosal disease, using intestinal MRI.
2. Using immune profiling of peripheral blood and mucosal biopsies to:
    a. Characterise immune re-constitution after HSCT, and assess impact of HSCT on disease activity
    b. Assess immunological events that precede disease recurrence post HSCT
    c. Assess the mechanism of restoration of responsiveness to anti-TNF therapies
    d. Serum will be stored for future assessment of response to vaccination post HSCT

5.1 Processes for data collection and processing

5.1.1 MRI

Intestinal MRI will be undertaken according to standard clinical protocols at participating centres, using standard bowel preparation, on a 1.5T scanner using gadolinium contrast (0.2mls/kg).

Intestinal MRIs at baseline and week 48 visits are to be read locally at participating centres, and read centrally (see section 9.3 for further details). MRIs at week 24 will also be read locally, informing decisions whether to restart anti-TNF therapy (section 9.3).

An additional intestinal MRI will be taken at the week 4 study visit for the purposes of the mechanistic sub-study. These scans will be recorded to CD by site staff, and read centrally by investigators. This will be done at the end of the study. Only participants receiving HSCTlite will have a week 4 study visit.

5.1.2 Blood samples and biopsies

In addition to locally processed standard blood tests, blood samples will be taken from each participant at six time points throughout their participation in the study; baseline, week 8, 14, 24, 32, 40 and 48 study visits. Each of these samples will be sent by the site to the John Van Geest Cancer Research Centre for processing and analysis. Intestinal biopsies will also be taken during the endoscopy / colonoscopy performed at baseline, week 24 and week 48 visits.

Biopsies

A minimum of four biopsies will be collected in formalin and sent to the local laboratory as part of routine clinical care (one each from inflamed and non-inflamed small bowel / ileum and inflamed and non-inflamed colon). Additional biopsies will be taken if required for clinical management. These will be paraffin embedded and processed for H/E routine histology.

In addition, four biopsies from the small bowel / ileum, and four biopsies from the colon will be collected at the time of endoscopy /colonoscopy for research, at each of the baseline, week 24 and week 48 study visits, according to standard clinical protocols. At each timepoint, two samples will be taken from an inflamed area, and two from a non-inflamed area of the small bowel / ileum and colon. The research biopsies will be stored locally in RNAse reagent overnight and then stored at -80 on site before being batch sent to the Nottingham lab four times a year. Two of the samples will be analysed using nanostring technology and the remaining samples will be stored for use in future research.

If it is not clinically possible to take the biopsies defined above, this will be recorded on the CRF, and biopsies will be taken as clinically appropriate.

Whole blood

This will be collected into commercially available lithium heparin vacutainers. Samples will be sent at ambient temperature on a per patient basis. An additional 5ml sample will be taken in EDTA and a Tempus tube for subsequent RNA extraction at each study visit. These will be frozen on site and sent in batches on dry ice to the central laboratory.

Peripheral blood mononuclear cells (PBMCs)

These samples will be collected in lithium heparin vacutainers at each visit. Samples will be sent at ambient temperature on a per patient basis. On receipt at the John van Geest Cancer Research Centre, they will be separated to extract the PBMCs ready for processing, analysis of immune system reconstitution and subsequent storage for future research.

Serum

Serum samples will be collected, frozen at -80˚C and stored at the site for use in future studies. Samples will be aliquoted by the site and will be batch-sent to the John van Geest Cancer Research Centre on dry ice, four times per year, per centre. Aliquots of serum will be used for the measurement of pro- and anti-inflammatory-related analytes, including cytokines, using relevant immunoassays. These analyses will determine the level of inflammatory markers and the response to vaccinations. Samples will also be stored for future assessment of anti TNF drug levels and anti-drug antibodies where appropriate.

Stool

Stool will be collected at each visit, separated into aliquots and stored at -80 on site for subsequent analysis in future research. Samples will be sent on dry ice to the central laboratory in batches.

Monitoring safety

The blood results from the above sample analysis will be uploaded to the Prospect database every two months. Any abnormal results that could have a clinical impact on the patient's safety or wellbeing will be checked against ad hoc reports from analysts at the John van Geest Cancer Research Centre, and any issues not

previously reported will be identified to the Haematologist at the appropriate site. This process aims to ensure that any adverse events relating to the samples collected, are identified, recorded and acted upon as necessary.

Full details of the mechanistic sub study, and the requirements for participating sites in terms of processing, storage and shipping, are documented in study specific standard operating procedures.

5.2 Future research

Whilst taking the samples for use in this study, whole blood, serum and biopsy samples will also be taken in addition to those defined in this protocol. Participants will also be invited to provide stool samples at the same time points in the study, which will be frozen and stored. These additional samples will be stored for use in future ethically approved research.

Most transplant centres retain a small portion of stem cells harvested from a patient, for quality purposes. Where possible, and with the participant's consent, a small sample of these stem cells will be stored and shipped to the John van Geest Cancer Research Centre along with the other samples, for use in future ethically approved research.

6. Selection and withdrawal of participants

6.1 Recruitment

We aim to randomise 99 patients with refractory CD to the ASTIClite study. From previous experience in the ASTIC trial (9), we anticipate approximately 198 patients will need to be discussed with the TMG and 116 consented (to account for screen failures) in order to achieve 99 eligible patients for randomisation. In this setting, screen failures are defined as those patients who appear to be initially eligible, provide informed consent, and then following subsequent eligibility assessments and investigations, do not meet the full eligibility criteria.

The site Principal Investigator (PI) will identify potential patients from the local population of patients with CD refractory to biologic therapy. The IBD BioResource may also be used to identify potentially eligible participants. In addition, each site may undertake a networked MDT with regional research active IBD centres that have recruited patients to the PANTS cohort of patients commencing anti-TNF therapy. All patients will be discussed on a case-by-case basis, via email or teleconference, by a panel comprising of the clinical members of the TMG. At least 2 members of the panel, in addition to the referring investigator, must agree that the patient is suitable to proceed with transplant in order for the patient to be entered into the trial.

6.2 Inclusion Criteria

In order to be eligible to take part, potential participants must meet all of the following inclusion criteria, and must not meet any of the exclusion criteria.

A participant is eligible for the study if the following criteria are met:

1. Participant of any gender, aged between 18 – 60
2. Participants must be willing and able to provide full informed consent.

3. Participants should be well nourished and of healthy weight in the opinion of the PI (typically BMI >18.5).
4. Diagnosis of CD using colonoscopy, histology and/or radiology.
5. Disease duration of at least six months.
6. Disease distribution accessible to endoscopic assessment (jejuno-ileal, ileo-caecal, or colonic).
7. Active clinical CD activity with impaired quality of life at any time within 3 months prior to randomisation into the trial, as assessed by a gastroenterology clinician.
8. Participants will be refractory or intolerant to azathioprine, mercaptopurine or methotrexate.
9. Participants will be refractory or intolerant to at least two classes of biologic therapy (currently anti-TNF therapy, Vedolizumab or Ustekinumab) despite dose optimisation.
10. Participants where surgery is considered not appropriate or has been declined.
11. Endoscopic evidence of active disease in screening (SES CD ulcer size sub-score of 2 or more in at least one segment). SES-CD will be used as standard for patients with disease in the ileum and/or colon. Should the disease only be proximal to the ileum, the SES-CD will still be used to score the relevant bowel segment.
12. Satisfactory EBMT Autoimmune Disease Working Party (ADWP) recommended screening assessment prior to HSCT.
13. Willingness to discontinue all immunosuppressant medication after randomisation if allocated to HSCT arm.
14. Participants, who, in the opinion of the TMG, are fit enough to undergo treatment.

6.3 Exclusion Criteria

A participant is not eligible for the study if any of the following criteria are met:

1. Diagnosis of ulcerative colitis or indeterminate colitis.
2. No evidence of active CD on screening endoscopic assessment.
3. Inability to assess for endoscopic active disease due to strictures.
4. Undrained perianal fistulae (patients with previous perianal disease or perianal disease adequately drained with a seton in situ are eligible).
5. Presence of undrained perianal sepsis on screening pelvic MRI.
6. Evidence of intra-abdominal sepsis on abdominal MRI.
7. Active or latent mycobacterial infection.
8. Prior exposure to Hepatitis B, Hepatitis C or HIV.
9. Evidence of an enteric or systemic infection.
10. Participant is currently pregnant or breastfeeding, or planning pregnancy within the study duration. Current pregnancy will be confirmed with a pregnancy test at screening assessment.
11. Unwilling to use adequate contraception (if appropriate) until at least 12 months after the last dose of study drug.
12. Contraindication to the use of cyclophosphamide, fludarabine, filgrastim or rabbit ATG.
13. Participants with significant medical co-morbidity that precludes HSCT adjudicated by the TMG.
14. Participants with significant psychiatric co-morbidity.
15. Significant language barriers, which are likely to affect the participant's understanding of the study, or ability to complete outcome questionnaires.

16. Concurrent participation in another interventional clinical trial.
17. Participants who are not considered medically fit for HSCT defined by any of the following:
    a. Renal: creatinine clearance <40ml/min (measured or estimated)
    b. Cardiac: clinical evidence of refractory congestive heart failure, left ventricular ejection fraction <45% by multigated radionuclide angiography (MUGA) or cardiac echo; uncontrolled ventricular arrhythmia; pericardial effusion with haemodynamic consequences as evaluated by an experienced echo cardiographer
    c. Hepatic: AST > two times the upper limit of normal
    d. Concurrent neoplasms or myelodysplasia
    e. Bone marrow insufficiency defined as neutropenia with an absolute neutrophil count $<1x10^9$/l, or thrombocytopenia with a platelet count $<50x10^9$/l, or anaemia with a haemoglobin <80g/l
    f. Uncontrolled hypertension, defined as resting systolic blood pressure >= 140mmHg and/or resting diastolic pressure >= 90mmHg despite at least 2 anti-hypertensive agents (subject to discussion at TMG).
    g. Uncontrolled acute or chronic infection with HIV, HTLV – 1 or 2, hepatitis viruses or any other infection the investigator or TMG consider a contraindication to participation.
    h. Other chronic disease causing significant organ failure, including established cirrhosis with evidence of impaired synthetic function on biochemical testing and known respiratory disease causing resting arterial oxygen tension <8kPa or carbon dioxide tension >6.7kPa. FEV_1/FVC <50%. Patients not known to have respiratory disease need not have blood gas measurements.

6.4 Informed Consent Process

Potential participants will receive an approved participant information sheet and be given the opportunity to ask questions from both the gastroenterology and haematology specialist teams. Potentially eligible patients will be invited to provide their consent to be referred to the TMG for discussion around eligibility. If the TMG agree that the potential participant appears initially eligible, the patient will be invited to give full written consent. Only those who have been provisionally approved for inclusion by the TMG will proceed to full screening investigations. Patients will have the opportunity to visit their local transplant centre, and also the opportunity to receive counselling from an independent clinician who is not a study investigator. Contact details for this independent clinician will be provided to the patient in the participant information sheet. Patients will be given sufficient time to read and understand the information provided to them, and ask further questions as required. They will be advised that they are free to withdraw from the study at any time, without obligation, with no impact on subsequent clinical care. They will also be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for study purposes by authorised individuals other than the treating physicians.

No study related procedures will occur before the approved consent form is signed, other than initial case note review by the referring clinician, and with the patient's permission, an initial discussion with the TMG adjudication panel. As the study is a Clinical Trial of an Investigational Medicinal Product (CTIMP) a medically qualified individual (site PI or other with delegated responsibility) will confirm eligibility and provide clinical oversight for the consent process. In addition, in line with EBMT

guidelines, patients will sign a local consent form confirming their understanding of the risks and benefits of HSCT for autoimmune disease. This EBMT consent form will also include an agreement to safety and outcome data being collected in the EBMT registry, and used for the purposes of the research (see related research in section 15). The Consultant Haematologist undertaking HSCT along with the study Research Nurse will supervise this process in line with Human Tissue Authority and JACIE requirements. The patient's GP will be informed of their participation in the study, as will their referring gastroenterologist (if appropriate).

For each participant, the original copies of the signed consent forms will be retained by the Investigator in the Site File but must be made available for inspection by the Study Monitor. Patients will also receive a copy of the Participant Information Sheet and their signed consent form to keep, and a copy will be filed in their medical notes. Consent will be reconfirmed at each study visit and documented in the medical notes.

A screening log will be maintained for each site, to document all potential participants screened, whether they were recruited, and any reasons for non-recruitment where this information is available.

6.5 Screening Procedures and Pre-randomisation Investigations

Consented patients will undergo screening and baseline assessment to ensure eligibility. This will include:

1. Standard pre-HSCT work, including one chest x-ray and one MUGA scan or cardiac echo (EBMT ADWP guidelines (15))
2. Genetic / functional assessment for monogenic cause of disease in patients with very early onset disease or with atypical phenotypes for whom this has not previously been excluded.
3. Assessment of clinical disease activity (CDAI and HBI)
4. Assessment of quality of life using patient completed questionnaires (IBDQ, IBD-Control, EQ5D, WPAI & Healthcare resource utilisation)
5. Endoscopic assessment of disease (endoscopic disease activity scored using SES CD)
6. MRI small bowel to record disease activity (MaRIA score)
7. MRI pelvis in patients with previous perianal disease to exclude perianal sepsis
8. Confirmation of eligibility by nominated members of TMG
9. Criteria for fitness for HSCT as per exclusion criteria 16 (see section 6.3). Participants who meet one of more of these exclusion criteria, but in the opinion of the PI are medically fit enough to undergo HSCT, may be put forward to TMG for discussion about eligibility.

Assessment of disease activity (MaRIA score, SES CD and CDAI), and screening blood tests should occur within 8 weeks of randomisation. If the exact required screening tests have been taken for clinical reasons since the date of consent, these results can be used for the CRF. However, this must also take into account the window between MaRIA score, SES CD, CDAI and blood tests and randomisation as above.

The patient will be asked to complete a symptom diary for a week prior to assessment of the CDAI; this cannot be taken immediately preceding a colonoscopy and patients should finish the diary prior to starting bowel prep for colonoscopy.

Prior to randomisation, delegated clinical members of the TMG will review all available screening information to confirm eligibility. TMG adjudication of eligibility is likely to occur with only partial screening information available, as patients would not undergo colonoscopy or MRI unless other information indicated likely eligibility, and this was agreed at the TMG. After initial TMG approval, additional screening investigations will be carried out. Once all eligibility assessment information is available, patients will be referred back to the adjudication panel for final approval for inclusion, prior to randomisation.

Anonymised data on patients who are screened but not randomised will be collated, in line with the Consolidated Standard of Reporting Trials (CONSORT) guidelines. Once eligibility has been confirmed and baseline data recorded, the participant will be randomly allocated to either the HSCTlite arm (n=66) or usual care arm (n=33).

6.6 Long term infertility

As expected of the normal standard of care, there should be a full discussion regarding the potential of the chemotherapy used in mobilisation and transplant leading to irreversible infertility and gonadal failure. Patients should be counselled and referred to local facilities for semen/oocyte/embryo cryopreservation if appropriate. Following transplantation, gonadal function should be assessed according to local SOPs and hormone replacement offered as appropriate.

6.7 Pregnancy & contraception

Patients are not eligible to take part in this trial if they are pregnant or breastfeeding at the time of screening. Pregnancy tests will also be performed prior to mobilisation, and prior to conditioning for those participants allocated the HSCTlite intervention.

It is possible that if the treatment is given to a pregnant woman, it will harm the unborn child. Pregnant women must not therefore take part in this study; neither should women who plan to become pregnant during the study. Although the HSCT regimen often results in infertility during this time, it is possible that women may become pregnant during the study follow-up period. Women who could become pregnant must use an effective contraceptive during the course of this study. Recommended effective contraception is combined hormonal contraceptive (oral, intravaginal, transdermal) or progestogen-only hormonal contraceptive (oral, injectable, implantable) initiated at least one month prior to baseline, intrauterine device, intrauterine hormone-releasing system, or bilateral tubal occlusion/ligation. This should be in addition to a barrier method, such as condom use. For male participants with female partners of childbearing age, participants should practice true abstinence, or use a condom along with their female partner using at least one of the measures described above. Abstinence is acceptable only as true abstinence, when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. The method(s) of contraception used must be stated in the patient medical notes.

Prior to entry into the trial, potential participants should be counselled about the importance of using adequate contraception for at least twelve months after the last dose of study drug.

If a female participant, or the female partner of a male participant becomes pregnant between the initiation of cyclophosphamide and 12 months post HSCT, they should

inform their local research team immediately. See section 10.8 for details on the reporting procedure for pregnancy. Pregnant participants will continue to be followed up as per protocol.

6.8 Co-enrolment guidelines

Concurrent participation in any other clinical study is not allowed for the duration of the study (i.e. until the week 48 study visit), with the exception of the ASTIClite EBMT Registry follow up study. At the point of entry into the trial, patients should not already be taking part in an interventional trial.

6.9 Early stopping of Protocol Treatment

Given the patient population, the nature of the intervention and the pre-specified endoscopic assessment at week 24 with introduction of anti-TNF therapy for patients with endoscopic evidence of disease recurrence, it is felt that withdrawal from the intervention group for medical reasons will be rare. Likewise, patients in the usual care arm can receive best standard of care including any licensed biologic or nutritional therapy for CD available at the time. As such there are no specific medical criteria for patient withdrawal. If there is a requirement for surgical treatment, or any additional medical treatment in the intervention arm (other than the re-introduction of anti-TNF therapy at week 24), the participant will remain in the study, but by definition such a patient will fail the primary endpoint. Any decision to withdraw a patient's treatment on medical grounds will be made by the local investigator. Participants who have treatment withdrawn will still remain in the trial and be followed up at scheduled time points.

However, patients may choose to withdraw from the trial visits and study related procedures at any time without prejudice to future clinical care; where possible the reasons for withdrawal will be recorded.

6.10 Early stopping of Follow-up

Excessive participant withdrawal from follow-up has a negative impact on a study. Centres will explain the importance of remaining on study follow-up to participants, and that changes to planned treatment need not imply withdrawal from the study. Nevertheless, if participants do not wish to remain in the study their decision must be respected.

If the participant explicitly states their wish not to contribute further data to the study, this will be recorded on a Study Completion/Discontinuation form. However, data up to the time of consent withdrawal will be included in the data reported for the study. This is made clear in the participant information sheet. Patients who have received a stem cell transplant as part of the trial, will be advised that they should continue with their clinical care in relation to this transplant, even if they no longer wish to contribute data to the study.

Participants who stop study follow-up early will not be replaced.

6.11 Participant Registration

Once a patient has been confirmed as eligible and has been registered onto the trial, they must be provided with the following:

- A copy of their signed consent form
- A copy of the participant information sheet
- A patient contact card. Site on-call contact details for 24 hour medical care must be added to this card, and participants advised to carry this with them at all times whilst participating in the trial.

7. Randomisation

Once eligibility has been confirmed and baseline data recorded (see section 6.5 and 9), participants will be centrally randomised using the CTRU online randomisation system (SCRAM). Participants will be randomly allocated to either the HSCTlite arm or usual care, in the ratio 2:1. The doctor or nurse will access the web-based randomisation system, patient details (ID, date of birth) will be entered and the treatment allocation will be returned. Randomisation will be stratified by centre, using permuted blocks.

Day 0 for the group allocated to receive usual care will be calculated as 49 days after the date of randomisation, to try and align the length of time both groups are within the trial.

8. Treatment of Participants

8.1 IMP Details

The investigational medicinal products (IMPs) and non-investigational medicinal products (NIMPs) for this study will be sourced from local hospital stock within the eight participating centres. A separate IMP manual will detail IMP handling and recording requirements.

IMPs are the products in the study whose effects are being studied. NIMPs are products given to participants in the study to mitigate some of the effects of the IMPs. The drugs classified as NIMPs are standard supportive therapies but all trial patients must be treated according to the following schedule in order to isolate the effects of the IMP. The products used in the ASTIClite study, and their classification is stated in Table 2.

Table 2: *Categorisation of each product in the ASTIClite trial.*

Product	Category
Cyclophosphamide	IMP
G-CSF (filgrastim)	IMP
Fludarabine	IMP
Rabbit ATG	IMP
Mesna	NIMP
Methylprednisolone	NIMP

The intervention group will receive enhanced supportive care throughout the mobilisation and conditioning phase with all appropriate prophylactic and therapeutic antimicrobial cover and intensive care support as required (13,15). Current immunosuppressive therapy will be discontinued prior to mobilisation and steroids will be weaned as appropriate (see section 8.7). Participants will be admitted to

hospital during the conditioning phase, although depending on usual local practices, some sites may have facilities to carry out mobilisation as a day case, if hospital accommodation is available. Individual decisions will be made by the local investigators dependent on clinical and geographical factors.

Doses should be calculated using actual body weight, and actual m^2, unless otherwise indicated in this section.

A pregnancy test will be carried out for all female participants undergoing HSCTlite, both prior to mobilisation, and prior to conditioning.

8.1.1. Mobilisation

Cyclophosphamide (IMP)
All participants in the intervention arm will undergo peripheral blood stem cell mobilisation. This regimen will consist of a one-hour infusion of cyclophosphamide 1g/ m^2.

Mesna (NIMP)
Mesna will be given during the mobilisation phase, to prevent haemorrhagic cystitis caused by the chemotherapy, and dose and administration will be in line with local Trust procedures.

G-CSF (filgrastim) (IMP)
This is followed by G-CSF (filgrastim) 5µg/kg, rounded according to local practice to the nearest vial size, given subcutaneously, and commencing 4 days after the cyclophosphamide infusion, until the day of stem cell harvest.

Stem cell harvest
Monitoring of full blood count and CD34+ counts will be carried out according to local standard practice, ideally from day 8, during the mobilisation phase. Participants will undergo stem cell harvest once peripheral blood CD34+ levels are shown to have exceeded $10x10^6$/L. This is expected to occur onwards from day 10 following cyclophosphamide (and after 5 days filgrastim). Once achieved, participants will undergo leukapheresis according to standard operating procedures in their local participating transplant centre until a minimum of 2.0 $x10^6$/kg CD34+ are collected for cryopreservation according to local protocols. Centres should allow for 10% wastage through quality assessment in this calculation.

The decision to admit the patient for the mobilisation and give the patient prophylactic antibiotics and other supportive care measures rests with the local supervising physician.

Requirements for mobilisation
In order to avoid a theoretical risk of cumulative cardiac and other toxicities from cyclophosphamide and to allow reporting of microbiological cultures prior to infusion of the stem cells, a minimum of three weeks must separate the administration of cyclophosphamide for mobilisation and commencement of transplant conditioning. To benefit from the potential stabilising effect of the mobilisation cyclophosphamide on CD activity the commencement of transplant conditioning should aim to occur within around 6 weeks of the date of administration of cyclophosphamide for mobilisation, unless there are clinical reasons (such as infections) that should be discussed with the TMG on an individual basis and treatment planned accordingly. Reharvesting

may be permitted in instances of mobilisation failure, microbiological contamination of harvests and other issues influencing the scheduling of transplant, and will be documented on the CRF and in the patient's medical notes. Decisions will be made on an individual basis by the site Haematologist, in discussion with clinical coordinators. The TMG should also be informed, as additional mobilising cyclophosphamide and/or G-CSF may impact on outcomes. The use of lower dose or no cyclophosphamide may be considered for patients in whom a second mobilisation regimen is attempted.

Summary of mobilisation regimen

The table below details the timing of doses of each product during the mobilisation phase. These have been mapped to example days of the week, but the process does not have to start on a Monday, this is an example only.

Table 3: *Timing of administration of IMP during mobilisation phase*

Day	1	2	3	4	5	6	7	8	9	10	11	12
Example day of the week	Mon	Tues	Wed	Thur	Fri	Sat	Sun	Mon	Tue	Wed	Thur	Fri
Cyclophosphamide 1g/m^2	✓											
G-CSF (filgrastim) 5µg/kg					✓	✓	✓	✓	✓			
Mesna (dose as per local practice)	✓											
PB CD34 count								✓	✓	✓	✓	✓
Stem cell harvest								✓*	✓*	✓*	✓*	✓*

*Stem cell harvest is approximate, the day of this will depend on adequate CD34+ counts, as described above.

8.1.2 Conditioning

Participants will be required to go into isolation during the conditioning and transplant procedure. This is made clear in the patient information sheet, and patients are reassured that visitors are still permitted, whilst in isolation.

Fludarabine (IMP)

Participants will then start the conditioning regimen. Fludarabine 25mg/m^2 (reduced in the presence of impaired renal function, see section 8.5), will be given IV on days -6, -5, -4, -3 and -2.

Cyclophosphamide (IMP)

Cyclophosphamide 60mg/kg/day IV over 1 hour will be given in 500ml of normal saline on days -3 and -2.

Mesna (NIMP)

Mesna will be given as a continuous IV infusion on days -3 and -2, to prevent haemorrhagic cystitis, and dose and administration will be in line with local Trust procedures.

Supportive care

Standard hydration will be given throughout the administration of cyclophosphamide, and diuretics will be used and fluids decreased as necessary to maintain baseline

weight. Any other medication that would usually be given as part of normal supportive care during stem cell transplant will be prescribed and administered in line with local Trust practices.

Rabbit ATG (IMP)
Rabbit ATG (Thymoglobulin; Genzyme) doses will be given IV (2.5mg/kg) on days -3, -2 and -1. A test dose of ATG is permitted if this is standard local practice.

Methylprednisolone (NIMP)
Methylprednisolone will be given intravenously at 2mg/kg per day for three days to cover the three doses of ATG; additional doses of methylprednisolone of up to 500-1000mg intravenously per day can be given at clinical discretion in the setting of a reaction to ATG..After the third day, methylprednisolone will be tapered as per local practice to cover febrile or other reactions due to ATG, whilst protecting against adrenal insufficiency in patients who have long-term steroid dependency.

The following dosing is a suggested tapering scheme for steroids (oral prednisolone or IV methylprednisolone): 40mg daily for day 0 to +2, 30mg daily from day +3 to day +4, 20mg daily from day +5 to engraftment, 10mg daily for 2 days post engraftment, then stop or resume pre-transplant dose in patients where a prolonged taper is appropriate. Dosing and administration of steroids is at the discretion of the treating physician and dependent on the individual patient's condition, but the doses should be documented on the participant's case report form to reflect their medication charts and medical records.

Stem cell reinfusion
Stem cells will be re-infused at day 0. G-CSF 5µg/kg/day (rounded to the nearest vial) will be started on day +5 in the conditioning regimen, and continued until the absolute neutrophil counts reach >1.0x10^9/L for 2 consecutive days.

Summary of conditioning regimen

Table 4: *Timing of administration of IMP during conditioning phase*

Day	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5
Fludarabine 25mg/m^2/day	✓	✓	✓	✓	✓							
Cyclophosphamide 60mg/kg/day				✓	✓							
Mesna (dose as per local practice)				✓	✓							
Standard hydration (as per local practice)				✓	✓							
Rabbit ATG (Thymoglobulin; Genzyme) (2.5mg/kg/day)				✓	✓	✓						
Methylprednisolone (2mg/kg/day)				✓	✓	✓						
Stem cell reinfusion							✓					
G-CSF (filgrastim) (5µg/kg/day)												✓ (continued until

												absolute neutrophil count >1.0x10^9/L for 2 days)

8.1.3 Supportive care

Supportive care should follow local standard operating procedures and is at the discretion of the transplant physician, but should include prophylactic broad spectrum antibiotics (intravenously in patients where absorption is potentially impaired) for the duration of the period when neutrophil count is less than 0.5 x10^9/L, transfusion of platelets to maintain a platelet count of >20 x10^9/L, and transfusion of red cells to maintain a haemoglobin concentration of >80g/L. Antifungal prophylaxis (according to site preference), and antiviral prophylaxis (e.g. aciclovir) (dose in line with local practice) should continue from the start of conditioning for at least 3 and 12 months post-transplant respectively. After stable engraftment, pneumocystis prophylaxis should commence and continue for at least 12 months, as per local policy (e.g. co-trimoxazole, nebulised pentamidine or atovaquone).

CMV- and EBV-related disease are recognised, potentially fatal, but preventable, complications following autologous transplantation in patients with Crohn's Disease and active surveillance is mandatory.

For CMV reactivation, CMV Ab-positive participants should undergo CMV PCR screening for the first 100 days post-transplant, according to local standard operating procedures (SOPs) for allogeneic transplantation. As a minimum this should be weekly until day +60 and, if consistently negative, may be reduced to 2-weekly until day +100 post-transplant. Rising EBV PCR levels should be initially investigated according to clinician discretion and local protocols, usually with LDH levels and clinically appropriate imaging (e.g. CT scan, PET-CT scan). Cases considered at risk of EBV-driven post-transplant lymphoproliferative disorder (EBV-PTLD) or other complications should be discussed with the TMG and/or clinical coordinators, irrespective of the EBV PCR level before administration of rituximab or other directed therapy, although treatment for symptom control may be administered in line with local clinical practice.

Local clinical practice will be followed in relation to prophylaxis for Varicella Zoster Virus (VZV) and Herpes Simplex Virus (HSV).

8.1.4 Week 24

Patients with evidence of disease activity at scheduled ileo colonoscopy / endoscopic assessment (SES CD ulcer size score 1 or more in any segment), or MRI scan as assessed by the local investigator will be started on maintenance anti-TNF therapy.

8.1.5 Vaccination

As routine standard of care based on EBMT and IDSA protocols (23) patients will receive pneumococcal conjugate vaccine at 3, 4 and 5 months, followed by conjugate HIB, DTP and inactivated polio vaccine at 6, 7 and 8 months and pneumococcal polysaccharide vaccine at one year. Table 5 summarises these standard vaccinations. A window of + 2 weeks will be permitted for vaccinations to occur.

Table 5: *Standard vaccinations following stem cell transplant.*

Vaccination	Months after stem cell transplant											
	1	2	3	4	5	6	7	8	9	10	11	12
Pneumococcal conjugate			✓	✓	✓							
Conjugate HIB (Haemophilus influenza type b)						✓	✓	✓				
DTP (diphtheria, pertussis & tetanus						✓	✓	✓				
Inactivated polio						✓	✓	✓				
Pneumococcal polysaccharide												✓

All patients who are not on immunosuppressive therapy will have serology for measles and varicella tested at 24 months (as per routine policy). All those who are negative will be immunised with 2 doses of MMR and varicella vaccine at least 4 weeks apart as per routine practice. Pre-vaccination and post-vaccination (at least 1 month after final dose) samples will be tested for pneumococcal serotype-specific antibodies, DTP, HIB and polio titres. Patients will have an annual Influenza vaccine.

Vaccinations will be carried out according to local practice at each site, which is likely to be by primary care teams. However, it is important that the vaccinations occur at the timings specified in this study protocol ("due" date + 2 weeks), and that these are recorded for the immune reconstitution analysis.

Participants will be provided with a vaccination proforma which they should take to their GP or Nurse to record the dates of each vaccination. Participants will be asked to bring this proforma with them to the next study visit after these vaccinations, and the Research Nurse will transfer the information to the study database.

Participants will also be provided with information regarding the need for vaccination for other members of their household. Their treating Haematologist will provide full information as to which vaccinations may be required, and the timing for these.

8.1.6 Participants randomised to Usual Care (control group)

The control group will continue current gold standard medical care until the primary endpoint at 48 weeks plus 49 days (24). Day 0 is calculated as 49 days post randomisation for the Usual Care group, with the aim of mirroring the timeframe for follow ups in the intervention arm, which take into account the length of the HSCT regimen. Usual care can include steroid therapy, immunomodulators, any licensed/approved biologic therapy and enteral or intravenous nutrition. After the week 48 assessment of the primary outcome they will be offered ongoing standard care which may include HSCT if permitted by NHSE specialist commissioning at that time.

8.2 Dispensing

This study does not mandate the use of a specific brand of each IMP, and due to the slow accrual (0.3 per month per site), and small numbers of participants overall at each site, the IMPs and NIMPs will be taken from local hospital stock. Labels for IMPs will not be required.

Site staff should follow the IMP manual for this study. However, whilst dose and exact chemotherapy regimen is specified, the exact brand is not. The IMP manual provides further instruction on dispensing requirements, and handling the IMPs.

In this study, SmPCs replace the Investigator's Brochure (IB) and IMP dossier, and this is reviewed annually and updated as required. The specific SmPCs used for the IMPs in this study are as follows:

- Cyclophosphamide 1000 mg Powder for Solution for Injection or Infusion
- Neupogen 30 MU (0.3 mg/ml) solution for injection (Filgrastim)
- Fludarabine 25 mg/ml Concentrate for Solution for Injection or Infusion
- Thymoglobuline 25 mg powder for solution for infusion

8.3 Accountability

As the products are not labelled, traceability will not be recorded specifically for the trial. Sites are permitted to use either the study-specific accountability log, or their own documentation, providing this covers the product prescribed, the product dispensed, and the batch number.

Site pharmacies will be checked, along with local procedures, at the site initiation visit. Dispensing records are also required to be retained for 25 years, in line with the retention period for this study. Site monitoring will check dispensing records against information recorded in the patient notes.

8.4 Adherence

As the trial treatment is administered by clinical staff, there is no opportunity in this trial for patient-related non-adherence. Records will be maintained in the CRF and in the patient's medical notes to document that doses and regimens are correctly administered.

8.5 Dose Modifications and Interruptions

Dosage is calculated per m^2, although in very overweight patients, recommendations from the American Society of Bone Marrow Transplantation on dose adjustments will be followed, as would be the case in routine HSCT (25).

No formal dose capping will be used as there is reportedly insufficient pharmacokinetic data to suggest that a full weight-based dosing schedule for chemotherapy agents in obese patients should not be used (25). Dose banding is permissible according to local pharmacy policy.

The dose of fludarabine should be reduced if a participant had reduced renal function. In patients with moderate impairment of renal function (creatinine clearance between 30 and 70 ml/min) the dose should be reduced by up to 50% and the patient should be monitored closely. Fludarabine treatment is contraindicated if creatinine clearance is <30 ml/min, and these patients would not meet eligibility criteria if this is the case at the time of screening.

All dose modifications or interruptions require approval from the lead Haematologist (or deputy), and this must be documented on the CRF and in the patient's medical notes.

Although considered unlikely, participants experiencing adverse events relating to an IMP or NIMP used in the study, which are not tolerable, will have this medication discontinued. This may result in a participant being unable to receive the planned intervention, depending on the reaction, and which product this relates to. Any decision to discontinue the study medication would be made by the local investigator and documented.

8.6 Overdose of Study Treatment

An overdose of study treatment is considered unlikely in this study, as participants will be in hospital at the time of receiving medication. In the unlikely event of an error in the dose calculation or administration of the study products, this will be reported to the CTRU and the Sponsor as a protocol non-compliance, as soon as it is identified. This is likely to be assessed as major non-compliance, and the Sponsor will advise on the appropriate action to be taken. The incident will also be reported through normal local Trust reporting procedures.

All medications taken by the participant will be recorded in the CRF, including dosage information, where specified, or overdose.

8.7 Concomitant Medications

Participants will maintain their current schedule of treatment up to the point of randomisation. At this point, those randomised to the usual care arm will continue on their current medication. In the intervention arm, participants will stop any immunosuppressant medication prior to mobilisation. Because mobilisation and conditioning are intensely immunosuppressive, additional immunosuppression is likely to be unnecessary and may pose additional risks. The last dose of infliximab, vedolizumab or ustekinumab should be at least 4 weeks before mobilisation and adalimumab, azathioprine and mercaptopurine should be at least 2 weeks before mobilisation. Immunosuppressive drugs (e.g. methotrexate, cyclosporine) should be stopped at least 1 week before mobilisation. Steroids will be reduced according to a standard regimen after mobilisation; 5mg/day/week up until 5mg, with further reduction or withdrawal based on serum cortisol. Re-introduction of steroids in the conditioning phase of trial treatment will be in line with the guidance in section 8.1.2. In exceptional circumstances, deviations from these arrangements may be permitted by the adjudication panel of TMG members. To ensure this happens in a timely fashion, this decision may be agreed and documented via email rather than at a monthly meeting.

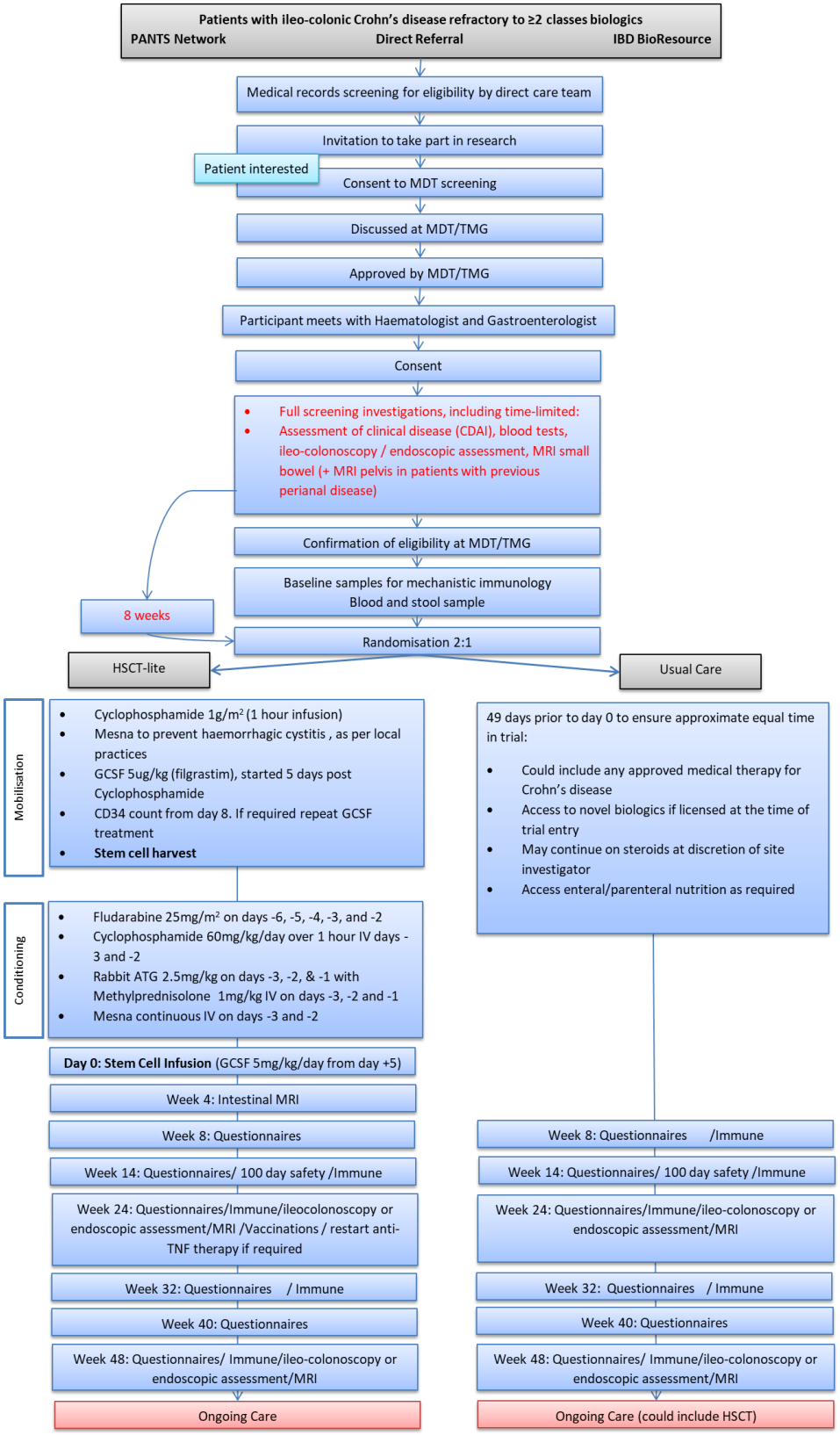
In order to protect against temporary liver function test abnormalities, azoles used for antifungal prophylaxis should be withheld until cyclophosphamide conditioning has been completed.

Participants are permitted to continue on any other medications they may be taking for conditions other than CD, for the duration of the trial. Participants will be monitored for adequate cardiac function during the intervention regimen, as they would in standard care.

Any changes to concomitant medications will be documented on the CRF at each study visit.

9. Assessments and Procedures

Figure 1: *Flow of assessments and participant visits*



9.1 Study Assessment Schedule

The study assessment schedule below details the assessments required during the course of the study. All participants will undergo these assessments regardless of which treatment arm they are randomised to, unless otherwise indicated. A window of +/- 1 week is permitted for each study visit to take place.

Following stem cell transplant, patients will be required to attend hospital between the study visits below. These additional visits will be as per routine practice following stem cell transplants, and will allow ongoing safety monitoring following the procedure.

For those participants receiving the stem cell transplant, the Harvey Bradshaw Index will also be recorded between successful mobilisation, and conditioning.

Day 0: For participants receiving stem cell transplant, day 0 is the date on which stem cells are reinfused. For those in the usual care group, day 0 is calculated as 49 days after randomisation, to align the timescales in both trial groups as much as possible.

Table 6: *Study assessment schedule*

Assessments	Screening[1]	Baseline		Week 4 (HSCT only)	Week 8	Week 14	Week 24	Week 32	Week 40	Week 48
Eligibility assessment	✓	✓	HSCT procedure (intervention) or continuation on current treatment							
Consent	✓									
Standard Pre-HSCT work (including chest x-ray and MUGA scan/Echo)	✓									
Serology for HBV, HCV, HIV	✓									
Demographics	✓									
Medication history	✓									
Concomitant medications	✓	✓		✓	✓	✓	✓	✓	✓	✓
Adverse events				✓	✓	✓	✓	✓	✓	✓
General Medical History	✓									

History of CD	✓									
General Physical Examination	✓	✓			✓	✓	✓	✓	✓	✓
Urinalysis	✓	✓								
Assessments	**Screening[1]**	**Baseline**	**HSCT procedure (intervention) or continuation on current treatment (control)**	**Week 4 (HSCT only)**	**Week 8**	**Week 14**	**Week 24**	**Week 32**	**Week 40**	**Week 48**
Pregnancy test	✓	✓								
Smoking History	✓									
Crohn's Disease Activity Index (CDAI)	✓	✓			✓	✓	✓	✓	✓	✓
Harvey Bradshaw Index	✓	✓			✓	✓	✓	✓	✓	✓
Karnofsky Performance Status	✓									✓
Patient Reported Outcome 2 questionnaire (PRO2)	✓				✓	✓	✓	✓	✓	✓
Ileo-colonoscopy (Simple Endoscopic Score for Crohn's Disease (SES CD)) / endoscopic assessment	✓						✓			✓
Biopsies[2]	✓						✓			✓
MRI Intestine	✓			✓			✓			✓
MRI Pelvis	✓									
Routine Clinical Care blood test		✓			✓	✓	✓	✓	✓	✓
Serum[3]		✓			✓	✓	✓	✓	✓	✓
Whole Blood[3]		✓			✓	✓	✓	✓		✓
Peripheral Blood mononuclear cells (PBMCs)[3]		✓			✓	✓	✓	✓		✓
Stool sample[3]		✓			✓	✓	✓	✓		✓
Inflammatory Bowel Disease Questionnaire (IBDQ)	✓				✓	✓	✓	✓	✓	✓
Inflammatory Bowel Disease Control Questionnaire (IBD-Control)	✓				✓	✓	✓	✓	✓	✓
100 day safety (collection of Adverse Events for transplant endpoint)						✓				
EQ-5D-5L	✓				✓	✓	✓	✓	✓	✓
Work Productivity and Activity Impairment questionnaire (WPAI)	✓				✓	✓	✓	✓	✓	✓
Health care resource use questionnaire	✓				✓	✓	✓	✓	✓	✓

Patient Global Impression of Change (PGIC)										✓
For participants in HSCTlite arm only:										
JACIE and HTA recommended routine tests		✓								
Anti-TNF therapy initiated (if required)							✓			
Adherence to re-vaccination policy						✓	✓	✓	✓	✓

1 – Screening assessments will take place over a few weeks, any investigations or tests required as part of eligibility assessment will only take place once a participant has provided written consent. Standard pre-HSCT work will include all investigations recommended by the EBMT, including chest x-ray, MUGA or cardiac echo, ECG, pulmonary function tests and blood tests, in addition to the MRIs and colonoscopy defined in this protocol.

2 – A minimum of 12 biopsies will be taken at each ileo colonoscopy / endoscopy (3 samples from an inflamed and a non inflamed segment of ileum and colon, unless there is a clinical reason this is not possible). One biopsy from each site at each colonoscopy (n=4) will be sent to the local laboratory for analysis and reporting, and the remaining samples sent to the John van Geest Cancer Research Centre, Nottingham, as part of the mechanistic study, and for future research.

3 - Serum, whole blood, and stool samples will be frozen as appropriate, and stored in the local laboratory for batch sending to the John van Geest Cancer Research Centre. Stabilised whole blood will be packaged and sent to the central lab on a per patient basis.

9.2 Unscheduled Study Visits

Participants' local care team may also be part of the research team for ASTIClite. Therefore, participants may be seen at additional visits outside those scheduled for the study, but these visits would be part of usual care. Any adverse events identified at additional usual care visits, will be documented in the CRF.

9.3 Procedures for Assessing Efficacy

Blood samples

Routine blood tests will be analysed in local laboratories. Samples for mechanistic studies will be processed on site according to study-specific standard operating procedures and shipped to the John van Geest Cancer Research Centre for analysis (see section 5).

Ileo-colonoscopy (baseline, 24 & 48 weeks):

Ileo-colonoscopy will be performed according to local practice using a standard bowel preparation and conscious sedation. If the disease is limited to the small intestine ileoscopy / enteroscopy may be performed. Videos of withdrawal from all endoscopies will be recorded. Eligibility for trial inclusion at baseline and the requirement for anti TNF therapy at week 24 in the intervention group will be based upon local PI assessment of endoscopic assessment using SES CD. In patients where disease is proximal to the ileum, the SES CD will still be used to score the diseased bowel segment present. All videos will be scored using the SES CD by investigators blinded to site, treatment allocation and timing of procedure for analysis of primary and secondary outcomes. Biopsies for routine histology will be sent to local laboratories in formalin. Biopsies for mechanistic analysis will be placed into RNAse reagent overnight and stored on site in -80 freezers. Shipments will be sent every three months as above. Full details of the ileo colonoscopy procedures are documented in the study-specific SOP.

MRI scan

MRI scans will be undertaken according to standard clinical protocols, using at a minimum, a 1.5T scanner using gadolinium contrast.

MRIs will be undertaken at baseline and week 48 in all participants, and will be scored using the validated MaRIA tool for disease activity. These scans will be read locally by a radiologist at each of the participating centres, as well as sequences being recorded to CD. A sample of these scans will also be scored centrally, by an investigator who is blind to the timing of assessment and treatment assignation, to confirm consistency. All site radiologists who will score the MRI scans will be trained in using the MaRIA tool, and guidance for the scoring system will be provided to all sites.

Participants in the intervention group will undergo an MRI scan at week 4, as part of the mechanistic analysis, to determine the early impact of the intervention on mucosal disease.

A further MRI scan is completed at the week 24 visit. The main purpose of this MRI is to inform the decision as to whether participants undergoing HCST will restart anti-

TNF therapy. The week 24 MRIs may also be recorded to CD to allow central reading.

The full procedure for MRI and the minimum sequences required are specified in the study-specific SOP.

Crohn's Disease Activity Index (CDAI)
The CDAI will be calculated using the standard scoring criteria. A cap of minus 10 will be used in relation to minus scores for those participants exceeding standard weight ranges.

The patient will be asked to complete a symptom diary for a week prior to assessment of the CDAI; this cannot be taken immediately preceding a colonoscopy. Patients should finish the diary prior to starting bowel prep for colonoscopy.

Harvey Bradshaw Index and PRO2 questionnaires
Both the Harvey Bradshaw Index and PRO2 questionnaires will be completed by the Research Nurse at each study visit, based on reports from the patient, and information from their medical notes.

9.4 Procedures for Assessing Safety

In accordance with EBMT and NHS England guidelines, decisions about patient eligibility and clinical management decisions will be made by an expert MDT comprising of clinical members of the TMG. Decisions on eligibility will be made either at the monthly MDT meeting, or via email to ensure timely decisions. At least two members of the panel must agree on eligibility in order for a patient to be included in the trial.

If there are any clinical concerns about a participant, identified through any of the research procedures or assessments, these will be referred to the appropriate clinical team for further investigation. This includes abnormal blood results, responses to questionnaires that cause concern about the participant's wellbeing, and any other concerns aside from the expected course of CD.

The DMEC will assess safety of the HSCTlite mobilisation regimen after the first 10 patients, and subsequently at each DMEC meeting. Should the protocol fail to mobilise 2 x 10^6/kg CD34+ cells (haematopoietic stem and progenitor cells) in more than 10% patients, or if greater than 10% patients experience a disease flare up (increase in Harvey Bradshaw Index of >30% from baseline associated with a rise in CRP) during mobilisation, a protocol amendment will be submitted to modify the mobilisation regimen for subsequent patients.

Responsibility for GCP adherence and recording adverse events will be the delegated responsibility of the PI and research team at each site documented in the signed clinical trial agreement. All adverse events will be recorded in the CRF and will use the NCI classification of toxicity for 100-day safety post HSCT for assessment of grade.

An adverse event will be defined as any untoward or unfavourable medical occurrence during the course of the study, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not

considered related to participation in the research. All adverse events (AE), serious adverse events (SAE) and Suspected Unexpected Serious Adverse Reactions (SUSAR) will be captured. All SAEs and SUSARs will be reported in accordance with the CTRU's standard operating procedure. Delegated site trial staff will be responsible for recording all adverse events and making them known to the Principal Investigator. Causality will be assigned by the PI or delegated deputy. The Summary of Product Characteristics (SmPCs) for relevant products will be used as the reference safety information for reporting SAEs. Adverse events will be recorded from consent throughout the mobilisation and conditioning period and at every visit after that time until the study closes.

The PI is responsible for ensuring the assessment of expectedness and relatedness for all SAEs (except those exempt from expedited reporting, see section 10.5) is completed within 24 hours of identification of the event, and reported to the CTRU. The CTRU will ensure all events are reported on to the Sponsor within 24 hours of the investigator becoming aware of the event. SUSARs are subject to expedited electronic reporting as indicated in the CTRU's SOP and section 10.5 of the protocol. The DMEC will receive a report of SAEs at a frequency agreed with the committee, and an annual safety report will be submitted to the MHRA and the Research Ethics Committee.

Any instances where the study protocol is not followed, either in error, or otherwise, should be documented using a protocol non-compliance form. Some pre-specified minor protocol non-compliances will be agreed with the Sponsor prior to the start of the study, and documented in the Trial Monitoring Plan. Occurrences of these pre-specified non-compliances may be signed off by the Study Manager. Any other protocol non-compliances will be reported to the Sponsor on an individual basis, and an assessment of the severity, and requirements for corrective and preventative action agreed. A summary of the protocol non-compliances to date will be provided at each TMG, TSC and DMEC meeting.

9.5 Procedures for Assessing Quality of Life

At baseline, week 8, 14, 24, 32, 40 and 48, participants will be invited to complete questionnaires to assess their quality of life, and health care resource utilisation, as described in section 9.1.

Analysis of this data in relation to cost effectiveness is not currently part of this study protocol. However, this analysis may be performed at a later date, subject to availability of funding to do so.

Questionnaires will be provided in print to the participant for self-completion. Data from the paper questionnaires will be entered onto the study database by the local research team. At the time of completion, questions may be clarified for the participant if the question is not understood, but the researcher will not provide any bias towards any of the answer options. The questionnaires must be completed by the participant themselves, and all will be completed at face-to-face study visits. In the case of the IBDQ, a stoma version of the questionnaire is available for patients where this is relevant.

9.6 Loss to Follow-up

Participants will be defined as lost to follow up if they do not attend or contribute data at the week 48 visit. If a participant is lost to follow up, this will be recorded in the CRF using the study completion/discontinuation form.

9.7 Participant Withdrawals

Participants may withdraw their consent for the study at any time, without providing a reason for this. If this occurs, this will be documented on a study completion/discontinuation form and the patient notes, and no further data will be collected for this participant for the study. If a participant does volunteer a reason for their withdrawal of consent, this will be documented on the form. Any data collected up to the point of the participant's withdrawal will be retained, and used in the final analysis, and this is made clear to the patient at the time of consent.

Participants may also wish to withdraw from the intervention, or there may be a clinical need to withdraw the participant (see section 6.9). Participants will be considered as having received the intervention, if they receive the stem cell transplant on Day 0. Participants withdrawing from the intervention prior to stem cell re-insertion will be followed up at subsequent time points, unless they withdraw from the trial.

9.8 Site and study closure procedures

The study will end after the last follow-up visit of the last study participant. Sites will be closed once data cleaning is completed and the regulatory authority and ethics committee have been informed.

10. Safety Reporting

ICH-GCP requires that both investigators and sponsors follow specific procedures when reporting adverse events/reactions in clinical studies. These procedures are described in this section.

For this study, the products have been categorised as either Investigational Medicinal Product (IMP), or Non-Investigational Medicinal Product (NIMP) (see section 8.1). NIMPs are medicinal products which are not the object of investigation, but which are specified in the protocol. These include any specified medicinal product given to participants to mitigate the effects of an IMP, or support/rescue medication.

10.1 Definitions

The definitions of the EU Directive 2001/20/EC Article 2 based on the Principles of ICH-GCP apply to this protocol. These definitions are given in Table 7 below.

Table 7: *Definitions of Adverse Events and Reactions*

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical study patient to whom a medicinal product has been administered irrespective of relationship
Adverse Reaction (AR)	Any AE that is judged, in the opinion of the PI, to be related to an investigational medicinal product or a non-investigational medicinal product.

Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SmPC).
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: • Results in death • Is life-threatening* • Requires hospitalisation or prolongation of existing hospitalisation** • Results in persistent or significant disability or incapacity • Congenital anomaly/birth defect • Is another important medical event***

**The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.*

***Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, that has not worsened or for an elective procedure do not constitute an SAE.*

****Other important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event/experience when, based upon appropriate medical judgement, they may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.*

10.2 Recording and reporting

All AEs and ARs will be recorded on the adverse event report form, within the participant CRF, including those that fulfil the criteria for being serious (see section 10.1). Sites are asked to enter all available information onto the ASTIClite study database as soon as possible after the site becomes aware of the event.

SAEs, SARs and SUSARs will require more detailed information to be recorded. In such cases, the event must also be reported to the Sheffield CTRU within 24 hours of the site becoming aware of the event. The CTRU will notify the Sponsor of each of these events. There are some events which do not require immediate reporting (see section 10.5).

10.3 Study Centre/Investigator Responsibilities

All AEs and ARs, whether expected or not, will be recorded in the participant's medical notes and recorded on an adverse event form within the CRF. SAEs and SARs will be notified to the CTRU within 24 hours of the investigator becoming aware of the event, unless these are any of the expected SARs defined in section 10.5.

For this study, the NIMPs are Mesna and methylprednisolone.

Where there is a possibility of an interaction between a NIMP and an IMP, the MHRA require these to be reported as SUSARs. There are no known interactions between any of the IMPs and NIMPs used in this study, however, if an investigator suspects an interaction has occurred between the two types of products, Sheffield CTRU should be contacted within 24 hours of becoming aware of the event.

If an adverse reaction associated with a NIMP is likely to affect the safety of the trial participants, the site must inform the CTRU, within 24 hours of becoming aware of the event. The decision as to whether the reaction is likely to affect the safety of the trial participants is a chief investigator decision, or a delegated deputy in the CI's absence.

10.3.1 Assessment of relatedness

The investigator should make an assessment of relatedness prior to sending the SAE form to the CTRU. For this study, the Reference Safety Information (RSI) will comprise of the Summary of Product Characteristics (SmPCs) for the IMPs and NIMPs to be used. The appropriate SmPCs should be referred to when making the assessment of relatedness.

For the standard care arm, participants could be taking a variety of medications. As would happen in usual clinical practice, the relevant SmPC should be referred to in these cases, although these SmPCs will not form the RSI.

10.4 SAE Notification Procedure

CTRU will be notified of all SAEs, within 24 hours of the investigator becoming aware of the event (except for expected SARs, see 10.5). Investigators must notify CTRU of all SAEs occurring for each participant from the time of consent until the participant has completed the trial (i.e. 48 week follow-up period)

The SAE form must be completed by the investigator (a clinician named on the delegation log who is responsible for the participant's care). In the absence of the investigator the form will be completed by a member of the study team and faxed/emailed as appropriate. The responsible investigator will subsequently check the SAE form, make changes as appropriate, sign and re-send the form to CTRU as soon as possible.

All SAE forms must be sent by fax to 0114 222 0870 or email to ctru-saes-group@sheffield.ac.uk. Receipt of the initial report should be confirmed within one working day. The site research team should contact the study team at CTRU if confirmation of receipt is not received within one working day.

Concomitant medications are recorded throughout the study and will not be collected on AE/SAE forms as standard. However for any event classified as a SAR or SUSAR CTRU may request additional information on concomitant treatments to facilitate onward reporting.

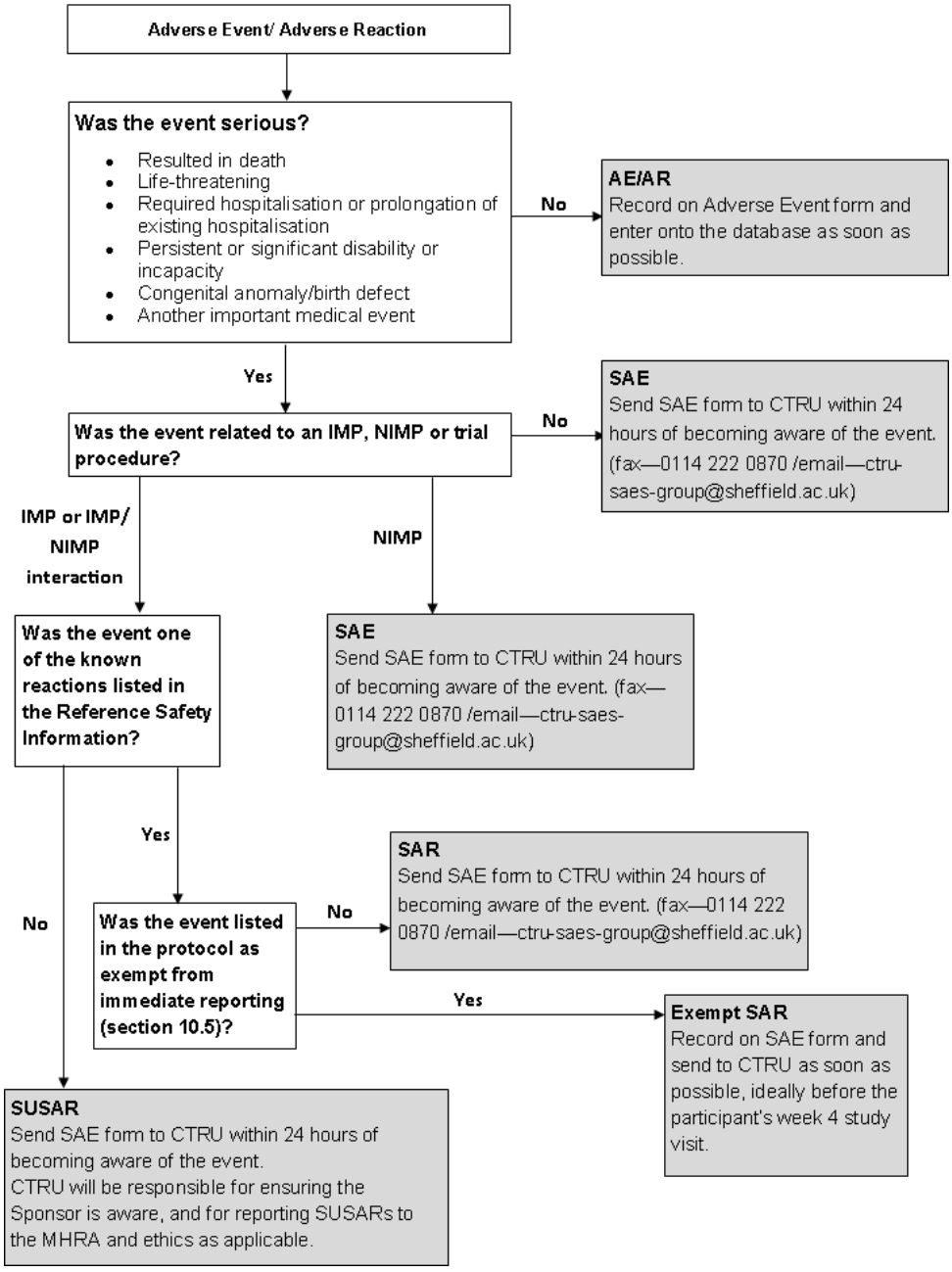
10.4.1 Follow up

Initial SAE reports must be followed by detailed reports when further information becomes available.

Participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow up information will be provided on an SAE report marked as such.

Further clarification on the reporting process can be seen in Figure 2.

Figure 2: *Procedure for AE/SAE reporting*



10.5 SARs that do not require immediate reporting

Patients receiving chemotherapy may require admission to hospital for appropriate medical intervention following development of some of the more severe known side effects of treatment. Where these events are confirmed as reactions (see section 10.3.1 for assessment of relatedness) these are regarded as expected Serious Adverse Reactions (SARs) for the purposes of this study, and do not require immediate reporting by site.

The following SARs are exempt from immediate reporting, for the purpose of this study:

- Admissions to control symptoms of vomiting and diarrhoea, unless the condition requires admission to a high dependency or intensive care facility, or is life threatening or proves fatal (i.e. grade 4 or above, according to NCI CTCAE criteria)
- Admissions for supportive treatment during an episode of febrile neutropenia, unless this proves fatal or requires admission to a high dependency or intensive care facility (i.e. grade 4 or above, according to NCI CTCAE criteria)
- Admissions relating to myelosuppression unless the condition requires admission to a high dependency or intensive care facility, or is life threatening or proves fatal (i.e. grade 4 or above, according to NCI CTCAE criteria)
- Admissions relating to skin reactions and abnormal liver function tests caused by supportive care medications, unless the condition requires admission to a high dependency or intensive care facility, or is life threatening or proves fatal (i.e. grade 4 or above, according to NCI CTCAE criteria)

10.5.1 Timelines for reporting expected SARs

The expected SARs defined in section 10.5 should be reported on an SAE form. SAE forms should be completed and returned to Sheffield CTRU via fax or email as soon as possible, ideally before the participant's week 4 study visit.

10.6 CTRU Responsibilities

The Chief Investigator or delegate will be responsible for the assessment of expectedness to confirm agreement with the site investigator. An unexpected adverse reaction is one not defined in the protocol as expected (section 10.5), not previously reported in the Reference Safety Information (RSI) used in the study, or one that is more frequent or more severe than reported in the RSI. If a SAR is assessed as 'unexpected', it is classified as a SUSAR.

The CTRU is responsible for reporting each SAE to the Sponsor, so that these can be reported to the MHRA and REC as appropriate.

If an adverse reaction associated with a NIMP is likely to affect the safety of the trial participants, the CTRU must inform the Sponsor as soon as the notification is received from the site, so that this can be reported to the MHRA and REC as an urgent safety measure, a substantial amendment or via a notification to terminate the trial early, as applicable. The decision as to whether the reaction is likely to affect the safety of the trial participants is a chief investigator decision, or a delegated deputy in the CI's absence.

The DMEC and TSC will also receive information on all AEs and SAEs, at a frequency agreed with each committee and documented in the appropriate charter/terms of reference.

10.7 SUSARs

All SUSARs should be recorded on an SAE form, and faxed or emailed to the CTRU within 24 hours of discovery. The CTRU will be responsible for reporting SUSARs to the Sponsor, for notification to the MHRA. Each site will be informed of SUSARs occurring across the study.

10.8 Reporting pregnancies during the trial

Female participants, and male participants with female partners, will be advised to use an adequate form of contraception during the course of the study; however, it is possible that women could become pregnant during the follow-up phase of the study. If this occurs, this will be recorded using the Pregnancy Report form within the CRF, and also (for participants in the intervention arm) notified to the EBMT. The Chief Investigator and the TMG will be informed, so that a discussion can take place regarding the participant's continuation in the study. Any such discussions will be documented, and recommendations for continuation or discontinuation in the study will be made according to clinical judgement.

Any participant who becomes pregnant during the course of the study will be followed up, irrespective of any treatment withdrawal or changes.

The DMEC and TSC will be advised at each meeting, of any pregnancies reported since their previous meeting.

11. Statistical Considerations

11.1 Study Hypothesis

There is limited evidence for haematopoietic stem cell transplant with low dose cyclophosphamide/G-CSF mobilisation and low intensity conditioning (HSCTlite) as a treatment for CD, and its use will continue to be infrequent unless its benefit is demonstrated. The study will assess whether HSCTlite is superior to standard care by assessing disease activity, quality of life and safety following HSCTlite in comparison to that observed following standard care. A mechanistic substudy will undertake immunological profiling of peripheral blood and mucosal biopsies before and after HSCTlite in order to give insight into its mechanism of action and CD pathogenesis as well as identify the mechanism of subsequent responsiveness to anti-TNF therapy.

11.2 Sample Size

The values in the calculations are based upon the endoscopic assessment post HSCT reported in the ASTIC trial program (9,10). For the primary outcome, to detect a significant difference in the proportion of patients with absence of ulceration on endoscopic assessment of 35%, based on 50% in the HSCT group and no more than 15% in the control group, with 90% power at 5% significance level requires 62 patients in the HSCT group, and 31 in the control group. Therefore, 93 patients will be recruited at baseline, using 2:1 randomisation. Due to the nature of the condition, the design of the intervention and control group, the definition of the primary endpoint and our experience in the ASTIC trial, we anticipate a 6% drop out rate and will therefore recruit 99 patients (66 in the intervention group and 33 in the control group).

Based on experience in ASTIC, recruitment is anticipated to take 36 months. Patients will be recruited at 8 UK NHS Trusts, at an anticipated rate of 2.75 per month across all sites, or approximately 4 patients per site per year.

11.3 Statistical Analysis

Clinical Trial

Analyses will be reported in line with the Consolidated Standards of Reporting Trials (CONSORT) 2010 Statement. Confidence intervals will be two-sided, 95% intervals and hypothesis tests will use a two-sided 5% level of significance. The primary analyses will be carried out using the intention to treat principle with data from all participants included in the analysis, including those who do not complete therapy and with participants analysed by the group to which they were randomised. The trial may terminate early on futility or safety grounds but no formal stopping rules are defined.

Analysis will be conducted in Stata version 14 or other validated statistical software as agreed by the study statisticians. Descriptive statistics within each randomised group will be presented for baseline values. These will include counts and percentages for binary and categorical variables and means and standard deviations, or medians with lower and upper quartiles, for continuous variables, along with minimum and maximum values and counts of missing values. There will be no tests of statistical significance or confidence intervals for differences between randomised groups on any baseline variable. Descriptive statistics will be used to summarise assessments of feasibility and acceptability in terms of recruitment, drop-out and completeness of therapy.

To test the primary hypothesis of a between group difference in the proportion of patients with absence of ulceration, we will estimate the proportions for each group. A mixed effects logistic regression will be used to estimate odds ratios for the disease remission in HSCTlite in comparison to conventional therapy. Baseline SES-CD ulcer subscore will be included as a fixed effect and study centre as a random effect. A number of sensitivity analyses will be carried out on the primary outcome, including assessing the impact of missing outcome data, adjustment for baseline predictors of missing outcome data and complier average causal effect (CACE) analysis.

For the secondary outcomes, analogous parametric regression models will be used as appropriate to the distributional form of the outcome, controlling for study centre, and the corresponding baseline assessment for the outcome under investigation where appropriate.

We will check for differential predictors of missing outcomes by comparing responders to non-responders on key baseline variables. Any predictors that qualitatively appear imbalanced will be included in the analysis models. This accounts for missing outcome data under a missing at random assumption, conditional on the covariates included in the model. As a sensitivity analysis, we will assess whether non-receipt of treatment in the intervention arm is associated with participant characteristics, and if it is associated, use inverse probability weights or multiple imputation to calculate a “protocol-compliant adjusted” treatment effect which we will compare against the primary analysis. For the purpose of these analyses, any participant who has received the stem cell transplant will be considered to have undergone treatment.

A secondary mediation analysis will investigate putative mediational factors using modern causal inference methods. This involves using parametric regression models to test for mediation of HSCT on treatment success through biomarkers. Analyses will adjust for baseline measures of the marker, and possible measured confounders.

Mechanistic immunology

The complex datasets will be integrated, analysed and interpreted using established artificial neural network (ANN) and computational intelligence-based approaches. The expertise is available in the John van Geest Cancer Research Centre and has been used by them previously (26,27). We will use adaptions of existing neuro-fuzzy computational intelligence models (28) to answer the questions posed. Importantly, these approaches will provide mechanistic insight into underlying responsiveness to anti-TNF and events that are associated with patients becoming refractory to it after HSCT.

Further details of statistical analysis for the ASTIClite study will be provided in a detailed Statistical Analysis Plan.

12. Study Supervision

The ASTIClite study will be led by the Chief Investigator working in co-ordination with the co-applicants and Sheffield CTRU. The Sponsor will be Bart's Health NHS Trust. Sheffield CTRU will take responsibility for project management and have set up a collaborator agreement for governance and safety reporting with the Sponsor. Specialist support for set up of a CTIMP involving HSCT will be provided by both Birmingham CRCTU and Miranda Clark (the trial co-ordinator for the ASTIC trial). There is a dedicated trial manager who is supervised by the CI and the Assistant Director of Sheffield CTRU, meeting at weekly intervals, and will liaise with the whole study team. Dr Hind will provide oversight for delivery of all CTRU support including trial management, data management, QA, randomisation, statistics, health economics, analysis reporting and dissemination. Health Research Authority (HRA) approval will be sought prior to commencement of the trial at participating centres.

Three committees will govern study conduct, deliver the trial, monitor study performance and ensure its safety; TSC, DMEC and Trial Management Group (TMG). The committees will function in accordance with Sheffield CTRU standard operating procedures. We intend to ensure appropriate expertise by inviting members of the EBMT autoimmune disease working party to serve on these committees.

12.1 Trial Steering Committee (TSC)

The TSC will consist of an independent chair, gastroenterologist and haematologist and two patient representatives identified through the ASTIC trial. The role of the TSC is to provide supervision of the protocol and statistical analysis plan, to provide advice on and monitor progress of the study, to review information from other sources and consider recommendations from the DMEC. The TSC can prematurely close the trial, should this be recommended by the DMEC. The TSC will meet at six monthly intervals as outlined in the TSC terms of reference.

12.2 Data Monitoring and Ethics Committee (DMEC)

The DMEC will consist of an independent statistician, gastroenterologist and haematologist with clinical trial expertise. The DMEC will review reports provided by the CTRU to assess the progress of the study, the safety data and the critical

endpoint data as required. No formal interim analyses and stopping guidelines are set in advance. The DMEC will assess the accumulating data, and in particular any data related to the safety of low dose cyclophosphamide/GCSF mobilisation, in accordance with the DMEC charter.

The DMEC will meet at least yearly with meetings comprising an open session to which members of the study team may attend, followed by a closed session with independent members only and to which unblinded data will be available. The DMEC may recommend the trial is stopped or modified on the basis of the data, in writing, to the chair of the TSC.

12.3 Study Management Group (TMG)

The Study Management Group (TMG) consists of the CI, other site PIs, collaborators and staff from CTRU. The CI will chair monthly meetings to discuss the day-to-day running of the study, including any implementation issues. The TMG will receive reports from the TSC and DMEC to manage trial progress. All potential participants will be discussed with clinical members of TMG to confirm eligibility prior to randomisation. The clinical panel will complete case evaluation of each potential participant and recommend patients for screening investigations for trial entry as appropriate. This may be at a scheduled TMG meeting, or outside the meetings, as appropriate within the study timelines. At least two clinical members of the TMG must agree eligibility before a patient can be included in the trial. Once TMG members have confirmed a potential participant appears eligible, consent will be taken, followed by further eligibility investigations, before full eligibility is reconfirmed with TMG members. This confirmation of eligibility may take place via email, and be confirmed by the CI (or delegate), to avoid delays in randomisation.

13. Data Handling and record keeping

Participant confidentiality will be respected at all times and the principles of the UK Data Protection Act (DPA) will be followed. The investigator will ensure that identifiable data is kept securely and protected from unauthorised parties.

Data management will be provided by the University of Sheffield Clinical Trials Research Unit (CTRU) who adhere to their own Standard Operating Procedures (SOPs) relating to all aspects of data management, including data protection and archiving. A separate data management plan (DMP) will detail data management activities for the study in accordance with SOP (Shef/CTRU/DM009), and the contract between CTRU and Bart's Health will document which areas of the Bart's Health Data Management SOPs are being followed in addition to the requirements of the Sheffield CTRU SOP.

The investigator or delegate at each site will maintain comprehensive and accurate source documents to record all relevant study information regarding each participant. The CTRU will provide worksheets (shadow CRFs) to allow the site staff to check what is required for a visit. The worksheets do not need to be completed if alternative source documentation is provided. However, they must be completed for data points where source documentation is not collected elsewhere and where completed, worksheets must accurately reflect the database as they form part of the source data.

Participants will only be identified on the study database by their names, if they consent to being sent information about the study, such as being informed of the results once the study is complete. Their name and email address and/or postal

address will be collected on the Contact Details CRF. All other CRFs will only identify the participant by their study ID number. All participants will be assigned a unique study ID number at screening that will link all of the clinical information collected for them on the study database. It will also be used in all correspondence between CTRU and participating centres.

Study records, including source data, will be stored for 25 years after the completion of the study by participating sites, before being destroyed. Each investigator is responsible for ensuring records are retained and securely archived during the retention period and information supplied to the Chief Investigator and Sponsor. Where trial related information is documented in the medical records, those records will be retained for at least 25 years after the last patient last visit. Access will be restricted to authorised individuals.

Data held by the CTRU will be stored in accordance with the archiving Standard Operating Procedure (CTRU SOP PM012) for 25 years following completion. Archived documents will be logged on a register which will also record items retrieved, by named individuals, from the archive. Electronic data will be stored in an 'archive' area of the secure CTRU server for a minimum of 25 years to ensure that access is future-proofed against changes in technology. Electronic data may also be stored (e.g. on a compact disc or USB flash drive) with the paper files. Archived documents will be transferred to the Sponsor before destruction.

Laboratory specimens will be labelled without the use of patient identifiable information. Labels will contain study ID, type of sample, and the date the sample was taken, and will be cryo-labels to withstand freezing of the sample.

14. Data access and quality assurance

The study will use the CTRU's in-house data management system (Prospect) for the capture and storage of study specific participant data. Access to Prospect is controlled by usernames and encrypted passwords, and a comprehensive access management feature will be used to ensure that users have access to only the minimum amount of data required to complete tasks relevant to their study role. This feature can also be used to restrict access to personal identifiable data.

The study nurse at each site will enter data from source documents into the study specific Prospect database when available. After data have been entered, electronic validation rules are applied to the database on a regular basis; discrepancies are tracked and resolved through the Prospect database. All entries and corrections are logged with the person, date and time captured within the electronic audit trail.

Participant confidentiality will be respected at all times. All research data will be anonymised, and will only be identifiable by the participant's study ID number. No patient identifiable data will be transferred from the database to the statistician.

Participating investigators shall agree to allow study-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required. Participants' consent for this will be obtained as part of the consent process.

14.1 Site Assessment

Throughout this protocol, the trial 'site' refers to the hospital at which trial-related activities are conducted. Participating sites must be able to comply with:

- Trial treatments, imaging, clinical care, follow up schedules and all requirements of the trial protocol
- Requirements of the Research Governance Framework and the Medicines for Human Use (clinical trials) Act (SI 2004/1031 and all amendments)
- Data collection requirements
- HSCT procedures must be undertaken at a transplant centre accredited by JACIE for allogenic transplants in adults, or for autologous transplants in adults if they have previous experience of autologous HSCT for CD

All participating sites must have an appropriate Principal Investigator (PI) i.e. a health care professional authorised by the site, ethics committee and regulatory authority to lead and coordinate the work of the trial on behalf of the site. Other investigators at site wishing to participate in the trial must be trained and approved by the PI, and this must be documented on the site delegation log. All investigators must be medical doctors and have experience in either autologous stem cell transplants, or working with patients with CD.

All site staff, including research staff, must be appropriately qualified by education, training and experience to perform the trial related duties allocated to them, which must be recorded on the site delegation log. CVs for all staff must be kept up to date, and copies held in the Investigator Site File (ISF), and the Trial Master File (TMF). Staff should also have completed GCP training within the last 2 years, ensure this is renewed every 2 years, and copies of the GCP certificate are held within the ISF and TMF.

Before each site is activated, capability to conduct the trial will be assessed and documented using a site assessment form. The CTRU will arrange a site initiation visit with each site, site staff will be trained in the day-to-day management of the trial and essential documentation required for the trial will be checked. Once all the required documentation is in order, and site staff have been trained, CTRU will formally activate the site to start recruitment. Sites should not open to recruitment until CTRU have provided this confirmation of activation.

14.2 Risk Assessment

Risk assessment will be performed by the Sponsor and CTRU prior to the start of the study, in accordance with Sheffield CTRU Standard Operating Procedures. The level of risk will be agreed with the Sponsor. Central and on-site monitoring (including Pharmacy) will be undertaken at a level appropriate to the detailed risk assessment, and will be documented in the Trial Monitoring Plan (TMP). This will include (at a minimum):

1. Source Data Verification (SDV)
2. SAEs/SUSARs – reported to the Sponsor and followed up to resolution
3. Resolution of data queries
4. Investigator site file maintenance
5. Training records for site staff (trial specific and GCP) and appropriate delegation of duties
6. Patient consent procedures
7. Reporting of protocol non-compliances

14.3 On-site Monitoring

On-site monitoring will be performed according to the ASTIClite TMP and in line with the Sheffield CTRU Study Monitoring SOP. The overall principles of the Bart's Health Monitoring SOP will be adhered to, where this is possible within Sheffield CTRU's SOP, and this is documented in the contract between CTRU and Bart's Health.

A site initiation visit will be performed at each participating site before each site recruits their first participant. During this visit, the Monitor will review with site staff the protocol, study requirements and their responsibilities to satisfy regulatory, ethical and Sponsor requirements.

Regular site monitoring visits will occur throughout the study and additional visits will be undertaken where required. At these visits, the Monitor will review activity to verify that the:

1. Data are authentic, accurate and complete.
2. Safety and rights of the patient are being protected and
3. Study is conducted in accordance with the approved protocol and study agreements, GCP and all applicable regulatory requirements.

Accurate and reliable data collection will be assured by verification and cross-check of the eCRF against Investigator's records by the Study Monitor (source document verification) (see section 13 for further details on data collection). Study Monitor will contact and visit sites regularly to inspect CRFs throughout the study, to verify adherence to the protocol and completeness, consistency and accuracy of the data being entered on the CRFs. Monitoring visits will also include a pharmacy visit to review processes, documentation and accountability of study drug.

A close-out visit will be performed after the last patient last visit at each site. Further close-out activities may be carried out remotely after this time, up to database freeze.

14.4 Central Monitoring at CTRU

CTRU staff will review entered data for possible errors and missing data points. A central review of consent forms will also be completed, and sites will be requested to post consent forms to CTRU on an ongoing basis. This will be made clear to the participant prior to their consent to the trial. CTRU will receive pharmacy dispensing logs centrally, which will be taken to on-site monitoring visits to allow full source data verification. Details will be included in the pharmacy manual.

14.5 Regulatory information

As a CTIMP, the trial will be conducted in accordance with ICH GCP and the Clinical Trials and Medicine for Human Use (Clinical Trials) Regulations 2004. A site agreement between the Sponsor, participating sites and Sheffield CTRU outlines responsibilities of all parties and is to be signed prior to commencement of recruitment at sites. All clinicians responsible for recruiting patients to the trial will be required to complete training in International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP).

Although ASTIClite involves autologous HSCT, all HSCT procedures will be undertaken at sites accredited in accordance with the international quality standards for clinical and laboratory practice in Haematopoietic Cell Therapy of the Joint

Accreditation Committee for ISCT and EBMT (JACIE), either for allogeneic HSCT or for autologous HSCT if there is previous experience in autologous HSCT for CD (e.g. in the ASTIC trial). As part of usual clinical practice, the United Kingdom National External Quality Assessment Scheme for Leucocyte Immunophenotyping, will ensure participating centres achieve national quality standards for stem cell harvest and storage.

15. Other Related Research

As part of normal standard of care following HSCT, patients are required to undergo an annual assessment as part of registry based follow up through the EBMT. An additional project; the ASTIClite EBMT Registry Follow up study (IRAS number: 228818) intends to use this resource to obtain long term follow up data on participants from the ASTIClite study, for a minimum of 4 years.

After the 48 week visit that forms the primary endpoint for the ASTIClite study, all participants will be followed up to assess disease activity, adverse events, quality of life (IBDQ, IBD-Control and EQ5D) and healthcare resource utilisation for at least four years after HSCT through an annual assessment. Data will be collected on site using the disease specific MED B form for CD and stored on the EBMT PROMISE database.

In addition, patients in the control arm in ASTIClite will also be followed up through the EBMT registry. After the week 48 visit, some participants may choose to take up HSCT post trial, and data from these participants will be included on the PROMISE database. For any participants in the control arm who do not go on to have HSCT, they will still complete annual follow up visits, and data will be stored within a separate database within the EBMT.

The main objective of the ASTIClite EBMT Registry Follow up study is to investigate the long term benefit and safety of HSCTlite using registry based follow up for a further four years.

The associated endpoints are:

- For four years after the trial:
    - Long term efficacy
    - Safety (through documentation of adverse events)
    - Quality of life (EQ-5D-5L,IBDQ and IBD-Control)
    - Health care resource utilisation (using patient reported questionnaires)
    - Late effects of both HSCT and best available therapy as defined in Table 1

16. Publication

Results of the study will be disseminated through peer reviewed scientific journals and at clinical and academic conferences, as well as submission of a final report to the funder, which will be made available online.

Details of the study will also be made available on the Sheffield CTRU website. Summaries of the research will be updated periodically to inform readers of ongoing progress.

Information throughout the course of the study may be disseminated at conferences and other events, providing this does not relate to any endpoint, but these must be with the approval of the Chief Investigator, and the funder must be informed with sufficient notice.

The study will also be added to the EudraCT trial repository.

The results will be published on a freely accessible database within one year of completion of the trial. Anonymised datasets will be made available after publication of the main trial results.

Full details, including guidance on authorship are documented in the ASTIClite Publication and Dissemination Plan.

17. Finance

ASTIClite is funded by the UK NIHR Efficacy and Mechanism Evaluation (EME) Programme (project number 15/178/09) and details have been drawn up in a separate agreement. Participants can be reimbursed for the cost of reasonable travel expenses. Further details are included in the site agreement.

18. Ethics approval

Before initiation of the study at participating sites, the protocol, informed consent forms, and information materials to be given to the participants will be submitted to an NHS Research Ethics Committee for approval. Any further amendments will be submitted and approved by the ethics committee.

In addition, the study will be submitted for HRA review and approval. Recruitment of study participants will not commence until the letter of approval has been received from the HRA.

19. Regulatory approval

The study will be conducted in accordance with the UK Clinical Trials Regulations 2004 and as such will be submitted to the Medicines and Healthcare Regulatory Agency (MHRA) for review. The study will not commence recruitment until a Clinical Trial Authorisation (CTA) has been granted by the MHRA.

20. Indemnity / Compensation / Insurance

The University of Sheffield has in place clinical trials insurance against liabilities for which it may be legally liable, and this cover includes any such liabilities arising out of this clinical study.

Standard NHS indemnity operates in respect of the clinical treatment which is provided.

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