ASTIC

Autologous Stem Cell Transplantation for Crohn's Disease:
Autologous Stem Cell Transplantation International Crohn's Disease Trial

A multicentre, prospective, randomised phase III study conducted by the European Crohn's and Colitis Organisation (ECCO), sponsored by the Autoimmune Disease Working Party of the European Group for Blood and Marrow Transplantation (EBMT)

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Protocol Approval Form

ASTIC:

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EudraCT Number: 2005-003337-40

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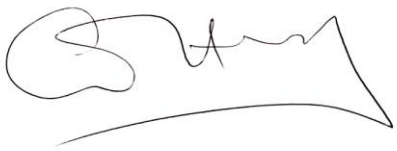
Signature:  Date: 18 March 2009

Principal Investigator Acceptance Form

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Principal Investigator at Study Site:

By signing this protocol, the named investigator takes responsibility for the conduct of the research as detailed in this protocol at the site mentioned below, and is accountable for this to their employer, and, through them, to the study sponsor.

Name of Hospital:

Name of Principal Investigator:

Signature of Principal Investigator:

Date:

A copy of this page is to be signed by the Principal Investigator at each participating site.

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ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
ASTIC	Autologous Stem Cell Transplantation International Crohn's Disease Trial
ATG	Antithymocyte Globulin
BMI	Body Mass Index
CD34	Glycoprotein 34
CDAI	Crohn's Disease Activity Index
CDEIS	Crohn's Disease Endoscopic Index of Severity
CMV	Cytomegalovirus
CRF	Case Report Form
CRP	C-reactive protein
CNS	Central Nervous System
CXR	Chest x-ray
DEXA	Dual energy X-ray absorptometry
DLCO	Diffusing Capacity of the Lung for Carbon Monoxide
DSMC	Data Safety Monitoring Committee
EBMT	European Group for Blood & Marrow Transplantation
EBV	Epstein Barr Virus
ECCO	European Crohn's and Colitis Organization
ECG	Electrocardiogram
EULAR	European League against Rheumatism
EuroQol (EQ-5D)	European Questionnaire of Life Quality
ESR	Erythrocyte Sedimentation Rate
FACs	Fluorescence-activated cell sorter
FBC	Full Blood Count
FEV_1	Forced Expiratory Volume in the first second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GVHD	Graft Versus Host Disease
Hb	Haemaglobin
Ht	Haematocrit
HIV	Human Immunodeficiency Virus
HSCT	Hematopoietic Stem Cell Transplantation
HSV	Herpes Simplex Virus
HTLV	Human T Lymphocyte Virus
IB	Investigator's Brochure
IBDQ	Inflammatory Bowel Disease Questionnaire
ICH	International Conference on Harmonisation
Ig	Immunoglobulin
MMF	Mycophenolate Mofetil
MNC	Mononuclear cells
MUGA	Multi-gated Radionuclide Angiography
NOD-2	Nucleotide-binding oligomerization domain 2
OCT	Embedding Compound OCT (Optimum Cutting Temperature)

PBSC	Peripheral Blood Stem Cells
PCR	Polymerase Chain Reaction
rbATG	Rabbit Antithymocyte Globulin
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAO	Study Administration Office
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SF36	36-item Short Form Health Survey
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TPMT	Thiopurine methyltransferase
TH	T-helper cell
VAS	Visual Analogue Scale
VDRL	Venereal Disease Research Laboratory
VZV	Varicella-zoster virus
WBC	White Blood Cell

1 INTRODUCTION AND RATIONALE

Chronic inflammatory bowel disease comprises Crohn's disease and ulcerative colitis, which are both relapsing and remitting disorders most commonly presenting in the 2nd or 3rd decade and causing life long impairment of health and quality of life. Both Crohn's disease and ulcerative colitis have a strong genetic component as evidenced by a high proportion of patients with a family history and, in the case of Crohn's disease, the specific contribution of mutations of the NOD2-gene, which detects and responds to intracellular bacterial lipopolysaccharide. Not all individuals with Crohn's disease have mutations in the NOD2-gene and the disorder should be viewed as a polygenic one and / or as a collection of syndromes.

Several environmental factors are associated with Crohn's disease[1], most prominently, smoking which enhances the risk of developing Crohn's disease and increases disease activity in those with established disease. Crohn's disease is also associated with early hygiene and with consumption of a diet high in unrefined carbohydrate although whether the latter is cause or effect is debated. Whilst the mechanisms by which interactions between genetic and environmental factors lead to Crohn's disease are unclear, Crohn's disease involves a loss of immune tolerance in the gastrointestinal tract, characterised by an overactive TH-1 immune response[2, 3]. Given that bone marrow and stem cell transplantation have been of some value in other diseases characterised by loss of immune tolerance and or a TH-1 predominant immune response, it is possible that these procedures could be of value in Crohn's disease.

On such an analysis, allogeneic transplantation could be of benefit by replacing the genetic predisposition to Crohn's disease in circulating leucocytes. Autologous transplantation might also be of benefit because clearing the body of committed lymphocyte clones might restore the patient to the *status quo ante* of being predisposed to Crohn's disease but not suffering from it.

In other diseases, full immune reconstitution has been shown to take months or years and clones underlying disease reactivity may, in theory, never re-emerge. An alternative explanation is that autologous stem cell transplantation simply allows more intense immunosuppression to be given. Immunosuppressive agents are of considerable value in Crohn's disease although relapse occurs on their cessation. It is possible that more intense immunosuppression in some way achieves a more fundamental switch in immune activity. On this basis, the transplant plays a secondary role in enabling intense immune suppression to be given relatively safely.

1.1 Evidence for the effectiveness of stem cell transplantation in Crohn's disease

The possibility that stem cell transplantation might be an effective treatment for inflammatory bowel disease, particularly Crohn's disease, initially arose from reports of improvements that occurred in patients with Crohn's disease who had stem cell or bone marrow transplants for other reasons[4].

1.1.1 Allogeneic Transplantation

In 1993 there was a report of a patient with Crohn's disease receiving an allogeneic bone marrow transplant for lymphoma[5]. Following the transplant the patients Crohn's disease improved although evaluation was not fully systematic and the follow up restricted to 6 months. Five years later a report of 6 patients undergoing allogeneic transplantation, who incidentally had Crohn's disease, substantially increased interest in the possibility that stem cell transplantation could be of

value in this disease[6]. Of 5 patients whose Crohn's disease was active before transplantation, 3 achieved long lasting remission for up to 10 years after transplantation, despite discontinuation of immunosuppression. Other patients did less well. One patient developed recurrent Crohn's disease approximately 18 months after transplantation and required surgery 3-4 months later. From these data it is clear that allogeneic transplantation could benefit Crohn's disease but that benefit is not universal.

Recently data concerning 11 patients (7 Crohn's disease, 4 ulcerative colitis) in Germany has been published[7]. These patients had had inflammatory bowel disease for a median of 10 (range 0.5 – 22) years with a post-transplant follow-up of 34 (range 3 – 117) months. Six of the 11 patients had active disease at conditioning and 4 of these were receiving sulfasalazine and/or steroids. Ten of the 11 patients became, and remained, inactive following transplantation (3 had initial symptoms or atypical histology, CMV colitis, GVHD).

Because these studies were not designed to investigate Crohn's disease itself, follow-up was less systematic than if they had been. However, they support the concept that the patients' immune system plays a central role in Crohn's disease and that replacing it with a transplanted one may be beneficial. This concept is further supported by another recent case report in which a healthy patient undergoing allogeneic transplantation developed aggressive Crohn's disease soon afterwards[8]. Investigations showed that the transplanted stem cell had a pathogenetic mutation of the NOD2-gene, compatible with the notion that the Crohn's disease developed when a healthy immune system was replaced by one, which, from the point of view of Crohn's disease, was an unhealthy one.

1.1.2 Autologous Transplantation

Several studies have reported the clinical course of patients with Crohn's disease receiving autologous transplantation for another condition[9-12]. Perhaps the most impressive concerned a patient who developed symptoms at the age of 9, who was diagnosed with Crohn's disease 4 years later and who required substantial treatment over the next 7 years[9]. Following autologous stem cell transplantation for non-Hodgkin's lymphoma it was reported that there was no clinical or laboratory evidence of recurrence of his Crohn's disease in the next 7 years. Another reported case of Crohn's disease had diffuse pan colitis before transplantation and was asymptomatic (but had inflammation) at endoscopy 3 years later[10]. Another patient who had had symptomatic Crohn's disease for 2 years and had undergone surgery was free of disease 5 years after transplantation[11]. Systematic colonoscopic evaluation showed persisting subclinical inflammation initially with progressive clearance. There are also case reports both of improvement in patients with ulcerative colitis but also of new development of ulcerative colitis or colonic ulceration following transplantation, although these have not been related to genetic polymorphisms.

1.1.3 Nottingham patient

We have recently been able to study a patient in Nottingham with Crohn's disease who also had incidental transplantation. This 41 year old man had had symptoms of intermittent diarrhoea because of diffuse colonic Crohn's disease since 1994. This had not required surgery and had been maintained on mesalazine and azathioprine. He developed worsening diarrhoea, loss of weight and fever and was found to have a small bowel lymphoma initially treated by chemotherapy but then by stem cell transplantation upon relapse. Immediately prior to transplantation he agreed to undergo colonoscopy. This showed patchy inflammatory changes compatible with mild active Crohn's disease. Following transplantation he went into remission

from his lymphoma. His bowel symptoms ceased and he was able to stop all immunosuppressive drugs. Eighteen months after transplantation he underwent colonoscopic re-evaluation of the large bowel. This showed no evidence of Crohn's disease.

1.1.4 Stem cell transplants done specifically for Crohn's disease

The first report of stem cell transplantation for Crohn's disease concerned 2 patients from the Chicago group[13]. This has been supplemented by a report mentioning 2 further patients[14].

In both of the initial cases Crohn's disease activity index was more than 250 (normal range <150, pathological range 220 - 600) despite treatment with infliximab. In both patients, peripheral blood stem cells (PBSC) were mobilised using cyclophosphamide and granulocyte colony stimulating factor (G-CSF), enriched ex-vivo by CD 34+ selection. Cyclophosphamide and anti thyomcyte globulin (ATG) were used for immune conditioning prior to transplantation. The first patient was a 22 year old woman with a past history of a right hemicolectomy with severe ileocolic Crohn's disease causing intractable diarrhoea with 25 bowel actions per day, fistula and peri-anal sepsis. The second was a 16 year old boy who had been unwell for 6 years requiring tube feeding and receiving methotrexate, 6-mercaptopurine and 5 amino acyclic acid with Crohn's colitis. In both of these cases, following transplantation, diarrhoea resolved and the Crohn's disease activity index normalised. In the first patient the haemoglobin rose, whereas in the second it remained at a slightly sub-normal level. Post-transplantation CRP was within normal limits and the albumin remained or became normal.

These cases were accompanied by extensive colonoscopic evidence. Prior to transplantation both cases had areas of severely active Crohn's disease with cobble stoning, fissuring and deep ulceration. Following transplantation the bowel remained abnormal though changes were much more trivial with superficial erosions only.

1.1.5 Europe

In Europe, a small number of patients have also undergone transplantation specifically for Crohn's disease and one has been reported in the literature[15]. A 36 year old patient developed ileocolic Crohn's disease in 1988. Despite multiple courses of corticosteroids, sulfasalazine, mesalazine, azathioprine, ciprofloxacin, metronidazol, formula diet and infliximab, he had a poor clinical course, requiring 4 operations between 1992 and 2000. In March 2001 when the patient was in clinical remission but had active histology in the small bowel and colon, he underwent stem cell mobilisation chemotherapy with cyclophosphamide, 4g/sqm. Under maintenance treatment on azathioprine and corticosteroids the patient was in endoscopic remission for 9 months. After a relapse one year after mobilisation, the patient was conditioned with cyclophosphamide and autologous peripheral blood CD 34+ stem cells were re-infused. Following this procedure the patient was reported to be in complete clinical endoscopic and histological remission for 9 months without additional treatment but then developed aphthous lesions with inflammatory changes on histology. At the time of the report (2003) the patient was asymptomatic on low dose prednisolone and methotrexate.

This case illustrates many of the issues surrounding Crohn's disease and stem cell transplantation in that on the one hand the patient achieved clinical improvement following mobilisation and on the other that complete histological remission was not maintained following transplantation without additional treatment. However, it should be pointed out the unusually prolonged interval between mobilization regimen and transplant conditioning might have reduced the effectiveness

of the procedure as a consequence of a lower dose intensity. Overall, the prospect of transplantation for Crohn's disease ranges from no difference compared to conventional management through substantial improvement to possible cure.

1.2 Practical and ethical issues surrounding transplantation for inflammatory bowel disease

Existing evidence therefore suggests that stem cell transplantation could be justified in some patients with inflammatory bowel disease. Whilst substantial improvements are reported, it is clear that not all patients achieve a cure. This raises the question of whether it is the transplantation or the intense immunosuppression surrounding it that is the critical event. Certainly many patients have remained in remission following withdrawal of immunosuppressive drugs. Whether a new clone of uncommitted lymphocytes, a reset of the immune system or long lasting effects of more intense immunosuppression than could have been given if not covered by stem cell transplantation are responsible is not clear. A concept much favoured is that stem cell transplantation enables immunosuppression to be more intense than would otherwise be the case and that consequently some long-term benefit arises through reinstated immunoregulation.

1.3 Alternative mechanisms for how stem cell transplantation could work

Recently, interesting insights into possible unsuspected mechanisms by which stem cell transplantation could affect the gut have emerged. In both animal and patient studies, sex mismatched transplants have been given, enabling donor cells to be identified by immuno histochemical staining for the Y chromosome. These have shown in both mice and women that a population of myofibroblast derived from the donor populates the intestinal mucosa, particularly in the sub-epithelial segment[16]. Given the importance of myofibroblasts in orchestrating the function of epithelial cells, these data suggest a mechanism other than one targeted at immuno suppression that could beneficially reset patient functions, for example enhancing barrier function following stem cell transplantation.

2 TRIAL SYNOPSIS

Title	Autologous Stem Cell Transplantation - International Crohn's Disease Trial (ASTIC)
Indication	Crohn's disease
Principle	Transplant study for patients with relapsing Crohn's disease demonstrating clear intolerance or toxicity to conventional treatment
Primary Objective	To evaluate the potential clinical benefit of hematopoietic stem cell mobilisation followed by high dose immuno-ablation and autologous stem cell transplantation versus hematopoietic stem cell mobilisation only followed by best clinical practice in patients with Crohn's disease.
Secondary Objectives	• To evaluate the safety of Hematopoietic Stem Cell Transplantation (HSCT) in Crohn's disease patients who have not responded to immunosuppressant medication • To evaluate the impact of HSCT on health related, and generic, quality of life measures • To identify factors predictive of success
Primary Endpoint	The primary endpoint will be the proportion of patients in sustained disease remission at one year. Sustained disease remission, based upon ECCO consensus is defined as: A minimum of a 3 month period of Crohn's disease activity index (CDAI) $\leq$ 150 without steroids or immunosuppressive drugs and no mucosal erosion or ulceration at ileocolonoscopy and no definite evidence of small bowel Crohn's Disease on barium studies.
Secondary Endpoints	The secondary endpoints are listed in full in Section 4
Study Design	Open label, randomised, multicentre study comparing early transplantation procedure with transplantation carried out to the same protocol but delayed by one year. The status of patients undergoing early HSCT will be evaluated after one year and compared to those about to undergo delayed HSCT
Medication	All medication used in the study is currently marketed and will be prescribed through the hospital pharmacies according to normal practice
Study Sites	This is a multi-centre study involving hospitals in a number of European hospitals approved by the Trial Steering Committee as having appropriate clinical experience
Sample Size & Power	Minimum 48 patients, 24 per treatment arm. The study will have 90% power to detect a rise in the rate of sustained remission from 30% to 75% (80% power for 70%).

3 TRIAL DESIGN

The considerations outlined in the introduction have led to the present proposal for a European trial of stem cell transplantation in Crohn's Disease (ASTIC).

In summary, this trial is a multicentre, prospective, controlled, open-label randomized study comparing early transplantation procedure with transplant delayed by one year.

Patients will be randomised to:

A. Hematopoietic stem cell mobilisation followed, within 4 weeks, by high dose immunoablation and autologous stem cell transplantation

B. Hematopoietic stem cell mobilisation followed, after 59 weeks, by high dose immunoablation and autologous hematopoietic stem cell transplantation

All patients will be mobilised prior to randomisation. Those receiving early transplantation will be compared over the first year with those whose transplant has been delayed.

See also Figure 1 (outline of trial), below.

3.1 Reasons for choice of design:

Although there are suggestions that stem cell mobilisation maybe of benefit to patients with Crohn's disease, there are few grounds to believe this benefit would be long-term. Two trial designs were possible:

- mobilisation for all with randomisation to early and delayed transplantation.
- a comparison of early mobilisation and transplantation with delayed mobilisation and transplantation.

We have chosen the former design for the following reasons:

a) A positive result clearly establishes the efficacy of transplantation.

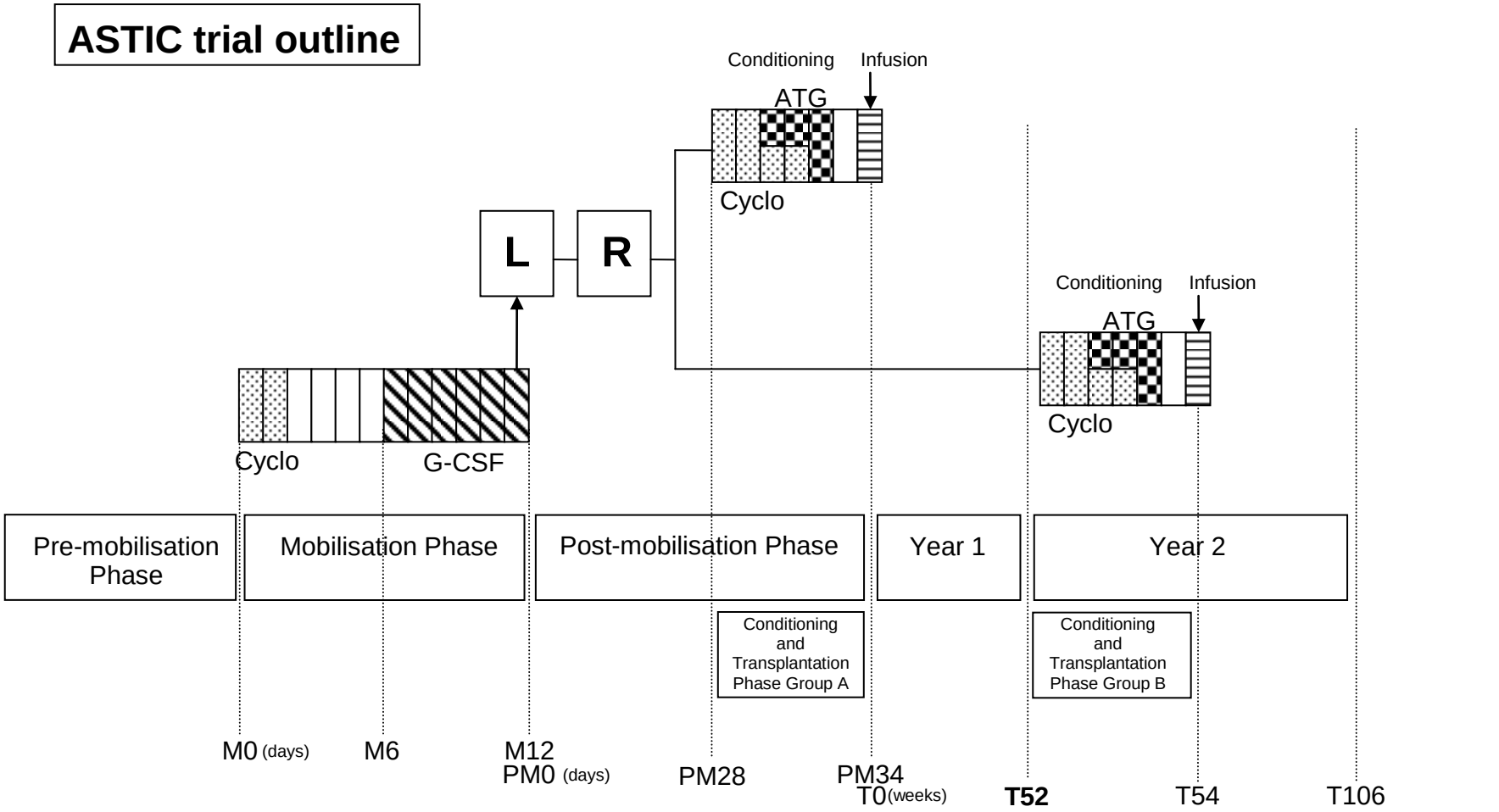
b) The finding of no difference at one year between the groups with the high response rate would imply persistent value of mobilisation and indicate a need for a trial, whilst no difference with a low response rate would indicate neither component of the treatment was worthwhile.

c) Any value of mobilisation regimes alone would be more effectively investigated in a protocol not involving a transplantation option.

3.2 Choice of trial centres

The trial will only be performed in units that are approved by trial Steering Committee to ensure the highest possible local standards. The Steering Committee will consider whether centres have appropriate stem cell transplantation and gastroenterology experience, and whether they are likely to be able to identify patients who fulfil the inclusion criteria.

The trial will be conducted in compliance with the protocol, ICH GCP and regulatory requirements. All necessary regulatory and ethical approvals will be obtained for each centre before patient recruitment begins at that centre.



Key:
L=leukapheresis, R=randomisation, Cyclo=cyclophosphamide, G-CSF=filgrastim, ATG=antithymocyte globulin, M=mobilisation phase time point, PM=post-mobilisation time point.
(M and PM time points are flexible but every attempt should be made to make sure that M34 is simultaneous with visit T0 (which must be no more than 3 days before the first conditioning dose of cyclophosphamide))

Figure 1. Outline of Trial

3.3 Methods

Suitable patients	will have relapsing disease (>1 exacerbation/year) despite attempts at control with thiopurines, methotrexate and infliximab maintenance therapy given alone or on separate occasions or clear demonstration of intolerance/toxicity to these drugs but be in otherwise good clinical condition, to minimise risks from HSCT. They will undergo extensive phenotyping and genotyping at baseline.
Stem cell mobilisation	will be achieved using cyclophosphamide 4g/m² ($2g/m^2$ on 2 consecutive days) followed 5 days later by filgrastim (G-CSF)10 μg/kg injection. This will be done daily until the day before the last day of leukapheresis.
Leukapheresis	will be performed to a target of 3-8 $x10^6$ CD34+ cells/kg Approximately 1 month later patients randomised to early HSCT will undergo:
Immunoablative conditioning	with cyclophosphamide 200 mg/kg (50mg daily on 4 consecutive days) and anti-thymocyte globulin (ATG) 2.5 mg/kg/day and intravenous methylprednisolone 1 mg/kg for 3 days, starting on the third day of cyclophosphamide. One day later, thawed stem cells (3-8 x 10^6/kg CD34 $^{+ve}$) will be reinfused.

3.4 Genotyping

Patients will be given the option to consent to genotyping for the NOD2 gene and for other genes discovered to be associated with the risk of Crohn's disease. This consent is not mandatory for the patient to enter the ASTIC trial, and the genotyping will be carried out as subsequent research rather than as part of this trial.

Patients will be asked to indicate whether they wish to be informed of their genotyping results. If they do wish to be informed, this will be done via their local Principal Investigator using the patient's most recent available contact details.

4 OBJECTIVES AND ENDPOINTS

4.1 Primary objective

To evaluate the potential clinical benefit of hematopoietic stem cell mobilisation followed by high dose immuno-ablation and autologous stem cell transplantation versus hematopoietic stem cell mobilisation only followed by best clinical practice in patients with Crohn's disease.

4.2 Secondary objectives

The secondary objectives are:

1. To evaluate the safety of hematopoietic stem cell transplantation (HSCT).
2. To evaluate the impact of HSCT on health related, and generic, quality of life measures.
3. To identify factors predictive of success.

4.3 Primary Endpoint

The primary endpoint will be the proportion of patients in sustained disease remission at one year. Sustained disease remission, based upon ECCO consensus is defined as:
A minimum of a 3 month period of Crohn's disease activity index (CDAI) $\leq$ 150 without steroids or immunosuppressive drugs and no mucosal erosion or ulceration at ileocolonoscopy and no definite evidence of small bowel Crohn's Disease on barium studies.

4.4 Secondary Endpoints

Secondary endpoints will be:

1) Proportion of patients in alternative sustained disease remission as measured by a minimum of 3 months with the alternative Harvey Bradshaw score ≤ 3 (Appendix 8), without steroids or immunosuppressive drugs* and no mucosal erosion or ulceration at ileocolonoscopy and no definite evidence of small bowel Crohn's Disease on barium studies.
2) Weeks in symptomatic remission (CDAI ≤ 150) over one year.
3) Weeks in alternative symptomatic remission (Harvey Bradshaw score ≤ 3) over one year.
4) Mean change from baseline in CDAI at one year
5) Mean change from baseline in Harvey Bradshaw score at one year.
6) Mean CDAI over months 3-12 after transplant date, regardless of treatment.
7) Median Harvey Bradshaw score over months 3-12 after transplant date, regardless of treatment.
8) Total steroid use over one year
9) Steroid use over months 3-12.
10) Number of days in clinical remission (CDAI ≤150 and on prednisolone ≤10mg/day)
11) Number of days in alternative clinical remission (Harvey Bradshaw score ≤ 3 and on prednisolone ≤10mg/day)
12) Days in drug free remission (CDAI ≤150 and not on steroids or any immuno-suppressive drugs*):
13) Days in alternative drug free remission (Harvey Bradshaw score ≤ 3 and not on steroids or any immuno-suppressive drugs*)
14) Time to and days in sustained disease remission

15) CRP at one year and average over months 3-12.

16) Platelet count at one year and average over months 3-12.

17) Change in CDEIS over 12 months, based on blinded videotape comparisons

18) Histology, based on blinded comparisons

* Infliximab is an immunosuppressive drug. For the purposes of calculating these endpoints, patients are considered to be on infliximab for 42 days after an infusion.
Time of onset or duration of clinical status as defined above will be based upon patient diary for symptom assessments and the next examination for objective measures

4.5 Endpoints at year 2

The main comparison will be the proportion of patients in each group in a potentially cured state. A potentially cured state is defined as: A minimum of a 6 month period of CDAI < 150 without steroids or immunosuppressive drugs and no mucosal erosion or ulceration at ileocolonoscopy and no definite evidence of small bowel Crohn's Disease on barium studies.

Time to and days in potentially cured state will be recorded, and other measures will be as for the first year. For all measures, comparisons will be between the 2 groups and also between year 1 and year 2.

4.6 Endpoints to Assess Secondary Objectives

1. The safety of HSCT will be evaluated in terms of Adverse Events (AEs, see Section 15). All adverse events (including Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs)) will be recorded in a standardised way and their relationship to study treatment will be assessed according to the criteria in Section 15.3 and will be presented descriptively.

2. The impact of HSCT on health related, and generic, quality of life measures will be evaluated in terms of.

- Change from baseline in Inflammatory Bowel Disease Quality of Life Questionnaire (IBDQ) at one year (Appendix 5)
- Average IBDQ over months 3-12.
- Change from baseline in EuroQol (EQ-5D) score over months 3-12. (Appendix 4)
- Average EuroQol (EQ-5D) score over months 3-12
- Change from baseline in Karnofsky Scale score over months 3-12. (Appendix 6)
- Average Karnofsky score over months 3-12

3. To identify factors predictive of success, the following factors will be entered into a multivariate logistic regression analysis: genotype, smoking status, location of disease, previous responsiveness to immunosuppressive drugs, family history number of CD34 cells engrafted and randomised treatment. The influence of other factors will be explored in a descriptive way.

4.7 Choice of Endpoints

At present the Crohn's Disease Activity Index[17] (CDAI, Appendix 3,) is used to measure clinical activity in therapeutic studies in Crohn's Disease (Singleton[18]), and is therefore used in the Primary Endpoint for this study. A pragmatic definition of sustained disease remission and a potentially cured state, based upon a consensus of interested GI trialists within European Crohn's and Colitis Organization (ECCO), is used.

However, the CDAI has limitations. It is cumbersome because it relies on laboratory as well as clinical data, although it is principally a symptom index. For this purpose, the Harvey Bradshaw index[19] (see Appendix 8) is easier to use (see Appendix 7 for patient diary). It is possible that the Harvey Bradshaw index will replace the CDAI as the preferred symptom index for trials so it is important to record both in the proposed study, and the Harvey Bradshaw index is included as a Secondary Endpoint.

Neither index measures inflammation nor are they good surrogates for this, so C reactive protein (CRP) will be followed and endoscopic appearances will be scored according to the Crohn's Disease Endoscopic Index of Severity[20,21] (CDEIS, see Appendix 9), as further Secondary Endpoints, to provide non-specific and specific objective measures of disease activity.

Health related quality of life will be assessed using the McMaster Inflammatory Bowel Disease Questionnaire[22] (IBDQ, see Appendix 5). The European Questionnaire of Lifequality (EuroQol)[24] (Appendix 4) and Karnofsky Scale[25] (Appendix 6) will be used as measures of generic quality of life. Licenses will be obtained for all questionnaires before use if required.

5 PATIENT SELECTION CRITERIA

5.1 Inclusion criteria: mandatory

1) Age between 18 and 50 years
   a) Patients aged 50-65 can participate if specially approved by the Trial Steering Committee
2) Confirmed diagnosis of active Crohn's Disease:
   a) Diagnosis of Crohn's disease based on typical radiological appearances and / or typical histology
   b) Active disease at the time of registration to the trial, defined as
      i) Crohn's disease activity index (CDAI) ≥ 250 at any time within 3 months prior to trial entry

      and ≥ 2 of the following:
      ii) raised CRP,
      iii) endoscopic evidence of active disease confirmed on histology
      iv) clear evidence of active small bowel Crohn's disease on small bowel barium study.
3) Unsatisfactory course despite 3 immunosuppressive agents (usually azathioprine, methotrexate and infliximab) in addition to corticosteroids. Patients should have relapsing disease (i.e. ≥ 1 exacerbation/year) despite thiopurines, methotrexate and/or infliximab maintenance therapy or clear demonstration of intolerance / toxicity to these drugs.
4) Impaired function and quality of life, compared to population means, on at least one of the following:
   a) IBDQ (Appendix 5)
   b) European Questionnaire of Lifequality (EuroQol–5D, Appendix 4)
   c) Impaired function on Karnofsky index (Appendix 6)
5) Current problems unsuitable for surgery and patient at risk for developing short bowel syndrome.
6) Informed consent
   (Sample Information Sheet and Consent Form given in Appendices 1 and 2 respectively)
   a) Prepared to enter controlled study.
   b) Prepared to undergo additional study procedures as per trial schedule
   c) Patient has undergone intensive counselling about risks
   d) Consent to future genotyping assessments is optional, but is not required for the patient to enter the trial

5.2 Inclusion criteria: discretionary

1. Wherever possible, diseased tissue should be accessible endoscopically for objective histological study
   a. Small bowel disease that is extensive but does not extend to duodenum or terminal ileum is an exception, which will allow participation without endoscopy of diseased areas. All patients will however undergo flexible sigmoidoscopy
2. Smokers may enter the study provided they have received intensive counselling about smoking.
3. Patients with an ileostomy or colostomy may enter the study. Clinical activity should be

assessed using modified CDAI and Harvey Bradshaw scoring method (See Appendix 3 and 8).

5.3 Exclusion criteria

1) Pregnancy or unwillingness to use adequate contraception during the study, if a woman of childbearing age
2) Concomitant severe disease
   a) renal: creatinine clearance < 40 ml/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; chronic atrial fibrillation necessitating oral anticoagulation; uncontrolled ventricular arrhythmia; pericardial effusion with hemodynamic consequences as evaluated by an experienced echo cardiographer
   c) psychiatric disorders including active drug or alcohol abuse
   d) concurrent neoplasms or myelodysplasia
   e) bone marrow insufficiency defined as leucocytopenia <3.0 x 10^9/l, thrombocytopenia <50 x 10^9/l, anaemia < 8 g/dl, $CD4^+$ T lymphopenia < 200 x 10^6/l
   f) uncontrolled hypertension, defined as resting systolic blood pressure ≥ 140ml and/or resting diastolic pressure ≥ 90ml mercury despite at least 2 anti-hypertensive agents.
   g) Uncontrolled acute or chronic infection with HIV, HTLV – 1 or 2, hepatitis viruses or any other infection the investigator or Steering Committee 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 <8 kpa or carbon dioxide tension >6.7 kpa. Patients not known to have respiratory disease need not have blood gas measurements.
   i) Crohn's Disease symptoms predominantly due to fibrotic stricturing and unlikely to respond to immune manipulation, in the opinion of any of the investigators or the steering committee
3) Infection or risk thereof
   a) Current abscess or significant active infection.
   b) Perianal sepsis is not an exclusion provided there is natural free drainage or a Seton suture(s) have been placed.
   c) History of tuberculosis or at current increased risk of tuberculosis
   d) Mantoux test result or other investigations that the investigator or Steering Committee regard as evidence of active tuberculosis.
   e) Abnormal chest x ray (CXR) consistent with active infection or neoplasm.
4) Significant malnutrition: Body Mass Index (BMI) ≤ 18, serum albumin ≤ 20 g/l
5) Previous poor compliance
6) Concurrent enrolment in any other protocol using an investigational drug or hematopoietic growth factor up to four weeks before study entry.

7) Lack of funding

6 INFORMED CONSENT AND PATIENT CONFIDENTIALITY

6.1 Informed Consent

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be advised that they are free to withdraw from the study without obligation, and informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than the treating physicians. They will be interviewed by both the caring gastroenterologist and a senior member of the transplant team, and will be given the opportunity to visit the transplant centre and meet patients who are both currently undergoing transplantation and who have previously undergone transplants for autoimmune disease.

If they still want to proceed, they will undergo counselling by a specialist who is not the investigator proposing to enter them into the study before giving informed consent. They must then be given ample time and opportunity to inquire about the details of the study and to decide whether or not to participate. The investigator shall seek consent only under circumstances that provide the patient with sufficient opportunity to consider whether or not to participate and that minimise the possibility of coercion or undue influence.

The information that is given to the patient shall be in language understandable to the patient. Patient Information Sheet and Consent Form are provided in Appendices 1 and 2 respectively. Written informed consent must be obtained for all patients considered for participation in the study, before they undergo any trial specific procedures. This must be done in accordance with the national and local regulatory requirements. For each patient, the original copy of the signed consent form 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 Patient Information Sheet and their signed Consent Form to keep, and a copy will be filed in their medical notes.

6.2 Patient confidentiality

The investigator must ensure that patient anonymity is maintained. On the case report forms (CRFs) or other documents, patients should be identified by their initials, date of birth and a study number only. Documents which contain the patient's full name should be kept in strict confidence by the Investigator and will not be removed from the Investigator's centre, although they will be reviewed by the Study Monitor during monitoring visits.

The patient will be asked to consent for their Primary Care Physician to be informed of their participation in the study.

7 PROCEDURES FOR PATIENT ENTRY INTO TRIAL

Before patients may be entered into the study, centres wishing to participate must acquire national and/or local research ethics committee approval and national Competent Authority approval. Participating UK centres, and depending on local requirements also in other countries, must also obtain approval from the local NHS Research and Development (R&D) department.

The study administration office in Nottingham must receive written confirmation of the documents listed below before the first patient can be enrolled.

7.1 Pre-study Documentation

The following documents will be required from each participating institution before the study can be started:

- Signed Clinical Trial Agreement
- Signed and dated protocol signature page (see page 2)
- Site Specific Assessment approval letter from local ethics committee
- Copy of institutional Research & Development Department approval letter (according to local regulations)
- Completed centre registration forms (site principal investigator and additional staff), including CV of principal investigator at site

The national lead investigators will organize the following documents:

- National (main) ethics committee approval letter
- Approval from Competent Authority

7.2 Patient consent

All patients who fulfil the eligibility criteria will be asked to give written informed consent to trial participation prior to the beginning of any trial procedures. Full details of the consent procedure are described in Section 6.1.

Patients will also be asked whether they wish to consent to genotyping procedures at this stage.

After patients have given informed consent they enter the pre-registration phase of the trial, and will be assigned a Patient Trial Number.

7.3 Patient investigations pre registration.

The following investigations will be completed before registration, and recorded in the Pre-Registration section of the Case Report Form (CRF). Patients will start to keep a daily diary to enable calculation of CDAI and Harvey Bradshaw index (Appendices 3 and 8 respectively).

- Demographics (date of birth, sex, smoking history and ethnic origin)
- Comprehensive patient's history with particular attention paid to the history of Crohn's disease, and to the treatment history
- Detailed physical examination with particular attention to the alimentary system
- Laboratory examinations comprising (also see table 1, below):
    - Haematology: including FBC with ESR and coagulation screen,

    - Clinical chemistry: including C reactive protein, 24-hour urinary protein and creatinine clearance, urine microscopy and culture.
    - Serology: including CMV, HSV, VZV, EBV, VDRL, HIV, (HTLV-1,2), Hepatitis B and C
- CDAI (Appendix 3)
- Harvey Bradshaw index (Appendix 8)
- IBDQ (Appendix 5)
- EuroQol (EQ-5D) (Appendix 4)
- Karnofsky index (Appendix 6)
- ECG with rhythm strip
- Cardiac echo or MUGA
- Pulmonary function tests: including FEV1, FVC, DLCO.
- Chest X-ray
- Mantoux test
- Pregnancy test
- Evaluation against inclusion/exclusion criteria
- Concomitant medication, including history of previous immunosuppressant use to treat Crohn's Disease
- Medical services used and employment status questionnaire
- Adverse events

The Investigator will record details of all patients who undergo pre-registration procedures in a Pre-Registration Log. All patients who meet the inclusion/exclusion criteria will start the registration procedure.

7.4 Patient registration procedure

All patients who fulfil the eligibility criteria, following the pre-registration assessments will be registered at the study administration office (SAO) in Nottingham (see contact details on cover page). The registration form is shown in Appendix 10. Details of these patients will be circulated to the other participants and the Trial Steering Committee, who will express a view about feasibility and suitability for the study and make suggestions for alternative treatments that should be tried prior to study entry. Proposed patients will be discussed at a regular teleconference of the Trial Steering Committee.

The Trial Steering Committee will inform the Investigator whether the patient is considered suitable to continue in the trial. The patient will then proceed to the Pre-mobilisation Phase.

Table 1: Procedures during pre-registration, pre-mobilisation, mobilisation and post-mobilisation phase

Phase	Pre-registration	Pre-mobilisation	Mobilisation	Post-Mobilisation			
Time points			**M0-M12**	**PM7**	**PM14**	**PM21**	**PM28**
Demographics	*						
History and examination	*	*		*	*	*	*
Medical services used & employment history	*	*		*	*	*	*
Haematology	*	*		*	*	*	*
Daily FBC			*				
Clinical Chemistry	*	*		*	*	*	*
Serology	*						
ECG	*						*
ECHO or MUGA	*						
Mantoux test	*						
Chest X-ray	*						*
Pulmonary function tests	*						
Pregnancy test	*	*					*
CDAI	*	*		*	*	*	*
Harvey Bradshaw	*	*		*	*	*	*
IBDQOL	*	*					*
EuroQOL	*	*					*
Karnofsky Index	*	*					*
Dexa scan.		*					
Lymphocyte subsets		*					*
Cytokines		*					*
Small bowel imaging		*					
Capsule Endoscopy, if available		*					
Colonoscopy		*					
Upper endoscopy		*					
Rectal Biopsy: Histology		*					
CDEIS		*					
Dental evaluation		*					
Bone marrow aspiration		*					
Leukapheresis			*				
Concomitant medication	*	*		*	*	*	*
Adverse events	*	*		*	*	*	*

Additional monitoring will be carried out according to clinical need

8 PRE-MOBILISATION PHASE

All patients will undergo extensive base line evaluation and stem cell mobilisation. See also Table 1.

8.1 Additional baseline evaluations

8.1.1 GI evaluations.

All patients will undergo the following evaluations, unless these have been done in the 3 months prior to registration and the Trial Steering Committee agrees to the use of those results. Details are given in Section 21.

- **Colonoscopy** with CDEIS score (Appendix 9). Colonoscopy must be conducted and a barium enema cannot be substituted.

  Biopsy samples of normal or abnormal mucosa from each of the 5 segments assessed for the CDEIS should be taken with 1-2 samples from each segment fixed in formalin and 1-2 samples from each segment fixed in RNA later and frozen.

  The maximum total number of samples per colonoscopy is 20.

- **Upper endoscopy** biopsy samples from oesophagus, stomach (body and antrum) and duodenum processed as for the colon. The maximum total number of samples per gastroscopy is 8

- **Small bowel imaging** by barium meal and follow through, or enteroclysis, according to local expertise. Where small bowel imaging has been done within the previous 6 months and the patient's symptoms do not suggest changes then the small bowel imaging should not be repeated and the results from the earlier small bowel barium imaging should be used.

Patients will undergo **capsule endoscopy** of the small intestine if available and not clinically contraindicated (results or procedure done in last 3 months need not be repeated)

8.1.2 Other baseline evaluations, to be done <u>< 4 weeks prior to mobilisation</u>

- PBMC Cell Surface Markers: Lymphocyte subsets on MNC obtained by Ficoll separation for EBMT/EULAR Consensus Core Set (immunophenotyping by FACS of peripheral blood mononuclear cells = $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+$ CD45RA, $CD4^+$ CD45RO, $CD3^-$ $CD56^+$ $CD16^+$, $CD19^+$, $CD14^+$).
- Serum IgG, IgA, IgM.
- Plasma samples in EDTA; heparinised blood samples and serum samples to be cryopreserved for genotyping and cytokine assays.
- Dental consultation and evaluation of teeth and gums.
- Bone marrow aspiration for morphology and clonogenic assay
- Baseline DEXA scan for bone mineral density

8.1.3 Investigations to be repeated no more than 3 days before the start of mobilisation

- Detailed physical examination with particular attention to the alimentary system
- Hematology: including FBC with ESR and coagulation screen,

- Clinical chemistry: including C reactive protein, 24-hour urinary protein and creatinine clearance, urine microscopy and culture.
- Pregnancy test
- CDAI (Appendix 3)
- Harvey Bradshaw index (Appendix 8)
- IBDQ (Appendix 5)
- EuroQol (EQ-5D) (Appendix 4)
- Karnofsky index (Appendix 6)
- Medical services used and employment status questionnaire
- Concomitant medication
- Adverse events

8.2 Immunosuppressive drug treatment

Because mobilisation and conditioning are intensely immunosuppressive, additional immunosuppression is likely to be unnecessary and may potentially pose additional risks. Infliximab should be stopped at least 4 weeks before mobilisation and adalimumab should be stopped at least 2 weeks before mobilisation. Azathioprine / mercaptopurine should be stopped at least 2 weeks before mobilisation. Other immunosuppressive drugs (e.g. methotrexate, cyclosporine etc) should be stopped at least 1 week before mobilisation. If the patient is taking a corticosteroid drug, it should be continued at a dose sufficient to prevent symptoms of adrenal insufficiency allowing for the stress of mobilisation and conditioning (prednisolone $\geq$ 10mg daily) until 3 weeks after transplantation (and according to clinical need otherwise). Exceptionally deviations from these arrangements can be sanctioned by the trial steering committee.

9 MOBILISATION PHASE

See Figure 1 for an illustration of the timing of events during this phase of the trial, and Table 1 for a summary of the assessments. The possibility that mobilisation is beneficial to disease activity will be evaluated during this phase. Additional monitoring will be carried out according to clinical need.

9.1 Mobilisation

All patients will undergo peripheral blood stem cell (PBSC) mobilisation, using a regimen consisting of:

- One-hour infusions of cyclophosphamide 4 g/m^2 (2g/m^2 on 2 consecutive days). Hyperhydration, alkalinisation of the urine and mesna will be given in order to prevent hemorrhagic cystitis according to local centre practice.
- Filgrastim (non-glycosylated G-CSF) 10 μg/kg/day subcutaneously. Administration of filgrastim commences 5 days after the last cyclophosphamide infusion and ends the day before the last leukapheresis.

Daily monitoring of full blood count for anaemia, neutropenia, thrombocytopenia and CD34+ counts is mandatory during mobilization, using validated by local protocols. General EBMT:

guidelines are as follows:
CD34+ monitoring should commence the latest when MNC exceed 2 x 10^9/l after nadir.
Patients should preferably undergo leukapheresis as soon as CD34+ blood levels exceed $20x10^3$/ml. This is expected to occur on day 5 or 6 of filgrastim treatment.

If the white blood cells (WBC) exceed 75 x 10^9/l or if intolerable adverse events (e.g. bone pain) are experienced, filgrastim dose reduction or appropriate treatment should be considered according to local practice. We recommend that mobilisation occurs under in-patient condition, although local out patient protocols may be followed if sanctioned by EBMT.

All patients will receive ciprofloxacin twice daily (or another antibiotic according to the Centre practice) as prophylaxis whilst neutropenic.

9.2 Leukapheresis

Leukapheresis will be performed on a continuous flow cell separator machine, to a target of 3-8 $x10^6$ CD34+ cells/kg body weight. PBSCs may be collected using a two arm venous access technique. If this is ineffective, a double lumen central catheter can be used. The endpoint of each leukapheresis collection should be the processing of 10 to 20 litres of whole blood in total. If mobilization needs to be repeated, G-CSF alone (10µg/ kg) will be used but not before 3 weeks from the last harvest. Repeated mobilisation failure will lead to withdrawal.

Cryopreserved cells will be stored in liquid nitrogen until reinfusion. Before cryopreservation, the CD3+, CD3-/CD16+/56+, CD4+, CD8+, CD19+, CD4/45+RA/RO positive cells will be determined.

Management of Crohn's disease symptoms after mobilisation is described in Section 13.

10 POST-MOBILISATION PHASE

10.1 Randomisation

Randomisation will be performed at least one day after confirmed successful mobilisation of an adequate number of CD34+ cells, and within 21 days, to ensure patients are treated in exactly the same way during the initial phase of the study. The Investigator will inform the SAO that this has been completed using the Randomisation Request Form, and the SAO will return the form telling the Investigator which treatment group the patient has been allocated. Treatment allocation is not blinded.

Patients may be randomised on working days between 08.30 and 17.00 (British Standard Time) at the SAO Nottingham (Fax: +44 115 9422232). Randomisation forms received after 17.00 will be processed first thing the following working morning.

A non-stratified randomisation will be used, with 50% of patients allocated to each treatment group. A randomisation list will prepared for each participating hospital before they begin recruiting patients to the study, using permuted blocks of 4 patients to ensure near-equal distribution of patients over the two treatment groups in each hospital. The random sequence of the permuted blocks will be generated using random number tables.

10.2 Assessments During the Post-Mobilisation Phase

Group A patients will enter the conditioning and transplantation phase as close to 4 weeks after successful leukapheresis as possible. There will be weekly post-mobilisation assessments (PM7, 14, 21 and 28) during the period until they enter the conditioning and transplantation phase. These are summarised in Table 1.

Group B patients will have weekly post-mobilisation assessments (PM7, 14, 21 and 28) for 4 weeks to enable comparison with Group A. They will then have one week lag (while Group A are undergoing conditioning and transplantation).

After transplantation of Group A, both groups will move on to have monitoring assessments for the next year, as described in Section 12.

11 CONDITIONING AND TRANSPLANTATION PHASE

The timing of assessments during the conditioning phase are summarised in Table 2.

T0 is the name given to the day that Group A undergo transplantation, and timing of subsequent trial assessments and procedures are calculated from this day.

For Group A, the time after leukapheresis when transplantation occurs is described in Section 11.3. For Group B, T0 is considered to be 5 weeks after leukapheresis, after patients have undergone the four weeks post-mobilisation assessments and one week lag described in Section 10.2. The timing of Group B transplantation is described in Section 12.2.

11.1 Pre-conditioning assessments

The following assessments will be carried out ≤ 3 days before the first conditioning dose of cyclophosphamide. For patients in Group A, these assessments may be done as the last of the four weekly assessments post-mobilisation (PM28). For Group B or if conditioning starts after the end of the post-mobilisation assessments, they must be repeated up to 3 days before the first conditioning dose of cyclophosphamide.

- Detailed physical examination
- Hematology: including FBC with ESR and coagulation screen,
- Clinical chemistry: including C reactive protein, 24-hour urinary protein and creatinine clearance, urine microscopy and culture.
- ECG and Chest X-ray
- Pregnancy test.
- Blood will be taken for lymphocyte subsets, cytokine measurements
- CDAI, Harvey Bradshaw index
- Medical services used and employment status questionnaire
- Concomitant medication
- Adverse events

11.2 Conditioning

Prior to conditioning, a central venous catheter will be placed e.g. in the subclavian vein under local anaesthesia. The conditioning regimen consists of:

- To achieve in vivo T cell depletion:
  - Intravenous cyclophosphamide 50 mg/kg/day for 4 consecutive days (total 200 mg/kg)
  - Intravenous rabbit antithymocyte globulin (rbATG: Thymoglobulin®, Genzyme) 2.5 mg/kg/day (total dose 7.5 mg/kg) starting 2 days after the first dose of cyclophosphamide, for 3 days.
- To improve tolerability:
  - Methylprednisolone 1 mg/kg/day (total 3 mg/kg) starting 2 days after the first dose of cyclophosphamide for 3 days

Infection prophylaxis whilst patients are neutropenic will be given also according to the local practice: (normally ciprofloxacin and an antimicotic such as fluconazole).

11.3 Transplantation

The day of transplantation will be 6 days after the start of cyclophosphamide administration, and 24 hours after the end of rbATG and methylprednisolone.

Stem cells are thawed and infused according to local standard operating procedures on the day of infusion. The number of CD34+ cells to be reinfused should be ≥ 3 x 10^6/kg. It is advisable to perform also a clonogenic test on the fraction to be reinfused. Hyperhydration, alkalinisation of the urine and mesna will be given to prevent hemorrhagic cystitis, according to local practice.

12 PROCEDURES FOLLOWING GROUP A TRANSPLANTATION

During these two years, patients will be managed according to best practice (surgery, prednisolone, immuno-suppressive drugs, infliximab or other treatments at the discretion of their physician). See Section 13 for further description of the management of Crohn's disease symptoms after mobilisation.

12.1 Year One Assessments

The schedule of assessments is shown in Table 2. Full details of the assessments are given in Section 22. During this period patients will also complete the patient diary weekly, and daily for the 7 days preceding each study assessment.

Both groups will have assessments at equivalent time-points approximately every 6 weeks during the first year after Group A undergo transplantation, to enable comparison between the groups. The exception to this is for Group A who have 8 weeks between the week 52 assessment and their next assessment at 60 weeks. During this time, patients in Group B, if suitable, will be undergoing conditioning and transplantation and will have their week 60 assessment post-transplantation.

The comparison of the groups at 52 weeks after T0 is the primary endpoint of the study, and a detailed assessment of patients' clinical state and quality of life is carried out (see Table 2). The acceptable time-range for this assessment is between 50 and 54 weeks after T0.

12.2 Group B Transplantation

After the 52 week assessment, the investigator should confirm that the patient remains suitable for transplantation by returning the Confirmation of Delayed Transplant form (Appendix 12) to the SAO. Group B patients then enter the conditioning and transplantation phase. A date should be identified well in advance for a delayed transplant to be completed on average 2 weeks after the week 52 assessment. This will require the process of conditioning to start approximately one week after the week 52 assessment i.e. week 53, which is now 58 weeks after successful mobilisation. Conditioning and transplantation will be carried out as described in Section 10.

12.3 Year Two Assessments

Approximately 6 weeks after transplantation of Group B, both groups will begin 6-weekly assessments equivalent to those during Year 1 (see Table 2 for details).

During this period patients will also complete the patient diary weekly, and daily for the 7 days preceding each study assessment.

Table 2: Assessments during the first 2 years following Transplantation (HSCT)

		Assessments for both Groups A & B									Assessments for both Groups A & B							
Weeks since Transplantation of Group A	**0**	**6**	**13**	**19**	**26**	**32**	**39**	**45**	**52**	**54**	**60**	**67**	**73**	**80**	**86**	**93**	**99**	**106**
Event	**HSCT Group A**									**HSCT Group B**								
History and examination	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Medical services used & employment history	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Haematology	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Clinical Chemistry	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Serology					*				*					*				*
ECG	*				*				*	*				*				*
Chest X-ray	*	*							*	*	*							*
Pulmonary function tests									*									*
Pregnancy test	*								*	*								
CDAI	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Harvey Bradshaw	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
IBDQOL					*				*					*				*
EuroQOL					*				*					*				*
Karnofsky Index					*				*					*				*
Dexa scan.									*									*
Lymphocyte subsets	*				*				*	*				*				*
Cytokines	*				*				*	*				*				*
Small bowel imaging									*									*
Upper endoscopy									*									*
Colonoscopy									*									*
Sigmoidoscopy #		*	*		*						*	*		*				
Rectal biopsy: Histology +		*	*		*				*		*	*		*				*
CDEIS									*									*
Concomitant Medication	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Adverse Events	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Group A patients only
Pre-conditioning assessments to be repeated only if T0 is > 3 days after PM28

Group B patients only
Note: At 54 weeks, this equates to 59 weeks post successful mobilisation

where clinical condition allows (for myofibroblast reconstitution studies)
+ in all patients undergoing colonoscopy or sigmoidoscopy
Note: Week 0 in this table may be the same visit as PM28 in Table 1, for patients in Group A
Additional monitoring will be carried out at the discretion of transplant centre according to the clinical need.

To summarise, table 3 shows the comparability of the assessment periods for year 1 and year 2.

Table 3 Comparability of the assessment periods

Year 1	**Week since Transplantation of Group A**	**0**	**6**	**13**	**19**	**26**	**32**	**39**	**45**	**52**
	Event	**HSCT Group A**								
Year 2	**Week since Transplantation of Group A**	**54**	**60**	**67**	**73**	**80**	**86**	**93**	**99**	**106**
	Event	**HSCT Group B**								

12.4 Assessments in years 3 to 5

The main assessments of this trial are in years 1 and 2. In years 3-5, patients are followed up and will make visits every 6 months, with assessments as shown in Table 4. During this period patients will complete the patient diary card on a weekly basis and also complete the daily diary card for the seven days before each scheduled study assessment.

Table 4 Assessments in years 3 to 5

Months since Transplantation of Group A	**30**	**36**	**42**	**48**	**54**	**60**
History and examination	*	*	*	*	*	*
Medical services used & employment history	*	*	*	*	*	*
Haematology	*	*	*	*	*	*
Clinical Chemistry	*	*	*	*	*	*
Lymphocyte subsets		*		*		*
Cytokines		*		*		*
ECG						*
CDAI	*	*	*	*	*	*
Harvey Bradshaw	*	*	*	*	*	*
IBDQOL	*	*	*	*	*	*
EuroQOL	*	*	*	*	*	*
Karnofsky Index	*	*	*	*	*	*
Upper endoscopy				*		
Colonoscopy & CDEIS				*		
Rectal biopsy: Histology+				*		
Concomitant Medication	*	*	*	*	*	*
Adverse Events	*	*	*	*	*	*

+ in all patients undergoing colonoscopy or sigmoidoscopy

13 PATIENT CARE AFTER TRANSPLANTATION

13.1 Supportive care

Supportive care measures, including prophylactic or therapeutic antibiotics, anti-viral or anti-fungal agents, will be taken according to local standard operating procedures for such patients. Prophylactic medication (e.g. oral acyclovir 400 mg bd, until 13 weeks after transplantation) is recommended for HSV prophylaxis. The choice of anti-emetic drugs after cyclophosphamide administration should follow local practice. Cell replacement therapy, including red cell and platelet transfusions, will be in accordance with local standard operating procedures for such patients.

Growth factors: G-CSF will be infused only in case of fever associated with prolonged neutropenia. Full blood count will be monitored daily and liver, kidney and coagulation function on alternate days. Parameters for hematologic recovery: neutrophils: $1x10^9$/l; platelets: $50x10^9$/l non-transfused for at least 3 days.

Oral sulphamethoxazole and trimethoprim is recommended to prevent pneumocystis carinii infection from discharge until 26 weeks after transplantation.

13.2 Monitoring after discharge

Haematological and biochemical parameters will be measured once a week from discharge until 30 days after the transplant, then once approximately every 6-7 weeks for 2 years, than every six months until 5 years after the transplant. The timing of the assessments is summarised in Table 2.

Monitoring of either CMV-associated pp65 antigen or PCR for virus-associated DNA fragments (or both) and EBV virus monitoring is strongly recommended until 90 days after transplant.

13.3 Management of Crohn's disease symptoms

Following mobilisation and/or HSCT, patients in either treatment group should receive any treatment deemed necessary for management of their Crohn's disease. This may include reinstatement of immunosuppressive drugs. In both treatment groups the goal will be to use the minimum amount of corticosteroids and immunosuppressive drugs necessary for the best possible clinical status, and to avoid adrenal insufficiency. All anti-inflammatory, immunosuppressive and analgesic drugs will be recorded at each assessment. Corticosteroids will normally be maintained at the same dose throughout the mobilisation phase and for 6 weeks thereafter or until recovery from immunoablation and HSCT allows weaning. Prednisolone can be increased at the discretion of the investigator for a flare up of symptoms or need to cover adrenal suppression.

Steroid weaning: If Crohn's disease activity is improved or stable at any point beyond 2 months after mobilisation, the daily dose of prednisolone will be reduced by 5mg every 1-2 weeks. Any immunosuppressive drugs will normally be maintained until prednisolone has been successfully weaned, followed by 2 step weaning of the immunosuppressive treatment. Patients requiring infliximab will however normally need to maintain a background treatment with immunosuppressive drugs or corticosteroids to maximise its effectiveness.

Treatment of a flare of activity (CDAI ≥220) Patients should receive best available care for active Crohn's disease regardless of the treatment group to which they were randomised. Treatment should be with the most effective regime for that patient based upon current

assessments and previous responses. Once the patient is in remission (CDAI $\leq$ 150), immunosuppressive drugs should typically be weaned over 8 weeks by reducing the dose and then stopping the drug. All anti-inflammatory, immunosuppressive and analgesic drugs will be recorded at each assessment.

14 TRIAL MEDICATION

All the medication to be used during the study is currently used for stem cell mobilisation or treatment of Crohn's disease, and will be prescribed by the Investigator and obtained from the hospital pharmacy in the normal way. No trial medication will be supplied by the SAO and none of the medication is blinded.

All medication taken by patients participating in the study will be recorded in the CRF.

15 SAFETY

15.1 Toxicity

High dose immunoablation and autologous stem cell transplantation can incur fatal complications. The treatment schedules used in this protocol can cause transient pancytopenia potentially resulting in septic and hemorrhagic complications and severe local tissue necrosis if leakage into the extravascular compartment occurs. Additional problems that commonly occur during high dose immunoablation and autologous stem cell transplantation are described below:

Oral mucositis: A sore mouth is a common complication.

Hyperuricaemia may occur, resulting in uric acid crystal formation in the urinary tract with associated renal dysfunction.

Nausea and vomiting cause considerable distress to many patients who receive chemotherapy, and to a lesser extent abdominal radiotherapy, and may lead to refusal of further. Drugs may be divided according to their emetogenic potential and some examples are given below, but the symptoms vary according to the dose, to other drugs administered and to individual susceptibility.
Mildly emetogenic treatment—fluorouracil, etoposide, methotrexate (less than 100 mg/m^2), the vinca alkaloids, and abdominal radiotherapy.
Moderately emetogenic treatment—doxorubicin, intermediate and low doses of cyclophosphamide, mitoxantrone (mitozantrone), and high doses of methotrexate (0.1–1.2 g/m^2).
Highly emetogenic treatment—cisplatin, dacarbazine, and high doses of cyclophosphamide.

Bone-Marrow Suppression: All cytotoxic drugs except vincristine and bleomycin cause bone-marrow suppression.

Fever in a neutropenic patient (neutrophil count less than 1.0×10^9/l) requires immediate broad-spectrum antibacterial therapy.

Alopecia: Reversible hair loss is a common complication, although it varies in degree between drugs and individual patients. No pharmacological methods of preventing this are available.

Reproductive function: Most cytotoxic drugs are teratogenic and should not be administered during pregnancy, especially during the first trimester. Alkylating drugs carry the risk of causing permanent male sterility (there is no effect on potency). For Women the span of reproductive life

may be shortened by the onset of a premature menopause.

Further potential toxicities of individual drugs used in mobilisation and conditioning, or toxicities seen with individual drugs that patients may receive during the course of the trial are described in Appendix 13.

15.2 Definition of Various Adverse Events

In order to comply with the standards for Good Clinical Practice it is essential that investigators are aware of the different definitions related to adverse events and how to record, report and review each of these specific occurrences. For the purpose of this protocol adverse events are classified in to the following categories:

Adverse Event (AE)
means any untoward medical occurrence in a clinical trial subject to whom an intervention or medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product or intervention. This includes abnormal laboratory findings, symptoms or disease temporally associated with the use of a medicinal product, whether or not related to the product or intervention.

Adverse Reaction (AR)
means any untoward and unintended response in a subject to any intervention or investigational medicinal product, which is related to that intervention or product.

Serious Adverse Event (SAE), Serious Adverse Reaction (SAR), or Unexpected Serious Adverse Reaction
means an adverse event, adverse reaction or unexpected adverse reaction respectively that does not necessarily have a causal relation ship to the treatment, and that at any dose:

- results in death
- is life threatening
- requires hospitalisation or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity or
- consists of a congenital anomaly or birth defect

Life threatening in the definition of a serious AE or AR refers to an event in which the patient *was at risk of death at the time of event*; it does not refer to an event, which hypothetically might have caused death if it were more severe.

***Suspected Unexpected* Serious Adverse Reaction (SUSAR)**
means an adverse reaction that is classed in nature as serious and which is not consistent with the information about the medicinal product or intervention in question set out

- in the case of a licensed product, in the summary of product characteristics (SmPC) for that product
- in the case of any other investigational medicinal product, in the IB relating to the trial in question

Therefore a serious event or drug reaction is not defined as a SUSAR when:

- it is serious but expected
- it does not fit the definition of a SAE or SAR, whether expected or not

15.3 Assessment of Causality

The relationship of adverse events to a medicinal product should be determined according to the following classification.

Not Related: the adverse event is not reasonably related to the medicinal product / intervention - or another cause can itself explain the occurrence of the event

Unlikely Related: the adverse event is doubtfully related to the medicinal product / intervention but can't be fully ruled out.

Possibly Related: the adverse event is reasonably related to the medicinal product / intervention, but the event could have been due to another, equally likely cause

Probably Related: the adverse event is reasonably related to the medicinal product / intervention, and the event is more likely explained by the drug than by any other cause

Definitely Related: the adverse event is clearly related to the medicinal product / intervention and there is no other cause to explain the event or a re-challenge (if feasible) is positive

All adverse events, whether or not considered related to the medicinal product or intervention, must be documented in the CRF. In addition to assessment of causality, severity of the adverse event should also be determined. Adverse events will be defined as mild, moderate or severe. The definition for each class of severity is documented in CRF8 (Medication Log and Adverse Event Forms).

15.4 Reporting of Serious Adverse Events (SAEs)

All events that fall into the SAE category must be reported (in as much detail as possible) to the Study Administration Office in Nottingham by telephone or by fax, **within 24 hours** of observing or learning of the event. This notification must be followed-up in writing using the SAE report form (Appendix 14) **within maximum 2 days** (for contact details see protocol title page). Medical terminology should always be used to describe any event. Investigators should avoid vague terms such as "sick". Where some information (e.g. date of resolution of event) is not available at the time of notification, the SAO should be notified of the event within the timelines above and supplementary information provided when available.

The following attributes must be assigned when reporting:

- Detailed description of the event
- Dates of onset and resolution
- Severity of the event

- Assessment of relatedness to treatment (see Section 15.3)
- Other suspect drugs/devices
- Action taken and outcome

The investigator may be asked to provide further information, and where a death has occurred autopsy reports and relevant medical reports should be sent with the notification or as soon as available.

The SAO, in conjunction with the Trial Steering Committee, will review all SAEs and classify them as SUSARs or non-SUSARs.

Patients experiencing serious adverse events considered 'related' to the study treatment will be followed up by the Principal Investigator until the event is resolved or considered stable. It will be left to the Principal Investigator's clinical judgment whether or not an adverse event is of sufficient severity to require that the patient should be withdrawn from study treatment. Patients may also voluntarily withdraw from the study due to what he or she perceives as an intolerable adverse event. All patient withdrawals due to adverse events should be documented and reported to the SAO without delay, and patients must be given appropriate care under medical supervision until adverse event symptoms cease or the condition becomes stable (see Section 16).

Any pregnancy occurring during the clinical study should be documented in the CRF and reported to the SAO, and withdrawal of the patient should be considered (see Section 16).

15.5 Expedited reporting of SUSARs

As of May 1st 2004, the sponsors (or their representatives) of clinical trials conducted in the EU and EEA must ensure that all relevant information regarding suspected unexpected serious adverse reactions (SUSAR) are recorded and reported in an expedited fashion.

It is a legal requirement of the sponsor to report *fatal or life-threatening SUSARs* within 7 calendar days to the relevant Regulatory Authorities after receiving first notification of the event. Non-fatal and non life-threatening SUSARs must be reported to the Regulatory Authorities within 15 calendar days. It will be the responsibility of the SAO to review all reported SAE's and evaluate them for expectedness, and to report SUSARs to the relevant Regulatory Authorities, Ethics Committees, and institutional bodies as required.

15.6 Data Safety Monitoring Committee (DSMC)

An Independent Data Safety Monitoring Committee with the power to stop the study at any time will review progress regularly and not less frequently than every 10 patients registered, or every one reported death, whichever is the sooner. The SAO will inform the committee when a meeting is required, and will ensure that the committee members have all details of all SAEs and SUSARs.

The DSMC will consider whether the study should continue according to the protocol, whether any modifications to the protocol are required, or whether the study should be discontinued. They will consider stopping the trial in the following circumstances:

1. If there are any deaths related to the treatment.
2. If there are more than 4 similar SUSARs following transplantation.

3. If any pattern of serious unexpected events occurs that places trial participants at risk.
4. Any other unexpected developments causing concern

The SAO will promptly implement any recommendations of the DSMC.

15.7 New Information regarding trial safety

In the event of any new information becoming available during the trial, which impacts on patient safety or on may affect patient's willingness to consent to the trial, the Trial Steering Committee shall as the SAO to inform all Principal Investigators participating in the trial, in writing. If necessary, Principal Investigators will ask ongoing patients to re-consent or withdraw from the trial.

16 PATIENT WITHDRAWAL

Patients may withdraw from the study at any time and for any reason. Reasons for withdrawal may include:

1. Death whatever the cause
2. Major organ failure
3. Non-compliance of the patient
4. Major protocol violations
5. Loss to follow-up
6. Repeated mobilization failure
7. Adverse events necessitating withdrawal
8. Patient's request, withdrawal of consent
9. Other

If a patient becomes pregnant between mobilisation and transplantation, and a delay to transplantation is required, patient withdrawal will be considered.

The date and reasons for patient withdrawal will be recorded in the CRF, and the SAO will be notified. Patients who withdraw following transplantation should be followed up according to EBMT standard patient care protocols.

17 STATISTICAL ANALYSIS

17.1 Sample Size Justification

Hemopoietic stem cell transplantation (HSCT) is a sufficiently intensive regimen that only large changes are of interest. Uncontrolled data imply a substantial affect[12]. Twenty four patients will undergo early HSCT. They will be compared to 24 patients who undergo mobilisation followed by best clinical practise. Because this phase is to assess a long-term change and because mobilisation may exert some benefit, which may or may not persist, this phase will be assessed as a comparison between groups at 1 year (52 weeks). The power of the study to detect differences in proportions in sustained clinical remission (2 sided chi square test) at 1 year, assuming 10%, 20% or 30% remission in the control group are shown in Table 5. Even with a less severe group of patients, in the ACCENT trial, only 30 per cent remained in remission after one year of intensive maintenance therapy with infliximab[26].

Table 5. Power of the study with various remission rate assumptions.

Control patients	HSCT patients	
	80% power	90% power
10%	48%	58%
20%	60%	65%
30%	70%	75%

Forty eight patients will undergo mobilisation. The effect of this manoeuvre will be assessed using the CDAI as the primary measure. There is 80% power to detect a change of 50% and 90% power to detect a change of 63% at 4 weeks. Additional descriptive evaluations will include CRP. The patients we intend to study are those that are kept under intensive follow-up because of the severity of their illness and we anticipate that none will be lost to follow-up.

17.2 Statistical Analysis

The primary analysis will be conducted per protocol using a chi-squared analysis.

For the secondary endpoints, the chi-squared technique will be used for comparison of proportions; log rank analysis for life table data; t-testing for parametric continuous variables; appropriate non-parametric analyses for continuous data that are not or cannot be normalised; analysis of variance for the influence of multiple factors on continuously variable endpoints, and logistic regression analysis for the influence of co-factors on binary endpoints. Multi-level logistic regression will be used to account for the fact that we will have hierarchical data (patients within sites). Safety data will be compiled and presented descriptively.

The primary analysis and related secondary analyses will be conducted as soon as all patients have completed one year in the study (i.e. covering the pre-transplantation period for patients randomised to delayed transplantation). Analysis of the follow-up data will be conducted after all patients have completed the five year follow-up period.

Sub-group analyses are not planned but the proposed statistical analysis will be revised prior to lock of the one year database.

Events occurring to patients in Group B during the 2 weeks while Group A undergo transplantation, and events occurring to patients in Group A during the corresponding 2 weeks after the 52 week assessment while Group B undergo transplantation, will be recorded but not included in the statistical analysis, to avoid bias. This period will be kept under review and adjusted if necessary to avoid assessment distortion by virtue of delay.

18 STUDY ORGANISATION AND ADMINISTRATION

18.1 Participating Centres

A participating centre can be any institution with expertise in the diagnosis and treatment of patients with severe Crohn's disease, and a registered hematopoietic stem cell transplantation centre, approved by the Trial Steering Committee as capable of handling the complexity and risks of stem cell transplantation in patients with autoimmune diseases. Therefore centres accredited for allogeneic transplantation will be allowed to join the trial. The possible inclusion of other centres with only autologous bone marrow transplantation (BMT) accreditation will be evaluated on a case by case basis by the Trial Steering Committee. Participating centres should aim to enrol at least 2 patients in 2 years.

Participating centres are required to submit study agreements and other study related documents before the first patient can be registered. For full details see section 5.1 (Pre-study Documentation Requirements)

18.2 Study Administration

A Trial Steering Committee (Appendix 16) will oversee trial conduct. Study organisation will be coordinated by the Study Administration Office (SAO) in Nottingham. An independent data safety monitoring committee (DSMC) has been set up, and their role is described in Section 15.6. The members of the DSMC are listed in Appendix 17.

18.3 Amendments to the protocol

All substantial changes to the protocol must be documented as a written amendment, and reviewed and approved by the trial Steering Committee and all bodies who approved the original protocol prior to implementation, unless they address an immediate safety risk. The SAO will distribute approved amendments to the participating centres.

18.4 Reasons for Terminating the Study

The study may be terminated for any of the following reasons:

- Completion of the study
- Failure to recruit sufficient subjects within a reasonable time period, as assessed by the Steering Committee
- Availability of new information which renders continuation of the study unethical
- Recommendation by the Data Safety Monitoring Committee

18.5 Trial Funding

A payment of €1250 per patient will be made to participating centres on randomisation of each patient, by the SAO. This payment will cover the administrative burden associated with randomising a patient into the trial. It will be the responsibility of the investigator to raise funds locally for all other medical costs associated with transplantation. However, whilst these are substantial, the case for funding may rest upon recognition that they are no more than the costs of regular treatment with infliximab, which costs €20-30,000 pa when used according to optimal protocols[27]. The availability of funding will be considered by the trial Steering Committee when considering potential patients put forward to be registered into the trial.

The Sponsor of the study is the EBMT Autoimmune Disease Working Party. The Broad

Foundation is providing funding for trial administration. The Horton Foundation (Switzerland) partly supports trial organisation and patient and medical costs within Switzerland.

18.6 Liability Insurance

The EU Clinical Trials Directive (2001/20/EC) requires that insurance or indemnity is in place to cover the liability of the investigator and sponsor. As the sponsor of this trial the EBMT offers indemnity cover via policies from Gerling Insurance Company in Cologne, Germany. This will provide compensation in the event of a patient sustaining an injury as a result of participation in the study, providing the protocol has been adhered to.

To ensure appropriate insurance coverage is in place, the centre registration form for trial participation should be submitted to the Study Administration Office in Nottingham (SAO) **before** the first patient is screened for trial participation. This enables the SAO to register the centre with the EBMT for inclusion into the insurance policy.

Copies of the insurance policy can be requested from the SAO (contact details on protocol cover), or directly from the EBMT by contacting: EBMT Trials Office (contact details are filed in the Trial Master File).

18.7 Trial duration

The trial is expected to start in autumn 2005, and recruit over 24 months. Primary trial endpoints will be available after 4 years. The end of trial is defined as: the last study visit (60 month follow-up visit) of the last patient enrolled into the trial.

19 ETHICAL CONSIDERATIONS

The EU commission Directive (2005/28/EC) declares that clinical trials shall be conducted in accordance with the declaration of Helsinki on Ethical Principals for Medical Research Involving Human Subjects, adopted by the General Assembly of the World Medical Association (1996). It shall be applied by all parties involved and at every step in the clinical investigation from the first recognition of the need and justification to the publication of results.

The protocol has been written, and the study will be conducted according to the guidelines for Good Clinical Practice issued by the European Union. The protocol will be approved by the EBMT/EULAR Working Party Autoimmune Diseases, ECCO Scientific Committee and by the relevant Competent Authorities, Ethical Committees and Institutional Review Boards. All changes to the protocol must be reviewed and approved prior to implementation. The Ethical Committees will be informed and consulted in case of unexpected serious adverse events including death.

Prior to initiation of the study at their centre, each potential investigator will submit the study protocol, consent form and any other documents as may be required, to their local Ethics Committee (EC) and Institutional Committee (where applicable) for review and approval. The investigator will request the Committees to provide a letter documenting approval, referring to the study by date, title, protocol number and the documents reviewed. This letter should be provided to the SAO before that centre begins to enrol patients into the study.

20 DOCUMENTATION & QUALITY ASSURANCE

Members of the Steering Committee, acting on behalf of ECCO and EBMT will act to ensure that the highest standards of care are afforded patients. Because of the sporadic way in which patients suitable for the trial will present, a systematic on-site monitoring approach will not be possible. However, the centres approved by EBMT as suitable for participation in the trial are by definition required to maintain the standards of care expected in this study.

20.1 Case Record Form Completion

All Investigators will be provided with copies of Case Report Forms (CRFs) to record the pre-registration assessments (CRF1) and separate forms to record data on patients successfully registered into the study (CRF2).

Data collected on each subject will be maintained as accurately and completely as possible with entries recorded in black ink on the Case Report Forms (CRFs). Any error should be crossed out with a single stroke and initialled and dated by the person making the correction (typing correction fluid must not be used). The personal data recorded on all documents will be regarded as confidential, and to preserve the subject's anonymity, only their initials, date of birth and unique trial number will be recorded on the CRF. The Principal Investigator at each centre will be responsible for the timing, completeness and accuracy of the CRFs and he/she will retain a copy of each CRF.

Completed CRF pages, patient diaries and questionnaires will be photocopied, and the original sent to the SAO for data entry. This should be done no less frequently than 6 monthly. Each Principal Investigator will retain a copy of the CRFs, patient diaries and questionnaires of patients enrolled at their centre, and will be responsible for archiving this at the end of the trial.

20.2 Site Initiation and Monitoring

The study monitor will be responsible for establishing the schedules and procedures to be followed for initiation of centres and monitoring the study, and these will be agreed with the Chief Investigator and documented in a Standard Operating Procedure (SOP). Monitoring visits will normally take place in the period around mobilisation and transplantation and less frequently thereafter. The monitor will also maintain telephone and written contact between visits as appropriate.

At each monitoring visit the investigator will be make the completed CRFs, patient notes and any other source data available to the monitor, and will be expected to allocate adequate time for the review and verification of records with the monitor. Time will also need to be spent with other centre personnel for the review of study procedures and facilities at the centre.

For the purpose of this study, the study Site File, signed Informed Consent forms, the CRFs, hospital notes and original reports of test results will be the source documents, and the Source Data Verification (SDV) plan will be documented and agreed with the Principal Investigator and form part of the Initiation Visit Report.

20.3 Curriculum Vitae (CV)

The Trial Steering Committee will be responsible for ensuring all Principal Investigators are

appropriately qualified to take part in the study, when considering which centres will participate.

All Principal Investigators will provide the SAO in Nottingham with an up-to-date copy of their CV, personally signed and dated. Furthermore, the principal investigator will sign a statement confirming that up-to-date copies of CVs of their co-investigators are kept in the trial file at the centre. Study activities delegated to other centre staff will be documented in the study file and investigator contract, and the monitor may request CVs of personnel responsible for other activities, either for review during monitoring or to file at the SAO, if considered necessary.

20.4 Quality Assurance

A quality assurance (QA) audit may be conducted by or on behalf of to ensure compliance of the study with GCP. The investigator must allow the QA auditor access to all relevant medical records, study related files and CRFs.

20.5 Archiving of Trial Related Documentation

All study documentation will be archived. The investigator must retain his/her copies of the CRFs, investigator Study File and all source documentation, for at least 15 years after completion of the study. If the investigator moves/retires he/she should inform trial Steering Committee as to whom will take over responsibility for the study documentation.

The SAO will arrange for all the central study documentation to be securely archived for at least 15 years.

20.6 Use of Information

After completion or termination of the trial a final clinical report – the Sponsor's Report - will be prepared by the Trial Steering Committee. This report will be submitted to the Chief Investigator to review and confirmation that it accurately represents the study. A copy will be made available to all participating Principal Investigators.

20.7 Publication Procedures

Investigators are requested not to publish or present results from their centre until the definitive report has been written. The definite report(s) must include all data from all centres and will be written by the Trial Steering Committee with involvement of participating centre trialists, data manager(s) and statistician, according to standard requirements for authorship, and will acknowledge all participating centres to this trial. Subsequently individual investigators may make local reports describing their own centre's experience with the permission of the Steering Committee.

21 EXPERIMENTAL LABORATORY INVESTIGATIONS AND SIDE STUDIES

Additional laboratory investigations and side-studies may be performed subject to the required additional ethical approval and informed consent. These may be either procedures additional to those described in this protocol, or a new use of material collected as specified here. In the case of the latter, patients may have already consented to use of the material for future studies (see Section 3.4, describing consent to genotyping work).

All blood and tissue samples taken from the patients for research purposes will be locally stored for complementary experimental studies, unless described otherwise in future approved protocols.

22 NOTES ON CERTAIN TRIAL PROCEDURES

- **Visualisation of the small bowel:** This can be done by barium meal and follow through or by small bowel infusion study according to local expertise. Double contrast techniques should be used as far as possible and views of the whole small bowel including terminal ileum obtained.
- **Colonoscopy:** This may be performed with or without sedation and analgesia according to local practice. The extent and severity of all diseased areas should be recorded in the CRF. At least four still photographs of each abnormal area should be taken and the procedure evaluated according to the CDEIS. The entire procedure will be recorded on digital videotape to enable blinded comparisons later. Where available, zoom views should be taken along with views employing image enhancement such as dye spraying. A barium enema cannot substitute for colonoscopy.
- **Gastroscopy:** This may be performed with or without sedation, analgesia and local anaesthesia according to local practise.
- **Biopsy Processing Protocol:** .One to two samples of normal or abnormal segments should be taken from each of the rectum/sigmoid the descending colon, the transverse colon, the caecum/ascending colon and the terminal ileum at standardised sites, as specified in the CDEIS (Appendix 9). A maximum number of 20 samples should be taken. In the case of upper endoscopy, biopsy samples should be taken from oesophagus, body, antrum and duodenum and any additional abnormal areas. A maximum number of 8 samples should be taken. These samples should be fixed in formalin, and fixed in RNA later and frozen.
- **Sigmoidoscopy:** In addition to the colonoscopy at the start and end of the study, all patients should undergo sigmoidoscopy at 6, 13 and 26 weeks after engraftment. Sigmoidoscopy should be deferred in patients who are neutropenic, thromobocytopenic or unwell in any way which, in the opinion of the investigator, would lead to significant risk. The purpose of sigmoidoscopy is to follow immune and myofibroblast reconstitution in the gut. These samples should be processed as described above.
- **Cytokines:** Blood will be collected into a heparinised tube, spun down, plasma removed and stored at –40°C.
- **Lymphocyte subsets:** these will be analysed by FACS analysis.
- **Dexa scan:** at least 3 lumbar vertebra and the neck of the femur will be examined and a T-score calculated for each

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Appendix 1: Patient Information Sheet

[local headed paper to be used]

Version Number: 1.0 Date:21 December 2005

Protocol Title: Autologous Stem Cell Transplantation for Crohn's Disease (ASTIC). A multicentre, prospective, randomised phase III study conducted by the European Crohn's and Colitis Organisation (ECCO), sponsored by the European Group for Blood and Marrow Transplantation.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the trial?
We would like to tell you about an international clinical study for patients suffering from severe Crohn's disease. Unfortunately, some patients fail to respond to best clinical treatment in Crohn's disease and some only experience temporary benefit, which is why the search for more effective treatments is continuing. Recently, an experimental treatment has been developed for severe Crohn's disease, called 'high dose immunoablation followed by autologous hematopoietic stem cell transplantation'. Hematopoietic blood stem cells are young, undifferentiated blood cells that can develop into differentiated ones, including lymphocytes, and over-reactive lymphocytes are thought to contribute to the development of Crohn's disease. These stem cells have nothing to do with embryonal stem cells or cloning of organs or individuals. This study involves removing your over-active lymphocytes (immunoablation) and replacing them using blood stem cells that had been taken (harvested) from your body earlier in the study. Conventional medication only temporarily suppresses the over-reactive lymphocytes.

By intervening at a relatively early stage in the disease, the likelihood of a long lasting beneficial response may increase. At present, about 30 patients suffering from Crohn's disease have been treated with stem cell transplantation worldwide. The results from those studies suggest that the therapy may be effective, but it cannot be concluded yet whether this treatment is better than any best clinical practice. An international collaborative group of medical specialists has agreed that this issue can only be solved by conducting a scientifically sound clinical study in which institutions from all over the world participate. This study is a European collaboration and we aim to treat a total of 48 patients in the different countries that are taking part.

What is being tested?
In the first stage of this study, stem cells will be removed (harvested) from all patients in a process called mobilisation. Drug treatment is used to mobilise the stem cells from your bone marrow, so that some of them can be harvested from the blood and stored for later use. It is useful to know that the drug used for mobilization your stem cells is also effective against

Crohn's disease, thus such preparatory phase resulting in a treatment by itself.

Patients will then be randomly assigned to undergo immunoablation and autologous stem cell transplantation either 4 weeks after mobilisation (early stem cell transplantation) or 59 weeks after mobilisation (delayed stem cell transplantation). Immunoablation means the elimination of the stem cells in your body, and is achieved by combining high doses of cyclophosphamide and antithymocyte globulin, both administered by infusion. Then the stem cells that were harvested earlier are transplanted (re-infused) into your blood, like a regular blood transfusion thereafter homing into the bone marrow. The re-infused stem cells give rise to a new generation of immune cells, replacing cells of the original 'sick' immune system.

By comparing the progress of patients who undergo early blood stem cell transplantation with those who receive late blood stem cell transplantation, the study will allow the value of immunoablation and stem cell transplantation to be assessed, whilst offering this new procedure to all patients that enter the study. It is possible that the process of mobilisation may give some benefit. The study will allow some assessment of this effect and control for it when assessing transplantation.

As well as the experimental treatment, all patients will receive any existing treatment they need, according to best current clinical practice. This will normally include the use of corticosteroids, immunosuppressive drugs and infliximab.

Genotyping:
There is some evidence that Crohn's disease has an underlying genetic cause. Some patients with Crohn's disease show mutations in the NOD2 gene and there may be other genetic polymorphisms associated with the disease. The group organising this study would like to investigate whether the patients with Crohn's disease, who enter the study, show abnormalities in these genes, as this information might help to develop future medicines. They would like your consent to use the blood samples taken from you during this study for genetic testing for the known mutations in NOD2 gene and other genes known to be associated with Crohn's, and for additional genetic testing in the future, when more information about genes that may cause Crohn's disease have been identified. It is up to you whether or not you agree to have this genetic testing done, and if you do agree to the testing you can decide whether you want to be told the results. You don't have to consent to the genotype testing to take part in the rest of this study.

If you do consent to the genotype testing, your blood may be stored indefinitely for this purpose. The stored blood may be used at any time in the future for genetic analysis as our understanding of the causes of Crohn's disease improves, although no analysis will happen without the approval of the investigators organising this study. While the blood samples are being stored you can request their withdrawal and destruction at any time.

In addition, we guarantee that any additional genetic information contained in your stored material will not be used for any other purpose and will not be communicated to or shared with any third party without your explicit knowledge and approval.

Storage of samples
During the course of this study we will collect blood samples, biological material and do other

tests to monitor your health and status of your Crohn's disease. With your permission we would like to store these samples to allow for the fact that they might be useful for new experiments that cannot be foreseen at present. If we or other investigators would like to use your samples for future experiments you will be contacted in advance and given a patient information sheet to explain what the samples will be used for. Your samples will not be used unless you have given consent for their use. It is up to you to decide if you would like your samples to be used in these future experiments. You should be aware that the researchers approaching you in the future may be different from the researchers in this study team and may include researchers in commercial companies. You do not have to give consent for your samples to be used in future to take part in this study and withholding consent when approached in the future will not affect the level care you will receive from your hospital.

Why have I been chosen?
You have been chosen because you have severe Crohn's disease and you are not responding to standard treatment.

Do I have to take part?
No, it is up to you to decide whether or not you want to take part in the study. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. When you are taking part, you may withdraw from the study at any time if you change your mind.

Whether or not you decide to take part in the study, your treating physician will discuss alternative treatments with you which will not be influenced by your decision regarding the study.

What will happen to me if I take part?
Should you be interested in participating, and give your consent, extensive pre-study investigations are needed to ensure that your medical condition meets the necessary requirements for the study. These investigations include blood tests, and evaluations of heart, lung and kidney function. If the results of these investigations show that you fulfil all the eligibility criteria to take part the study, and with your informed consent given, your physician will register you for the study at the central study management office in Nottingham University, England. The study management team will randomly allocate you to one of the two treatment options. Your treating physician does not have any influence regarding which of the two treatment options (early versus late transplantation) you will be allocated to.

Mobilisation: You will then undergo the process of mobilisation. You will have infusion of cyclophosphamide ($2g/m^2$/day) for two days, followed by daily subcutaneous injections of a specific growth factor (filgrastim) starting 5 days after the infusion of cyclophosphamide. As a result, stem cells will migrate from the bone marrow to the blood, and enough cells can usually be counted in blood samples after 5-8 days of filgrastim administration. Because mobilisation uses a powerful immunosuppressive drug, it may alone already induce an improvement in your clinical state. Your existing treatment other than corticosteroids (eg prednisolone) will be normally stopped for a period of about 2 months to reduce risks of treatment over-dosage. However, your doctor may be allowed to continue or re-start existing treatments according to clinical need, after consulting the group organising the study.

Leukapheresis: Once there are sufficient stem cells in the bloodstream, they will be harvested, a process called leukapheresis. This procedure requires you to tolerate two needles, one in each arm (or rarely the neck). One needle is used to transport your blood via a tube to the centrifuge, the machine that separates the stem cells from the blood itself; whereas the other needle and tube are used to re-infuse the reminder of the blood back to you. The whole procedure takes about 3 – 5 hours. The amount of blood in the infusion system is always less than ½ litre of blood. The collected cells will be cryopreserved (frozen) until later use at transplantation (see below).

A small number of patients experience a failure of blood stem cells collection. In this case your physician may decide either to repeat the procedure or to exclude you from the trial.

Randomisation: You will have an equal chance to be in either one of the two study treatments: Group A: Leukapheresis then early high dose immunoablation followed by autologous stem cell transplantation or Group B: Leukapheresis then delayed high dose immunoablation followed by autologous stem cell transplantation

Immunoablation and stem cell transplantation: If you are allocated to the early treatment option, autologous stem cell transplantation will be started approximately 4 weeks after leukapheresis (harvesting your cells). Immunoablation is achieved by infusions of high doses cyclophosphamide given on each of 4 days, with antithymocyte globulin ('ATG') on days 3, 4 and 5, and followed by re-infusion of the blood stem cells ('autologous stem cell transplantation') on the day after the last infusion of ATG. ATG is a protein derived from rabbits that is commonly used to effectively deplete immune cells in recipients of stem cell transplantation. You will need to be in hospital for several weeks to carry out these steps, and to cover any unwanted consequences that may occur as a result of the therapy. Antibiotic treatment and transfusions of red blood cells or platelets usually are necessary in the period immediately following the transplantation.

For patients allocated to delayed high dose immunoablation followed by autologous stem cell transplantation, these procedures will be carried out approximately 59 weeks after leukapheresis.

Your institute will provide you with more detailed information on the various aspects involved with transplantation. If needed, treatment with corticosteroids, immunosuppressive drugs such as azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, MMF and infliximab will be re-started by your doctor according to your clinical need, whichever treatment group you are in.

Study assessments: The main assessments of the study take place over 2 years. During this time, you will make clinic visits every 6-7 weeks (more regularly immediately after the transplant) for clinical assessment and tests. These will include blood tests, gastroscopy, colonoscopy (camera tests of the upper and lower bowel), barium X ray examinations of the small bowel and tests of heart lung and kidney function. You will be required to keep a weekly summary of your symptoms in a diary, and a daily symptom diary one week before every visit to the clinic, so that assessments of disease activity can be made. Furthermore we ask you to fill in questionnaires to assess the impact of your Crohn's disease and its treatment on the quality of your life a total of 13 times during the treatment and assessment period. In order to monitor long term effects, 6-monthly follow-up appointments are considered necessary for a minimum of 5 years after entry in the study, and you will be asked to continue keeping the weekly symptom diary during these 5 years.

What are the possible disadvantages and risks of taking part?
High dose immunoablation followed by autologous stem cell transplantation is an intensive treatment with risks of severe complications which on rare occasions have been fatal.

These complications may require hospitalisation at any time and include: infections, bleeding, heart failure, respiratory insufficiency (breathing difficulties), kidney failure, lymphoma. Less severe, but more frequent (reversible) toxicities include: nausea, fever, alopecia (hair loss), infertility, arthralgia (joint pain), myalgia (muscle pain), menstrual disorders and hematuria (blood in the urine) due to irritation of the lining of the bladder. Of course, the treating physicians will do their utmost to prevent these from occurring and treat them as best as they can when serious complications do occur.

The process of stem cell mobilisation is generally safer but can incur similar risks.

Existing drug treatments that you may receive also have side effects. Hospitalisation may be indicated when specific (rare) complications occur, such as severe infections. Other (reversible) side-effects include: nausea, alopecia (hair loss), anemia, bruising liver or kidney damage and infertility.

As part of this study you will have a number of X-ray examinations of different sorts. At least several of these you will have already experienced as part of your normal care. The purpose of these X-ray examinations is twofold: one, is to monitor for possible side effects or complications from your treatment; the other is to help assess how effective the treatment has been.

You should be aware that exposure to radiation similar to X-rays is a part of normal daily life. In Britain everyone receives on average about 2.2 units of radiation a year from *natural* sources (e.g. the sun, from the ground, in our food). In this study you will receive X-rays of up to about 50 units in total (possibly 90 units in exceptional cases). Of these you would have received about two thirds as part of your normal care, that is, if you had not entered the trial. Exposure to X-rays carries some risk of harm (the development of cancer later in life) proportional to the dose you receive. The dose of 50 units from this study carries about a 1 in 260 lifetime risk of suffering seriously from cancer (including fatal cancer) above the natural rate of cancer occurrence. This represents an increase of about 0.8% on the natural lifetime risk of developing cancer of around 1 in 2.

You will need to inform your private medical insurance company (if you have one) to make sure that your participation in the study does not affect your medical insurance.

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. Women who are at risk of pregnancy may be asked to have a pregnancy test before taking part to exclude the possibility of pregnancy. Women who could become pregnant must use an effective contraceptive during the course of this study. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.

What are the possible benefits of taking part?
High dose immunoablation followed by autologous stem cell transplantation may have beneficial

effects, although this must be balanced against potentially serious toxicity (side effects). There is some evidence that the treatment may offer long-term benefit from Crohn's disease.

What happens when the research study stops?
During or after either treatment, disease activity may progress or recur. If after the study period your condition deteriorates, your treating physician will discuss the available treatment options with you.

What is something goes wrong?
The sponsor of this research study (see below) will take out insurance coverage on your behalf with Gerling Insurance Company. This insurance covers the potential damage as a result of your participation to this study, which becomes evident during your participation in this study or within 5 years after your registration in this study. The insurance does not cover damage:

- Which occurs in offspring as a consequence of a negative impact of your treatment on your genetic material (DNA damage)
- Which was very likely to occur in view of the pre-treatment tests
- Which would have occurred if you would not have participated to the study

In order to keep the right of damage compensation it is important for you to follow the instructions of the investigators.

In case you believe to have suffered damage by your participation to this study, then you should report this as soon as possible to your treating physician in order to start the process of informing the insurance company. You will have to provide all necessary and required information to the insurance company regarding your claim. Omission of this obligation may lead to loss of damage reimbursement.

What will happen to data collected from me?
If you agree to take part in this research project, your study doctor will make a detailed record of your treatment in your hospital records and, with your permission, your primary care physician will be told about your involvement in the study. All information that is collected from you during the course of the research will be kept strictly confidential. Collected study data from you will be entered into an electronic database at the University of Nottingham, England, who are organising this study. Most information will also be shared with, and entered into the database of, the sponsor of this study – the EBMT (European group of Blood and Marrow Transplantation), as well as made available to the research group representatives of ECCO (European Crohn's and Colitis Organisation). This will involve your data being transferred between countries within the European Economic Area (EEA), who have similar data protection laws.

The EBMT is a charitable organisation that has been collecting data on donors and patients undergoing stem cell harvest and transplantation for over 20 years. The main EBMT database is held in the Netherlands. The purpose of the EBMT is to collect international transplant related information for research, and they may use the data from this study for future (as yet unspecified) research projects. With this information new and improved transplant procedures are developed with the aim to help improve the quality of transplant procedures, and the data from your participation in the study may be used to contribute to this. The EBMT also oversees hospital accreditation internationally.

All data stored in the above mentioned databases are non-identifiable. Only your initials, date of birth and hospital number are used for identification purpose. You will also be allocated a unique study number, which will be used for communication between the study management group in Nottingham and your treating team. Any information about you, which leaves the hospital, will have your name and address removed so that you cannot be recognised from it.

Representatives of the group organising the study will come to the hospital to look at your medical records to make sure that the study is being conducted properly. You should be aware that the Regulatory Authorities in your country also have the right to access your patient notes to see that the study is conducted according to procedures approved by the ethics committee. All persons viewing your hospital notes are bound by confidentiality laws.

You should be also be aware that all (paper) documentation collected during your participation in this study will be stored safely - in a way that ensures your confidentiality - for at least 15 years before being destroyed, in your treating hospital as well as in Nottingham. The information in the EBMT database would be stored for as long as the EBMT research organisation exists. To date the EBMT has collected valuable information on more than 200,000 stem cell donors and recipients.

What will happen to the results of the research study?
Some results will be available after all patients have finished their first year in the study, however, the final analysis of the study results will be done when all participating patients have completed 5-year follow-up. Once the results of the study have been analysed, after every patient has completed follow-up, Nottingham and the EBMT aim to publish the final study outcome in scientific medical journal(s) and in scientific meetings and conferences attended by physicians treating patients with conditions such as yours. Patients participating in the study will never be named and can never be identified in these publications and meetings.

Who is organising and funding the research?
The Sponsors of the study are the European Blood and Marrow Transplant (EBMT) Autoimmune Disease Working Party. *The study is supported financially by a grant from the Broad Foundation, with additional support in different countries (to be confirmed)*

Who has reviewed the study?
The protocol has been approved by the EBMT and European Crohn's and Colitis Organisation (ECCO) Clinical Trials Group. The study protocol has also been reviewed and approved by your national and local Ethics Committee, national regulatory authority and your hospital review board.

Contact for further information
Finally, in case some issues remain unclear to you, please contact your physician who will be happy to explain details of the study or treatment alternatives

Physician's Name: ……..

Contact Details: …….
…….
…….

Appendix 2: Patient Consent Form

USE HOSPITAL HEADED PAPER

PATIENT CONSENT FORM
ASTIC: Autologous Stem Cell Transplantation for Crohn's Disease
A trial conducted by the European Crohn's and Colitis Organisation (ECCO) and sponsored by the European Group for Blood and Marrow Transplantation (EMBT)

Centre Number: ___ | ___

Patient's Trial Number: ___ | ___ | ____ | ___

Principal Investigator: _____

	Consent for Participation in ASTIC Study	**Yes**	**No**
1)	I have read and understand the patient information sheet: Version ___, Date _____, and have been given a copy to keep	☐	☐
2)	I have been able to discuss the study with a Consultant Gastroenterologist and a Consultant Haematologist. I have been able to ask questions about the project and I understand why the research is being done and any risk involved.	☐	☐
3)	I have had sufficient time to reach my decision and I agree participate in this study	☐	☐
4)	I understand that my participation is voluntary and that I may withdraw from the study at any time I choose without explanation and without this affecting my future treatment.	☐	☐
5)	I give permission for someone from the research team to look at my medical records. I understand that the national regulatory authorities may review my hospital notes. I am aware that the information will be kept confidential by all persons reviewing my notes.	☐	☐
6)	I agree that my general practitioner be notified of my participation	☐	☐
7)	I understand that I will not benefit financially if this research leads to the development of a new treatment or medical test.	☐	☐
8)	I know how to contact the research team if I need to, and how to get information about the results of the research	☐	☐
9)	I consent to non-identifiable data being used in future EBMT research projects, provided the same level of protection for my privacy is applied and that the relevant regulatory authorities approve the future research.	☐	☐

PATIENT CONSENT FORM cont.

		Yes	No
10)	**Consent for sample storage and use in possible future research projects** I agree that the samples I have given and the information gathered about me can be stored by the research Investigators at this hospital for possible use in future projects, as described in the attached information sheet. I understand that some of these future projects may be carried out by researchers other than the investigators of this study team, including researcher working for commercial companies. I will be contacted prior to the use of my samples and informed about the studies intended. The samples will not be used unless I have given my consent for their use. I understand that am free to withdraw my approval for use of the samples at any time without giving a reason and without my medical treatment or legal rights being affected. *Note: You do not have to consent to sample storage and use in future research projects to take part in the ASTIC trial*	☐	☐
11)	**Consent for genetic research** I give permission to test my blood samples for mutations in the NOD2 gene and other mutations known to be associated with Crohn's disease, as part of this study. I **want / do not want** (*delete as applicable*) to be told the results of these tests. I understand I can change my mind about this later. *Note: You do not have to consent to genetic research to take part in the ASTIC trial*	☐	☐
12)	**Consent for future genetic research** I give permission to use my blood sample for additional genetic testing in the future, when more information about genes that may cause Crohn's disease has been identified. I **want / do not want** (*delete as applicable*) to be told the results of the future tests. I understand I can change my mind about this later. *Note: You do not have to consent to future genetic research to take part in the ASTIC trial*	☐	☐

SIGNATURES:		
Name of Patient (BLOCK CAPITALS)	Signature of Patient	Date
Name of person taking consent (BLOCK CAPITALS)	Signature of person taking consent	Date
Name of Consultant Haematologist (BLOCK CAPITALS)	Signature of Consultant Haematologist	Date
Name of Consultant Gastroenterologist (BLOCK CAPITALS)	Signature of Consultant Gastroenterologist	Date

Original version to be filed in Study file. One copy to be given to patient, one copy to be filed in patient notes.

Appendix 3: Crohn's Disease Activity Index (CDAI)

Crohn's Disease Activity Index (CDAI) is derived from 8 patient-derived parameters. The CDAI score is calculated by summation of the score for each parameter multiplied by the corresponding weighting/multiplication factor. The composite score ranges from 0 to 700. Index values of 150 or below are associated with clinical remission. Index values above 150 indicate active disease. Values above 450 are observed in patients with extremely severe disease. The ASTIC Trial enrols patients with a CDAI $\geq$ 250.

Crohn's Disease Activity Index (CDAI)

To be completed by the Investigator using the previous 7 days' patient diary data.

CROHN'S DISEASE ACTIVITY INDEX (CDAI)										
								Total in previous 7 days	**Multi-plication Factor**	**Sub-total**
NUMBER OF LIQUID OR VERY SOFT STOOLS *										
Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7			X 2	
ABDOMINAL PAIN/CRAMPS RATING 0 = None 1 = Mild – aware, but tolerable 2 = Moderate – interferes with usual activities 3 = Severe -– incapacitating									X 5	
Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7				
GENERAL WELL BEING 0 = Generally Well 1 = Slightly Under Par 2 = Poor 3 = Very Poor 4 = Terrible									X 7	
Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7				
TOTAL # OF LISTED CATEGORIES the patient now has experienced during the last 7 days ☐ A – Arthritis/Arthralgia ☐ B – Iritis/Uveitis, Erythema Nodosum or Pyoderma Gangrenosum ☐ C – Aphthous Ulcers ☐ D – Anal Fissure, Fistula, Abscess ☐ E – Other Fistula (Specify: _____) ☐ F – Fever over 100 F (37.8°C)									X 20	
ANTIDIARRHEAL DRUG THERAPY (i.e: loperamide, diphenoxilate, opiates) 0 = None 1 = Yes									X 30	
ABDOMINAL MASS 0 = None 2 = Questionable 5 = Definite									X 10	
ANAEMIA: (absolute haematocrit X100 = % haematocrit) Male: 47 - _____% = _____ Female: 42 - _____% = _____									X 6	
BODY WEIGHT: Standard body wt = _____kg Actual body wt = _____ kg Std. Wt – Acutal Wt. x 100% = ----------→ Std. Wt.									X 1	=
								CDAI SCORE = _____		

*For patients with an ileostomy or colostomy, please record the number of times the patient empties the bag.

APPENDIX 4: European Questionnaire of Lifequality (EUROQOL (Eq-5d))

Your Current State of Health

Please consider your state of health <u>today</u> and tick one box for each question.

Q.1: Your mobility….

- ❒ I have no problems in walking about.
- ❒ I have some problems in walking about.
- ❒ I am confined to bed.

Q.2: Your self-care…

- ❒ I have no problems with self-care.
- ❒ I have some problems with washing or dressing myself.
- ❒ I am unable to wash or dress myself.

Q.3: Your usual activities…(e.g. work, study, housework, family or leisure activities)

- ❒ I have no problems with performing my usual activities.
- ❒ I have some problems with performing my usual activities.
- ❒ I am unable to perform my usual activities.

Q.4: Pain / Discomfort…

- ❒ I have no pain or discomfort.
- ❒ I have moderate pain or discomfort.
- ❒ I have extreme pain or discomfort.

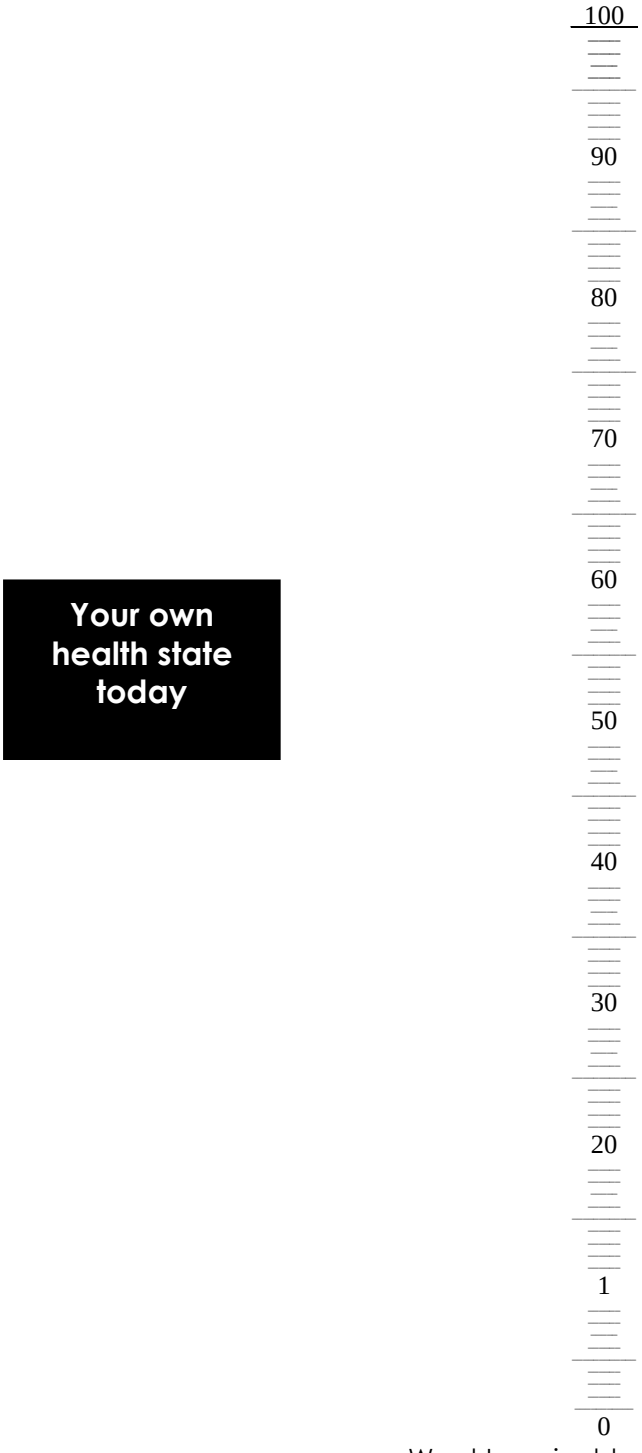
Q.5: Anxiety / Depression…

- ❒ I am not anxious or depressed.
- ❒ I am moderately anxious or depressed.
- ❒ I am extremely anxious or depressed.

How good or bad is your health today?

To help people say how good or bad a health state is, we have drawn a scale (like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0. We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health is today.

Best Imaginable Health State



Worst Imaginable Health State

Appendix 5: Inflammatory Bowel Disease Questionnaire

Inflammatory Bowel Disease Questionnaire (IBDQ)

*** Please read the entire introduction *before* you read the questionnaire.**

INTRODUCTION AND INSTRUCTIONS FOR QUESTIONNAIRE

This questionnaire is designed to find out how you have been feeling during the last week. You will be asked about symptoms you have been having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been.

On this questionnaire are 32 questions. Each has a graded response numbered from 1 through 7. Please read each question carefully and *circle* the answer that best describes how you have been feeling in the past 2 weeks.

EXAMPLE

How often have you felt unwell as a result of your bowel problem in the past two weeks?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

If you are having trouble understanding a question, STOP for a moment!

Think about what the question means to you. How is it affected by your bowel problem? Then answer as best you can. You will have the chance to ask the nurse questions after completing the questionnaire. This takes only a few minutes to complete.

Inflammatory Bowel Disease Questionnaire (IBDQ)

This questionnaire is designed to find out how you have been feeling during the last 2 week. You will be asked about symptoms you have been having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been.

1. How frequent have your bowel movements been during the last two weeks?

1. Bowel movements as or more frequently than they have ever been
2. Extremely frequent
3. Very frequent
4. Moderate increase in frequency of bowel movements
5. Some increase in frequency of bowel movements
6. Slight increase in frequency of bowel movements
7. Normal, no increase in frequency of bowel movements

2. How often has the feeling of fatigue or of being tired and worn out been a problem for you during the last two weeks?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

3. How often during the last two weeks have you felt frustrated, impatient, or restless?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

4. How often during the last two weeks have you been unable to attend school or do your work because of your bowel problem?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

5. How much of the time during the last two weeks have your bowel movements been loose?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

6. How much energy have you had during the last two weeks?

1. No energy at all
2. Very little energy
3. A little energy
4. Some energy
5. A moderate amount of energy
6. A lot of energy
7. Full of energy

7. How often during the last two weeks did you feel worried about the possibility of needing surgery because of your bowel problem?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

8. How often during the last two weeks have you had to delay or cancel a social engagement because of your bowel problem?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

9. How often during the last two weeks have you been troubled by cramps in your abdomen?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

10. How often during the last two weeks have you felt generally unwell?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

11. How often during the last two weeks have you been troubled because of fear of not finding a washroom?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

12. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have liked to have done during the last two weeks?

1. A great deal of difficulty; activities made impossible
2. A lot of difficulty
3. A fair bit of difficulty
4. Some difficulty
5. A little difficulty
6. Hardly any difficulty
7. No difficulty; the bowel problems did not limit sports or leisure activities

13. How often during the last two weeks have you been troubled by pain in the abdomen?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

14. How often during the last two weeks have you had problems getting a good night's sleep or been troubled by waking up during the night?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

15. How often during the last two weeks have you felt depressed or discouraged?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

16. How often during the last two weeks have you had to avoid attending events where there was no washroom close at hand?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

17. Overall, in the last two weeks, how much of a problem have you had with passing large amounts of gas?

1. A major problem
2. A big problem
3. A significant problem
4. Some trouble
5. A little trouble
6. Hardly any trouble
7. No trouble

18. Overall, in the last two weeks, how much of a problem have you had maintaining, or getting to, the weight you would like to be at?

1. A major problem
2. A big problem
3. A significant problem
4. Some trouble
5. A little trouble
6. Hardly any trouble
7. No trouble

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

19. Many subjects with bowel problems often have worries and anxieties related to their illness. These include worries about getting cancer, worries about never feeling any better, and worries about having a relapse. In general, how often during the last two weeks have you felt worried or anxious?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

20. How much of the time during the last two weeks have you been troubled by a feeling of abdominal bloating?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

21. How often during the last two weeks have you felt relaxed and free of tension?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

22. How much of the time during the last two weeks have you had a problem with rectal bleeding with your bowel movements?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

23. How much of the time during the last two weeks have you felt embarrassed as a result of your bowel problem?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

24. How much time during the last two weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels were empty?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

25. How much of the time during the last two weeks have you felt tearful or upset?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

26. How much of the time during the last two weeks have you been troubled by accidental soiling of your underpants?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

27. How much of the time during the last two weeks have you felt angry as a result of your bowel problem?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

28. To what extent has your bowel problem limited sexual activity during the last two weeks?

1. No sex as a result of bowel disease
2. Major limitation as a result of bowel disease
3. Moderate limitation as a result of bowel disease
4. Some limitation as a result of bowel disease
5. A little limitation as a result of bowel disease
6. Hardly any limitation as a result of bowel disease
7. No limitation as a result of bowel disease

29. How much of the time during the last two weeks have you been troubled by feeling sick to your stomach?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

30. How much of the time during the last two weeks have you felt irritable?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Inflammatory Bowel Disease Questionnaire (IBDQ) (Continued)

31. How often during the last two weeks have you felt a lack of understanding from others?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

32. How satisfied, happy, or pleased have you been with your personal life during the past two weeks?

1. Very dissatisfied, unhappy most of the time
2. Generally dissatisfied, unhappy
3. Somewhat dissatisfied, unhappy
4. Generally satisfied, pleased
5. Satisfied most of the time, happy
6. Very satisfied most of the time, happy
7. Extremely satisfied, could not have been more happy or pleased

To Be Scored By Investigator

Score: _____

Investigator signature: _____ Date of scoring: _____

Appendix 6: Karnofsky Scale

A 10-point scale developed by Karnofsky, Abelmann, Craver & Burchenal, 1948. An index for clinical estimation of a patient's physical state, performance, and prognosis after a therapeutic procedure.

100%	Perfectly Well. Able to work. Normal; No complaints; No evidence of disease.
90%	Can live a normal life. Able to work. Able to carry on normal activity; Minor symptoms.
80%	Normal activity with some effort. Able to work. Normal activity with effort; Some symptoms.
70%	Independent; not able to work. Cares for self; Unable to carry on normal activity.
60%	Requires occasional help with personal needs.
50%	Disabled; dependent. Requires considerable assistance and frequent care.
40%	Severely disabled; Patient needs nursing assistance and medical care but is not hospitalized.
30%	Severely disabled in hospital, death not imminent.
20%	Very sick. Active supportive treatment needed.
10%	Moribund. Fatal processes are rapidly progressing

Appendix 7: Weekly and Daily Patient Diary: Disease Activity Parameters

Patients will be asked to answer the following questions in a diary card weekly during their participation in the study, and daily during the week preceding each study visit.

Weekly Patient Diary Questions

Please complete this diary once a week on the same day each week, answering "Y" for yes, "N" for no, or using the rating scale given in the question. Your answers should describe your symptoms during the last 7 days. Thank you for your time.

Date: (Day/Month/Year)							
Average number of stools per 24 hour period, during the last week *For patients with ileostomy/colostomy, average no of times emptied bag per 24 hour period, during the last week*							
Average number of liquid or very soft stools per 24 hour period, during the last week							
Rate your abdominal pain/cramps since last filling in the diary (0=none, 1=mild, 2=moderate, 3=severe)							
Rate your general well being since last filling in the diary (0=well, 1=slightly below par, 2=poor, 3=very poor, 4=terrible)							
Have you had a fever over 100 F (37.8 C) since last filling in the diary?							
Have you had joint pain or problems since last filling in the diary?							
Have you had eye inflammation since last filling in the diary?							
Have you had skin problems since last filling in the diary?							
Have you had mouth ulcers since last filling in the diary?							
Have you had an anal fistula / fissure / abscess?							
Have you had any other type of fistula?							
Have you taken anti-diarrhoeal drugs since last completing the diary? If yes, please give the name of the drug and the average dose you took each day.							
Have you taken any other drugs since last completing the diary? If yes, please give the name of the drug and the average dose you took each day.							

Daily Patient Diary Questions

Please complete this diary each day for a week, at approximately the same time each day, answering "Y" for yes, "N" for no, or using the rating scale given in the question. Your answers should describe your symptoms during the previous 24 hours. Thank you for your time

Date: (Day/Month/Year)							
Number of stools in the last 24 hours *For patients with ileostomy/colostomy, average no of times emptied bag per 24 hour period*							
Number of liquid or very soft stools in the last 24 hours							
Rate your abdominal pain/cramps during the last 24 hours (0=none, 1=mild, 2=moderate, 3=severe)							
Rate your general well being during the last 24 hours (0=well, 1=slightly below par, 2=poor, 3=very poor, 4=terrible)							
Have you had a fever over 100 F (37.8 C) during the last 24 hours?							
Have you had joint pain or problems?							
Have you had eye inflammation?							
Have you had skin problems?							
Have you had mouth ulcers?							
Have you had an anal fistula / fissure / abscess?							
Have you had any other type of fistula?							
Have you taken anti-diarrhoeal drugs in the last 24 hours? If yes, please give the name of the drug and the dose you took.							
Have you taken any other drugs since last completing the diary? If yes, please give the name of the drug and the dose you took.							

Appendix 8: Harvey Bradshaw Index

1. Patients with Crohn's disease who scored 3 or less on the Harvey-Bradshaw index were very likely to be in remission according to the Crohn's disease activity index.
2. Patients who scored 7 or more on the Harvey-Bradshaw index were very likely to be in relapse according to the Crohn's disease activity index.

	Item	Rating/Score
1	**General wellbeing**	0 = very well 1 = slightly below par 2 = poor 3 = very poor 4 = terrible
2	**Abdominal pain**	0 = none 1 = mild 2 = moderate 3 = severe
3	**Number of liquid stools per day ***	
4	**Abdominal mass**	0 = none 1 = dubious 2 = definite 3 = definite and tender
5	**Complications (scored if present):**	
	Arthralgia	1
	Uveitis	1
	Erythema nodosum	1
	Aphthous ulcers	1
	Pyoderma gangrenosum	1
	Anal fissure	1
	New fistula	1
	Abscess	1

*For patients with an ileostomy or colostomy, please record the number of times the patient empties the bag.

Appendix 9: Crohn's Disease Endoscopic Index of Severity (CDEIS)

Endoscopic score of disease activity is assessed using a validated Simple Endoscopic Score for Crohn's Disease[21]. Selected endoscopic parameters (ulcer size ulcerated and affected surfaces, stenosis) are scored from 0 to 3. The scoring system and an example of how the data is collated, and as it appears in the CRF, is shown below:

How to score - For each segment score as follows:

Variable	0	1	2	3
1. Extent of affected surface:	Unaffected segment	0-50%	50-75%	> 75%
2. Presence and size of ulcers	None	Aphthous ulcers (< 5cm diameter)	Large ulcers (0.5-2cm diameter)	Very large ulcers (>2cm diameter)
3. Extent of ulcerated surface	None	<10%	10-30%	>30%
4. Presence and type of narrowings	None	Single, can be passed	Multiple, can be passed	Cannot be passed

Record the findings in the table below, using the guidance in the above table to score.

	Ileum	Right colon	Trans-verse colon	Left colon	Rectum	Total
Segment code	**G**	**H**	**I**	**J**	**K**	
Segment explored fully (+), partially (+/-) or not explored (0)						
Findings						
1. Extent of affected surface						
2. Presence and size of ulcers						
3. Extent of ulcerated surface						
4. Presence and type of narrowings						
CDEIS Total						

Appendix 10: Patient Registration Form

ASTIC STUDY: PATIENT REGISTRATION FORM

Page 1

Please complete pages 1 and 2 of the patient registration form, and fax to the Study Administration office (+44 (0)115 9422232). The Study Administration office will return the form confirming whether the patient is suitable to be registered for the ASTIC trial.

Centre Number	___ : ___
Principal Investigator (PI)	
Hospital name and address	
PI Phone Number	
PI Fax Number	
Patient's Initials	___ : ___ : ___ (First / Mid / Last initial)
Patient's Trial Number	___ : ___ : ___ : ___ (First two digits = centre number. Second two digits sequentially allocated at each centre, starting from 01)
Date of Birth	_____ : _____ : _____ (Day / Month / Year)
Sex	☐ Male ☐ Female

Inclusion Criteria (all inclusion criteria, or corresponding discretionary inclusion criteria, should be Yes)		
☐ Yes ☐ No	Age between 18 and 50 years. If No ↓	
	☐ *Yes* ☐ *No*	*Age between 50 and 65*
☐ Yes ☐ No	Confirmed diagnosis of Crohn's Disease (histology and/or radiological appearance), and confirmed active Crohn's disease (CDAI > 250 within the last 3 months, at least 2 of raised CRP, endoscopic evidence, evidence from barium study? If No ↓	
	☐ *Yes* ☐ *No*	*Diseased tissue can't be accessed endoscopically as doesn't extend to duodenum or terminal ileum?*
☐ Yes ☐ No	Unsatisfactory course despite 3 immunosuppressive agents	
☐ Yes ☐ No	Impaired function and quality of life	
☐ Yes ☐ No	Current problems unsuitable for surgery, risk of short bowel syndrome	
☐ Yes ☐ No	Informed consent	
Exclusion Criteria (all should be No)		
☐ Yes ☐ No	Pregnancy or unwillingness to use contraception	
☐ Yes ☐ No	Concomitant severe disease	
☐ Yes ☐ No	Significant infection or risk thereof	
☐ Yes ☐ No	Significant malnutrition	
☐ Yes ☐ No	Previous poor compliance	
☐ Yes ☐ No	Concurrent enrolment in another study	
☐ Yes ☐ No	Lack of funding	

See CRF1 pages 22-24 for more detailed inclusion/exclusion criteria

ASTIC STUDY: PATIENT REGISTRATION FORM	Page 2
Centre Number	___ : ___
Patient's Initials	___ : ___ : ___ (First / Mid / Last initial)
Patient's Trial Number	___ : ___ : ___ : ___
Narrative explanation of why patient should be considered for trial **Current Symptoms**	
Narrative history of patient's Crohn's disease. Summarise major events by date **Summary of relevant surgeries with dates**	
Principal Investigator signature	
Date	_____ : _____ : _____ (dd / mmm / yyyy)
Suitability for registration (to be completed by SAO)	
Is this patient suitable to be registered into the trial?	☐ Yes ☐ No
Authorised on behalf of Trial Steering Committee by (name):	
Signature	
Date	_____ : _____ : _____ (dd / mmm / yyyy)

Appendix 11: Randomisation Request Form

ASTIC STUDY: RANDOMISATION REQUEST FORM

Please complete this form after the patient has undergone successful leukapheresis and fax to the Study Administration office (+44 (0)115 9422232). The Study Administration office will return the form confirming whether which treatment group the patient has been allocated to.

Centre Number	
Principal Investigator (PI)	
Hospital name and address	
PI Phone Number	
PI Fax Number	
Patient's Initials	___ : ___ : ___ (First / Mid / Last initial)
Patient's Trial Number	___ : ___ : ___ : ___
Date of Birth	__ __ : __ __ __ :__ __ __ __ (Day / Month / Year)
Sex	☐ Male ☐ Female
Leukapheresis data (evaluated before cryopreservation)	
Date (of evaluation)	__ __ : __ __ __ : __ __ __ __ (Day / Month / Year)
Total no of nucleated cells (/kg) ***(kg of recipient body weight***	__ __ . __ x 10^8
CFU-GM (cells/kg) ***(methyl cellulose + cytokines)***	__ __ __ x 10^4
CD 34^+ (cells/kg)	__ __ . __ x 10^6
CD 3^+ (cells/kg)	__ __ . __ x 10^4
CD 14^+ (cells/kg)	__ __ __ x 10^4
CD 19^+ (cells/kg)	__ __ __ x 10^4

Randomisation (to be completed by the SAO)

Patient eligible to be randomised?	☐ Yes ☐ No
Randomised to:	☐ **Group A, Early Transplant** (high dose immunoablation and autologous hematopoietic stem cell transplantation (HSCT) 1 month later)
	☐ **Group B, Delayed Transplant** (high dose immunoablation and autologous hematopoietic stem cell transplantation (HSCT) 13 months later)
Authorised on behalf of Trial Steering Committee by (name):	
Signature	
Date	__ __ : __ __ __ : __ __ __ __ (Day / Month / Year)

Appendix 12: Confirmation of Delayed Transplantation

ASTIC STUDY: CONFIRMATION OF DELAYED TRANSPLANT	
Please complete this form before transplantation of Group B patients, and fax to the Study Administration office (+44 (0)115 9422232). The Study Administration office will return the form confirming whether the patient is still suitable for delayed transplantation.	
Centre Number	___ : ___
Principal Investigator (PI)	
Hospital name and address	
PI Phone Number	
PI Fax Number	
Patient's Initials	___ : ___ : ___ (First / Mid / Last initial)
Patient's Trial Number	___ : ___ : ___ : ___
Date of Birth	_____ : _____ : _____ (Day / Month / Year)
Sex	☐ Male ☐ Female
Leukapheresis data (evaluated before cryopreservation)	
Date (of evaluation)	_____ : _____ : _____ (Day / Month / Year)
Total no of nucleated cells *(/kg of recipient body weight)*	__ __ . __ x 10^8
CFU-GM (cells/kg) ***(methyl cellulose + cytokines)***	__ __ __ x 10^4
CD 34^+ (cells/kg)	__ __ . __ x 10^6
CD 3^+ (cells/kg)	__ __ . __ x 10^4
CD 14^+ (cells/kg)	__ __ __ x 10^4
CD 19^+ (cells/kg)	__ __ __ x 10^4
Has there been any significant change in the patient's condition?	☐ Yes ☐ No If Yes, give details:
DELAYED TRANSPLANT APPROVAL (to be completed by the SAO)	
Patient suitable to proceed to delayed transplant?	☐ Yes ☐ No
Authorised on behalf of Trial Steering Committee by (name):	
Signature	
Date	_____ _____ _____ (Day / Month / Year)

Appendix 13: Adverse Effects of Drugs/Agents to be Used in the Trial

Problems that commonly occur during high dose immunoablation and autologous stem cell transplantation are described in section 8 of the protocol:

Further potential toxicities of individual drugs used in mobilisation and conditioning are described in below

Cyclophosphamide:
Nausea, vomiting, loose stools, malaise, alopecia, local thrombophlebitis, hyperuricaemia and hemorrhagic cystitis. Whereas high cumulative doses of oral cyclophosphamide have been associated with a higher long-term risk on bladder- and skin cancer, data on such associations with intravenous high-dose cyclophosphamide are lacking. High cumulative doses of cyclophosphamide cause both male and female infertility, which may be irreversible in some patients. The amenorrhoea that is induced by cyclophosphamide in female patients requires hormonal replacement therapy (estradiol/dydrogesteron).

Filgastrim (non-glycosylated G-CSF)**:**
Toxicity of **G-CSF** mainly involves transient mild to moderate bone pain due to rapid expansion of bone marrow cells. No long-term toxicity of G-CSF has been reported, so far, when administered to patients with idiopathic neutropenia treated for almost five years. Other recorded AEs include allergic reactions, musculoskeletal pain, splenic enlargement, hepatomegaly, transient hypotension, epistaxis, urinary abnormalities (including dysuria, proteinuria, and haematuria), osteoporosis, exacerbation of rheumatoid arthritis, cutaneous vasculitis, thrombocytopenia, anaemia, transient decrease in blood glucose, raised uric acid
Rarely reported: exacerbation of rheumatoid arthritis, adult respiratory distress syndrome.

Rabbit anti-thymocyte globulin (ATG):
Toxicity of ATG consists of symptoms of cytokine release with arthralgia/arthritis, myalgia, fever chills and malaise. Serum sickness and anapylaxis are rare when prophylaxis with i.v. methylprednisolone is given. Thrombocytopenia, leukopenia, hemolysis and respiratory distress also occur
Some patients are susceptible to (iatrogenic) symptomatic fluid retention resulting from hyperhydration, probably related to diminished compliance of blood vessels and heart.
Mesna: AEs include nausea, vomiting, colic, diarrhoea, fatigue, headache, limb and joint pains, depression, irritability, rash, hypotension and tachycardia; rarely hypersensitivity reactions (more common in patients with auto-immune disorders)

The following additional AEs have been recorded with individual drugs that patients may receive during the course of the trial:

Acyclovir:
Nausea, vomiting, abdominal pain, diarrhoea, headache, fatigue, rash, urticaria, pruritus, photosensitivity; rarely hepatitis, jaundice, dyspnoea, angioedema, anaphylaxis, neurological reactions (including dizziness, confusion, hallucinations and drowsiness), acute renal failure, decreases in haematological indices; on *intravenous infusion*, severe local inflammation (sometimes leading to ulceration), fever, and rarely agitation, tremors, psychosis and convulsions

Sulphamethoxazole:
Nausea, vomiting; rash (including Stevens-Johnson syndrome, toxic epidermal necrolysis, photosensitivity)—discontinue immediately; blood disorders (including neutropenia, thrombocytopenia, rarely agranulocytosis and purpura)—discontinue immediately; rarely, allergic reactions, systemic lupus erythematosus, myocarditis, serum sickness, diarrhoea, glossitis, stomatitis, anorexia, arthralgia, myalgia; also reported, liver damage including jaundice and hepatic necrosis, pancreatitis, antibiotic-associated colitis, eosinophilia, cough and shortness of breath, pulmonary infiltrates, aseptic meningitis, headache, depression, convulsions, peripheral neuropathy, ataxia, tinnitus, vertigo, dizziness, hallucinations, megaloblastic anaemia, electrolyte disturbances, crystalluria, renal disorders including interstitial nephritis

Trimethoprim:
Gastro-intestinal disturbances including nausea and vomiting, pruritus, rashes, hyperkalaemia, depression of haematopoiesis; rarely erythema multiforme, toxic epidermal necrolysis, photosensitivity and other allergic reactions including angioedema and anaphylaxis; aseptic meningitis reported

Methylprednisolone:
IV pulse 1mg/kg *gastro-intestinal effects* include dyspepsia, peptic ulceration (with perforation), abdominal distension, acute pancreatitis, oesophageal ulceration and candidiasis; *musculoskeletal effects* include proximal myopathy, osteoporosis, vertebral and long bone fractures, avascular osteonecrosis, tendon rupture; *endocrine effects* include adrenal suppression, menstrual irregularities and amenorrhoea, Cushing's syndrome (with high doses, usually reversible on withdrawal), hirsutism, weight gain, negative nitrogen and calcium balance, increased appetite; increased susceptibility to and severity of infection; *neuropsychiatric effects* include euphoria, psychological dependence, depression, insomnia, increased intracranial pressure with papilloedema in children (usually after withdrawal), psychosis and aggravation of schizophrenia, aggravation of epilepsy; *ophthalmic effects* include glaucoma, papilloedema, posterior subcapsular cataracts, corneal or scleral thinning and exacerbation of ophthalmic viral or fungal disease; *other side-effects* include impaired healing, skin atrophy, bruising, striae, telangiectasia, acne, myocardial rupture following recent myocardial infarction, fluid and electrolyte disturbance, leucocytosis, hypersensitivity reactions (including anaphylaxis), thromboembolism, nausea, malaise, hiccups

Ciprofloxacin bd po:
Side-effects of the quinolones include nausea, vomiting, dyspepsia, abdominal pain, diarrhoea (rarely antibiotic-associated colitis), headache, dizziness, sleep disorders, rash (rarely Stevens-Johnson syndrome and toxic epidermal necrolysis), and pruritus. Less frequent side-effects include anorexia, increase in blood urea and creatinine; drowsiness, restlessness, asthenia, depression, confusion, hallucinations, convulsions, paraesthesia; photosensitivity, hypersensitivity reactions including fever, urticaria, angioedema, arthralgia, myalgia, and anaphylaxis; blood disorders (including eosinophilia, leucopenia, thrombocytopenia); disturbances in vision, taste, hearing and smell. Also isolated reports of tendon inflammation and damage (especially in the elderly and in those taking corticosteroids). Other side-effects that have been reported include haemolytic anaemia, renal failure, interstitial nephritis, and hepatic dysfunction (including hepatitis and cholestatic jaundice). The drug should be **discontinued** if psychiatric, neurological or hypersensitivity reactions (including severe rash) occur. Flatulence, dysphagia, tremor, hyperglycaemia, vasculitis, erythema nodosum, petechiae, haemorrhagic

bullae, tinnitus, tenosynovitis, tachycardia, oedema, syncope, hot flushes and sweating have also been reported

Azathioprine:
This is widely used for transplant recipients and it is also used to treat a number of auto-immune conditions, usually when corticosteroid therapy alone provides inadequate control. It is metabolised to mercaptopurine, and doses should be reduced when allopurinol is given concurrently. Blood tests and monitoring for signs of myelosuppression are essential in long-term treatment with azathioprine. The enzyme thiopurine methyltransferase (TPMT) metabolises azathioprine; the risk of myelosuppression is increased in those with a low activity of the enzyme, particularly in the very few individuals who are homozygous for low TPMT activity.
Side-effects: hypersensitivity reactions (including malaise, dizziness, vomiting, diarrhoea, fever, rigors, myalgia, arthralgia, rash, hypotension and interstitial nephritis—calling for immediate withdrawal); dose-related bone marrow suppression; liver impairment, cholestatic jaundice, hair loss and increased susceptibility to infections and colitis in patients also receiving corticosteroids; nausea; rarely pancreatitis, pneumonitis, hepatic veno-occlusive disease

Mycophenolate mofetil:
is metabolised to mycophenolic acid which has a more selective mode of action than azathioprine.
Side-effects : diarrhoea, vomiting, constipation, nausea, dyspepsia, pancreatitis, abdominal pain; hypertension, oedema, chest pain; dyspnoea, cough, rhinitis; dizziness, insomnia, headache, tremor; infection (including cytomegalovirus viraemia, herpes simplex, candidiasis, aspergillosis, sepsis, urinary-tract infection and pneumonia); leucopenia, anaemia, thrombocytopenia, leucocytosis, polycythaemia; electrolyte disturbances, hyperglycaemia, hypercholesterolaemia; asthenia; renal damage, haematuria; acne; lymphoproliferative disease; less frequently, gastro-intestinal perforation, colitis, abnormal liver-function tests, hepatitis, gingivitis, mouth ulceration, haemorrhage, influenza-like syndrome, hypotension, arrhythmias, tachycardia, hypoglycaemia, weight gain; allergic reactions, benign neoplasm of skin and skin carcinoma reported.

Methotrexate:
Anorexia, abdominal discomfort, intestinal ulceration and bleeding, diarrhoea, toxic megacolon, hepatotoxicity (see Cautions above); ecchymosis; pulmonary oedema, pleuritic pain, pulmonary fibrosis, interstitial pneumonitis (see Pulmonary Toxicity above); anaphylactic reactions, urticaria; dizziness, drowsiness, malaise, headache, mood changes, abnormal cranial sensations; precipitation of diabetes, osteoporosis; menstrual disturbances, vaginitis, impotence, loss of libido; haematuria, dysuria, renal failure; arthralgia, myalgia, vasculitis, blurred vision; rash, pruritus, Stevens-Johnson syndrome, toxic epidermal necrolysis, photosensitivity, changes of skin pigmentation, telangiectasia.

Cyclosporine:
Dose-dependent increase in serum creatinine and urea during first few weeks (see also under Cautions); less commonly renal structural changes on long-term administration; also hypertrichosis, headache, tremor, hypertension (especially in heart transplant patients), hepatic dysfunction, fatigue, gingival hypertrophy, gastro-intestinal disturbances, burning sensation in hands and feet (usually during first week); *occasionally* rash (possibly allergic), mild anaemia, hyperkalaemia, hyperuricaemia, gout, hypomagnesaemia, hypercholesterolaemia, hyperglycaemia, weight increase, oedema, pancreatitis, neuropathy, confusion, paraesthesia,

convulsions, benign intracranial hypertension (discontinue), dysmenorrhoea or amenorrhoea; myalgia, muscle weakness, cramps, myopathy, gynaecomastia (in patients receiving concomitant spironolactone), colitis and cortical blindness also reported; thrombocytopenia (sometimes with haemolytic uraemic syndrome) also reported; incidence of malignancies and lymphoproliferative disorders similar to that with other immunosuppressive therapy

Infliximab:
Hepatic impairment; renal impairment; monitor for infections before, during, and for 6 months after treatment (see also Tuberculosis below); heart failure (discontinue if symptoms develop or worsen; avoid in moderate or severe heart failure); demyelinating CNS disorders (risk of exacerbation)
Tuberculosis (often in extrapulmonary sites) has been reported. Patients must be evaluated for active and latent tuberculosis before treatment. Tuberculosis chemoprophylaxis must be started before initiating infliximab in latent tuberculosis (contra-indicated in active tuberculosis). Patients should be instructed to seek medical advice if they develop symptoms suggestive of tuberculosis (such as persistent cough, weight loss, fever). If active tuberculosis suspected, infliximab should be discontinued until infection either ruled out or treated
Hypersensitivity reactions (including fever, chest pain, hypotension, hypertension, dyspnoea, pruritus, urticaria, serum sickness-like reactions, angioedema, anaphylaxis) reported during or within 1–2 hours after infusion (risk greatest during first or second infusion or in patients who discontinue other immunosuppressants. All patients should be observed carefully for 1–2 hours after infusion and resuscitation equipment should be available for immediate use. Prophylactic antipyretics, antihistamines, or hydrocortisone may be administered. Readministration is not recommended after infliximab-free interval of more than 16 weeks—risk of delayed hypersensitivity reactions. Patients should be advised to keep Alert card with them at all times and seek medical advice if symptoms of delayed hypersensitivity develop
Other AEs: dyspepsia, diarrhoea, constipation, hepatitis, cholecystitis, diverticulitis, gastro-intestinal haemorrhage, flushing, bradycardia, arrhythmias, palpitation, syncope, vasospasm, peripheral ischaemia, ecchymosis, haematoma, interstitial pneumonitis or fibrosis, fatigue, anxiety, drowsiness, dizziness, insomnia, confusion, agitation, amnesia, seizures, demyelinating disorders, vaginitis, myalgia, arthralgia, endophthalmitis, rash, sweating, hyperkeratosis, skin pigmentation, alopecia.

Tacrolimus:
Adverse events include gastro-intestinal disturbances including dyspepsia, and inflammatory and ulcerative disorders; hepatic dysfunction, jaundice, bile-duct and gall-bladder abnormalities; hypertension (less frequently hypotension), tachycardia, angina, arrhythmias, thromboembolic and ischaemic events, rarely myocardial hypertrophy, cardiomyopathy; dyspnoea, pleural effusion, tremor, headache, insomnia, paraesthesia, confusion, depression, dizziness, anxiety, convulsions, incoordination, encephalopathy, psychosis; visual and hearing abnormalities; haematological effects including anaemia, leucocytosis, leucopenia, thrombocytopaenia, coagulation disorders; altered acid-base balance and glucose metabolism, electrolyte disturbances including hyperkalaemia (less frequently hypokalaemia); altered renal function including increased serum creatinine; hypophosphataemia, hypercalcaemia, hyperuricaemia; muscle cramps, arthralgia; pruritus, alopecia, rash, sweating, acne, photosensitivity; susceptibility to lymphoma and other malignancies particularly of the skin; less commonly ascites, pancreatitis, atelectasis, kidney damage and renal failure, myasthenia, hirsutism, rarely Stevens-Johnson syndrome

Warning. Cardiomyopathy has been reported in children given tacrolimus after transplantation. Patients should be monitored carefully by echocardiography for hypertrophic changes; dose reduction or discontinuation should be considered if these occur

Appendix 14: Serious Adverse Event form

ASTIC STUDY: SERIOUS ADVERSE EVENT (SAE) FORM **Page 1** (please complete pages 1-3)			
A full definition of a serious adverse event (SAE) is available in the ASTIC protocol, Section 15.2. All SAEs must be notified to the Study Administration Office **within 24 hours of learning of the event**, and the SAE form must be submitted within a maximum of 2 days, using fax number +44 (0)115 9422232.			
Report Type:	☐ Initial ☐ Follow-up	**Date of report:**	___ : ____ : ____ (DD / M M M / Y Y Y Y)
Centre Number:	___ : ___		
Principal Investigator (PI):			
Hospital name and address:			
Telephone:	**Fax:**	**Email:**	
Patient's Initials:	___ : ___ :___ (First / Mid / Last initial)		
Patient's Trial Number:	___ : ___ : ___ : ___ (First two digits = centre number. Second two digits sequentially allocated at each centre, starting from 01)		
Date of Birth:	_____ | _____ | _____ (DD / M M M / Y Y Y Y)		
Sex:	☐ Male ☐ Female		
Onset of first sign of SAE:	_____ : _____ : _____ (DD / M M M / Y Y Y Y)		
Adverse Event Term:			
Diagnosis and description of SAE in medical terms *(include relevant symptoms, body site, treatment received, dates of hospital admission and discharge)*			
Expedited SAE reporting criteria	**Tick all appropriate to event:** ☐ **Patient died** ☐ **Event was life-threatening** ☐ **In-patient hospitalisation or prolongation of hospitalisation** ☐ **Persistent or significant disability or incapacity** ☐ **Event caused a congenital anomaly or birth defect** ☐ **Is otherwise a significant medical event**		
Is the event likely to be due to progression of Crohn's disease?	☐ **Yes** ☐ **No**		
Outcome of the event at the time of this report	☐ **Completely recovered.** **Date of recovery: ___ / _____ / _____**(DD / MMM / YYYY) ☐ **Recovered with sequelae** ☐ **Condition improving** ☐ **Condition still present and unchanging** ☐ **Condition deteriorated** ☐ **Not assessable at this time** ☐ **Patient died. Date of death: ___ / ___ / _____** **NOTE: Provide follow-up report if SAE is ongoing at time of this report**		

ASTIC STUDY: SERIOUS ADVERSE EVENT (SAE) FORM Page 2	
Date of report:	_____ : _____ : _____ (DD / M M M / Y Y Y Y)
Centre Number:	___ : ___
Patient's Initials:	___ : ___ :___ (First / Mid / Last initial)
Patient's Trial Number:	___ : ___ : ___ : ___ (First two digits = centre number. Second two digits sequentially allocated at each centre, starting from 01)
Relatedness of the event to study treatment or procedures	☐ **Not Related:** the adverse event is not reasonably related to the medicinal product / intervention - or another cause can itself explain the occurrence of the event ☐ **Unlikely Related**: the adverse event is doubtfully related to the medicinal product / intervention but can't be fully ruled out ☐ **Possibly Related**: the adverse event is reasonably related to the medicinal product / intervention, but the event could have been due to another, equally likely cause ☐ **Probably Related**: the adverse event is reasonably related to the medicinal product / intervention, and the event is more likely explained by the drug than by any other cause ☐ **Definitely Related**: the adverse event is clearly related to the medicinal product / intervention and there is no other cause to explain the event or a re-challenge (if feasible) is positive
Action taken to treat the SAE	**Tick all appropriate:** ☐ No action taken ☐ Trial drug permanently discontinued due to this adverse event* ☐ Trial drug dosage adjusted or temporarily interrupted* ☐ Concomitant medication taken* ☐ Non-drug therapy given* ☐ Hospitalisation / prolonged hospitalisation * Provide further details of relevant treatment, dose changes and dates below:

Concomitant drugs relevant to SAE:

Drug 1	**23.1.1.1.1 Name**	**Brand**	**Indication**	**Dose**	**Frequency**	**Route**
	23.1.1.1.2					
Start Date: ☐☐ ☐☐ ☐☐ ☐☐ d d m m ...m y y		**Stop Date:** ☐☐ ☐☐ ☐☐ ☐☐ d d m m ...m y y		**Continuing:** ☐ No ☐ Yes		

Drug 2	**23.1.1.1.3 Name**	**Brand**	**Indication**	**Dose**	**Frequency**	**Route**
	23.1.1.1.4					
Start Date: ☐☐ ☐☐ ☐☐ ☐☐ d d m m ...m y y		**Stop Date:** ☐☐ ☐☐ ☐☐ ☐☐ d d m m ...m y y		**Continuing:** ☐ No ☐ Yes		

Drug 3	**23.1.1.1.5 Name**	**Brand**	**Indication**	**Dose**	**Frequency**	**Route**
	23.1.1.1.6					
Start Date: ☐☐ ☐☐ ☐☐ ☐☐ d d m m ...m y y		**Stop Date:** ☐☐ ☐☐ ☐☐ ☐☐ d d m m ...m y y		**Continuing:** ☐ No ☐ Yes		

ASTIC STUDY: SERIOUS ADVERSE EVENT (SAE) FORM **Page 3**	
Date of report:	_____ : _____ : _____ (DD / M M M / Y Y Y Y)
Centre Number:	___ : ___
Patient's Initials:	___ : ___ :___ (First / Mid / Last initial)
Patient's Trial Number:	___ : ___ : ___ : ___ (First two digits = centre number. Second two digits sequentially allocated at each centre, starting from 01)

Concomitant drugs relevant to SAE cont...:

Drug 4	**23.1.1.1.7 Name**	**Brand**	**Indication**	**Dose**	**Frequency**	**Route**
	23.1.1.1.8					

Start Date: ☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y) **Stop Date:** ☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y) **Continuing:** ☐ No ☐ Yes

Relevant Laboratory/Diagnostic Tests

Date	Test	Results
☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y)		☐ Results pending *(check box if applicable)*
☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y)		☐ Results pending *(check box if applicable)*
☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y)		☐ Results pending *(check box if applicable)*
☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y)		☐ Results pending *(check box if applicable)*
☐☐ ☐☐☐☐ ☐☐ (d d m m ...m y y)		☐ Results pending *(check box if applicable)*

Last study visit date before onset of SAE	_____ : _____ : _____ (DD / M M M / Y Y Y Y)	
Relevant medical history and additional information		
Form completed by (print name): _____	**Signed:** _____	**Date:** _____

Appendix 15: Declaration of Helsinki

Recommendations Guiding Medical Physicians in Biomedical Research involving Human Subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964; amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975; 35th World Medical Assembly, Venice, Italy, October 1983 and the 41st World Medical Assembly Hong Kong, September 1989 and South Africa (1997).

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration", and the International Code of Medical Ethics declares that "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient".

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease. In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research, a fundamental distinction must be recognised between medical research in which the aim is diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.
3. Biomedical research on human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility

for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interest of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimise the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports on experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.
11. In the case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

    Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current and diagnostic and therapeutic methods.
3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.
5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (Appendix I.2).
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

Non-Therapeutic Biomedical Research involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

Appendix 16: Trial Steering Committee

The Trial Steering Committee (TSC) is a panel of experts with a balance of Gastroenterologists and Haematologists to provide experience from both specialities involved in the study.

They will primarily be involved in:

a) Assessment of patient suitability for entry into the trial;
b) Providing advice for actions to be taken in the event of Serious Adverse Events (SAEs) and other significant patient events;
c) Any other significant trial conduct issues.

The composition of the TSC may change during the trial. The TSC composition history and current members is kept on file in the Trial Master File and updated by the Study Administration Office (SAO) when required.

Appendix 17: Independent Data Safety Monitoring Committee (DSMC)

The role of the Independent Data Safety Monitoring Committee (DSMC) is detailed in section 15.6 of the protocol.

The composition of the DSMC may change during the trial. The DSMC composition history and current members is kept on file in the Trial Master File and updated by the Study Administration Office (SAO) when required.