Safety and efficacy of autologous haematopoietic stem-cell transplantation with low-dose cyclophosphamide mobilisation and reduced intensity conditioning versus standard of care in refractory Crohn's disease (ASTIClite): an open-label, multicentre, randomised controlled trial

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Summary

Background A previous controlled trial of autologous haematopoietic stem-cell transplantation (HSCT) in patients with refractory Crohn's disease did not meet its primary endpoint and reported high toxicity. We aimed to assess the safety and efficacy of HSCT with an immune-ablative regimen of reduced intensity versus standard of care in this patient population.

Methods This open-label, multicentre, randomised controlled trial was conducted in nine National Health Service hospital trusts across the UK. Adults (aged 18–60 years) with active Crohn's disease on endoscopy (Simplified Endoscopic Score for Crohn's Disease [SES-CD] ulcer sub-score of ≥2) refractory to two or more classes of biological therapy, with no perianal or intra-abdominal sepsis or clinically significant comorbidity, were recruited. Participants were centrally randomly assigned (2:1) to either HSCT with a reduced dose of cyclophosphamide (intervention group) or standard care (control group). Randomisation was stratified by trial site by use of random permuted blocks of size 3 and 6. Patients in the intervention group underwent stem-cell mobilisation (cyclophosphamide 1 g/m² with granulocyte colony-stimulating factor (G-CSF) 5 µg/kg) and stem-cell harvest (minimum $2·0 \times 10^6$ $CD34^+$ cells per kg), before conditioning (fludarabine 125 mg/m², cyclophosphamide 120 mg/kg, and rabbit anti-thymocyte globulin [thymoglobulin] 7·5 mg/kg in total) and subsequent stem-cell reinfusion supported by G-CSF. Patients in the control group continued any available conventional, biological, or nutritional therapy. The primary outcome was absence of endoscopic ulceration (SES-CD ulcer sub-score of 0) without surgery or death at week 48, analysed in the intention-to-treat population by central reading. This trial is registered with the ISRCTN registry, 17160440.

Findings Between Oct 18, 2018, and Nov 8, 2019, 49 patients were screened for eligibility, of whom 23 (47%) were randomly assigned: 13 (57%) to the intervention group and ten (43%) to the control group. In the intervention group, ten (77%) participants underwent HSCT and nine (69%) reached 48-week follow-up; in the control group, nine (90%) reached 48-week follow-up. The trial was halted in response to nine reported suspected unexpected serious adverse reactions in six (46%) patients in the intervention group, including renal failure due to proven thrombotic microangiopathy in three participants and one death due to pulmonary veno-occlusive disease. At week 48, absence of endoscopic ulceration without surgery or death was reported in three (43%) of seven participants in the intervention group and in none of six participants in the control group with available data. Serious adverse events were more frequent in the intervention group (38 in 13 [100%] patients) than in the control group (16 in four [40%] patients). A second patient in the intervention group died after week 48 of respiratory and renal failure.

Interpretation Although HSCT with an immune-ablative regimen of reduced intensity decreased endoscopic disease activity, significant adverse events deem this regimen unsuitable for future clinical use in patients with refractory Crohn's disease.

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Research in context

Evidence before this study

Phase 3 and 4 clinical trials highlight that current conventional and biological or small molecule therapies, used either as monotherapy or in combination, do not achieve sustained clinical and mucosal remission in more than 50% of patients with Crohn's disease. Mucosal inflammation drives disease-related complications, the need for surgery, and impaired quality of life. A previous controlled trial of haematopoietic stem-cell transplantation (HSCT) in patients with refractory Crohn's disease was halted because of a patient death and did not meet its primary endpoint of sustained clinical remission off therapy with no evidence of active disease at 1 year. A heavy burden of serious adverse events presumed secondary to the high doses of cyclophosphamide used in stem-cell mobilisation and conditioning was reported. A systematic review and meta-analysis of eight prospective uncontrolled cohort studies and case series and one randomised controlled trial reported a high rate of endoscopic remission (81·9% [95% CI 0·603–0·931]). However, there was also a high incidence of transplantation-related mortality (6·4% [0·028–0·140]). A lower rate of transplantation-related mortality (1·2%) was reported in the largest registry-based analysis of 82 patients, in which treatment-free survival was reported in 55% patients 1 year after transplantation.

Added value of this study

We report the findings of an open-label, multicentre, randomised controlled trial assessing the safety and efficacy of HSCT with an immune-ablative regimen of reduced intensity versus standard of care in patients with endoscopically active Crohn's disease refractory to conventional and biological therapies, using centrally read endoscopic healing as the primary outcome. Doses of cyclophosphamide lower than those detailed in the European Society for Blood and Marrow Transplantation guidelines were used and fludarabine was added to the conditioning regimen for immunosuppression. The trial was halted early after the randomisation of 23 patients because of suspected unexpected serious adverse reactions, including renal thrombotic microangiopathy in three participants and one death due to pulmonary veno-occlusive disease. No patients in the control group met the primary endpoint, which was reported in three (43%) of seven patients in the intervention group. Patients in the intervention group showed improvements in clinical and endoscopic secondary endpoints. Mechanistic analysis showed the impact of HSCT on the immune phenotype of peripheral blood and mucosal immune cells.

Implications of all the available evidence

Two randomised controlled trials of HSCT with immune-ablative chemotherapy and anti-thymocyte globulin in patients with refractory Crohn's disease have been halted due to significant adverse events. Despite the reported benefits in some patients, HSCT with a conditioning regimen comprising cyclophosphamide, anti-thymocyte globulin (thymoglobulin), and fludarabine should not be used as a therapy for refractory Crohn's disease.

Introduction

Active Crohn's disease refractory to treatment results in severe and debilitating symptoms, recurrent hospitalisation, and disability.[1] In addition to a substantial impact on patients' quality of life, Crohn's disease is associated with high direct and indirect health-care costs.[2] Optimised use of currently licenced therapies is unable to deliver sustained clinical and mucosal remission in many patients.[3] Although a limited ileocaecal resection can result in enhanced quality of life in the short term,[4] Crohn's disease frequently recurs despite postoperative medication. Surgery for refractory colonic Crohn's disease or complications at critical parts of the bowel can require a permanent stoma.

Haematopoietic stem-cell transplantation (HSCT) has been shown to be effective in patients with other immune-mediated diseases, such as multiple sclerosis.[5] Published case series have suggested the efficacy of HSCT in patients with refractory Crohn's disease, with reports of treatment-free remission in the long term.[6] Although a substantial proportion of patients who achieved remission after HSCT had disease recurrence, many responded to therapies that they had previously been refractory to.[7,8]

A randomised controlled trial of autologous HSCT in patients with Crohn's disease (the ASTIC trial)[9] reported no benefit of HSCT over standard care in the primary endpoint of medication-free clinical remission for 3 months with no imaging or endoscopic evidence of disease activity. Furthermore, HSCT was associated with a high burden of serious adverse events (SAEs), including one patient death.[10] However, HSCT was significantly more efficacious than standard care in several secondary endpoints, including clinical remission and endoscopic disease activity. Several patients with complete endoscopic remission reported clinically significant symptoms, presumably related to previous intestinal damage, and did not meet the primary endpoint. On completion of the trial, patients in the control group also underwent HSCT using the same transplantation protocol. A subsequent analysis of the combined cohort reported a regression of all endoscopic ulceration in 19 (50%) of 38 patients 1 year following transplantation.[11]

Despite the potential efficacy of HSCT, toxicity remains a substantial barrier to its use in patients with Crohn's disease. An analysis that comprised eight cohort studies and the ASTIC trial estimated a treatment-related mortality rate of 6·4%.[12] A lower rate of transplantation-related mortality (1·2%) was reported in the largest registry-based analysis of 82 patients, in which treatment-free survival at 1 year occurred in

54·6% patients (95% CI 43·8–65·5%).[9] A specialist review of the ASTIC trial implicated the high dose of cyclophosphamide used in stem-cell mobilisation (4 g/m²) and conditioning (200 mg/kg) in the infectious SAEs observed among patients.[9,13,14] Reducing the dose of cyclophosphamide during mobilisation and conditioning might reduce morbidity in patients with autoimmune diseases,[8,15–17] and the importance of supportive care in reducing SAEs has been shown.[8,14,18] We aimed to compare the safety and efficacy of autologous HSCT with a reduced dose of cyclophosphamide during stem-cell mobilisation and conditioning versus standard of care in patients with refractory Crohn's disease.

Methods

Study design and participants

ASTIClite was an open-label, multicentre, two-arm, parallel, randomised controlled trial conducted in nine National Health Service (NHS) hospital trusts across the UK, which had either previously participated in the original ASTIC trial or had accreditation from the Joint Accreditation Committee International Society for Cell & Gene Therapy-Europe & European Society for Blood and Marrow Transplantation for performing allogeneic HSCT. Adult patients (aged 18–60 years) with significantly active Crohn's disease on endoscopy (Simplified Endoscopic Score for Crohn's Disease [SES-CD] ulcer sub-score of ≥2 in at least one segment) that was refractory to at least two classes of biological therapy, who reported impaired quality of life and in whom surgery was not appropriate or was declined, were eligible for inclusion. Patients with active perianal or penetrating intra-abdominal sepsis, a current infection, or a clinically significant comorbidity were excluded. All participants were offered referral to a fertility service to freeze eggs or sperm, and individuals with early-onset Crohn's disease underwent genetic screening for a monogenic cause at the discretion of the recruiting team. The full protocol and eligibility criteria have been published previously.[19]

All immunosuppressant medication was stopped with appropriate washout periods before stem-cell mobilisation (ie, infliximab, vedolizumab, or ustekinumab for >4 weeks; adalimumab, azathioprine, or mercaptopurine for >2 weeks; and methotrexate or ciclosporin for >1 week).[19] Concomitant steroids were weaned by 5 mg/day each week after mobilisation.

The trial received favourable opinion from the London–Chelsea Research Ethics Committee (reference number 17/LO/1690) and authorisation from the Medicines and Healthcare Products Regulatory Agency ([MHRA]; reference number 14620/0051/001-0001). The trial was conducted in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Good Clinical Practice guidelines, and the Declaration of Helsinki. Two patients who had previously undergone HSCT for Crohn's disease provided input into trial development and were on the Trial Steering Committee. A multidisciplinary team assessed patient eligibility before participants provided their written consent and again before they were randomly assigned.

Randomisation and masking

Eligible participants were centrally randomly assigned (2:1) at the Sheffield Clinical Trials Research Unit to either HSCT with a reduced dose of cyclophosphamide (intervention group) or standard of care (control group). The trial statistician, masked to treatment allocation, generated the randomisation schedule, which was stratified by site by use of random permuted blocks of size 3 and 6. The allocation sequence was concealed from all study staff, except for the statisticians who generated it, by use of a web-based randomisation platform hosted by the Sheffield Clinical Trials Research Unit. All participants were unmasked to group assignment.

Procedures

Participants randomly assigned to the intervention group underwent peripheral blood stem-cell mobilisation with cyclophosphamide 1 g/m² on day 1 and non-glycosylated granulocyte colony-stimulating factor (filgrastim) 5 µg/kg from day 5 until the day of stem-cell harvest (approximately day 9 [range 7–9]). Supportive therapy was given in line with local NHS trust procedures. Patients underwent leukapheresis when $CD34^+$ cell counts exceeded 10×10^6 cells per L, until a minimum of $2{\cdot}0\times10^6$ $CD34^+$ cells per kg were collected and cryopreserved. Patients were admitted to hospital for the conditioning and transplantation regimen after a minimum of 3 weeks to avoid the risk of cumulative cardiac toxicity. The conditioning regimen comprised fludarabine 25 mg/m² intravenously on days –6, –5, –4, –3, and –2; cyclophosphamide 60 mg/kg per day intravenously on days –3 and –2; and rabbit anti-thymocyte globulin (thymoglobulin; Sanofi–Genzyme, Reading, UK) 2·5 mg/kg intravenously on days –3, –2, and –1, with a test dose given as per local practice. Supportive care included intravenous mesna (based on individual unit policies); hydration; and methylprednisolone 2 mg/kg per day intravenously on days –3, –2, and –1 (additional doses of 0·5–1·0 g/day were permitted if a reaction to anti-thymocyte globulin was observed). There was no gut sterilisation before conditioning; however, prophylactic and therapeutic antimicrobial therapy was used in line with local standard practice. Unselected stem cells were reinfused on day 0 and administration of granulocyte colony-stimulating factor 5 µg/kg per day (to the nearest vial) began on day +5 and continued until absolute neutrophil counts reached more than $1{\cdot}0\times10^9$ cells per L for 2 consecutive days. Post-transplantation supportive care was provided, including monitoring for Epstein-Barr virus and cytomegalovirus reactivation by PCR, as per guidelines.[15,18] At week 24, patients in the intervention

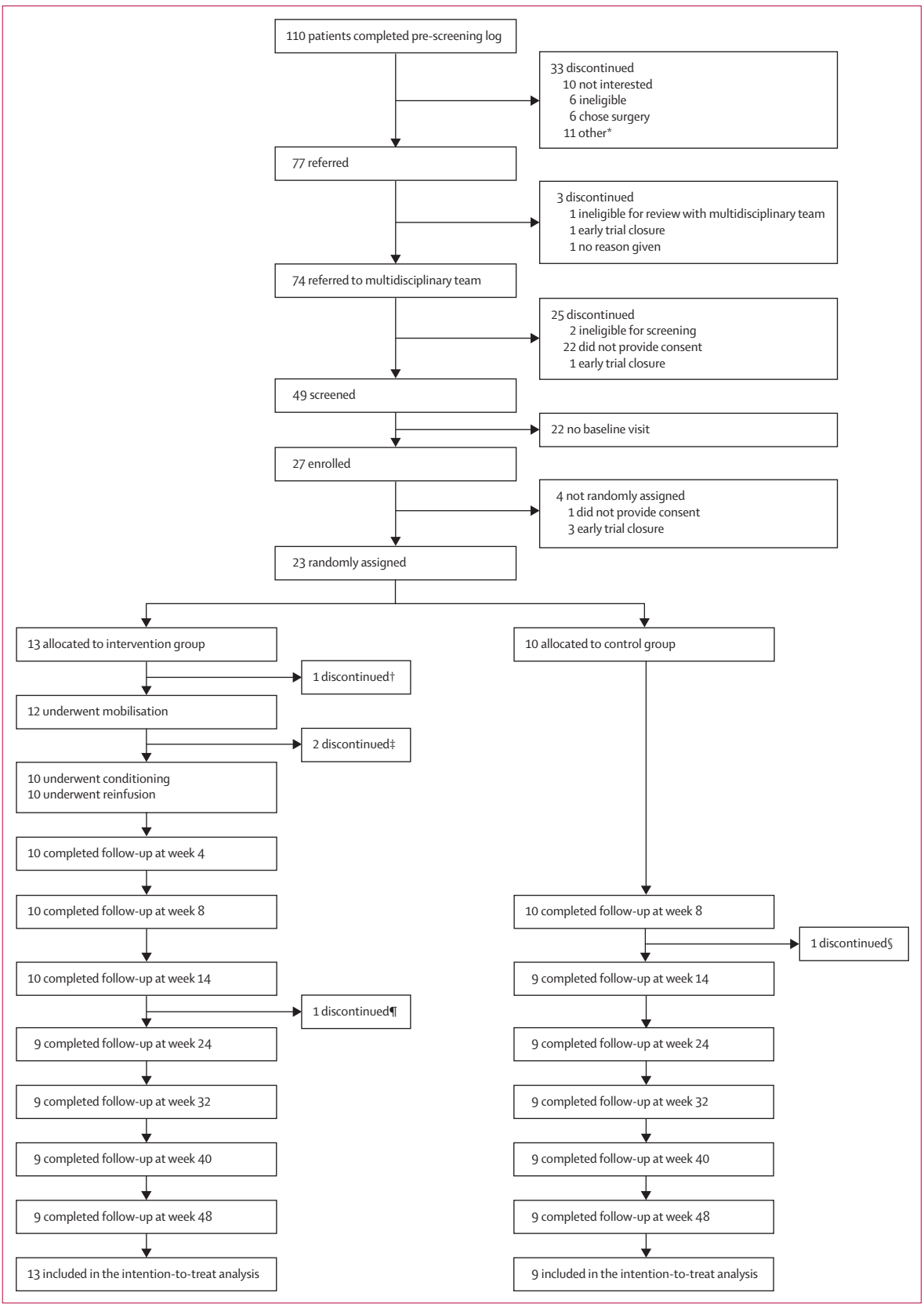

Figure 1: **Trial profile**
*Four illness-related reasons and seven timing-related reasons. †One participant withdrew consent. ‡Two patients were discontinued due to early study closure, one of whom had received stem-cell mobilisation. §Participant was found to be ineligible (SES-CD incorrectly scored) and was withdrawn. ¶One participant died. A further participant died after follow-up visit at week 48.

group could restart anti-TNF therapy if there was evidence of active disease, as assessed by the local gastroenterologist. Participants randomly assigned to the control group continued with any available conventional, biological, or nutritional therapy for Crohn's disease, with no restriction.

Participants had scheduled follow-up visits at weeks 8, 14, 24, 32, 40, and 48. Day 0 was the date of stem-cell reinfusion for participants in the intervention group and was 49 days after randomisation for participants in the control group, which was the median time from randomisation to stem-cell reinfusion in the ASTIC trial.[12] Although a visit window of 1 week before and after the due date for the appointment was initially permitted, a wider visit window was allowed because of the COVID-19 pandemic and some follow-up visits were conducted remotely. At each visit, patients were assessed for disease activity (Crohn's Disease Activity Index [CDAI][20] and Harvey-Bradshaw Index[21]), quality of life (EQ-5D-5L,[22] inflammatory bowel disease [IBD] Control,[23] and IBD Questionnaire[24]), and adverse events (AEs). Ileocolonoscopy or enteroscopy was performed at baseline, week 24, and week 48; and local endoscopy assessment established eligibility and the requirement for anti-TNF therapy at week 24. All AEs, SAEs, and suspected unexpected serious adverse reactions were captured from the time that consent was provided to study closure. AEs were recorded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE) criteria (version 4.03). A review by the Data Monitoring and Ethics Committee was scheduled after the first ten participants in the intervention group completed treatment; however, these safety assessments were accelerated after suspected unexpected serious adverse reactions were reported.

Outcomes

The primary endpoint was defined as the absence of endoscopic ulceration (SES-CD ulcer sub-score of 0) without surgery or death at week 52, assessed by the central reading of endoscopy videos by clinicians masked to the time of assessment and treatment using a validated bespoke IT platform.[25] Protocol-defined secondary disease activity endpoints, analysed at week 48, were CDAI clinical remission (CDAI <150); steroid-free clinical remission (CDAI <150 and no concomitant use of steroids); Harvey-Bradshaw Index clinical remission (score ≤4); patient-reported outcome (PRO2) of clinical remission (mean score for abdominal pain ≤1 and stool frequency ≤1·5; endpoint not reported); absolute CDAI; absolute SES-CD; change in CDAI and SES-CD between baseline and week 48; complete endoscopic remission (total SES-CD score 0); and absolute Magnetic Resonance Index of Activity score (not reported because of scarce availability of data). Protocol-defined safety endpoints were the toxicity of chemotherapy, measured with NCI CTCAE criteria, and AEs and SAEs, including

	Intervention group (n=13)	Control group (n=9)
Centre*		
Barts Health	5 (38%)	3 (33%)
Cambridge	0	1 (11%)
Edinburgh	1 (8%)	2 (22%)
Liverpool	1 (8%)	0
Nottingham	4 (31%)	2 (22%)
Oxford	1 (8%)	0
Sheffield	1 (8%)	1 (11%)
Sex		
Male	6 (46%)	4 (44%)
Female	7 (54%)	5 (56%)
Age, years	34·5 (9·5)	36·3 (10·1)
Ethnicity		
White†	10 (77%)	8 (89%)
Asian or Asian British‡	3 (23%)	1 (11%)
BMI, kg/m²	26·2 (6·0)	27·4 (6·2)
Smoking status§		
Never	7 (54%)	7 (78%)
Current	2 (15%)	1 (11%)
Previous (stopped for ≥5 years)	3 (23%)	1 (11%)
Perianal disease		
Yes	3 (23%)	5 (56%)
No	10 (77%)	4 (44%)
Stoma		
Yes	7 (54%)	2 (22%)
No	6 (46%)	7 (78%)
Disease behaviour		
B1 non-stricturing, non-penetrating	5 (38%)	0
B2 stricturing	7 (54%)	3 (33%)
B3 penetrating	1 (8%)	6 (67%)
Disease location¶		
L1 ileal	3 (23%)	0
L2 colonic	1 (8%)	1 (11%)
L3 ileocolonic	5 (38%)	3 (33%)
L4 isolated upper disease	0	1 (11%)
L1 L4	3 (23%)	2 (22%)
L3 L4	1 (8%)	2 (22%)
Montreal stage at onset classification		
A1 (>16 years)	5 (38%)	2 (22%)
A2 (17–40 years)	8 (62%)	7 (78%)
Previous operations for Crohn's disease		
Intestinal surgery	12 (92%)	8 (89%)
Perianal surgery	4 (31%)	4 (44%)
Extraintestinal manifestations		
Yes	4 (31%)	2 (22%)
No	9 (96%)	7 (78%)
Age at disease onset, years	20·7 (7·7)	22·6 (6·3)
Duration of disease, years	11 (10 to 16)	10 (10 to 19)
C-reactive protein, mg/L		
Participants with data	12 (92%)	8 (89%)
Median (IQR)	10·9 (7·5 to 14·2)	20·0 (4·8 to 34·2)

(Table 1 continues on next page)

	Intervention group (n=13)	Control group (n=9)
(Continued from previous page)		
Crohn's Disease Activity Index		
Participants with data	12 (92%)	8 (89%)
Score	354·5 (197·2 to 481·0)	290·5 (174·2 to 352·2)
Patient-reported outcome		
Participants with data	13 (100%)	8 (89%)
Score	23·0 (12·0 to 31·0)	17·0 (12·2 to 25·8)
Harvey-Bradshaw Index		
Participants with data	13 (100%)	9 (100%)
Score	10·0 (4·0 to 16·0)	13·0 (8·0 to 18·0)
Central SES-CD		
Participants with data	9 (69%)	8 (89%)
Score	8·0 (7·0 to 15·0)	8·5 (5·5 to 14·2)
Local SES-CD		
Participants with data	9 (69%)	8 (89%)
Score	8·0 (6·8 to 12·5)	9·0 (7·2 to 11·0)
Number of segments examined in colonoscopy	2·0 (1·0 to 4·0)	4·0 (2·0 to 4·0)
IBD Control		
Participants with data	11 (85%)	7 (78%)
Score	2·0 (1·0 to 4·0)	0·0 (0·0 to 1·5)
EQ-5D-5L		
Participants with data	13 (100%)	9 (100%)
Score	0·434 (–0·023 to 0·768)	0·582 (0·336 to 0·632)
IBD Questionnaire		
Participants with data	5 (38%)	7 (78%)
Score	85·0 (54·0 to 166·0)	89·0 (81·0 to 95·5)

Data are n (%), mean (SD), or median (IQR). SES-CD=Simplified Endoscopic Score for Crohn's Disease. IBD=inflammatory bowel disease. *One site did not recruit any patients in either group. †English, Welsh, Scottish, Northern Irish, British; Irish, Gypsy, or Irish Traveller; or any other White background. ‡Indian, Pakistani, Bangladeshi, Chinese, or any other Asian background. §One (8%) participant in the intervention group was a previous smoker; however, their smoking history, including when they stopped smoking, was not recorded, so they do not appear in the table. ¶Disease location with L4 present in addition to L1–L3 occurs when L4 is a modifier, accounting for the presence of concomitant upper gastrointestinal disease.

Table 1: **Baseline characteristics**

mortality. Protocol-defined quality-of-life endpoints were disease-specific quality of life measured by the IBD Questionnaire, disease-specific quality of life measured by the IBD Control, quality of life measured by the EQ-5D-5L, and use of health-care resources by questionnaire (not reported due to early trial closure).

See Online for appendix

Statistical analysis

Sample size calculations were based on the endoscopic outcome in the ASTIC trial.[10] To detect a 35% significant difference in the proportion of patients with no ulceration in endoscopy assessment based on 50% in the intervention group and 15% in the control group, with 90% power at a two-sided 5% significance level and assuming 6% attrition, 66 patients were required in the intervention group and 33 in the control group. The initial statistical analysis plan was modified considering the early termination of the trial, although all analyses were pre-planned.

The primary endpoint was analysed in the intention-to-treat population, which included all randomly assigned participants with valid data on the primary outcome and excluded participants found to be ineligible after randomisation (ie, randomised in error). All safety analyses were performed in the extended intention-to-treat population, which included all randomly assigned participants. Due to the reduced size of the dataset, no statistical models were fitted on any clinical or patient-reported outcomes; instead, outcomes are descriptively compared between treatment groups. Safety data were summarised by treatment group and by NCI CTCAE grade. All statistical analyses were performed with R (version 4.0.0). This trial is registered with the ISRCTN registry, 17160440.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The sponsor provided governance advice during the planning and delivery of the trial.

Results

Between Oct 18, 2018, and Nov 8, 2019, 49 (66%) of 74 patients who were referred to the multidisciplinary team were screened for eligibility, of whom 27 (55%) patients met eligibility criteria and completed a baseline visit (figure 1). 23 (85%) participants were randomly assigned: 13 (57%) to the intervention group and ten (43%) to the control group.

On Dec 30, 2019, the trial was paused to investigate suspected unexpected serious adverse reactions, including the death of one patient in the intervention group. On June 3, 2020, the Trial Steering Committee and Data Monitoring and Ethics Committee recommended that further participant recruitment should cease and that patients in screening or randomly assigned to the intervention group who had not yet received treatment should be withdrawn. Study follow-up concluded on Nov 13, 2020.

The intention-to-treat population included 22 participants, 13 (59%) in the intervention group and nine (41%) in the control group (figure 1). The mean age of participants was 35 (SD 10) years and mean duration of Crohn's disease was 13·8 (7·0) years, and all patients were refractory to conventional and biological therapy (appendix p 14). 20 (91%) patients had undergone at least one previous intestinal resection and nine (41%) had a current stoma, seven (78%) of whom were in the intervention group (table 1). All patients had a clinically significant burden of symptoms; median CDAI at baseline was numerically higher in the intervention group than in the control group (table 1). However, there was evidence of clinically significant endoscopic disease activity in both groups (table 1).

In the intervention group, two (15%) patients were withdrawn due to early study closure and one (8%)

withdrew consent (figure 1). 12 (92%) participants underwent stem-cell mobilisation with a median CD34+ stem-cell harvest of $5{\cdot}4\times10^6$ cells per kg (IQR $4{\cdot}3–6{\cdot}4\times10^6$). After a median of 36 days (IQR 30–41), ten (77%) patients underwent conditioning and reinfusion, with a median of $4{\cdot}6\times10^6$ CD34+ cells per kg (4·0–6·3) reinfused (appendix pp 15–16). The mean number of days between randomisation and stem-cell reinfusion (ie, time to day 0) was 99 days (range 71–185), which was considerably longer than the planned 49 days because of waiting lists for beds in the haematology department. One (8%) patient died at 24 weeks; the remaining nine (69%) participants completed the follow-up visit at week 48.

In the control group, one (10%) participant was withdrawn from the study at week 8 because of ineligibility and the remaining nine (90%) completed the study. Eight (89%) participants received ongoing biological therapy, two (22%) required intravenous nutrition, seven (78%) continued corticosteroids, and one (11%) received oral tacrolimus. One (11%) participant underwent small bowel resection and one (11%) underwent examination under anaesthetic with seton. The trial had been paused before the start of the COVID-19 pandemic; however, the pandemic still affected follow-up visits and prevented or delayed colonoscopy in some patients. No patient showed symptomatic SARS-CoV-2 infection during the trial.

In total, 13 participants across both groups contributed valid data on the primary outcome at week 48, including participants with treatment failure. Of the nine (69%) participants followed up at week 48 in the intervention group, one (11%) declined endoscopic assessment and the central readings for two (22%) participants were not recorded (local endoscopic scores were available). The participant who died at week 24 was classified as a treatment failure in line with the protocol definition of the primary outcome (ie, the absence of endoscopic ulceration [SES-CD ulcer sub-score of 0] without surgery or death). In the control group, five (56%) participants did not have colonoscopy data that were centrally read at week 48 (two [22%] due to the COVID-19 pandemic, one [11%] not recorded, and two [22%] not performed due to worsening disease or surgery). However, two (22%) patients without colonoscopy data were included in the analysis of the primary endpoint as protocol-defined treatment failures (appendix p 17).

Three (43%) of seven participants in the intervention group and none of six participants in the control group with available data on the primary outcome met the primary endpoint. Of the three participants with local colonoscopy scores alone, an absence of ulceration according to the local investigator was observed in two (67%) participants in the intervention group, whereas a clinically significant ulceration was observed in one (33%) participant in the control group and was classified as a treatment failure. Therefore, combining central and local colonoscopy scores, an absence of ulceration was reported in five (56%) of nine participants in the intervention group versus none in the control group (appendix p 18). However, one (11%) participant in

	Intervention group (n=13)	Control group (n=9)
Secondary categorical outcomes		
Clinical remission (CDAI <150)	4/7 (57%)	1/6 (17%)
Steroid-free clinical remission (CDAI <150)	4/7 (57%)	1/6 (17%)
Clinical remission (HBI <4)	3/8 (38%)	1/8 (13%)
Complete endoscopic remission (SES-CD 0)	2/5 (40%)	0/3
Secondary continuous outcomes		
CDAI		
Participants with data	7 (54%)	6 (67%)
Score	127·0 (108·5 to 422·0)	319·9 (179·8 to 443·7)
Change in CDAI from baseline to week 48		
Participants with data	7 (54%)	5 (56%)
Score	–82·0 (–149·0 to –19·0)	28·0 (–28·0 to 64·0)
Central SES-CD		
Participants with data	5 (38%)	3 (33%)
Score	3·0 (0·0 to 4·0)	15·0 (13·5 to 22·0)
Local SES-CD		
Participants with data	5 (38%)	4 (44%)
Score	1·0 (0·0 to 3·0)	10·5 (9·2 to 16·0)
Change in central SES-CD from baseline to week 48		
Participants with data	5 (38%)	2 (22%)
Score	–6·0 (–7·0 to –5·0)	7·5 (6·2 to 8·8)
IBD Questionnaire		
Participants with data	9 (69%)	6 (67%)
Total score	167·0 (100·0 to 198·0)	91·5 (81·0 to 124·5)
IBD Control		
Participants with data	9 (69%)	7 (78%)
Score	10·0 (6·0 to 14·0)	1·0 (0·5 to 5·5)
EQ-5D-5L		
Participants with data	9 (69%)	7 (78%)
Score	0·584 (0·516 to 0·720)	0·585 (0·380 to 0·723)

Data are n/N (%), n (%), or median (IQR). CDAI=Crohn's Disease Activity Index. HBI=Harvey-Bradshaw Index. SES-CD=Simplified Endoscopic Score for Crohn's Disease. IBD=inflammatory bowel disease.

Table 2: **Secondary outcomes at week 48**

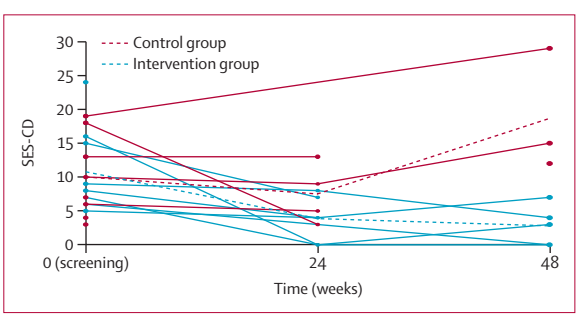

Figure 2: **Centrally read SES-CD over time in the intention-to-treat population**
Dashed lines represent the mean for each treatment group. Solid points and lines represent individual participants. SES-CD=Simplified Endoscopic Score for Crohn's Disease.

	Mobilisation		Transplantation		Follow-up		Total*		
	Intervention group (n=13)	Control group (n=10)	Intervention group (n=13)	Control group (n=10)	Intervention group (n=13)	Control group (n=10)	Intervention group (n=13)	Control group (n=10)	All (n=23)
Number of participants with ≥1 SAE	2 (15%)	2 (20%)	11 (85%)	3 (30%)	6 (46%)	3 (30%)	13 (100%)	4 (40%)	17 (74%)
Number of all SAEs (including repeated events)	4	3	24	3	8	9	38	16	54
Number of SAEs by seriousness									
Death	0	0	1	0	0	0	1	0	1
Life-threatening	1	0	3	1	0	0	4	1	5
Inpatient hospitalisation	3	3	10	2	6	9	21	14	35
Extended hospitalisation	0	0	3	0	2	0	5	1	6
Persistent or clinically significant disability or incapacity	0	0	3	0	0	0	3	0	3
Congenital abnormality or birth defect	0	0	0	0	0	0	0	0	0
Another important medical event	0	0	4	0	0	0	4	0	4
Number of SAEs by outcome									
Recovery	3	1	11	2	2	2	17	6	23
Improvement	1	2	7	1	3	7	12	10	22
No change	0	0	3	0	1	0	4	0	4
Deterioration	0	0	0	0	0	0	0	0	0
Persistence	0	0	0	0	1	0	1	0	1
Death†	0	0	3	0	1	0	4	0	4

Data are n (%) or n. SAE=serious adverse event. *Includes all SAEs, including those that occurred before mobilisation, hence the discrepancy between total SAEs and the sum of SAEs at each timepoint. †The four SAEs with the outcome of death relate to two patients, one who died during the trial and one who died after follow-up at week 48 following extended hospitalisation due to suspected infection. Eight SAEs were reported by three patients who were randomly assigned to the intervention group, but did not undergo transplantation.

Table 3: **SAEs by treatment group and time period**

	Time from stem-cell reinfusion, days
Respiratory failure	0
Acute oliguric renal failure	0
Renal failure	74
Thrombotic microangiopathy	90
Pulmonary veno-occlusive disease	93
Thrombotic microangiopathy	99
Acute kidney injury	133
Thrombotic microangiopathy	153
Unexplained significantly elevated C-reactive protein	281

Data presented for the ten participants in the intervention group who received treatment.

Table 4: **Suspected unexpected serious adverse reactions**

the intervention group who underwent HSCT and showed an absence of ulceration subsequently died after the final follow-up visit at week 48.

The few participants with valid outcome data precluded statistical comparisons between groups. However, participants in the intervention group had numerically higher quality-of-life scores in terms of IBD Questionnaire and IBD Control scores, with lower disease activity scores and endoscopic activity both in central and local assessments (table 2). The centrally read SES-CD for the intervention group decreased over time and was lower than that for the control group at week 48 (table 2); individual patient data for centrally read SES-CD over time are shown in figure 2.

AEs were reported by all 13 (100%) participants in the intervention group and by four (44%) participants in the control group (appendix p 19). In total, 38 SAEs were reported by 13 (100%) participants in the intervention group and 16 SAEs by four (44%) participants in the control group (table 3). Nine suspected unexpected serious adverse reactions occurred in six (46%) patients in the intervention group (table 4). Thrombotic microangiopathy proven by renal biopsy was reported in three participants between days 90 and 153. Two (15%) participants in the intervention group died: one (8%) at week 24 of pulmonary veno-occlusive disease that commenced on day 93, and one (8%) after the follow-up visit at week 48 (ie, 60 weeks after HSCT), having experienced respiratory and oliguric renal failure on the day of stem-cell reinfusion and remaining hospitalised during this time. Given the potential common mechanism of endothelial damage, additional tests were undertaken in patients affected by thrombotic microangiopathy (best fitting with atypical haemolytic uraemic syndrome) and veno-occlusive disease; however, no clear cause was identified. Possible explanations include viral reactivation, reduced ADAMTS13 (by autoantibody formation or other cause), or a consistent

relationship with the batch of anti-thymocyte globulin (ie, thymoglobulin).

No significant changes were observed in the population of peripheral blood immune cells in patients in the control group, as assessed by flow cytometry from baseline to week 48 (appendix pp 1–3). By contrast, HSCT induced profound reductions in many immune cell populations, which persisted for varying periods of time over the course of the 48-week follow-up (appendix pp 3, 20). These reductions were particularly marked and prolonged in populations of naive effector memory and gut homing CD4+ T cells across various cell phenotypes, although there was relative preservation of T-helper-22 lymphocytes. The effect on B cells was less persistent and populations of monocytes, innate lymphoid cells, and natural killer (NK) cells seemed to have largely recovered by week 14. There was a significant increase in NKp46+ innate lymphoid cells after HSCT. Consistent with previous reports of an impact of HSCT on thymic regeneration of lymphocyte populations, a persistent reduction in recent thymic emigrants was observed in the intervention group, as assessed by T-cell receptor excision circle quantitation (appendix p 4). In the intervention group, the percentage of stimulated CD4+ T cells producing IL-4, TNF, and IFN-γ was higher at early timepoints (ie, weeks 8–24) than at baseline. No differences were observed in the control group (appendix p 5).

Comparisons were made between baseline and pooled follow-up timepoints (appendix pp 1–2). No differences were observed in the predicted mucosal cellular composition between baseline and subsequent timepoints in patients in the control group. By contrast, there was a significant reduction in populations of monocytes, CD4+ memory cells, and CD8+ lymphocytes in mucosal biopsies following HSCT from participants in the intervention group, with an increase in the small proportion of follicular helper T cells (appendix p 6). 58 differentially expressed genes were found in biopsy samples taken at baseline and following HSCT (false discovery rate 0·05; appendix p 7). A pathway enrichment analysis showed reduced signalling in several inflammatory response pathways, including IFN-γ, IL-6, TNF, NF-κB, and JAK-STAT (appendix p 8).

Despite the low number of patients, there seems to have been differences in the recovery and function of populations of peripheral blood mononuclear cells after HSCT when comparing between patients in the intervention group who subsequently met the primary endpoint and those who did not. Patients who responded had early recovery of effector CD8+ T-cell populations, suppressed T-helper-22 lymphocytes, and delayed recovery of gut homing lymphocytes, monocytes, and myeloid-derived suppressor cells (appendix p 9); however, the few samples precluded statistical analysis. Furthermore, patients who responded showed a delayed recovery of thymic emigrants in T-cell receptor excision circle analysis and a reduction in the percentage of peripheral blood CD4+ cells producing IL-17 (appendix pp 10–11).

Discussion

The ASTIClite trial was designed to assess whether autologous HSCT with an immune-ablative regimen of reduced intensity would lead to endoscopic ulcer healing in patients with treatment-refractory Crohn's disease. Compared with the ASTIC trial, the transplantation-conditioning regimen comprised reducing cyclophosphamide to 60% of the original dose, maintaining the dose of anti-thymocyte globulin, and adding fludarabine, an immunosuppressive agent that has been used in combination with cyclophosphamide and other cytotoxic chemotherapy for HSCT conditioning in other settings, most commonly allogeneic HSCT,[26,27] but also in autologous HSCT.[28,29] All recruited participants had severe treatment-refractory active disease, and most had undergone at least one previous surgical resection.

The trial was stopped on the advice of the Data Monitoring and Ethics Committee and the Trial Steering Committee because of a high incidence of suspected unexpected serious adverse reactions and one death. No participant who continued standard care met the primary endpoint (absence of endoscopic ulceration [SES-CD ulcer sub-score of 0] without surgery or death), compared with three (43%) of seven participants undergoing HSCT. Likewise, there were clinically relevant improvements in total SES-CD among participants in the intervention group, which were not observed in the control group. However, the reported benefits were clearly outweighed by the number of SAEs and suspected unexpected serious adverse reactions in participants undergoing HSCT, including delayed renal failure due to proven thrombotic microangiopathy in three participants. Two participants in the intervention group died.

The trial was designed and powered with an endoscopic endpoint to provide an objective assessment of disease activity, which is now considered to be a standard in randomised controlled therapeutic trials for patients with Crohn's disease.[30–32] This study was the first investigator-led trial in the UK to use central reading for its primary endpoint. A bespoke web-based platform was developed to share anonymised videos, and the reliability and validity of the pool of central readers were verified.[25] In view of the early study termination and reduced recruitment, an insufficient number of participants were recruited to meet the original sample size target. The analysis was further limited by unavailable primary outcome data due to the COVID-19 pandemic, early trial termination, and unavailable source data, which could introduce bias. The most generous efficacy assessment with local disease assessment where available suggested that five (55%) of nine participants in the intervention group met the primary endpoint. The range of 40–55% patients showing endoscopic healing 1 year after HSCT is in line with our a

priori assumptions for the sample size calculation. In all analyses, the observation that no participants in the control group met the primary endpoint, despite all available licenced therapies, reflects the severe and refractory nature of the patient population studied.

The modified HSCT regimen used in this trial was not associated with a reduced burden of side-effects compared with previously reported regimens. Although the safety and efficacy of stem-cell mobilisation were acceptable, conditioning and HSCT treatment were associated with unacceptable toxicity; all participants undergoing HSCT reported at least one SAE and all NCI CTCAE grade 4 AEs occurred in the intervention group only. There were nine suspected unexpected serious adverse reactions reported in six participants in the intervention group, including thrombotic microangiopathy and pulmonary veno-occlusive disease. It is important to note that SAEs related to disease activity were also observed among participants in the control group, with complications from surgery, hospital admission for disease flare, and thromboembolic events reported. These life-threatening adverse events highlight the considerable impact of ongoing active disease on patient safety and the importance of effective therapy to control intestinal inflammation.

Although thrombotic microangiopathy and veno-occlusive disease are well recognised complications of allogeneic HSCT related to endothelial damage, they are rare in the setting of autologous HSCT. A comprehensive analysis of patients with thrombotic microangiopathy did not suggest that background comorbidity, disease phenotype, or previous drug history was relevant. Many patients undergoing HSCT showed viral reactivation following transplantation, but this observation was not restricted to patients who subsequently developed thrombotic microangiopathy. All patients with thrombotic microangiopathy received anti-thymocyte globulin from the same batch; however, working together with the manufacturer and regulators (the MHRA and European Medicines Agency), no issues with this drug batch were identified. In addition, reviewing the clinical course of patients undergoing autologous HSCT in other settings of autoimmune disease who had received the same batch of anti-thymocyte globulin at clinical sites participating in this trial did not identify any adverse renal outcomes. All participants undergoing HSCT (even those without reported thrombotic microangiopathy) showed a mild deterioration in renal function, which might suggest that aspects of the drug regimen used in this trial affect kidney function. One key difference between this regimen and that used in the ASTIC trial[10] and other studies in patients with Crohn's disease is the addition of fludarabine. Although fludarabine has not been associated with reports of thrombotic microangiopathy after autologous HSCT, it is commonly incorporated into conditioning regimens of reduced intensity for allogeneic HSCT, whereby endothelial damage and thrombotic microangiopathy are recognised complications with several potential causes.[33,34] Alternatively, it is plausible that the unexpected incidence of thrombotic microangiopathy and pulmonary veno-occlusive disease relates to aspects of Crohn's disease pathology, such as bacterial translocation, the impact of the HSCT on the microbiome,[35] or the various biological therapies to which patients had been exposed. Of note, a single transplantation-related mortality with veno-occlusive disease of the liver was reported in the ASTIC trial,[8] where fludarabine had not been used. The one agent in the transplantation-conditioning regimen that did not change was rabbit anti-thymocyte globulin, whereby a total dose of 7·5 mg/kg was used in both trials, which is at the upper end of the previously recommended range of 5·0–7·5 mg/kg in guidelines from the European Society for Blood and Marrow Transplantation.[18] Previous series of autologous HSCT in autoimmune disease have reported the use of reduced doses of rabbit anti-thymocyte globulin in the conditioning regimen with less toxicity.[5–9,18] Whether or not the dosing of anti-thymocyte globulin is clinically significant is not clear, particularly as there is considerable heterogeneity in pharmacokinetic exposure to anti-thymocyte globulin when used in HSCT.[36] The investigation of cases did not support viral reactivation consequent to immunosuppression or any other factor triggering thrombotic microangiopathy directly or indirectly (eg, through autoantibodies against ADAMTS13).[37] However, the potential contribution of rabbit anti-thymocyte globulin, its dosing, and its individual pharmacodynamics needs to be considered given the unexpected toxicities in these trials.

Although limited by the number of patients analysed, data from the flow cytometry immunophenotyping indicate that the reduced total lymphocyte count (ie, $CD3^+$) reflected a low $CD4^+$ cell count (and associated subsets) at all timepoints. By contrast, $CD8^+$ subsets recovered rapidly after transplantation, returning to similar counts to those observed in the control group. This observation has been reported previously[38] and is consistent with the repopulation of $CD8^+$ cells from peripheral regeneration, with limitations of the $CD4^+$ cell compartment to regenerate in the year after transplantation. Along with the T-cell receptor excision circle analysis, which was performed on the total lymphocyte population (not specifically $CD4^+$ cells), this finding supports limitations in regeneration via thymic pathways[39] in the first 48 months after transplantation.

There are several exploratory observations related to peripheral blood monocuclear cell cytokine release and mucosal gene expression. Generally, $CD4^+$ and $CD8^+$ lymphocytes from patients who underwent HSCT expressed higher levels of T-helper-1 and T-helper-17 cytokines (ie, IFN-γ, TNF, and IL-17) after stimulation at most timepoints than did lymphocytes from participants in the control group. HSCT resulted in the down-regulation of several mucosal gene signatures related to

disease. The clinical and scientific significance of this finding is uncertain, and the low number of patients studied means that it is not possible to associate this with a clinical outcome.

Given the reported impact of HSCT on mucosal disease activity and immunology in this population of patients with refractory Crohn's disease, future clinical trials of targeted conditioning regimens or cellular therapies might offer safer lympho-myeloablation with less systemic toxicity.[40] As such, there might be exceptional circumstances in which HSCT could be considered for patients with Crohn's disease that is refractory to all treatments, but only in expert centres after careful discussion of the risks with patients and with central registration of data in all cases.[18,41] Nevertheless, two randomised controlled trials of autologous HSCT have now been conducted in patients with treatment-refractory Crohn's disease. Both trials were halted before complete recruitment after a patient death. Despite thorough investigation, the cause of the higher than expected incidence of thrombotic microangiopathy and veno-occlusive disease observed in the ASTIClite and ASTIC trials has not been elucidated. Based on this trial, the future clinical use of autologous HSCT plus conditioning with cyclophosphamide, anti-thymocyte globulin, and fludarabine in patients with Crohn's disease is not considered to be appropriate.

Contributors

JOL, JAS, LS, HB, MB, LD, EL, AGP, and GAF produced the first draft of the manuscript. JOL, JAS, DH, DP, LS, MB, RE, AGP, MP, JS, ST, CH, YM, GWM, AL, and JG, on behalf of the ASTIClite research group (appendix p 22), conceived or designed the study. JAS, JG, JB, BU, AP, AP, and MK reviewed and amended the transplantation and supportive care regimen in the protocol. LS, HB, GAF, ET, AL, CC, UBC, JOL, JG, AL, JAS, GWM, JB, MP, BU, ST, AP, SD, SS, AP, PMI, and MK acquired the data. JOL, JAS, LS, HB, MB, LD, BD, EL, GAF, AGP, ET, AL, RE, and SR, on behalf of the ASTIClite research group, analysed the data. JOL, JAS, LS, HB, MB, LD, BD, GAF, EL, AGP, and SR, on behalf of the ASTIClite research group, interpreted the data. EL, MB, and LD directly accessed and verified the underlying data. All authors revised the work critically for important intellectual content. All authors were involved in the final approval of the version to be published. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Data sharing

De-identified participant data will be made available on appropriate request to the corresponding author after approval by the study sponsor. The study protocol and statistical evaluation plan are available on the NIHR website (https://fundingawards.nihr.ac.uk/award/15/178/09).

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