A multi-centre Phase I/II trial of granulocyte-augmented cord blood transplantation for young adults with very poor risk acute myeloid leukaemia.

STUDY PROTOCOL

Short Title: GRanulocyte Augmented Cord blood transplantation for poor risk leukaEmia (GRACE)

Chief Investigator: Dr Mark Williams
Sponsor: University of Manchester
Version 1.0: 02/07/2025
ClinicalTrials.gov number: <insert number>
IRAS number: 357519

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

For and on behalf of the Trial Sponsor:
Signature: Date:
..../...../.....
Name (please print):
.....
Position:
.....

Chief Investigator:
Signature: Date:
..../...../.....
Name: (please print):
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Statistician:
Signature: Date:
..../...../.....
Name: (please print):
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Position:
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AMENDMENTS

I. CONTENTS

II. ABBREVIATIONS

AE	Adverse Event
AML	Acute Myeloid Leukaemia
AR	Adverse Reaction
BSA	Body Surface Area
BSBMTCT	British Society of Blood and Marrow Transplantation and Cellular Therapy
BSH	British Society for Haematology
CA	Competent Authority
CI	Chief Investigator
CMV	Cytomegalovirus
CNS	Central Nervous System
CRF	Case Report Form
CRO	Contract Research Organisation
CRP	C-Reactive Protein
CRS	Cytokine-Release Syndrome
CT	Computed Tomography
CTA	Clinical Trial Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CTU	Clinical Trials Unit
DLT	Dose-Limiting Toxicity
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FBC	Full Blood Count
FEV1	Forced Expiratory Volume in the first second
FISH	Fluorescence In-Situ Hybridisation
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GMP	Good Manufacturing Practice
GvHD	Graft-versus-Host-Disease
HHV6	Human Herpesvirus 6
HLA	Human Leucocyte Antigen
HRA	Health Research Authority
IB	Investigator Brochure
ICC	International Consensus Classification
ICF	Informed Consent Form
IRAS	Integrated Research Application System
ISF	Investigator Site File (This forms part of the TMF)
ISRCTN	International Standard Randomised Controlled Trials Number
MHRA	Medicines and Healthcare products Regulatory Agency
MMF	Mycophenolate Mofetil
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NGS	Next-Generation Sequencing
NHSBT	National Health Service Blood & Transplant
NHS R&D	National Health Service Research & Development
NRM	Non-Relapse Mortality
OS	Overall Survival
PFS	Progression-Free Survival
PI	Principal Investigator
PIC	Participant Identification Centre

PIS	Participant Information Sheet
PPIE	Patient and Public Involvement and Engagement
PTLD	Post-Transplant Lymphoproliferative Disorder
QA	Quality Assurance
QC	Quality Control
QoL	Quality of Life
QP	Qualified Person
REC	Research Ethics Committee
RR	Response Rate
RP2D	Recommended Phase II Dose
RFS	Relapse-Free Survival
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SOP	Standard Operating Procedure
SOS	Sinusoidal Obstruction Syndrome
SmPC	Summary of Product Characteristics
SSI	Site Specific Information
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total Body Irradiation
TITE-CRM	Time-to-Event Continual Reassessment Method
TMF	Trial Master File
TMG	Trial Management Group
TRM	Transplant-Related Mortality
TSC	Trial Steering Committee
VAF	Variant Allele Frequency

III. TRIAL SYNOPSIS

Title
A multi-centre Phase I/II trial of granulocyte-augmented cord blood transplantation for young adults with very poor risk acute myeloid leukaemia.

Trial Design
This is a prospective phase I/II study of granulocyte-augmented cord blood transplantation for young adults (16-55 years) with very poor risk acute myeloid leukaemia. The target population is high-risk AML with TP53 mutations, MECOM rearrangements or chemoresistant phenotypes - defined by partial response to 2 cycles of induction chemotherapy or MRD positive disease by flow cytometry (>0.1%) after 2 cycles of induction for those with adverse risk AML or early relapse after intensive chemotherapy.

Participants will receive a T-replete cord blood transplant with a standardised protocol consisting of centralised cord unit selection, mid-intensity conditioning and GvHD prophylaxis. A single pool of irradiated granulocytes will be given daily for a variable number of days (1, 3, 5 or 7 days) starting on the day of transplant.

The study consists of two phases. Phase I has two components: dose-escalation and dose-optimisation to identify the Recommended Phase II Dose (RP2D) of granulocytes. Phase 2 will assess preliminary efficacy at the RP2D, based on relapse-free survival (RFS) at 1 year, using a single-stage Bayesian design. All patients will be followed-up for a minimum of one year.

Objectives
See Table 2 for full descriptions of objectives and endpoints.

Primary Objectives
Phase 1:
1. To determine the safety of peri-transplant granulocyte infusion for adult recipients of T replete cord blood transplants.
2. To determine the optimal dosing schedule (RP2D) for granulocyte administration using measures of activity and dose-limiting toxicity.

Phase 2:
3. To assess preliminary efficacy at the RP2D, based on relapse-free survival (RFS) at 1 year.

Secondary Objectives (Phases 1 & 2)
- To assess relapse and survival in terms of relapse-free survival (RFS), non-relapse mortality (NRM), overall survival (OS), cumulative incidence of relapse and GvHD-free and relapse-free survival (GRFS).
- To assess safety and tolerability in terms of the cumulative incidence of acute grade II-IV and III-IV GvHD, cumulative incidence of moderate or severe chronic GvHD, cytokine release syndrome rate, immune suppression-free rate, cumulative incidence of intestinal failure, number of inpatient days, QoL within the first 12 months, and the incidence of ≥ grade 3 toxicities. The measures of activity and dose-limiting toxicity assessed in Phase 1 will also be assessed in Phase 2.
- To assess engraftment and immune reconstitution in terms of the cumulative incidence of engraftment, incidence of full donor chimerism, cumulative incidence of infection requiring admission and cumulative incidence of viral infection or reactivation requiring treatment.

Exploratory Objectives
The scientific research associated with the study will attempt to describe the mechanism of peri-transplant granulocyte administration, identify key features of successful transplantation and investigate whether responders can be identified to allow targeted application of this approach. Specifically, the research will aim to address the following questions:
1. Does disease response require priming of donor T-cells against HLA mismatched shared between the recipient and the granulocyte product?
2. Does granulocyte-induced inflammation induce leukaemic differentiation?
3. Does pre-treatment sensitivity of leukaemia to interferon-gamma identify responders?
4. Can plasma proteomics identify novel biomarkers of treatment response?

5. When transplanting patients with detectable disease, can immune clearance of residual disease be detected using cell-free DNA methylation analysis?

Patient Population

Young adults (16-55 years) with acute myeloid leukaemia with TP53 mutations, MECOM rearrangements or chemoresistant phenotypes, defined by poor response to induction chemotherapy or early relapse.

Sample Size

The trial will enrol up to 30 patients across 4 dose levels in Phase I and up to 20 patients in Phase 2. Notably, evaluable patients who received RP2D in phase I will also contribute to the phase 2 activity evaluation.

Inclusion Criteria

1. Availability of a suitable cord blood unit
2. Age between 16 and 55 years
3. Primary diagnosis of Acute Myeloid Leukaemia (AML) or MDS/AML (as defined by ICC 2022) fitting one or more of the following criteria:
    a. TP53 mutation (single- or multi-hit)
    b. Presence of inv(3) (q21.3q26.2) or t(3;3)(q21.3;q26.2)
    c. Adverse risk (as per ICC 2022) **and** >0.1% MRD by flow cytometry after 2 cycles of induction
    d. AML (any risk) with partial remission (<10% blasts) after 2 cycles induction
    e. Early relapse (<6 months) after chemotherapy alone (excluding t(16;16), inv(16) or t(8;21))
4. Disease status at transplant (disease assessment will be performed within 28 days of starting conditioning chemotherapy)
    a. All patients must have <10% blasts
    b. >10% blasts with a hypocellular background may be discussed with the trial team
5. Suitable fitness and organ function as per the following criteria:
    a. Glomerular filtration rate >50 mL/min/1.73m^2
    b. Ejection fraction >50%
    c. FEV1 >65% *without* dyspnoea on mild activity
    d. AST/ALT <3 x ULN
    e. Bilirubin <1.5 x ULN (excluding Gilbert's syndrome)
    f. Performance Status (ECOG) of 0 or 1
6. Females of and male patients of reproductive potential (i.e., not post-menopausal or surgically sterilised) must agree to use appropriate, highly effective, contraception from the point of commencing therapy until 12 months after transplant

Exclusion criteria

- AML secondary to a myeloproliferative neoplasm
- Active CNS disease (extramedullary disease at other sites should be discussed with the trial team)
- Prior allogeneic stem cell transplant
- Participation in another clinical trial that would alter any aspect of the transplant protocol or that aims to reduce the subsequent risk of relapse (discuss with trial team if unsure)
- History of cardiac arrhythmia
- Ischaemic heart disease, valvular heart disease or congestive cardiac failure
- Transient ischaemic attack or cerebrovascular accident
- Rheumatologic disease (SLE, RA, polymyositis, mixed CTD or polymyalgia rheumatica)
- Ulcerative colitis or Crohn's disease
- Liver cirrhosis
- Presence of an active second malignancy
- Uncontrolled infection, including viral reactivation (CMV, EBV)
- HIV positive
- Hepatitis B/C active infection with measurable viral load (patients with chronic hepatitis B or C infection require clear documentation of absence of cirrhosis by either fibroscan or biopsy, regardless of viral load)
- Pregnancy, breastfeeding, unwilling to use contraception

- Contraindications to administration of pooled granulocytes
- Previous history of sensitivity to granulocytes
- Inability of patient to give informed consent
- Any other organ dysfunction or co-morbidity that precludes transplant in the opinion of the investigator
- Any concern by PI

Trial Duration
Patients will be recruited over 36 months. Patients will be followed up for a minimum of 1 year.

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IV. FUNDING
Funding for this study was provided by Blood Cancer UK (Grant reference: 25003).

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V. ROLE OF SPONSOR AND FUNDER

Sponsor
The trial will be sponsored by the University of Manchester. The responsibilities of the sponsor are as defined in the UK Policy Framework for Health and Social Care Research. The University of Manchester, as the sponsor, has delegated a number of responsibilities to King's College Hospital (KCH), these are trial management, monitoring and data management. Full details of the role and responsibilities of KCH are outlined in the Delegation of Responsibilities section of the research agreement signed between the Sponsor and KCH.

The sponsor has legal responsibilities that cannot be delegated.

Funder
GRACE is funded by Blood Cancer UK. The roles and responsibilities of the funder are defined within the funding agreement.

VI. ROLES & RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITTEES, GROUPS AND INDIVIDUALS

As this is a Phase I/II study, the Trial Steering Committee (TSC) and the Data Monitoring and Ethics Committees (DMC) will be combined to aid decision making during the adaptive phase, which includes both safety and preliminary efficacy assessments.

Joint committee (TSC/DMC)
The joint committee will provide overall supervision for the trial and provide advice through its independent chair. Recommendations for the continuation of the trial lies with the joint committee. Data analyses will be supplied in confidence to the committee, which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further patients.

Additional meetings may be called if recruitment is faster than anticipated and the committee may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The joint committee will

report directly to the TMG who will convey findings to the funders, and/or sponsors as applicable. The committee may recommend discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise patient safety.

The joint committee will operate in accordance with a trial specific charter based upon the template created by [insert details]. The role of the joint committee will vary according to the phase of the study:

Phase 1: In the safety phase of this study the joint committee will meet with members of the TMG to discuss safety data and make decisions regarding dosing and study continuation. These meetings will be open, with discussion and consensus decisions reached via voting.

Phase 1 to 2 Transition Review Meeting: Once phase I has been completed and an RP2D identified, we will convene a special "Phase I to II Transition Review Meeting" to serve as a checkpoint for progression from phase I to II. This meeting will include the TSC/DMC and a representative from the grant body to review important factors including:

- The number of evaluable patients treated at the RP2D during Phase I
- Remaining grant duration
- Available resources
- Any other important parameters

Phase 2: As the second phase of the study has the potential to be practice changing, the TMG (except the statistician(s)) will remain blinded to the long-term efficacy data. In phase II, there will be open and closed sessions. Members of the TMG will attend open sessions where safety data will be shared. Efficacy data regarding RFS, or other outcome measures, will only be showed in the closed session to the independent members.

Trial Management Group (TMG)
The TMG will be responsible for the set up and management of the clinical trial. The group will meet regularly to ensure that all practical details of the trial are progressing, working well and that everyone within the trial understands them. The TMG will closely monitor toxicity and adverse events during Phase 1. The TMG includes the PIs and clinicians from all three study sites. Sharing direct clinical experience and discussing study data will be important to inform decisions about dose escalation/reduction and study continuation. Clinical experience may also have implications for the management of treatment complications and will be shared.

VII. PROTOCOL CONTRIBUTORS

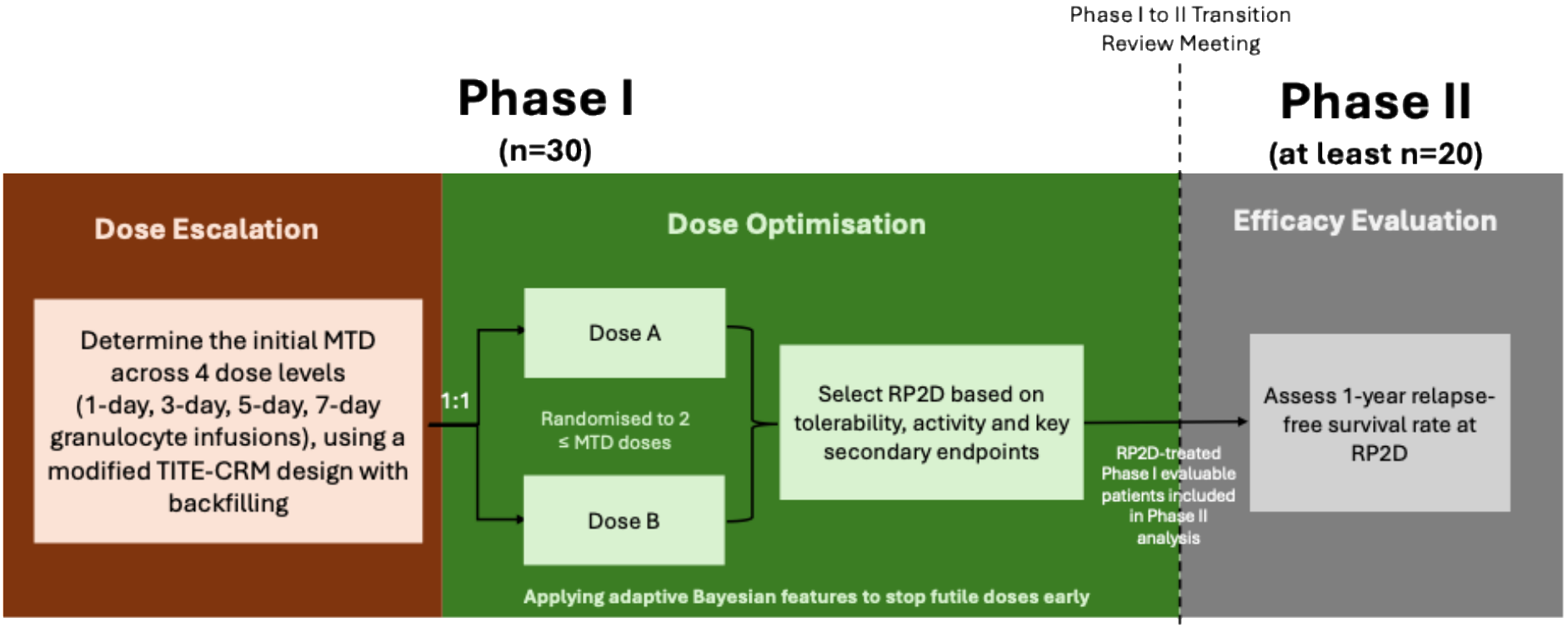
This protocol was written by the study Trial Management Group which included expertise in the original paediatric study that led to this study (Professor Rob Wynn), cord blood transplantation (Professor Rob Wynn, Dr Chloe Anthias and Professor Kay Poulton), acute myeloid leukaemia and adult stem cell transplantation (Dr Victoria Potter, Dr Mili Shah, Dr Mark Williams and Dr Emma Nicholson), granulocyte supply and biology (Professor Simon Stanworth), translational research (Dr Mark Williams), early phase and adaptive trials methodology (Professor Christina Yap) and statistics (Professor Christina Yap and Xinjie Hu).

Patient and Public Involvement and Engagement
During the initial design phase, a virtual event was held with 25 attendees including leukaemia patients, friends and families of those who had died, and patient representatives from Anthony Nolan, MDS UK, and Blood Cancer UK. Feedback from this group led to broadening the inclusion criteria and raising of the upper age limit. The patient representative lead reviewed the funding application documents and the patient information leaflet. The latter has also been reviewed and edited by the patient representative team from Anthony Nolan.

VIII. KEYWORDS

Cord blood transplantation; acute myeloid leukaemia; graft-versus-leukaemia; granulocytes.

IX. TRIAL SCHEMA



Occurrence of dose-limiting toxicities (DLTs). Patients would be considered to have experienced a DLT if they experience any of the toxicity events:

I. Severe CRS (intensive care admission requiring intubation >7days)
II. Primary graft failure (failure of count recovery and absent donor DNA at day 28 bone marrow)
III. Severe acute graft-versus-host disease (death from GvHD in the first 100 days)

Evidence of clinical activity. Patients would be considered to show early indicators of clinical activity if they experience any of the events:

I. CRS (fever in the first 7 days following stem cell infusion)
II. Lymphocyte expansion (>0.5 x10^9/L in the first 12 days)
III. Maximum CRP >150 mg/L on days 5-10 following stem cell infusion
IV. Early evidence of disease response (MRD negative on day 28 bone marrow)

MTD: Maximum Tolerated Dose
RP2D: Recommended Phase 2 Dose
TITE-CRM: Time-to-event Continual Reassessment Method
Backfilling: Enrol additional patients at tolerable doses

X. SCHEDULE OF EVENTS

Assessment	Baseline	D-6 to D-1	D0	D1-14	D21	D28	D56	D100	D180	D270	D360
Informed consent	X										
Medical history	X										
Transplant workup[1]	X										
Pregnancy test[2]	X										
Physical examination, vital signs, weight and ECOG PS	X		X	X	X	X	X	X	X	X	X
Full blood count, biochemistry[3]	X		X	X	X	X	X	X	X	X	X
Disease assessment	X					X		X	X	X	X
Lymphocyte subsets[4]						X	X	X	X	X	X
Quality of life assessments[5]	X							X	X		X
Cytokine Release Syndrome assessment[6]			X	X	X	X					
GvHD assessment[7]				X	X	X	X	X	X	X	X
Chimerism						X	X	X	X	X	X
Concomitant medications	X		X	X	X	X	X	X	X	X	X
Research samples (PB)[8]	X		X	X	X	X	X	X			
Research samples (BM)[9]	X			X		X		X	X	X	X
MID INTENSITY CONDITIONING REGIMEN (FLUDARABINE, CYCLOPHOSPHAMIDE, THIOTEPA, TBI) GRANULOCYTE ADMINISTRATION AS PER PROTOCOL											
		D-6	D-5	D-4	D-3	D-2	D-1	D0	D1-14	D21	D28	D56	D100	D180	D270	D360
Cyclophosphamide 50mg/kg +mesna 20mg/kg +pre- and post-hydration		X														
Fludarabine 30mg/m²		X	X	X	X	X										
Thiotepa 5mg/kg				X	X											
TBI 4Gy							X									
Cord infusion								X								
Immunosuppression: Ciclosporin + MMF					Start on D-3, stop MMF at D35 or 7d post engraftment in absence of GvHD. Start CSA wean at D50-60 in absence of GvHD.											
Granulocyte administration (see protocol)[10]								X	X							

1. All baseline assessments and transplant work-up investigations should be performed within 8 weeks of initiating conditioning chemotherapy
2. Pregnancy test for female patients with reproductive potential should be performed using serum
3. Full blood count should include white blood cell differential. Biochemistry should include urea, electrolytes, creatinine, LDH, CRP, magnesium, bilirubin, AST/ALT and ALP. Lipid profile testing to include total cholesterol, high density lipoprotein cholesterol, non-DL cholesterol and triglycerides
4. Lymphocyte subsets for CD3, CD4, CD8, CD19 and CD56
5. Quality of life assessments as per FACT-BMT
6. Cytokine Release Syndrome (CRS) assessment as per ASTCT Consensus Grading for CRS (see study CRS SOP for more information)
7. GvHD (acute and chronic) should be assessed continually until the end of the trial, with formal assessments weekly for the first month post-transplant (day 7, day 14, day 21, day 28), day 56, day 100 and months 6, 9 and 12 post-transplant. GvHD grading as per the modified Glucksberg criteria (revised by MAGIC) and the NIH criteria (see Appendix 2 for more information)
8. Peripheral blood research samples will be collected at baseline, D0, D1, D3, D5, D7, D14, D21, D28, D56, D100 and at disease relapse (if applicable).
9. Bone marrow aspirate research samples will be collected at the time of clinical bone marrow assessment (at baseline, D28 and months D100, D180, D270 and D360 post-transplant, and when there is suspicion of disease recurrence). An additional bone marrow procedure will be performed in the first few days post-transplant (see lab manual for details).
10. As granulocyte supply is limited to particular days of the week, the day of stem cell infusion must be a Tuesday to accommodate the granulocyte treatment schedules shown below. Participants should therefore be admitted the day before conditioning begins, to avoid delays. Patients will be allocated to one of the following granulocyte treatment schedules:

	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed
	D0	D1	D2	D3	D4	D5	D6	D7	D8
Schedule 1 (1 dose)	1st dose								
Schedule 2 (3 doses)	1st dose	2nd dose	3rd dose						
Schedule 3 (5 doses)	1st dose	2nd dose	3rd dose	4th dose	5th dose				
Schedule 4 (7 doses)	1st dose	2nd dose	3rd dose	4th dose	5th dose	X	X	6th dose	7th dose

1. BACKGROUND AND RATIONALE

1.1 Background

Allogeneic stem cell transplantation is the only potentially curative therapy for patients with high-risk acute myeloid leukaemia (AML), but relapse is common and remains the leading cause of death. Patients with TP53 mutations, MECOM rearrangements and those transplanted without first achieving remission have especially poor outcomes, with 5-year survival of less than 10-20%[1-4]. Most relapse within the first 100 days following transplant and then face a life expectancy of weeks to months. Due to these poor outcomes, many centres do not offer transplants to this group of patients.

Transplantation succeeds when donor immune cells eliminate residual disease, a process termed the graft-versus-leukaemia effect. Recent observations suggest that this therapeutic effect can be enhanced to deliver durable responses for those with highly resistant disease. Umbilical cord blood is an alternative stem cell source with unique properties, including low rates of chronic graft-versus-host disease and reduced relapse for those with residual disease. Our group has reported leukaemia-free survival of ~50% in children with residual disease who received T-replete cord blood transplant, compared to ~10% using other cell sources[5]. Similar results have been described for adults[6-8].

In addition, we recently reported that the administration of third-party granulocytes following cord blood transplant caused a systemic inflammatory response with rapid expansion of donor-derived T cells and induction of durable responses in children with highly refractory AML[9,10]. We have since expanded our cohort and of 28 children, many referred from palliative care pathways, 24 achieved molecular remission and 14 remain alive and disease free after a median follow-up of 19 months (Figure 1).

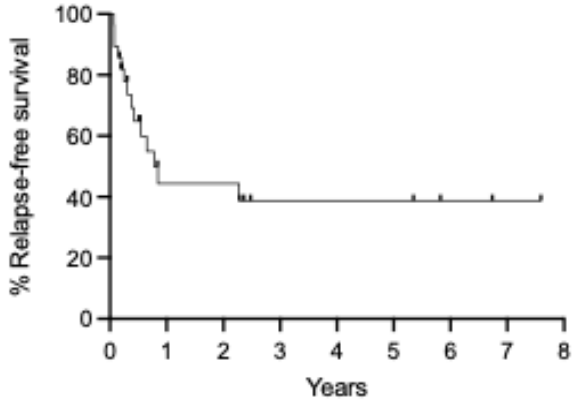


Figure 1. Relapse-free survival for 28 children with relapsed-refractory AML treated with granulocyte-augmented T-replete cord blood transplantation. 24 achieved molecular remission and 14 remain alive and disease free after a median follow-up of 19 months.

Our peri-transplant granulocyte approach has been reproduced by a team in Seattle (US), that used pooled, expanded cord blood units to generate a mismatch myeloid product that could be given as a single dose immediately following cord blood transplant (pooled granulocytes are not routinely available in North America). The Seattle cohort consisted of 15 adult patients, 10 with AML and 5 with acute lymphoblastic leukaemia. They observed a similar response to infusion of unmatched myeloid cells, with fever and transient lymphocyte expansion. All patients remain alive and in remission with a median follow-up of ~1 year, with no severe acute GvHD and no chronic GvHD[11]. This cohort had less resistant disease than our original paediatric patients, who had relapsed/refractory disease and most had already received and failed a first transplant. Whereas the Seattle cohort had not been previously transplanted and most had standard risk disease, with all being in morphological remission and 75% being minimal residual disease (MRD) negative at the point of transplant. Nonetheless, these results support the potential utility of this approach and demonstrate the safety and feasibility of delivering this treatment to adults.

This multi-centre, phase I/II trial will assess the safety and effectiveness of granulocyte-augmented cord blood transplantation in young adults (<55 years) with very high-risk AML.

1.2 Trial Rationale

1.2.1 Justification for patient population

Relapse remains the leading cause of death following allogeneic stem cell transplantation. Patients with TP53 mutations, MECOM rearrangements and those transplanted without first achieving remission have especially poor outcomes, with 5-year survival of less than 10-20%[1-4]. Recent studies suggest that AML patients with 'single-hit' TP53 alterations have similar outcomes to those with 'multi-hit' or biallelic alterations, in contrast to previous findings in myelodysplastic syndrome[12-14]. Relapse of non-core binding factor AML within 6 months of intensive chemotherapy identifies another group of patients that have dismal outcomes with conventional transplantation[15].

The addition of peri-transplant granulocyte infusions to T-replete cord blood transplantation led to remarkable outcomes in children with highly resistant AML[9,10]. This trial will assess the safety and efficacy of this approach in young adults (<55 years) with very high-risk AML. This trial therefore addresses a major unmet need and has the potential to change clinical practice if the approach is well tolerated and the outcomes observed in children are replicated.

1.2.2 Justification for design

In our original paediatric cohort, peri-transplant granulocyte infusions were associated with a transient inflammatory state characterised by high fever, rash and peripheral blood lymphocyte expansion[9,10]. The magnitude and timing of granulocyte-induced inflammation was associated with transplant outcome, suggesting that reliable induction of this reaction is a key part of the therapeutic strategy (Figure 2). The first part of this trial therefore aims to determine a granulocyte dosing schedule that reliably induces this inflammatory response whilst being safe and tolerable for patients.

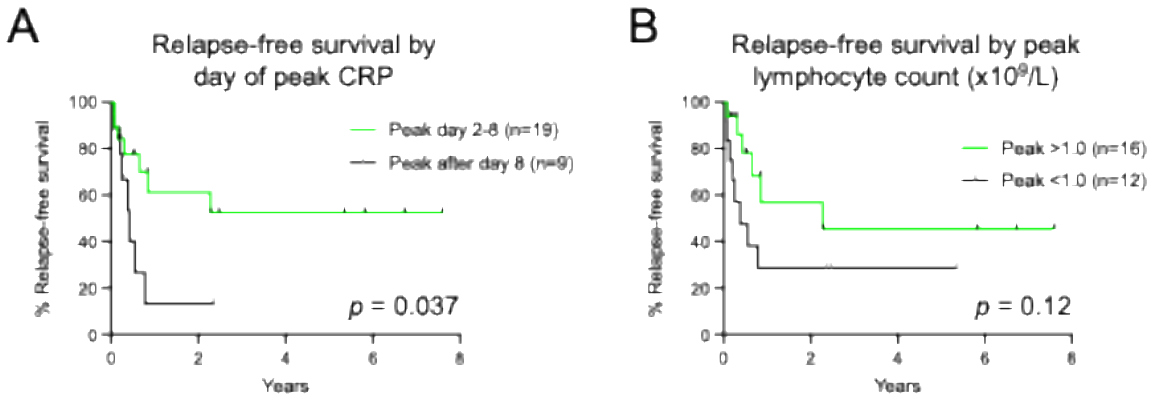


Figure 2. Peri-transplant granulocytes were administered to 28 children. Most experienced a putative cytokine release syndrome (CRS), with high fever and elevated CRP in the days following stem cell infusion. This was well tolerated, with most requiring either no additional support or fluid boluses and/or oxygen delivered in a ward setting. CRS was accompanied by a transient, early lymphocyte expansion and was closely correlated with disease response. 3 children who did not experience CRS did not remit, whilst an early CRP rise (A), CRP peak >200 and lymphocyte peak >1.0 (B) was associated with relapse-free survival (RFS).

This trial utilises an innovative early phase seamless design, integrating dose-escalation and randomised dose-optimisation, which is built on the latest FDA and MDICT guidance[16,17]. This design maximises efficiency and leverages all key data in real-time, ensuring identification of an optimal dose, whilst also providing the flexibility to rigorously assess treatment efficacy within the same trial framework.

Phase I (up to 30 patients) will include two components: dose-escalation and dose-optimisation to identify the Recommended Phase II Dose (RP2D). In the dose-escalation component, a modified 2-stage Bayesian Time-to-event Continual Reassessment Method (TITE-CRM) will be used to determine the maximum tolerated dose and tolerable doses among 4 dose schedules (daily granulocyte infusions for 1, 3, 5 or 7 days)[18,19]. Once there are 9 patients at the proposed initial maximum tolerated dose

(MTD) and the model still recommends the next cohort to be recruited at that initial MTD, dose optimization will be triggered. In the dose optimisation component, subsequent patients will be randomised to selected tolerable doses to determine the RP2D based on both treatment tolerability and activity. A Bayesian framework will be used, including an interim futility analysis after the first 6 patients at each dose to potentially eliminate any dose deemed futile. The final RP2D would be selected based on tolerability, activity and other key secondary endpoints, in consultation with the joint committee (TSC/DMC).

Phase 2 (20 patients) will assess preliminary efficacy at the RP2D, based on relapse-free survival (RFS) at 1 year, using a single-stage Bayesian design. Notably, evaluable patients who received RP2D in phase I will also contribute to the phase 2 activity evaluation. We have based our power calculations on the expected outcomes for the target population as reported in the literature[1-4,12-15]. However, we will also conduct an additional analysis in which we will construct a historical comparator group matched for the patient, transplant and disease characteristics of our study population. Whilst universally poor, the outcomes for patients of differing ages with specific high-risk mutations or varying levels of residual disease nonetheless vary. There is a risk that our study population becomes more or less high-risk than our power calculation assumes. It would be unethical to randomise patients to receive the investigational transplant protocol, because the target population has such poor outcomes with conventional transplantation that many clinicians advocate palliation. In collaboration with The British Society of Blood and Marrow Transplantation and Cellular Therapy (BSBMTCT), we will therefore construct a comparator cohort that is well matched for other patient and transplant characteristics. In the absence of randomisation, this represents a robust method for determining the efficacy of peri-transplant granulocytes.

1.2.3 Choice of treatment

Participants will receive a T-replete cord blood transplant with a standardised protocol consisting of centralised cord unit selection, mid-intensity conditioning (cyclophosphamide 50 mg/kg, fludarabine 150 mg/m^2, thiotepa 10 mg/kg, total body irradiation 4Gy[20]) and graft-versus-host disease (GvHD) prophylaxis (ciclosporin and MMF). A single pool of irradiated granulocytes will be given daily for a variable number of days (see above) starting on the day of transplant. Children in our original cohort ranged from 2 to 17 years of age and granulocyte dose was 10ml/kg capped at a single pool (~200mls). We found no effect of age (and hence granulocyte dose per kilo) on the magnitude or timing of granulocyte-induced inflammation, engraftment or outcome (Table 1). However, we did observe that some children who did not receive all 7 doses of granulocytes nonetheless responded, suggesting that 7 daily doses may not be necessary. The study will therefore assess the safety and activity of four different granulocyte administration schedules that vary the number of daily granulocyte doses (1, 3, 5 and 7 days). The design does not assume that higher doses will be more effective, because prolonged antigen exposure has the potential to induce T-cell anergy.

	AGE (years)	GRANULOCYTE-INDUCED CRS					ENGRAFTMENT			OUTCOME	
		Highest CRP	Day of highest CRP	Peak ALC	Day of peak ALC	Highest fever	Graft failure	Day of neutrophil engraftment	Day of platelet engraftment	Remission induction	Molecular relapse
<10yrs (n=12)	4 (2-9)	221 (55-465)	10 (2-18)	1.42 (0.27-4.0)	16 (5-30)	40.37 (39.2-41.2)	3/12 primary graft failure	19 (12-24)	66 (31-153)	7/12 remit	2/7 relapse
>10yrs (n=12)	14 (11-17)	262 (167-530)	12 (5-33)	1.49 (0.47-3.05)	12 (6-27)	40.07 (38.9-41.2)	1/12 primary graft failure	21 (13-30)	50 (27-101)	12/12 remit	5/12 relapse
p-value	0.00	0.45	0.44	0.88	0.18	0.28	NA	0.51	0.35	NA	NA

Table 1. Data from 24 evaluable patients (those with complete CRS and engraftment data) split into 2 cohorts to examine the effects of age on granulocyte-induced CRS, engraftment and outcome. Data displayed as mean (range) where appropriate. Children in our original cohort ranged from 2 to 17 years of age and granulocyte dose was 10ml/kg capped at a single pool (~200mls). Per kilogram granulocyte dose therefore varied significantly from 10ml/kg to <2.5ml/kg. However, we found no effect of age (and hence granulocyte dose per kilo) on the magnitude or timing of granulocyte-induced inflammation, engraftment or outcome.

2. OBJECTIVES & ENDPOINTS

2.1 Table 2. Objectives & endpoints

Primary objectives	Primary endpoints
Phase 1: To determine the safety of peri-transplant granulocyte infusion for adult recipients of T replete cord blood transplants	Safety will be determined by assessing the frequency, causality and severity of the following: I. Cytokine Release Syndrome (CRS) II. Acute Graft vs Host Disease (GvHD) III. Primary graft failure IV. Transplant Related Mortality (TRM) defined as death due to any transplantation-related cause other than disease relapse within the first 100 days following stem cell infusion V. Other adverse events
Phase 1: To determine the optimal dosing schedule (RP2D) for granulocytes administration using measures of activity and dose-limiting toxicity	**Occurrence of dose-limiting toxicities (DLTs).** Patients would be considered to have experienced a DLT if they experience any of the toxicity events: I. Severe CRS (intensive care admission requiring intubation >7days) II. Primary graft failure (failure of count recovery and absent donor DNA at day 28 bone marrow) III. Severe acute graft-versus-host disease (death from GvHD in the first 100 days) **Evidence of clinical activity.** Patients would be considered to show early indicators of clinical activity if they experience any of the events: I. CRS (fever in the first 7 days following stem cell infusion) II. Lymphocyte expansion (>0.5 x10^9/L in the first 12 days) III. Maximum CRP >150 mg/L on days 5-10 following stem cell infusion IV. Early evidence of disease response (MRD negative on day 28 bone marrow) The maximum tolerated dose (MTD) will be the dose with an estimated DLT rate closest to 30% using a Bayesian Time-to-Event Continual Reassessment Method (TITE-CRM). The final optimal dosing schedule (RP2D) will be based upon an integrated assessment of the MTD or the maximum administered schedule (if no DLTs occurs), clinical activity, tolerability and other key secondary endpoints, in consultation with the Joint TSC/DMC.
Phase 2: To assess preliminary efficacy at the RP2D, based on relapse-free survival (RFS) at 1 year	Relapse-free survival (RFS) rate is defined as proportion of patients who remain relapse-free and alive within 1 year from transplant.

Secondary objectives	Secondary endpoints
Relapse and survival	
Phases 1 & 2: To assess relapse and survival in terms of relapse-free survival (RFS), non-relapse mortality (NRM), overall survival (OS), cumulative incidence of relapse and GvHD-free and relapse-free survival (GRFS)	Relapse-free survival (RFS) is defined as the time from day 0 to date of first relapse or death from any cause. Patients who are alive and relapse free will be censored at the date of last follow-up.
	Non-relapse mortality (NRM), defined as the time from day 0 to date of death without relapse. Patients who relapse will be considered a competing risk at their date of relapse will be considered a competing risk at their date of relapse and patients alive and relapse free will be censored at the date of last follow-up.
	Overall survival (OS), defined as the time from day 0 to date of death, from any cause. Patients who are alive will be censored at the date of last follow-up.
	Cumulative incidence of relapse, defined as the time from day 0 to date of relapse.
	GvHD-free, relapse-free survival (GRFS) defined as the time from day 0 (ie. the day of stem cell infusion) to the first occurrence of any of the following events: acute grade III-IV and/or chronic GvHD requiring systemic immune suppressive treatment, disease relapse or progression, or death from any cause. Patients who are alive and free of any of these event will be censored at the date of last follow-up.
Safety and tolerability	
Phases 1 & 2: To assess safety and tolerability in terms of the cumulative incidence of acute grade II-IV and III-IV GvHD, cumulative incidence of moderate or severe chronic GvHD, cytokine release syndrome rate, immune suppression-free rate, cumulative incidence of intestinal failure, number of inpatient days, QoL within the first 12 months and the incidence of ≥ grade 3 toxicities. The measures of activity and dose-limiting toxicity assessed in Phase 1 will also be assessed in Phase 2.	Cumulative incidence of acute grade II-IV and III-IV GvHD, defined as time from day 0 to date of onset of aGvHD. Patients who relapse/progress or die without relapse, progression or aGvHD will be considered a competing risk at date of relapse/progression for the former and date of death for the latter. Patients, who are alive, relapse and aGvHD free will be censored at the date of last follow-up.
	Cumulative incidence of moderate or severe chronic GvHD, defined as time from day 0 to date of onset of cGvHD. Patients who relapse/progress or die without relapse, progression or cGvHD will be considered a competing risk at date of relapse/progression for the former and date of death for the latter. Patients, who are alive, relapse and cGvHD free will be censored at the date of last follow-up.
	Cytokine release syndrome (CRS) rate defined as proportion of patients who developed grades 1, 2, 3 and 4 CRS within 28 days of transplant.
	Immune suppression-free rate at 1 year, defined to be patients who are alive, relapse free and do not require ongoing immune suppression to control or suppress GvHD at 1-year post transplant. Patients who discontinue immune-suppression within 15 days or less prior to the 1-year time point will not be considered immune-suppression free.
	Cumulative incidence of intestinal failure at 1 year

	The number of inpatient days during first 12 months
	QoL measured by FACT-BMT questionnaire at baseline, 6 months and 12 months
	Incidence of ≥grade 3 toxicities reported as per the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) V5.0
	Measures of activity and dose-limiting toxicity as defined above
Engraftment and immune reconstitution	
Phases 1 & 2: To assess engraftment and immune reconstitution in terms of the cumulative incidence of engraftment, incidence of full donor chimerism, cumulative incidence of infection requiring admission and cumulative incidence of viral infection or reactivation requiring treatment	Cumulative incidence of engraftment defined as time from day 0 to date of engraftment (Neutrophil engraftment defined to be the first of 3 consecutive days a neutrophil count ≥0.5 x10^9/L is reached and platelet engraftment defined to be the first of 3 consecutive days an unsupported platelet count ≥ 20 x10^9/L is reached). Patients who relapse/progress or die prior to relapse, progression or engraftment will be considered a competing risk at their date of relapse/progression for the former and date of death for the latter. Patients alive and engraftment free will be censored at the date of last follow-up.
	Incidence of full donor chimerism (lineage specific chimerism will be determined at the indicated time points by local testing and % donor chimerism in each lineage will be recorded on the appropriate CRF), at 100 days.
	Cumulative incidence of infection requiring inpatient admission at 1 year, defined as the time from day 0 to date of inpatient admission due to infection.
	Cumulative incidence of viral infection or reactivation requiring treatment, defined as time from Day 0 to date of commencing anti-viral treatment. Patients who die without viral infection or reactivation requiring treatment will be considered a competing risk at their date of death. Patients alive and free of viral infection or reactivation requiring treatment will be censored at the date of last follow-up.

2.2 Exploratory Objectives

The scientific research associated with the study will attempt to describe the mechanism of peri-transplant granulocyte administration, identify key features of successful transplantation and investigate whether responders can be identified to allow targeted application of this approach. Specifically, the research will aim to address the following questions:

1. Does disease response require priming of donor T-cells against HLA mismatched shared between the recipient and the granulocyte product?
2. Does granulocyte-induced inflammation induce leukaemic differentiation?
3. Does pre-treatment sensitivity of leukaemia to interferon-gamma identify responders?
4. Can plasma proteomics identify novel biomarkers of treatment response?

5. When transplanting patients with detectable disease, can immune clearance of residual disease be detected using cell-free DNA methylation analysis?

3. TRIAL DESIGN

This is prospective phase I/II study of granulocyte-augmented cord blood transplantation for young adults (16-55 years) with very poor risk acute myeloid leukaemia. The target population is high-risk AML with TP53 mutations, MECOM rearrangements or chemoresistant phenotypes (defined by partial response to 2 cycles of induction chemotherapy or MRD positive disease by flow cytometry (>0.1%) after 2 cycles of induction for those with adverse risk AML or early relapse after intensive chemotherapy).

Participants will receive a T-replete cord blood transplant with a standardised protocol consisting of centralised cord unit selection, mid-intensity conditioning and GvHD prophylaxis. A single pool of irradiated granulocytes will be given daily for a variable number of days (1, 3, 5 or 7 days) starting on the day of transplant.

The study consists of two phases. Phase I (up to 30 patients) has two components: dose-escalation and dose-optimisation to identify the Recommended Phase II Dose (RP2D). Phase 2 (20 patients) will assess preliminary efficacy at the RP2D, based on relapse-free survival (RFS) at 1 year, using a single-stage Bayesian design. All patients will be followed-up for a minimum of one year.

Further details about the trial design are provided in section 12.

Starting dose & dose levels
Children in our original cohort ranged from 2 to 17 years of age and granulocyte dose was 10ml/kg capped at a single pool (~200mls). We found no effect of age (and hence granulocyte dose per kilo) on the magnitude or timing of granulocyte-induced inflammation, engraftment or outcome (Table 1). However, we did observe that some children who did not receive all 7 doses of granulocytes nonetheless responded, suggesting that 7 daily doses may not be necessary. The study will therefore assess the safety and activity of four different granulocyte administration schedules that vary the number of daily granulocyte doses (1, 3, 5 and 7 days). The design does not assume that higher doses will be more effective, because prolonged antigen exposure has the potential to induce T-cell anergy. However, it is possible that fewer days of granulocyte exposure will reduce the duration of the associated inflammatory state. Given that tolerance of granulocyte-induced CRS is the major safety concern of this trial, the starting dose will be the 3-day regimen.

4. ELIGIBILITY

4.1 Inclusion criteria

7. Availability of a suitable cord blood unit
8. Age between 16 and 55 years
9. Primary diagnosis of Acute Myeloid Leukaemia (AML) or MDS/AML (as defined by ICC 2022) fitting one or more of the following criteria:
    a. TP53 mutation (single- or multi-hit)
    b. Presence of inv(3) (q21.3q26.2) or t(3;3)(q21.3;q26.2)
    c. Adverse risk (as per ICC 2022) **and** >0.1% MRD by flow cytometry after 2 cycles of induction
    d. AML (any risk) with partial remission (<10% blasts) after 2 cycles induction
    e. Early relapse (<6 months) after chemotherapy alone (excluding t(16;16), inv(16) or t(8;21))
10. Disease status at transplant (disease assessment will be performed within 28 days of starting conditioning chemotherapy)
    a. All patients must have <10% blasts
    b. >10% blasts with a hypocellular background may be discussed with the trial team
11. Suitable fitness and organ function as per the following criteria:
    a. Glomerular filtration rate >50 mL/min/1.73m^2
    b. Ejection fraction >50%
    c. FEV1 >65% *without* dyspnoea on mild activity
    d. AST/ALT <3 x ULN
    e. Bilirubin <1.5 x ULN (excluding Gilbert's syndrome)
    f. Performance Status (ECOG) of 0 or 1
12. Females of and male patients of reproductive potential (i.e., not post-menopausal or surgically sterilised) must agree to use appropriate, highly effective, contraception from the point of commencing therapy until 12 months after transplant

4.2 Exclusion criteria

- AML secondary to a myeloproliferative neoplasm
- Active CNS disease (extramedullary disease at other sites should be discussed with the trial team)
- Prior allogeneic stem cell transplant
- Participation in another clinical trial that would alter any aspect of the transplant protocol or that aims to reduce the subsequent risk of relapse (discuss with trial team if unsure)
- History of cardiac arrhythmia
- Ischaemic heart disease, valvular heart disease or congestive cardiac failure
- Transient ischaemic attack or cerebrovascular accident
- Rheumatologic disease (SLE, RA, polymyositis, mixed CTD or polymyalgia rheumatica)
- Ulcerative colitis or Crohn's disease
- Liver cirrhosis
- Presence of an active second malignancy
- Uncontrolled infection, including viral reactivation (CMV, EBV)
- HIV positive
- Hepatitis B/C active infection with measurable viral load (patients with chronic hepatitis B or C infection require clear documentation of absence of cirrhosis by either fibroscan or biopsy, regardless of viral load)
- Pregnancy, breastfeeding, unwilling to use contraception
- Contraindications to administration of pooled granulocytes
- Previous history of sensitivity to granulocytes
- Inability of patient to give informed consent
- Any other organ dysfunction or co-morbidity that precludes transplant in the opinion of the investigator
- Any concern by PI

5. RECRUITMENT, SCREENING AND CONSENT

5.1 Recruitment pathway

The aim of this section is to give referring clinicians and study centres guidance as to when patients should to be referred and when/where study investigations should be performed. The study envisages recruiting patients who are already being treated at study centres as well as those referred from secondary care or other UK transplant centres. When and where investigations are performed will therefore vary, and the study aims to avoid duplication and disruption to patients. A key goal is to avoid patients travelling to study centres for assessment who are subsequently found to be ineligible, as this would be distressing for patients and create additional workload for study centres. In practice, this means ensuring that patients meet study disease eligibility criteria, are likely to be fit enough and have suitable single cord options **before** assessment at a study centre.

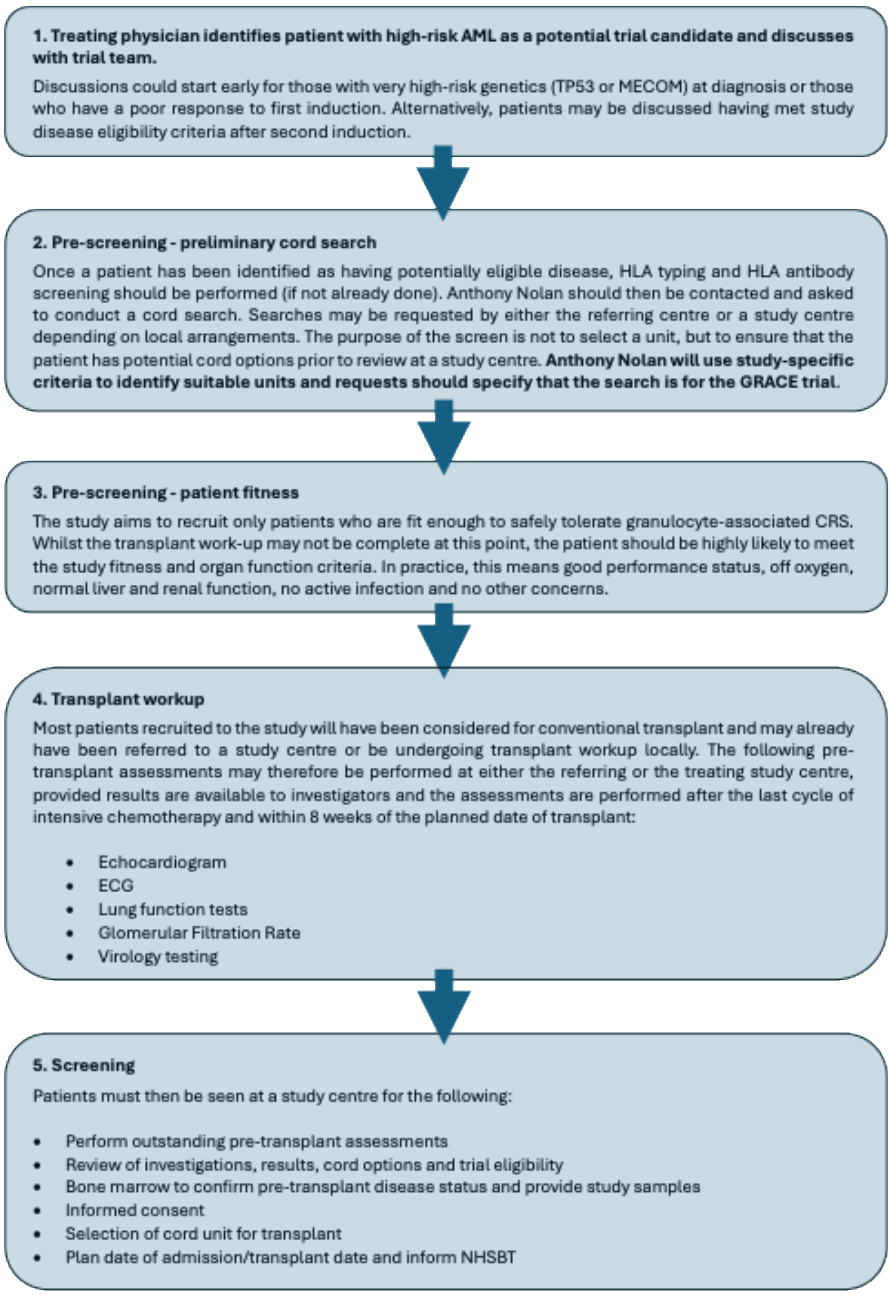


Figure 3. Flow chart illustrating the identification, pre-screening and screening of potential study participants.

5.2 Participant identification

The study will be discussed regularly at AML and transplant sub-groups and clinical networks to ensure that all UK centres are familiar with the study.

Treating centres will be:

- The Christie NHS Foundation Trust
- Kings College Hospital NHS Foundation Trust
- The Royal Marsden NHS Foundation Trust

Following initial discussion at local multidisciplinary team meetings, patients will likely be highlighted at the following timepoints:

- Initial diagnosis for those with poor-risk genetics (TP53 or MECOM)
- After poor response to first induction chemotherapy
- When meeting study disease eligibility criteria after second induction

These patients will be referred to the 'GRACE Clinical Team' for initial review of eligibility and the patient information leaflet will be sent to the patient, where appropriate. Pre-screening will then ensure that patients are likely to meet study fitness/organ function criteria and that suitable single cord units are available. The patient will then be reviewed at one of the above centres for further assessment and suitability review. Cord blood options will be reviewed, and a suitable unit requested.

5.3 Pre-screening

Preliminary cord search
Once a patient has been identified as having potentially eligible disease, HLA typing and HLA antibody screening should be performed (if not already done). Anthony Nolan should then be contacted and asked to conduct a cord search. Searches may be requested by either the referring centre or a study centre depending on local arrangements. The purpose of the screen is not to select a unit, but to ensure that the patient has potential cord options prior to review at a study centre.

Anthony Nolan will use study-specific criteria to identify suitable units. **Search requests must therefore specify that the search is for the GRACE trial**. This will also allow the proportion of study searches that yield suitable units to be reported, information that is crucial for understanding both study recruitment and the wider applicability of this approach once the study concludes.

Patient fitness
The study aims to recruit only patients who are fit enough to safely tolerate granulocyte-associated CRS. Whilst the transplant work-up may not be complete at this point, the patient should be highly likely to meet the study fitness and organ function criteria. In practice, this means good performance status (ECOG 0-1), off oxygen, normal liver and renal function, no active infection and no other concerns.

5.4 Screening

Participating centres are required to maintain a Screening Log of all potential study candidates. A patient information sheet (PIS) will be given to potential trial patients and sufficient time should be given for patients to make an informed decision about entering the study. The Investigator will then formally assess whether the patient fully satisfies the inclusion and exclusion criteria. Assessments that are performed as standard of care for transplant work-up do not require informed consent.

Patients who meet initial eligibility, will go on to be screened for the trial. The following procedures should be performed:

- Medical history and demographics
- Disease assessment (see below)

- Transplant workup (see below)
- Virology testing of donor and recipients should be performed as per local transplant policy and requirements of the Human Tissue Authority but should include appropriate surveillance for CMV, EBV and adenovirus. Patients with active HBV or HCV infection are excluded from the trial.
- Pregnancy test for women of childbearing potential (serum)
- Physical examination, ECOG evaluation vital signs, height, weight and body
- Surface area measurements (as per institutional guidelines)
- Haematology - FBC with differential

5.4.1 Disease assessment

Disease assessment should be via bone marrow aspirate and trephine biopsy within 28 days of starting conditioning chemotherapy. **This must be performed at the treating study centre**. The following investigations should be performed:

1. Aspirate for morphology assessment
2. Aspirate for flow cytometry in accredited laboratory
3. Aspirate for flow MRD (all patients) and molecular MRD (where available)
4. Aspirate for the following genomic testing (if not available at baseline and residual disease is anticipated):
    a. FISH
    b. Cytogenetics
    c. Myeloid Gene Panel by NGS
5. Aspirate for research (see Section 7.4)
6. Trephine biopsy (at least 2cm in size) for histology assessment

5.4.2 Patient fitness for transplant

All patients will be assessed to determine fitness for transplant. This will be done by an in-person assessment by the treating study centre as well as the investigations below. These investigations can be performed at either the referring or the treating study centre, provided results are available to investigators and the assessments were performed after the last cycle of intensive chemotherapy and within 8 weeks of the planned date of transplant:

1. Echocardiogram
2. ECG
3. Lung function tests
4. Glomerular Filtration Rate (GFR)

5.4.3 Cord unit selection

Cord unit selection will be performed by the local transplant team using study-specific guidance (see Cord Selection SOP) and consulting with the trial team, when required. The selected cord must be on site at the treating centre at least 14 days prior to starting conditioning chemotherapy.

5.5 Consent

It is the responsibility of the Investigator to obtain written informed consent for each patient prior to performing any trial related procedure. A PIS is provided to facilitate this process. Investigators must ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time. The patient should be given ample time (at least 24 hours) to read the PIS and to discuss their participation with others outside of the site research team. The patient must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate in the trial without giving a reason must be respected.

If the patient expresses an interest in participating in the trial they should be asked to sign and date the latest version of the Informed Consent Form (ICF). The Investigator must then sign and date the form. When complete, copies should be 1.) provided to the patient, 2.) placed in the medical notes, 3.) sent to the MCRC Biobank, 4.) sent to the central trial office. The copy sent to the central trial office should have patient identifiable information redacted. The original should be kept in the Investigator Site File.

The informed consent process is expected to involve an interview between the investigator team and the patient which should facilitate two-way communication. It is possible for this interview to be conducted remotely. Where this occurs, the patient can be sent the Patient Information Sheet in advance in the post. The Informed Consent Form should be wet-ink signed by the patient and the Investigator when the patient attends for their first clinic appointment, but this must be prior to their entry onto the trial.

Once the patient is entered into the trial the patient's trial number should be entered on the ICF maintained in the ISF and the copy sent to the MCRC Biobank. Details of the informed consent discussions should be recorded in the patient's medical notes, this should include date of, and information regarding, the initial discussion, the date consent was given, with the name of the trial and the version number of the PIS and ICF.

Throughout the trial the patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log and, with the patient's prior consent, their General Practitioner (GP) should also be informed that they are taking part in the trial. A GP Letter is provided for this purpose.

It is expected that patients will undergo the standard local transplant consent process in addition to consenting to the study.

6. TRIAL ENTRY

Patients must be allocated to a treatment schedule (below) prior to admission for transplant. An eligibility checklist must be completed via the electronic case report form (eCRF) prior to treatment allocation.

Patients will be allocated to one of the following granulocyte treatment schedules:

	Tues	Wed	Thurs	Fri	Sat	Sun	Mon	Tues	Wed
	D0	D1	D2	D3	D4	D5	D6	D7	D8
Schedule 1 (1 dose)	1st dose								
Schedule 2 (3 doses)	1st dose	2nd dose	3rd dose						
Schedule 3 (5 doses)	1st dose	2nd dose	3rd dose	4th dose	5th dose				
Schedule 4 (7 doses)	1st dose	2nd dose	3rd dose	4th dose	5th dose	X	X	6th dose	7th dose

Table 3. Granulocyte dosing schedules.

Patients will be allocated to treatment based on an algorithm prepared by the trial statistician and following review of safety data by the TMG and joint committee, where appropriate.

Eligible patients should be allocated to treatment before admission for transplant, but ONLY after transplant work up has been completed and the relevant disease evaluations have been performed.

Treatment allocation will be conducted via the eCRF by logging onto:
[insert study website]

Login details will be provided by the Trials Office as part of the Site Initiation.

GRACE Trials Office Contact Details

[insert address]
[insert telephone]
[insert study email]

7. TREATMENT DETAILS

7.1 Trial treatment

7.1.1 Granulocytes

Optimised pooled granulocytes will be provided by NHS Blood & Transplant (NHSBT). This is a standardised component, available to participating hospitals with ordering through the Blood Bank. A single pool of irradiated granulocytes will be given daily for a variable number of days (see Table 3) starting on the day of transplant.

7.1.2 Cord blood stem cells

Un-manipulated cord blood stem cells will be infused on day 0 according to local transplant policy.

7.2 Treatment schedule

There should be no change in the timing, dose and route of administration of the specified transplant protocol. Dose modifications for organ dysfunction can be made according to local policies, but any other changes to the schedule must be agreed with the CI prior to commencing the transplant schedule. Where drugs are to be administered by IV, infusion rates should be as per local policy.

As granulocyte supply is limited to particular days of the week, the day of stem cell infusion must be a Tuesday to accommodate the granulocyte treatment schedules shown in Table 3. Participants should therefore be admitted the day before conditioning begins, to avoid delays. For all schedules, day 0 is the day of cord blood stem cell infusion, days before this are marked as negative.

7.2.1 Transplant Conditioning Regimen

Mid-intensity conditioning (cyclophosphamide 50 mg/kg, fludarabine 150 mg/m^2, thiotepa 10 mg/kg, total body irradiation (TBI) 4Gy, single fraction[20]). G-CSF will be given from Day +5. TBI should be administered on a Monday with stem cell infusion the following day (Tuesday).

7.2.2 Granulocyte administration

A single pool of irradiated granulocytes will be given daily for a variable number of days (Table 3) starting on the day of transplant (D0). Patients with a previous history of sensitivity to granulocyte transfusion will be excluded from the study. Patients with HLA-antibodies will not be excluded, but cases will be discussed with clinical staff at NHSBT. Granulocytes will be prescribed for patients on the fluid and blood component prescription charts in accordance with local policy for blood component prescription and administration.

7.2.2.1 Component ordering

- Notice to request granulocytes should be provided a soon as possible to relevant liaison staff with NHSBT [insert contact details]. This will support early advanced planning and advice in unexpected situations of lack of availability.
- Standard hospital and blood bank procedures will be followed for ordering granulocytes.
- Donations from CMV-seronegative donors are required for CMV-seronegative recipients.
- NHSBT aims to provide the granulocyte component daily up to 6 days a week from Monday to Saturday. Whole blood donations are routinely collected from Monday to Friday with fewer collections taking place at weekends. Granulocyte availability is likely to be lower from Sunday until late Monday. Days where daily transfusion is not possible will be recorded.
- Granulocytes often arrive in the evening (~18-19:00) and participating centres must make provision to administer on the day of receipt.

7.2.2.2 Issue and Prescribing

The hospital blood bank will process granulocytes as per existing local practices, including red cell compatibility testing and labelling. Optimised pooled granulocytes must be prescribed using the usual ordering and prescribing pathways in participating hospitals (electronic patient records or manually). Local guidelines for safe administration of granulocytes must be followed.

Prescribing will define:

- A single pool of irradiated granulocytes will be given on each of a variable number of days starting on the day of transplant (D0), as defined by the trial (Table 3).
- The first dose of granulocytes (D0) should be given **after** stem cell infusion
- For Phase 1, the granulocyte dosing schedule will be allocated by the trial office and communicated pre-transplant.
- The Phase 2 granulocyte dosing schedule will be the RP2D determined by Phase 1.

7.2.2.3 Administration Schedule

- The granulocyte component will be transfused to patients in accordance with BSH guidelines and the approved Trust standard operating procedure (SOP) for infusion at the bedside.
- The component will be administered intravenously over 30-60 minutes, via a dedicated infusion line. The product may by infused via a peripheral IV device or via a central IV device. Granulocytes must be transfused at least 6 hours apart from the administration of amphotericin.
- All local processes for documentation of transfusion, and occurrence of transfusion reactions (and responses) will be followed.
- Further guidance on the recognition and management of granulocyte transfusion reactions are detailed in a separate document that describes the study approach to both granulocyte-induced CRS and transfusion reactions.

7.2.3 Graft-versus-host-disease prophylaxis

Ciclosporin (1.5mg/kg IV BD) and Mycophenolate Mofetil (MMF) (1gram IV BD) will start on D-3.

- Ciclosporin target trough levels are 150-250 µg/L.
- In the absence of GvHD, MMF should be stopped at D35 or 7 days after engraftment - whichever is later.
- Ciclosporin wean should be initiated at D50-D60, with an aim to stop by D90-D100 in the absence of GvHD.

7.2.4 Cytokine-release syndrome (CRS) management

In our original paediatric cohort, peri-transplant granulocyte infusions were associated with a transient inflammatory state characterised by high fever, rash and peripheral blood lymphocyte expansion[9,10]. Plasma levels of CRP, IFNγ and IL-6 were increased, and we term this reaction granulocyte-associated cytokine release syndrome (grans-CRS). The magnitude and timing of grans-CRS was associated with transplant outcome, suggesting that reliable induction of this reaction is a key part of the therapeutic strategy. Supressing this reaction may compromise treatment, but it is also unclear how grans-CRS will manifest in adults and how well it will be tolerated. Ultimately it will be important to define an approach that avoids overtreatment whilst ensuring patient safety, but currently optimum management has not been defined. We have therefore created a guideline that describes the previous clinical experience in children and the rationale for a suggested management approach in adults (see Grans-CRS guideline). **The guidance is not prescriptive, the treatment suggestions are not protocol-mandated and are not intended to replace clinical judgement.**

7.3 Assessments

Every effort should be made for participants to attend on the scheduled visit days, however, if a participant is unable to attend on the specified day, visits may be arranged at ±3 days for the first 28 days and ±10 days for months 3 (D100), 6 (D180), 9 (D270) and 12 (D360). In the event a visit is moved, subsequent visits should be performed on the days/months specified by the protocol.

7.3.1 Haematology
Haematology will be assessed at baseline, daily from D0 to D14, then at D21, D28, D56, D100, D180, D270 and D360. A full blood count (haemoglobin, white blood cells (with differential), neutrophils, platelets and lymphocytes) should be assessed.

7.3.2 Blood chemistry
Blood chemistry will be assessed at baseline, daily from D0 to D14, then at D21, D28, D56, D100, D180, D270 and D360. Assessments should include urea, electrolytes, creatinine, LDH, CRP, magnesium, bilirubin, AST or ALT and ALP.

7.3.3 Virology
Patients should be monitored for EBV and CMV reactivation as per local policy and reported in the event of a re-activation.

7.3.4 Physical examination/symptom assessment
A physical examination, vital signs (blood pressure, pulse, oxygen saturation and temperature), weight, and assessment of ECOG performance status (Appendix 1) at baseline, daily from D0 to D14, then at D21, D28, D56, D100, D180, D270 and D360.

7.3.5 Cytokine release syndrome (CRS) assessment
CRS assessment should be performed daily from D0 to D14 then at D21 and D28.

7.3.6 Disease assessment (including MRD)
Disease assessment (bone marrow aspirate and trephine) will be performed at baseline, D28, D100, D180, D270 and D360 post-transplant. These assessments should include flow cytometric MRD for all patients and molecular MRD where available.

7.3.7 Graft-versus-host-disease (GvHD) assessment
GvHD (acute and chronic) should be assessed continually until the end of the trial; with formal assessment weekly for the first month post-transplant (day 7, day 14, day 21, day 28), day 56, day 100 and months 6, 9 and 12 post-transplant. aGvHD will be assessed using the modified Glucksberg criteria[21] and cGvHD will be assessed using the National Institutes of Health (NIH) criteria[22], see Appendix 2. The aGvHD and cGvHD score is recommended to be calculated using the eGvHD App (www.uzleuven.be/egvhd)[23]. The app can be accessed as a webpage or as an app for both android and apple devices. The use of the app is intended as a tool to aid with the calculation of the GvHD score. Information (including all organ specific source information) should be documented in the patient's notes and treatment decisions should be based on the Investigator's assessment.

7.3.8 Chimerism assessment
Engraftment will be assessed by lineage specific chimerism measurements. Lineage specific chimerism in both whole blood and T-cell compartments (where possible) will be assessed at D28, D56, D100, D180, D270 and D360. Tests should be performed in local laboratories.

7.3.9 Quality of Life assessments
Quality of Life will be assessed using the FACT-BMT questionnaire (Appendix 3) at baseline, D100, D180 and D360 post-transplant.

7.3.10 Collection of lymphocyte subsets
Numbers of CD3, CD4, CD8, CD19 and CD56 cells should be collected at D28, D56, D100, D180, D270 and D360 post-transplant.

7.3.11 Pregnancy testing
For women of childbearing potential, a pregnancy test should be performed at baseline (serum).

7.3.12 Concomitant medication assessment

All concomitant medications, including blood transfusions (platelet and red cell) and G-CSF administration, to be recorded at baseline, daily from D0 to D14, then at D21, D28, D56, D100, D180, D270 and D360.

7.4 Research sample collection

In addition to clinical assessments, the following research samples will be taken. All samples will be shipped to the Manchester Cancer Research Centre Biobank for processing and storage (see lab manual for detailed instructions).

7.4.1 Bone marrow

Bone marrow aspirate research samples will be collected at the time of clinical bone marrow assessment (at baseline, D28 and months D100, D180, D270 and D360 post-transplant, and when there is suspicion of disease recurrence). Participants will also be consented to allow routinely stored bone marrow trephine material that is surplus to clinical requirement to be used for research, including samples taken prior to recruitment (where available). An additional bone marrow procedure will be performed in the first few days post-transplant to address the trial's exploratory endpoints. Samples will be shipped to the Manchester Cancer Research Centre biobank for processing and storage (see lab manual for detailed instructions).

7.4.2 Peripheral blood

Peripheral blood research samples will be collected at baseline, D0, D1, D3, D5, D7, D14, D21, D28, D56, D100 and at disease relapse (if applicable). Samples will be processed for storage of cryopreserved peripheral blood mononuclear cells and plasma (see lab manual for detailed instructions).

7.5 Supportive Treatment

- Patients should receive appropriate supportive care measures (including blood product support and anti-emetics) at the discretion of the local Investigator.
- Infectious disease prophylaxis/therapy to be provided as below:
    - Antibacterial prophylaxis: Quinolone or suitable alternative, as per local policy
    - Antifungal prophylaxis: Start from D0 until neutrophils >1.0x10^9/l (or longer if on steroids)
        - Recommend: Posaconazole 300mg BD (loading) followed by 300mg OD or alternative as per local policy
    - Antiviral prophylaxis: Aciclovir 400mg BD
    - CMV prophylaxis: Letermovir 480mg OD (240mg OD in patients taking ciclosporin) PO/IV from D0 to at least D100 for recipients who are CMV seropositive at transplant. Consider extended Letermovir prophylaxis if ongoing need for immunosuppression
    - PCP prophylaxis: Pentamidine (nebulised) at D28, and every 4 weeks until counts stable
        - Consider switching to co-trimoxazole (960mg Mon/Wed/Fri) when Platelets >75 and Neutrophils >1.0 unsupported
        - Alternatives as per local policy if intolerant of the above
- Sinusoidal obstruction syndrome (SOS) prophylaxis (if required): Ursodeoxycholic acid
- Filgrastim start at D+5 - dose based on body weight as below:
    - 300 micrograms (<80kg)
    - 480 micrograms(>/= 80kg)
- In the event of hypomagnesaemia, magnesium supplementation should be given.
- Patients developing acute-pattern GvHD grade II-IV or moderate-severe chronic GVHD should be treated as per local policy.

7.6 Concomitant medication

Concomitant medication may be given as medically indicated. Administration of live vaccines is prohibited throughout the trial.

7.7 Patient follow-up

Patients will be followed-up for a minimum of 1-year post-transplant according to the trial assessment schedule. Follow-up visits are scheduled at day 100 and then at months 6-, 9- and 12-months post-transplant. In the event of relapse/progression before 12-months post-transplant patients will be followed up for survival information only, at the time-points specified in the assessment schedule. Where appropriate, care may be shared with the referring centre with scheduled trial visits to the treating hospital and routine clinical care delivered locally.

7.8 Patient withdrawal

The Investigator will make every reasonable effort to keep each patient on trial treatment. However, if the Investigator removes a patient from the trial treatment or if the patient declines further treatment the patient should be followed-up according to the trial schedule unless they withdraw specific consent. All results of the evaluations and observations, together with a description of the reasons for withdrawal from treatment, must be recorded in the eCRF.

~~If a patient were to lose capacity during the trial (for example, requiring sedation for intubation and ventilation), clinical data collection should continue, but research sample collection should cease until the patient regains capacity and is willing/able to reconsent.~~
If a patient were to lose capacity during the trial (for example, requiring sedation for intubation and ventilation), all trial procedures (administering granulocytes and collecting research samples) should cease until the patient regains capacity and is willing/able to reconsent. If a patient were to permanently lose capacity all trial procedures (administering granulocytes and collecting research samples) should cease, but clinical data should continue to be collected from the medical record. Research samples already collected will be retained and used in the study.

Patients who stop study therapy due to adverse experiences (clinical or laboratory) will be treated and followed according to the trial schedule where possible. All pertinent information concerning the outcome of such treatment must be recorded in the eCRF.

The following are justifiable reasons for the Investigator to stop study treatment:
- Unacceptable toxicity
- Unforeseen events: any event which in the judgement of the Investigator makes further treatment inadvisable
- Withdrawal of consent for treatment
- Serious violation of the trial protocol
- Clinical reasons not related to the trial treatment

Patients must stop study treatment in the event of:
- Unacceptable toxicity
- SAE requiring permanent discontinuation of treatment
- Pregnancy

In the event of a patient's decision to withdraw from the trial, the Investigator should ascertain from which aspects of the trial the patient wishes to withdraw and record the details on the appropriate eCRF. All information and blood/tissue samples collected up until point of retraction will be retained and analysed. If a patient chooses to withdraw from treatment only, the patient should discontinue treatment and continue to be assessed in accordance with the protocol.

If a patient wishes to withdraw from the trial (i.e. including trial specific assessments) but is willing for further data to be supplied to the Trials Office, then further routine "follow-up" data (e.g. survival and further treatment data) will continue to be supplied by the Investigator to the Trials Office on a follow-up form.

8. ADVERSE EVENT REPORTING

Definitions of different types of AE are listed in Appendix 4. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient (this should be documented in the source data).

8.1 Reporting requirements

8.1.1 Adverse Events

AEs (see Appendix 4 for definition) are commonly encountered in patients undergoing transplant, only AEs that are equal to or greater than Grade 3 of the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be reported (unless the event meets the definition of an SAE).

Please note this does not include abnormal laboratory findings. An abnormal laboratory value is only considered to be an AE if the abnormality:

- Results in early discontinuation of study treatment and/or
- Requires study treatment dose modification or interruption, any other therapeutic intervention or is judged to be of significant clinical importance

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded. Pre-existing conditions should only be reported if the condition worsens by at least 1 CTCAE grade. Details of all AEs experienced by the patient should be recorded in the hospital notes.

8.1.2 Serious Adverse Events

Investigators should report AEs that meet the definition of an SAE (see Appendix 4 for definition) and are not excluded from the reporting process as described below.

8.1.2.1 Events that do not require expedited reporting

Patients receiving chemotherapy may require admission to hospital for appropriate medical intervention following development of some of the more severe known side effects of treatment. For this reason, the following SAEs do not require expedited (immediate) reporting by site and are not regarded as unexpected for the purpose of this trial:

- Admissions for supportive treatment during an episode of myelosuppression unless this proves fatal or requires admission to a high dependency or intensive care facility.

An SAE Form should still be completed for these events but can be emailed to the Trials Office (as described in Section 8.2) at any time prior to completion of chemotherapy treatment.

8.1.2.2 Events that do not require reporting on a Serious Adverse Event Form

The following events should not be reported on an SAE Form:

- Hospitalisations for:
    - Protocol defined treatment (including admission for transplant)
    - Pre-planned elective procedures unless the condition worsens
    - Treatment for progression of the patient's cancer

- Progression or death due to the patient's cancer, as this information is captured elsewhere

8.1.3 Reporting period

Details of all AEs (except those listed above) will be documented and reported from the date of commencement of protocol defined treatment until 28 days after the administration of the last dose of granulocytes. SAEs will be reported from the date of consent.

8.2 Reporting procedure

8.2.1 Site

8.2.1.1 Adverse Events

AEs should be reported on an AE Form (and where applicable on an SAE Form). An AE Form should be completed at each visit.

AEs will be reviewed using the CTCAE version 4.0 (see Appendix 5). Any AEs experienced by the patient but not included in the CTCAE should be graded by an Investigator and recorded on the AE Form using a scale of (1) mild, (2) moderate or (3) severe. For each sign/symptom, the highest grade observed since the last visit should be recorded.

8.2.1.2 Serious Adverse Events

For more detailed instructions on SAE reporting refer to the SAE Form Completion Guidelines contained in the ISF.

AEs defined as serious and which require reporting as an SAE (excluding events listed in Section 8.1 above) should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 4.0.

On becoming aware that a patient has experienced an SAE, the Investigator (or delegate) must complete, date and sign an SAE Form. The form should be sent together with a SAE Cover Sheet to the Trials Office using the options listed below as soon as possible and no later than 24 hours after first becoming aware of the event:

To report an SAE, send the SAE Form with an SAE Cover Sheet to:
Email: [insert study email address]
Please ensure to add “GRACE SAE” as the subject line

On receipt the Trials Office will allocate each SAE a unique reference number. This number will be transcribed onto the SAE Cover Sheet which will then be sent back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day, please contact the Trials Office. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE. The SAE Cover Sheet completed by the Trials Office should be filed with the SAE Form in the ISF.

For SAE Forms completed by someone other than the Investigator, the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the Trials Office by post or secure email and a copy kept in the ISF.

Investigators should also report SAEs to their own Trust in accordance with local practice.

8.2.1.3 Provision of follow-up information

Patients should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form (refer to the SAE Form Completion Guidelines for further information).

8.2.2 Trials Office

On receipt of an SAE form, seriousness and causality will be determined independently by a Clinical

Coordinator. An SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the trial treatment will be regarded as a Serious Adverse Reaction (SAR). The Clinical Coordinator will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the Reference Safety Information) it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

8.2.3 Reporting to the Health Research Authority (HRA) and Research Ethics Committee (REC)

8.2.3.1 Suspected Unexpected Serious Adverse Reactions

The Trials Office will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to the HRA and main REC within 7 days. Detailed follow-up information will be provided within an additional 8 days.

All other events categorised as SUSARs will be reported within 15 days.

8.2.3.2 Serious Adverse Reactions

The Trials Office will report details of all SARs (including SUSARs) to the HRA and main REC annually.

8.2.3.3 Adverse Events

Details of all AEs will be reported to the HRA on request.

8.2.3.4 Other safety issues identified during the course of the trial

The main REC will be notified immediately if a significant safety issue is identified during the course of the trial.

8.2.4 Investigators

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to Principal Investigators. A copy of any such correspondence should be filed in the ISF.

8.2.5 Data Monitoring Committee

The Joint Committee (TSC/DMC) will review all SAEs.

9. DATA HANDLING AND RECORD KEEPING

9.1 Data collection

This trial will use an electronic data capture (EDC) system which will be used for completion of eCRFs.

Access to the EDC system will be granted to individuals via the Trials Office. SAE reporting and Notification of Pregnancy will be paper-based. The EDC system will comprise a set of forms capturing details of eligibility, baseline characteristics, treatment and outcome details. The eCRFs must be completed by the Investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log).

Certain CRFs, including the Eligibility Form and SAE form, will require Investigator review and sign off. Entries on the CRF should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change.

Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning. QoL will be recorded directly onto the questionnaires provided.

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate. The completed originals should be sent to the Trials Office and a copy filed in the Investigator Site File.

Trial forms may be amended by the Trials Office, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

Further details can be found in the study data management plan.

9.2 Electronic data capture (EDC)

A web based electronic data capture (EDC) system will be created in collaboration with the CI and trial analyst(s), using the MACRO 4 system. This will be maintained by the King's Clinical Trials Unit (KCTU) for the duration of the project. It will be hosted on a dedicated server within King's College London (KCL).

Data entry
Source data will be entered in the EDC by authorised [site staff / central staff within the co-ordinating study team]*delete as appropriate, typically within [insert X] days of data collection by going to www.ctu.co.uk and clicking the link to access the MACRO 4 EDC system.

A full audit trail of data entry and any subsequent changes will be automatically date and time stamped, alongside information about the user making the entry/changes within the system.

Participant initials and possibly date of birth will be entered on the EDC. Whereas NHS number, email addresses, participant names, addresses and full postcodes will not be entered into the EDC system.

EDC Access
No data will be entered onto the EDC system unless a participant has signed a consent form to participate in the study.

The CI or delegate (e.g., Trial Manager) will request usernames and passwords from KCTU for new staff members joining the study and will request access removal when staff members leave the project.

EDC access will be strictly restricted through user-specific passwords to the authorised research team members. It is a legal requirement that passwords to the EDC are not shared, and that only those authorised access the EDC.

EDC Troubleshooting
Site staff experiencing issues with the EDC system should contact the CI or delegate (e.g., Trial Manager).
MACRO training videos are available at www.ctu.co.uk under 'Resources – Events & Training' tab.

Data Quality Processes
Site staff will respond to data queries (DCRs) within the EDC as required.
[No data will be amended independently of the study site responsible for entering the data]* delete if single site or if central team responsible for data entry
The KCTU will provide the study team with a Data management plan for MACRO EDC.

Database Lock
At the end of the trial, the site PI will review all the data for each participant [and provide electronic sign-off]*delete if not using this functionality to verify that all the data are complete and correct. At this point, all data can be formally locked for analysis.

9.3 Archiving

Recruiting centres are responsible for archiving trial documents at their sites or at a secure records facility. All essential documents (including original consent forms) required to be held by the Investigator should be stored in such a way that ensures that they are readily available for 10 years. Destruction of essential documents requires authorisation from the Sponsor. The medical files of trial subjects should be retained in accordance with national legislation and the minimum/maximum period of time permitted by the hospital.

All other essential documents and the trial database will be archived in University of Manchester repositories for 10 years from the date of the final publication in a way that will facilitate the management of the trial, audit and inspection. Documents will be securely stored, and access restricted to authorised personnel.

Once the study is completed, the cleaned and locked version of the dataset will be transferred to the Chief Investigator and maintained on secure University of Manchester servers and computers in access-controlled areas, in line with the Sponsor's (University of Manchester) policy regarding long term storage of research data.

10. QUALITY MANAGEMENT

10.1 Site set-up and initiation

All sites will be required to sign a Clinical Study Site Agreement prior to participation. In addition, all participating Investigators will be asked to sign the necessary agreements, registration forms and supply a current CV to the Trials Office. All members of the site research team will also be required to sign the Site Signature and Delegation Log, which should be returned to the Trials Office. Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, Adverse Event reporting, collection and reporting of data and record keeping. Sites will be provided with an Investigator Site File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The Trials Office must be informed immediately of any change in the site research team.

10.2 On site monitoring

Monitoring will be carried out as required following a risk assessment and as documented in the trial monitoring plan. Additional on-site monitoring visits may be triggered for example by poor CRF return, poor data quality, low SAE reporting rates, excessive number of patient withdrawals or deviations. If a monitoring visit is required, the Trials Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the GRACE trial staff access to source documents as requested.

10.3 Central monitoring

Trials staff will be in regular contact with the site research team to check on progress and address any queries they may have. Trials staff will check incoming CRFs for compliance with the protocol, data consistency, missing data and timing. Sites will be sent Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the Trial Management Group (TMG), the Joint Committee (TSC/DMC) and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the main REC.

10.4 Audit and inspection

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

11. END OF TRIAL DEFINITION

The end of trial will be 12 months following the last data capture (the last patient visit, as per the schedule of events). This will allow sufficient time for the completion of protocol procedures, data collection and data input. The Trials Office will notify the HRA and main REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

12. STATISTICAL CONSIDERATIONS

12.1 Trial design

This trial utilises an innovative early phase seamless design that integrates dose-escalation with randomised dose-optimisation to identify a RP2D, followed by a single arm expansion cohort at the RP2D. This design maximises efficiency and makes use of all emerging data to determine an optimal dose that is safe, tolerable and shows preliminary evidence of activity. It also provides the flexibility to rigorously assess treatment efficacy at RP2D within the same trial framework.

(1) Phase I: Dose Escalation and RP2D Determination

The primary objective of Phase I is to identify a granulocyte dosing schedule that is both tolerable and shows early evidence of clinical activity. The phase utilises a seamless design, built on latest FDA and MDICT guidance[12,13], combining dose-escalation and randomised dose-optimisation. In this setting, higher number of total granulocyte infusions (from 1 to 7 days) may increase DLT risk, while activity might plateau, so the RP2D could be the MTD or a lower dose.

Phase I (up to 30 patients) consists of two components to identify the Recommended Phase II Dose (RP2D).

- **Part 1: Dose escalation:**

  A two-stage modified Bayesian TITE-CRM design will be used, targeting a 30% target DLT rate[14,15]. Four dose schedules will be evaluated: daily granulocyte infusions for 1, 3, 5 or 7 days. The DLT assessment period includes a 28-day acute toxicity window and an extended 100-day period to capture risks of graft failure/CRS and acute GvHD, respectively.

  Key design features include:
  - **No dose skipping** during escalation or de-escalation
  - **Backfilling** permitted at lower doses (up to three patients) of lower dose levels in Stage 1 to further explore activity.
  - **Safety stopping rule**: If all dose levels are deemed excessively toxic – defined as P(true DLT rate at lowest dose>0.3|data)>0.9 – the trial will stop early for safety. This will be evaluated using a beta-binomial conjugate analysis with a Beta(0.3,0.7) prior. For example, early stopping would be triggered by 3 DLTS in 3 patients or ≥ 4 DLTs in 6 patients at the lowest dose.

  Stage 1: Patients will be recruited in cohorts of 3, starting at dose 2, d(2). If no DLT are observed at any tested levels (d(2), d(3) and d(4)), subsequent patients may be recruited continuously at the highest dose - d(4) until an initial MTD is determined, with optional backfilling at lower doses (see Figure 4). If a DLT occurs, Stage 2 will commence.

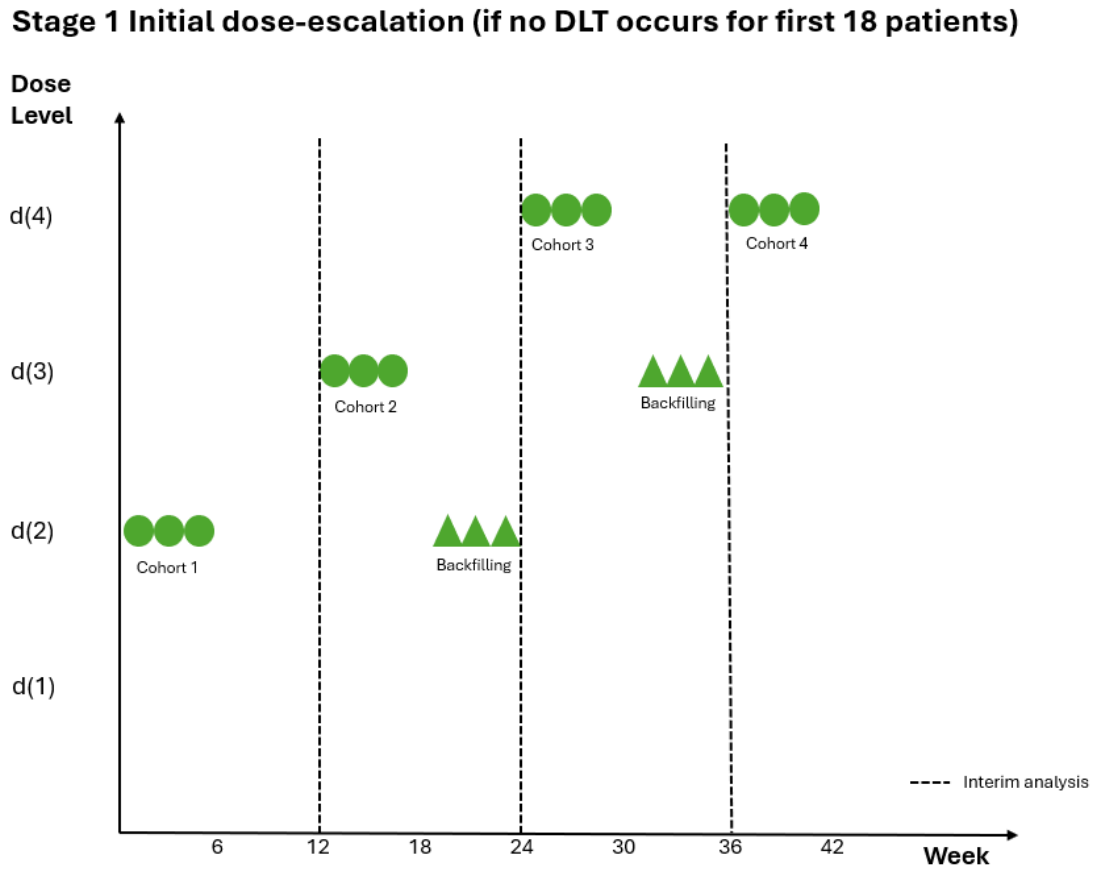


Figure 4: This figure illustrates the operation of the Stage 1 dose-escalation design in the absence of any DLTs among the first 18 patients, including backfilling at lower dose levels previously deemed tolerable.

Stage 2: Once a DLT occurs, the TITE-CRM model will guide subsequent dosing. With a 28-day acute and 100-day full DLT periods, we use a piecewise linear weighted measure assigning 90% weight to days 1-28 and 10% to days 28-100.

Once 9 patients are treated at the proposed initial MTD (and the next recommended dose is the same dose), we will proceed to Part 2. The TITE-CRM model will continue to be updated throughout Part 2 to adjust the initial MTD if needed.

- **Part 2: Dose optimisation**:

In consultation with the Joint TSC/DMC, doses selected from Part 1 – typically including the MTD and one lower dose – will undergo further evaluation. In this dose optimisation component, patients will be randomised equally between the selected doses to avoid selection bias and allow a fair comparison of tolerability and preliminary efficacy, ultimately to identify the RP2D.

A Bayesian framework will be used, incorporating an interim futility analysis after the first 6 patients at each dose arm. If fewer than 4 responses are observed among the initial 6 patients, that dose arm may be discontinued due to futility. This decision rule is based on a response rate (RR) at 28 days being considered promising, aligned with the high rate of early remission observed in our paediatric cohort.

Due to the trial's adaptive nature, the number of patients at each dose will vary, depending on observed DLTs and patients treated in Part 1. We expect 9-15 patients will be treated at the RP2D. With 12 patients, a dose will be declared promising if we observe at least 8 responses. The decision boundary is derived from a beta-binomial conjugate analysis (interim: $P(RR>0.5|data) < 0.34$, final:

P(RR>0.5|data) ≥ 0.69) with a weaky informative (0.5,0.5) prior, giving 87% power and 16% type 1 error.

The final RP2D would be selected based on an overall assessment of tolerability, activity and other key secondary endpoints, in consultation with the Joint TSC/DMC. Full technical details and simulation results are provided in the Statistical Simulation Plan.

The key measures of safety, tolerability and activity used in Phase I are:

Safety: Safety will be determined by assessing the frequency, causality and severity of the following:
1. Cytokine Release Syndrome (CRS)
2. Acute Graft vs Host Disease (GvHD)
3. Primary graft failure
4. Transplant Related Mortality (TRM) defined as death due to any transplantation-related cause other than disease relapse within the first 100 days following stem cell infusion
5. Other adverse events

Toxicity: Patients would be considered to have experienced a DLT if they experience *any* of the toxicity events:
1. Severe CRS (intensive care admission requiring intubation >7days)
2. Primary graft failure (failure of count recovery and absent donor DNA at day 28 bone marrow)
3. Severe acute graft-versus-host disease (death from GvHD in the first 100 days)

Activity: Patients would be considered to show early indicators of clinical activity if they experience *any* of the events:
1. CRS (fever in the first 7 days following stem cell infusion)
2. Lymphocyte expansion (>0.5 x10^9/L in the first 12 days)
3. Maximum CRP >150 mg/L on days 5-10 following stem cell infusion
4. Early evidence of disease response (MRD negative on day 28 bone marrow)

(2) Phase II: Efficacy assessment at RP2D based on relapse-free survival (RFS) at 1 year

Phase 2 (minimum 20 evaluable patients, including evaluable patients from Phase 1) will assess preliminary efficacy at the RP2D, based on relapse-free survival (RFS) at 1 year, using a single-stage Bayesian design. Notably, evaluable patients who received RP2D in phase I will also contribute to the phase 2 activity evaluation. We have based our power calculations on the expected outcomes for the target population of 1-year RFS of 20% as reported in the literature[1-4]. The decision criterion is to declare treatment as promising if $Pr(\theta > 0.2|data) \geq 0.82$ *(GO if ≥6 successes/20 patients)*, ensuring 87% power (with 1-year RFS=40%) and 20% type I error. This uses a weakly informative prior of Be(0.2, 0.8). Because evaluable patients at RP2D in Phase I will also be evaluated in Phase II, we will only need to recruit additional (20–number at RP2D) patients. Continuation to the full planned total of 50 patients (Phase 1 and 2 combined) may be considered in consultation with the TSC/DMC if results are encouraging and where feasible within the available time and funding, as this would add value to the exploratory subgroup analyses.

We will also construct a historical comparator group that is matched for the patient, transplant and disease characteristics of our study population. Whilst universally poor, the outcomes for patients of differing ages with specific high-risk mutations or varying levels of residual disease nonetheless vary. There is a risk that our study population becomes more or less high-risk than our sample size assumes. It would be unethical to randomise patients to receive the investigational transplant protocol, because the target population has such poor outcomes with conventional transplantation that many clinicians advocate palliation. In collaboration with The British Society of Blood and Marrow Transplantation and Cellular Therapy (BSBMTCT), we will therefore construct a comparator cohort that is well matched for other patient and transplant characteristics. In the absence of randomisation, this represents a robust method for determining the efficacy of peri-transplant granulocytes.

12.2 Analysis population

Analysis Set	Description
Safety	All participants who received at least one dose of study intervention
DLT	The DLT analysis set will include all patients who received at least one dose of study intervention and meet at least one of the following criteria: • Experienced a DLT within 100 days of treatment initiation, regardless of the number of doses received or whether the DLT period was completed. Patients will generally be analysed according to the dose level of the total doses received (i.e. 1, 3, 5 or 7 doses). • Received the planned total doses of the study intervention within the initial 28-day DLT period, completed at least 28 days of follow-up, and did not experience a delay of more than 1 day in administration. • Did not receive the planned total dose, but received the full dose corresponding to a lower dose level and had at least 28 days of DLT follow-up. These patients will be eligible for DLT analysis at that lower dose level. Patient evaluability for DLT and DLT outcomes will be reviewed and confirmed by the Joint TSC/DMC, considering any relevant deviations such as from the planned dosing schedule and clinical circumstances. Non-evaluable patients will be replaced.
Response	All participants who received at least one dose of study intervention and had a 28-day bone marrow response assessment. Participants will be analysed per the actual dose level (total doses) received.
Efficacy	All participants who received at least 80% of intended doses and had a 1-year bone marrow response assessment.

12.3 Analysis of outcome measures

A separate statistical analysis plan (SAP) which includes a more technical and detailed description of the statistical analyses described in this section will be provided and finalised prior to the first interim analysis. This section is a summary of the planned statistical analyses of the most important endpoints, including primary and key secondary endpoints.

In general, analysis will primarily be descriptive in nature. Continuous variables will typically be summarised using appropriate measures of central tendency (e.g., mean or median) and variability (e.g., standard deviation, interquartile range (25^{th} to 75^{th} percentile), minimum and maximum). Categorical variables will be described using frequency counts and percentages. Patients will be analysed according to the total number of doses received.

1. Safety Analyses

Safety variables will be summarised by descriptive statistics and based on the safety analysis set. Laboratory variables will be described using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Please see section 9 for exceptions to this.

Adverse events (AEs) will be reported for each dose level and presented as tables of frequency of AEs by body system and by worst severity grade observed. Tables should indicate related and unrelated events. Laboratory data will be presented by dose level at each observation time. Values outside normal limits will be identified and summarised by frequency.

2. Phase I Dose-Escalation and Dose-Optimisation

Analysis for the Bayesian dose escalation (Part 1) will be performed using the DLT analysis set. Dose escalation decisions will typically be made after all participants in the most recent cohort have completed the initial 28-day DLT period or dropped out, incorporating mature data from any backfill patients. However, the Joint TSC/DMC committee may recommend an earlier model update – for example, after at least two participants have completed their 28-day DLT period, without waiting for 28-

day follow-up data from a third participant. However, data of such evaluable participants will be included in the subsequent Joint TSC/DMC meeting.

At the end of Part 1, the MTD (defined as the dose level with estimated DLT rate closest to the target toxicity level of 30%) will be reported with its associated DLT rate and 90% credible intervals.

Activity analysis will be performed on the response-evaluable analysis set. The number of patients achieving a response (any of the activity outcomes specified above) will be presented, and the overall response rate will be presented with its 95% confidence interval.

An interim futility analysis will be conducted after the first 6 evaluable patients at each dose level. The posterior probability P(RR>0.5|data) will be computed using a weaky informative (0.5,0.5) prior. If this probability is <0.34 (equivalent to observing fewer than 4 observed responses among the initial 6 evaluable patients) the corresponding dose arm may be discontinued for futility.

For the final activity analysis at the RP2D, the posterior probability P(RR>0.5|data) will again be computed. If this probability is at least 69%, the dose arm will be considered promising.

The final RP2D will be selected based on an integrated assessment of tolerability, activity and other key secondary endpoints, in consultation with the Joint TSC/DMC.

3. Phase II Efficacy Analysis

The primary efficacy endpoint is the proportion of patients who remain relapse-free and alive at 1-year. The primary analysis will be conducted using the Efficacy-evaluable analysis set. A secondary analysis will include all treated patients (i.e. the safety analysis set).

Other time-to-event endpoints, including GvHD-free, relapse-free survival and overall survival probabilities, will be calculated using the Kaplan-Meier estimator and displayed graphically. Median progression-free survival and median overall survival will be reported with their 95% confidence intervals.

Full details will be specified in a Statistical Analysis Plan, which will be developed by the study statisticians and approved before any formal interim analysis is conducted.

12.4 Planned subgroup analysis

Sub-group analysis will be conducted on the primary outcome by the stratification factors of CRS grade and disease risk score. This analysis has not been powered and therefore, due to the lack of power, will be interpreted with caution and considered as hypothesis generating.

12.5 Planned interim analysis

The joint committee (TSC/DMC) will convene a pre-planned interim analysis during Phase I after 6 patients are evaluable for response at each dose arm, as detailed in Section 12.2.

Once phase I has been completed and an RP2D identified, we will convene a special “Phase I to II Transition Review Meeting” to serve as a checkpoint for progression from phase I to II. This meeting will include the TSC and a representative from the grant body to review important factors including:

- The number of efficacy-evaluable patients treated at the RP2D during Phase I
- Remaining grant duration
- Available resources
- Any other important parameters

12.6 Planned final analysis

The final analysis will be conducted after one of the following conditions is met.

- The trial is terminated early (for example, due to toxicity or futility).
- All patients have completed their 'off-study' visit and have been followed up for a minimum period of 12 months, and data collection has been completed.

12.7 Sample size

The trial will enrol up to 30 patients across 4 dose levels in Phase I and up to 20 patients in Phase II, using an adaptive seamless design that combines dose-finding (based on tolerability and activity) and efficacy analysis for enhanced speed and efficiency.

A target sample size of 30 evaluable patients will be recruited in Phase I and this target sample size is anticipated to be sufficient to determine the RP2D as per the modified 2-stage TITE-CRM seamless design.

With patients dosed at RP2D in phase 1, we will anticipate up to 20 additional patients in Phase II to assess the 1-year RFS rate based on a single-stage Bayesian design. A weakly informative prior of (0.2, 0.8) will be used. The decision criterion is to declare treatment as promising if $Pr(\theta > 0.2|data) \geq 0.82$ *(GO if ≥6 successes/20 patients)*, ensuring 87% power (with 1-year RFS=40%) and 20% type I error. Because evaluable patients at RP2D in Phase I will also be evaluated in Phase II, we will only need to recruit additional (20–number at RP2D) patients. Continuation to the full planned total of 50 patients (Phase 1 and 2 combined) may be considered in consultation with the TSC/DMC if results are encouraging and where feasible within the available time and funding, as this would add value to the exploratory subgroup analyses.

Full simulation results are provided in the Statistical Simulation Plan.

13. TRIAL ORGANISATIONAL STRUCTURE

13.1 Sponsor

The trial is sponsored by the University of Manchester.

13.2 Co-ordinating centre

The trial is being conducted under the auspices of King's College Hospital NHS FT, according to their local procedures and in line with University of Manchester (Sponsor) processes.

13.3 Trial Management Group (TMG)

The TMG will be responsible for the set up and management of the clinical trial. The group will meet regularly to ensure that all practical details of the trial are progressing, working well and that everyone within the trial understands them. A subgroup of the TMG, the cord selection committee, will also meet regularly to identify suitable cord units for participants. The TMG will closely monitor toxicity and adverse events during Phase 1. The TMG includes the PIs and clinicians from all three study sites. Sharing direct clinical experience and discussing study data will be important to inform decisions about dose escalation/reduction and study continuation. Clinical experience may also have implications for the management of treatment complications and will be shared.

13.4 Trial Steering Committee and Data Monitoring Committee

As this is a Phase I/II study, the Trial Steering Committee (TCS) and the Data Monitoring and Ethics Committees (DMC) will be combined to aid decision making during the adaptive safety phase.

Joint committee (TSC/DMC)

The joint committee will provide overall supervision for the trial and provide advice through its independent chair. The ultimate decision for the continuation of the trial lies with the joint committee. Data analyses will be supplied in confidence to the committee, which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further patients.

Additional meetings may be called if recruitment is faster than anticipated and the committee may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The joint committee will report directly to the TMG who will convey findings to the funders, and/or sponsors as applicable. The committee may recommend discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise patient safety.

The joint committee will operate in accordance with a trial specific charter based upon the template created by [insert details]. The role of the joint committee will vary according to the phase of the study:

Phase 1: In the safety phase of this study the joint committee will meet with members of the TMG to discuss safety data and make decisions regarding study continuation. These meetings will be open, with discussion and consensus decisions reached via voting.

Phase 1 to 2 Transition Review Meeting: Once phase I has been completed and an RP2D identified, we will convene a special "Phase I to II Transition Review Meeting" to serve as a checkpoint for progression from phase I to II. This meeting will include the TSC/DMC and a representative from the grant body to review important factors including:

- The number of evaluable patients treated at the RP2D during Phase I
- Remaining grant duration
- Available resources
- Any other important parameters

Phase 2: As the second phase of the study has the potential to be practice changing, the TMG (except the statistician) will remain blinded to the long-term efficacy data. In phase II, there will be open and closed sessions. Members of the TMG will attend open sessions where safety data will be shared.

Efficacy data regarding RFS, or other outcome measures, will only be showed in the closed session to the independent members.

13.5 Finance

This is a clinician-initiated and clinician-led trial funded by Blood Cancer UK. No individual per patient payment will be made to NHS Trusts, Investigators or patients. This trial is an NIHR CRN portfolio study.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Regulatory compliance and REC review

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (website: http://www.wma.net/en/30publications/10policies/b3/index.html).

The trial will be conducted in accordance with the Research Governance Framework for Health and Social Care, the applicable UK Statutory Instruments (the General Data Protection Regulation (GDPR) and Human Tissue Act 2008) and Good Clinical Practice (GCP). The protocol will be submitted to and approved by the main Research Ethics Committee (REC) prior to circulation.

Before any patients are enrolled into the trial, the Principal Investigator at each site is required to confirm local capability and capacity. Sites will not be permitted to enrol patients until written confirmation of local capability and capacity is received by the Trials Office.

14.2 Peer review

The study background, trial design, aims and objectives were peer reviewed by the funder (Blood Cancer UK). Review consisted of assessment and feedback from four independent experts and two lay assessors.

14.3 Patient and Public Involvement and Engagement (PPIE)

During the initial design phase, a virtual event was held with 25 attendees including leukaemia patients, friends and families of those who had died, and patient representatives from Anthony Nolan, MDS UK, and Blood Cancer UK. Feedback from this group led to broadening the inclusion criteria and raising of the upper age limit to 55 years. The patient representative lead reviewed the funding application documents and the patient information leaflet. The latter has also been reviewed and edited by the patient representative team from Anthony Nolan.

14.4 Notification of serious breaches

The Sponsor of the trial is responsible for notifying the HRA and main REC in writing of any serious breach of:

- The conditions and principles of GCP in connection with that trial or;
- The protocol relating to that trial, within 7 days of becoming aware of that breach

For the purposes of this regulation, a “serious breach” is a breach which is likely to effect to a significant degree:

- The safety or physical or mental integrity of the subjects of the trial; or
- The scientific value of the trial

Sites are therefore requested to notify the Trials Office of a suspected trial-related serious breach of GCP and/or the trial protocol. Where the Trials Office is investigating whether or not a serious breach has occurred sites are also requested to cooperate with the Trials Office in providing sufficient information to report the breach to the HRA where required and in undertaking any corrective and/or preventive action.

14.5 Confidentiality and data protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation and the Data Protection Act (2018). With the patient’s consent, their initials and date of birth will be collected at trial entry. Patients will be identified using only their unique trial number, initials and date of birth in correspondence between the Trials Office and participating sites. It is expected that trial number alone will be sufficient in most cases, but date of birth may be required in some instances (eg. SAE forms). Signed consent

forms that have had patient identifiable information redacted will be collected by the central trial office to allow in-house monitoring of the consent process.

The Investigator must maintain documents not for submission to the Trials Office (e.g. Patient Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

The Trials Office will maintain the confidentiality of all patient's data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and organisations for which the patient has given explicit consent for data transfer (e.g. laboratory staff). Representatives of the GRACE trial team may be required to have access to patient's notes for quality assurance purposes, but patients should be reassured that their confidentiality will be respected at all times.

14.6 Insurance and indemnity

University of Manchester employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake whilst in the University's employment.

In terms of liability at a site, NHS Trust and non-Trust hospitals have a duty to care for patients treated, whether or not the patient is taking part in a clinical trial. Compensation is therefore available via NHS Resolution in the event of clinical negligence having been proven.

The University of Manchester cannot offer indemnity for non-negligent harm. The University of Manchester is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation.

14.7 Protocol compliance

As stated in the UK Clinical Trials Regulations, planned deviations or waivers to the trial protocol are not permitted, unless the deviation/non-compliance is being performed as an urgent safety measure to protect a participant from immediate harm.

Accidental protocol non-compliances can happen at any time. Non-compliances vary in incidence and impact and are classified accordingly as minor, major or as a serious breach. The sponsor will subsequently advise on any further action or information required.

The trial team will maintain a log of all protocol non-compliances to enable these events to be monitored for frequency.

15. PUBLICATION POLICY

Results of this trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the Trial Management Group (TMG) and authorship will be determined by mutual agreement and will usually be in accordance with ICMJE guidance. The current plan is to publish the combined results of Phase 1 and 2 together at the end of the study. However, Phase I results may be published separately if they provide important insights into the safety profile, optimal dosing, or preliminary signs of clinical activity that could inform ongoing or future research. In the meantime, Phase 1 safety and activity data (once the phase is completed) may be shared with other investigators where it would inform the design of related studies.

Any secondary publications and presentations prepared by Investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for

publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of the University of Manchester.

Intellectual property rights will be addressed in the Clinical Study Site Agreement between Sponsor and site.

16. REFERENCE LIST

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17. APPENDICES

Appendix 1 Eastern Cooperative Oncology Group (ECOG) Performance Status

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

Appendix 2 Assessment of GVHD

Acute GvHD Scoring – modified Glucksberg criteria[21]

Stage	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	< 34 micromol/L (or < 2mg/dl)	No or intermittent nausea or vomiting or anorexia	< 500 ml/day or < 3 episodes/day
1	Maculopapular rash < 25% of body surface area (BSA)	34-50 micromol/L (or 2-3mg/dl)	Persistent nausea or vomiting or anorexia	500–999 ml/day or 3–4 episodes/day
2	Maculopapular rash 25-50% of BSA	51-102 micromol/L (or 3.1-6mg/dl)		1000–1500 ml/day or 5–7 episodes/day
3	Maculopapular rash > 50% of BSA	103-255 micromol/L (or 6.1-15mg/dl)		>1500 ml/day or >7 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation > 5% BSA	> 255 micromol/L (or > 15mg/dl)		Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

Overall clinical grade (based upon most severe target organ involvement):

Grade 0: No stage 1–4 of any organ

Grade I: Stage 1–2 skin without liver, upper GI or lower GI involvement

Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI

Grade III: Stage 2–3 liver and/or stage 2–3 lower GI, with stage 0–3 skin and/or stage 0–1 upper GI

Grade IV: Stage 4 skin, liver or lower GI involvement, with stage 0–1 upper GI

Chronic GVHD Scoring - National Institutes of Health criteria[22]

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† ☐ **SCORE % BSA** *GVHD features to be scored by BSA:* **Check all that apply:** Maculopapular rash/erythema; Lichen planus-like features; Sclerotic features; Papulosquamous lesions or ichthyosis; Keratosis pilaris-like GVHD	No BSA involved	1-18% BSA	19-50% BSA	>50% BSA
SKIN FEATURES SCORE:	No sclerotic features		Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** Deep sclerotic features; "Hidebound" (unable to pinch); Impaired mobility; Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** Hyperpigmentation; Hypopigmentation; Poikiloderma; Severe or generalized pruritus; Hair involvement; Nail involvement; *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
MOUTH *Lichen planus-like features present:* **Yes** **No**	No symptoms	Mild symptoms **with** disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs on examination **with** major limitation of oral intake
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* **Yes** **No** **Not examined**	No symptoms	Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
Abnormality present but explained entirely by non-GVHD documented cause (specify):				
GI Tract ***Check all that apply:*** Esophageal web/ proximal stricture or ring Dysphagia Anorexia Nausea Vomiting Diarrhea Weight loss ≥5%* Failure to thrive	No symptoms	Symptoms without significant weight loss* (<5%)	Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
Abnormality present but explained entirely by non-GVHD documented cause (specify):				
LIVER	Normal total bilirubin and ALT or AP < 3 x ULN	Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	Elevated total bilirubin > 3 mg/dL
Abnormality present but explained entirely by non-GVHD documented cause (specify):				
LUNGS** **Symptom score:**	No symptoms	Mild symptoms (shortness of breath after climbing one flight of steps)	Moderate symptoms (shortness of breath after walking on flat ground)	Severe symptoms (shortness of breath at rest; requiring O_2)
Lung score: % FEV1 ☐ *Pulmonary function tests* Not performed	FEV1≥80%	FEV1 60-79%	FEV1 40-59%	FEV1 ≤39%
Abnormality present but explained entirely by non-GVHD documented cause (specify):				

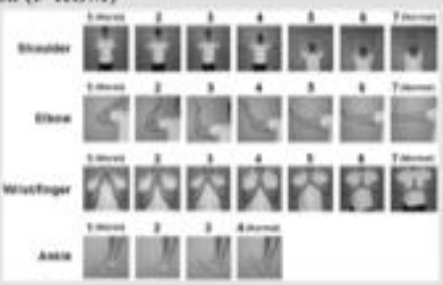
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*[2]) Not examined *Currently sexually active* Yes No	No signs	Mild signs[2] and females with or without discomfort on exam	Moderate signs[2] and may have symptoms with discomfort on exam	Severe signs[2] with or without symptoms
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

Ascites (serositis)___	Myasthenia Gravis___	
Pericardial Effusion___	Peripheral Neuropathy___	Eosinophilia > 500/µl___
Pleural Effusion(s)___	Polymyositis___	Platelets <100,000/µl ___
Nephrotic syndrome___	Weight loss>5%* without GI symptoms ___	Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ❑ **No GVHD** ❑ **Mild** ❑ **Moderate** ❑ **Severe**

Photographic Range of Motion (P-ROM)



† Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring.

* Weight loss within 3 months.

**Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); ULN (normal upper limit).

‡ To be completed by specialist or trained medical providers (see Supplemental Figure).

Appendix 3 FACT-BMT Questionnaire

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4
	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
BMT1	I am concerned about keeping my job (include work at home)	0	1	2	3	4
BMT2	I feel distant from other people	0	1	2	3	4
BMT3	I worry that the transplant will not work	0	1	2	3	4
BMT4	The effects of treatment are worse than I had imagined	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
C7	I like the appearance of my body	0	1	2	3	4
BMT5	I am able to get around by myself	0	1	2	3	4
BMT6	I get tired easily	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
BMT7	I have concerns about my ability to have children	0	1	2	3	4
BMT8	I have confidence in my nurse(s)	0	1	2	3	4
BMT9	I regret having the bone marrow transplant	0	1	2	3	4
BMT10	I can remember things	0	1	2	3	4
Br1	I am able to concentrate	0	1	2	3	4
BMT11	I have frequent colds/infections	0	1	2	3	4
BMT12	My eyesight is blurry	0	1	2	3	4
BMT13	I am bothered by a change in the way food tastes	0	1	2	3	4
BMT14	I have tremors	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
BMT15	I am bothered by skin problems (e.g., rash, itching)	0	1	2	3	4
BMT16	I have trouble with my bowels	0	1	2	3	4
BMT17	My illness is a personal hardship for my close family members	0	1	2	3	4
BMT18	The cost of my treatment is a burden on me or my family	0	1	2	3	4

Appendix 4 Definition of Adverse Events

Adverse Event
Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Comment: An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product.

Adverse Reaction
All untoward and unintended responses to an IMP related to any dose administered.

Comment: An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event
Any untoward medical occurrence or effect that at any dose:
- Results in death
- Is life-threatening*
- Requires hospitalisation** or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Or is otherwise considered medically significant by the Investigator***

Comments:
The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria.

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

**Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE.

*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Serious Adverse Reaction
An Adverse Reaction which also meets the definition of a Serious Adverse Event.

Suspected Unexpected Serious Adverse Reaction
A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information.

A SUSAR should meet the definition of an AR, UAR and SAR.

Unexpected Adverse Reaction
An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SmPC) for a licensed product).

When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.

Appendix 5 Common Toxicity Criteria Gradings

Toxicities will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. The full CTCAE document is available on the National Cancer Institute (NCI) website, the following address was correct when this version of the protocol was approved:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Appendix 6 WMA Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Recommendations guiding physicians
in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964
and amended by the
29th World Medical Assembly, Tokyo, Japan, October 1975
35th World Medical Assembly, Venice, Italy, October 1983
41st World Medical Assembly, Hong Kong, September 1989
and the
48th General Assembly, Somerset West, Republic of South Africa, October 1996

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 fulfillment 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 recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying 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.

I. 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 involving 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 interests 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 minimize 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 of 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 should be informed that 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 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.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical Research)

13. 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.
14. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.
15. 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. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.
16. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.
17. 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 (I, 2).
18. 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.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-Clinical Biomedical Research)

19. 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.
20. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
21. 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.
22. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.