BMJ Open

# Intestinal Microbiota Transplant Prior to Allogeneic Stem Cell Transplant - (MAST) trial: Study Protocol for a Multi-Centre, Double-Blinded, Placebo-Controlled, Phase IIa Trial

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2024-093120 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 31-Aug-2024 |
| Complete List of Authors: | Mullish, Benjamin; Imperial College London Faculty of Medicine, Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction<br>Innes, Andrew J; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Ghani, Rohma; Imperial College London, Department of Metabolism, Digestion and Reproduction; Imperial College Healthcare NHS Trust, Department of Infectious Diseases<br>Anim-Burton, Shian; Imperial College London, Department of Surgery and Cancer<br>Webber, Lee; Imperial College London, Department of Surgery and Cancer<br>Roberts, Lauren A; Imperial College London, Department of Metabolism, Digestion and Reproduction<br>Farshi, Pakhshan; Manchester Royal Infirmary, Department of Haematology<br>Khan, Anjum B; Leeds Teaching Hospitals NHS Trust, Department of Haematology<br>Kinsella, Francesca; University Hospitals Birmingham NHS Foundation Trust, Department of Haematology<br>Kottaridis, Panagiotis; University College London Hospitals NHS Foundation Trust, Department of Haematology<br>Krishnamurthy, Pramila; King's College Hospital NHS Foundation Trust, Department of Haematology<br>Nicholson, Emma; The Royal Marsden Hospital, Department of Haematology<br>Palanicawandar, Renuka; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Davies, Frances J; Imperial College Healthcare NHS Trust, Department of Infectious Diseases<br>Marchesi, Julian R.; Imperial College London, Department of Metabolism, Digestion and Reproduction<br>Pavlů, Jiří; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Johnson, Nicholas; Imperial College London School of Public Health, |

| | |
|---|---|
| | Department of Public Health<br>Wheeler, Graham; Imperial College London, Imperial Clinical Trials Unit |
| Keywords: | Bone marrow transplantation < HAEMATOLOGY, Leukaemia < HAEMATOLOGY, Transplant medicine < INTERNAL MEDICINE, Microbiota |
| | |

*BMJ Open – Protocol:*

# Intestinal *M*icrobiota Transplant Prior to *A*llogeneic *S*tem Cell *T*ransplant - (MAST) trial: Study Protocol for a Multi-Centre, Double-Blinded, Placebo-Controlled, Phase IIa Trial

Benjamin H Mullish[1,2], Andrew J Innes[3,4], Rohma Ghani[1,5], Shian Anim-Burton[6], Lee Webber[6], Nicholas A Johnson[7], Lauren A Roberts[1], Pakhshan Farshi[8], Anjum B Khan[9], Francesca Kinsella[10], Panagiotis Kottaridis[11], Pramila Krishnamurthy[12], Emma Nicholson[13], Renuka Palanicawandar[3,4], Graham M Wheeler[7,14], Frances J Davies [5], Julian R Marchesi[1]*, Jiří Pavlů[3,4,]*

*Affiliations:*

[1]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, United Kingdom

[2]Department of Hepatology, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

[3]Centre for Haematology, Department of immunology and inflammation, Faculty of Medicine, Imperial College London, London, United Kingdom

[4]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom.

[5]Department of Infectious Diseases, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom.

[6]Cancer Research UK Imperial Centre, Clinical Trials Section, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, United Kingdom

[7]Imperial Clinical Trials Unit, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom

[8]Department of Haematology, Manchester Royal Infirmary, Manchester, United Kingdom

[9]Department of Haematology, Leeds Teaching Hospitals, Leeds, United Kingdom

[10]Department of Haematology, University Hospital Birmingham, Birmingham, United Kingdom

[11]Department of Haematology, University College London Hospital, London, United Kingdom

[12]Department of Haematology, King's College Hospital, London, United Kingdom

*BMJ Open – Protocol:*

[13]Department of Haematology, The Royal Marsden Hospital, London, United Kingdom

[14]Statistics and Data Science Innovation Hub, GSK, London, United Kingdom

*Joint senior authors and trial co-chief investigators

Correspondence to: Prof Julian R Marchesi

Postal address: Department of Metabolism, Digestion and Reproduction
St Mary's Hospital Campus, Imperial College London
10th Floor, QEQM Wing
South Wharf Road
Paddington, London
W2 1NY
United Kingdom

Email: j.marchesi@imperial.ac.uk

Word count: 5589

Financial support: This study is funded via the Medical Research Council (MRC) Developmental Pathway Funding Scheme (DPFS) (grant number: MR/X004996/1). The Division of Digestive Diseases and Centre for Haematology receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre (BRC) based at Imperial College London and Imperial College Healthcare NHS Trust. BHM is the recipient of a Medical Research Council (MRC) Clinician Scientist award (MR/Z504002/1) and was the recipient of an NIHR Academic Clinical Lectureship (CL-2019-21-002).

Conflicts of interest: GMW is an employee of and holds share in GSK. All other authors have nothing to report.

*BMJ Open – Protocol:*

**Abstract:**

**Introduction:** Lower diversity of the gut microbiome pre-allogeneic haematopoietic cell transplantation (HCT) correlates with reduced survival after the intervention. Most patients undergoing HCT for a haematological malignancy have previously received intensive chemotherapy, resulting in prolonged neutropenic episodes requiring broad-spectrum antibiotics; use of these has been linked to reduced microbiome diversity. Intestinal microbiota transplant (IMT) is a novel treatment approach that restores this diversity. We hypothesised that IMT performed prior to initiation of HCT conditioning restores microbiome diversity during the early stages of HCT, leading to decreased frequency of complications and improved outcomes of HCT.

**Methods and analysis:** Fifty adult patients receiving allogeneic HCT will be recruited into this phase 2a trial and randomised 1:1 to receive capsulised IMT or matched placebo shortly prior to initiation of HCT conditioning and followed for up to twelve months. The primary outcome will be to assess the increase in alpha diversity between pre-IMT and that measured at ~42 days after the IMT administration (day +28 of HCT), comparing the difference between patients receiving IMT compared to placebo. Secondary outcomes will include tolerability, and the dynamics of gut microbiome diversity metrics and taxonomy over all timepoints assessed, as well as clinical outcomes (including burden of invasive infections, days of fever, admission to intensive care, development of graft-vs-host disease, and mortality).

**Ethics and dissemination:** This study was approved by a UK Research Ethics Committee (REC reference: 23/NE/0105). Dissemination of results will be in concert with patient and public involvement (PPI) group input and is expected to be primarily via abstract presentation at conferences and manuscripts in peer-reviewed journals.

**Trial registration number:** ClinicalTrials.gov ID: NCT 6355583; ISRCTN: https://doi.org/10.1186/ISRCTN13241761; EudraCT: 2022-003617-10

**Keywords:** Bone marrow transplantation; Leukaemia; Transplant medicine; Gut microbiome; Faecal microbiota transplant.

**Article Summary:**

- The use of IMT pre-HCT by ourselves and others has shown promising signals related to gut microbiome changes, infection burden, and haematological outcomes from HCT in a case series, and this will allow exploration of this in a randomised controlled trial setting. One particular distinctive aspect of study design here compared to other studies is the use of IMT *pre*- rather than *post*-HCT, with the aim of 'prehabilitating' the gut microbiome prior to HCT.
- Our prior experience in this area has used a conventional slurry IMT, but this study will use a novel capsulised multi-donor IMT preparation, based upon patient preference during our patient and public involvement (PPI) group feedback. We do not have direct head-to-head experience of this IMT product compared to conventional IMT slurry in this setting.
- The study has been robustly powered for its primary outcome, which is a biological, in order to confirm the potential of this approach to pre-habilitate the microbiome. While the relatively small number are participants will limit exploration of clinical outcomes, the multi-centre design will test the feasibility and deliverability for future larger studies. Furthermore, the multi-centre design is also expected to ensure that participants include both sexes, from a range of socio-ethnic backgrounds, and with a range of underlying haematological diagnoses, making this more representative of a 'real world' population.
- Blood, urine and stool samples collected during this trial will be utilised for extensive multi-omic profiling (shotgun metagenomics, metabolomics, PBMC analysis, etc) to begin to explore potential mechanisms of efficacy.

## 1. Introduction:

Allogeneic haematopoietic cell transplantation (HCT) is a powerful therapeutic modality for patients with acute leukaemia and certain other haematological malignancies. Furthermore, with the advent of reduced intensity protocols, and approaches that permit safer use of mismatched donors, its frequency is increasing. In preparation for transplant, patients receive a conditioning regimen of high-dose chemotherapy and/or total-body irradiation, followed by infusion of compatible haematopoietic cells; the engraftment of these cells restores the recipient's haematopoesis, and exerts long-term remission from the graft-versus-leukaemia effect of the donor immune effector cells. However, this process is associated with marked perturbation of the gut microbiome, including reduced gut barrier integrity, loss of gut microbiome diversity, and microbiome enrichment in pathobiont bacteria[1] [2]. The immunosuppressive nature of both the underlying haematological malignancy, together with the treatments used, collectively result in a markedly increased risk of infections in these patients. More specifically, the increase in susceptibility to infection leads to an increase in antibiotic exposure, driving the dominance of pathobionts, and a further selection pressure for overgrowth of antimicrobial resistance (AMR) genes in the gut[3]. In this setting, the impact of antibiotics, and multidrug-resistant organism (MDRO)-associated infection, is associated with poorer clinical outcomes in patients[4]; for example, use of imipenem-cilastatin or piperacillin-tazobactam use to treat neutropenic fever has been associated with increased graft-versus-host disease (GvHD) mortality up to even five years post-HCT[5].

A large observational study of more than 1,300 patients from four centres observed that patients with patterns of microbiota disruption characterised by loss of diversity had a higher risk of transplantation-related death, and death attributable to GvHD[6]. Baseline samples obtained before HCT already showed evidence of microbiome disruption, and lower diversity before transplantation was closely associated with poor survival. Specific gut taxonomic features have also been linked with allo-HCT outcome; specifically, expansion of *Enterococcus*

(particularly *Enterococcus faecium*) was observed across the period of having allo-HCT. *Enterococcus* was found to associate closely with GvHD and mortality, with presence of the disaccharide lactose identified as a factor that promoted *Enterococcus* expansion[7].

These data support the hypothesis that a microbiome-based intervention, performed prior to initiation of HCT conditioning, may improve microbiome diversity during transplant, and has the potential to impact upon clinical outcomes. A number of approaches have been considered in this setting[8], including dietary/prebiotic interventions, probiotics, and non-absorbable antibiotics (such as rifaximin). In this study we have opted for a biological approach that attempts to restore the whole gut ecosystem, using intestinal microbiota transplant (IMT; also known as 'faecal microbiota transplant'). IMT consists of transferring minimally processed stool, from a healthy screened donor, into the gut of an recipient. This approach was pioneered in patients with recurrent *Clostridioides difficile* infection (rCDI), in which the major risk factor is recurrent antibiotic exposure[9]. The success in this setting had led to exploration of IMT in other conditions which the gut microbiome appears contributory to their aetiopathogenesis[10], with promising early data. Despite initial concerns about safety of IMT in immunocompromised patients - driven in part by descriptions of pathogen transmission via IMT in such patients[11] - adherence to strict screening protocols results in a safety profile comparable to that in immunocompetent recipients[8 9 12].

After previously reports that the use of IMT for rCDI was also associated with reduced antibiotic resistance genes within the gut microbiome[13], and our own observation of a clinical case where IMT seemed to show clinical benefit when used prior to HCT in a patient colonised with MDROs[14], we completed a cohort study of IMT performed prior to initiation of HCT conditioning in patients colonised and/or previous infected with MDROs. While we observed that rates of decolonisation of intestinal MDROs were comparable to that observed spontaneously, we saw a significant reduction in rates of bloodstream infection (including MDRO-related), length of stay, and days of carbapenem use, compared to a matched historical control arm[15]. With longer follow-up, these benefits translated to improvement in overall survival, such that the poor outcome associated with MDRO colonisation could be negated with IMT[16].

*BMJ Open – Protocol:*

In this clinical trial, we will investigate the broader role of IMT in HCT, both with and without MDRO colonisation. By randomising patients to receive IMT or placebo prior to HCT conditioning, and measuring microbiota diversity, in stool, as a surrogate for its impact upon gut ecology, we will determine the capacity of capsule IMT to restore a normal microbiome, and track the impact during the course of HCT. Using multi-omic profiling of stool, urine and blood, we will investigate the wider impact of IMT in HCT patients, while clinical parameters will explore the potential to overall outcome.

## 2. Methods and analysis:

### 2.1. Design and objectives:

The MAST study is a multi-centre, randomised, phase 2a double-blind placebo-controlled trial**.** The major objective of this trial is to determine the ability of capsulised IMT given prior to allogeneic HCT to increase and maintain stool microbiota diversity after HCT from baseline. Secondary objectives include determination of clinical tolerability, effects of capsule IMT upon clinical outcomes, and to explore microbiome and immune dynamics related to IMT use. The primary outcome is the change in alpha diversity (measured as inverse Simpson's index) after IMT administration measured at immediately prior to IMT (at 14 +/- 3 days prior to HCT) and 28 +/-3 days after HCT, comparing the change between patients receiving capsulised IMT *versus* placebo. The study is sponsored by Imperial College London. This is an investigator-led study; while funding for the study was only awarded after peer review, the funder, sponsor and industrial partner have had no role in any aspect of study design. We have used the SPIRIT checklist in writing this report[17].

### 2.2. Patient and public involvement:

From its inception, the MAST trial was co-developed with the patient and public involvement group based around NCRI AML Supportive care group. The group refined the protocol and participant-facing documents and provided input into the design.

### 2.3. Consent:

Patients will be identified as per site established processes and invited to participate by their primary haematology team. Eligible patients will be provided with the patient information sheet (PIS) (**Supplementary Material 1**), and given sufficient time to consider the study, with

opportunities to discuss and ask questions. Investigators will ensure that they adequately explain the study, including the aims, trial treatment, anticipated benefits and potential risks of participation. The right of the patient to refuse participation in the trial, or withdraw at any point, without giving explanation will be respected. Informed consent is requested from the patient by the investigator who has been delegated the responsibility on the delegation log. Consent will be obtained no earlier than 24 hours after receiving the PIS to give them time to read and understand what their participation in the study entails (consent form provided as **Supplementary Material 2**). With patient consent, it is the investigator's responsibility to inform the patient's General Practitioner regarding study participation.

### 2.4. Study setting and participants:

This trial will be performed across seven Haematology Units in the United Kingdom which regularly undertake HCT. The study will recruit adults with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), acute leukaemia (AL) of ambiguous lineage, high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML), and chronic myeloid leukaemia (CML) in blast phase, considered suitable/fit for allogeneic haematopoietic stem cell transplantation HCT. Patients will be eligible to enter the study if they achieved complete remission (defined as < 5% blasts), have received a minimum of two cycles of intensive chemotherapy (**Supplementary Material 3, Appendix 1**), and have received broad-spectrum antibiotics within three months of HCT**.** Inclusion and exclusion criteria are summarised below:

#### 2.4.1. Inclusion criteria:

1. Patients aged 18 years and over with a morphological documented diagnosis of ALL, AML, AL of ambiguous lineage, MDS, CMML, and CML in blast phase (**Supplementary Material 3, Appendix 2**) who are deemed fit for allogenic HCT with one of the following disease characteristics:

   *ALL, AML, AL of ambiguous lineage*

   - Patients in first complete remission (CR1) or second complete remission (CR2) including complete remission with incomplete blood count recovery with < 5% blasts (**Supplementary Material 3, Appendix 2**)

- Secondary leukaemia (defined as previous history of MDS, antecedent haematological disease or chemotherapy exposure) in CR1 or CR2 defined as < 5% blasts (**Supplementary Material 3, Appendix 2**)

*MDS and CMML*

- Patients with advanced or high risk MDS with an IPSS-M moderate high or higher including intermediate or high risk CMML who have < 5% blasts at the time of randomisation (**Supplementary Material 3, Appendix 2**)

*CML in blast phase*

- Patients with Philadelphia or BCR:ABL1 positive chronic myeloid leukaemia (CML) in blast phase defined by the presence of ≥ 20% blasts in blood or bone marrow who have achieved second chronic phase with < 5% blasts (**Supplementary Material 3, Appendix 2**).

2. Patients must have completed minimum of two cycles of intensive chemotherapy prior to trial enrolment (**Supplementary Material 3, Appendix 1**).
3. Patients must have received broad-spectrum antibiotics within 3 months prior to trial enrolment
4. Patients must be considered suitable/fit to undergo allogeneic HCT, as clinically judged by the Local investigator
5. Patients with a Karnofsky performance status score 60 or above (**Supplementary Material 3, Appendix 3**).
6. Females of and male patients of reproductive potential (i.e., not post-menopausal or surgically sterilised) must use appropriate, highly effective, contraception from the point of commencing therapy until 6 months after treatment
7. Patients have given written informed consent
8. Patients willing and able to comply with scheduled study visits and laboratory tests

**2.4.2. Exclusion criteria:**

1. Patients with contraindications to receiving allogeneic HCT.
2. Female patients who are pregnant or breastfeeding. All women of childbearing potential must have a negative pregnancy test before commencing treatment.
3. Adults of reproductive potential not willing to use appropriate, highly effective, contraception during the specified period.

4. Patients with renal or hepatic impairment as clinically judged by the Local Investigator.
5. Patients with active infection, HIV-positive or chronic active HBV or HCV.
6. Patients with a concurrent active malignancy or a prior malignancy, except lobular breast carcinoma *in situ*, fully resected basal cell or squamous cell carcinoma of skin or treated cervical carcinoma *in situ*, incidental histologic finding of prostate cancer (T1a or T1b using the tumour, node, metastasis (TNM) clinical staging system), previous MDS, CMML, MPN resulting in secondary AML. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed.
7. Swallowing difficulties that may preclude safe use of IMT capsules.
8. Administration of IMT within 3 months prior to enrolment (probiotic administration prior to enrolment is allowed but should be recorded at screening).
9. Patients taking probiotics after enrolment to the trial.
10. Gastrointestinal disorders and diseases, including delayed gastric emptying, coeliac disease, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, chronic diarrhoea, and colonic perforation or fistula.
11. Any autoimmune disease requiring, or that may require, systemic treatment with steroids and/or other immunosuppressants/immunomodulators.
12. Significant bleeding disorder (ALL, AML, AL of ambiguous lineage, MDS, CMML, and CML satisfying inclusion criteria are not excluded).
13. Anaphylactic food allergy.
14. Requirement for vasopressors.
15. Valvular heart disease or known structural defects of the heart.
16. Known severe allergy to capsule components.

**2.5. Interventions:**

**2.5.1. Allocation:**

50 adult patients will be allocated 1:1 between two groups:

- Capsulised IMT – as a single oral dose of 10 capsules of EBX-102-02 (i.e., dried full spectrum microbiota product derived from pooled human stool from screened donors), administered within two weeks of the initial study screening visit. Given the

immunosuppressed nature of the recipients, out of an abundance of caution, IMT material will be prepared from CMV-negative donors, as per suggestions from current guidelines[9].

- Matched capsulised placebo – containing microcrystalline cellulose and magnesium stearate; administered at the same point as capsulised IMT.

Randomisation will be performed centrally by the Imperial College Trials Unit – Cancer using OpenClinica electronic data capture system (EDC). To reduce relevant imbalances and increase statistical power, randomisation will be stratified by disease history (either: 1. patients known to have intestinal colonisation or blood-borne infection with multidrug-resistant organisms during previous therapy; or: 2. patients without this history).

Treatment with either IMT or placebo will take place at 14 (+/- 2) days prior to HCT. Both IMT and placebo will be stored in a refrigerator (at 2 – 8$^{o}$C) until administration, with temperature monitoring of the investigational medicinal product prior to administration. Study participants will be nil by mouth for at least 30 minutes prior to – and one hour after – each course of IMT/ placebo capsule administration. They will be asked to take each capsule with sips of water, and will be monitored for at least 15 minutes after capsule administration for complications (e.g. nausea).

**2.5.2. Blinding:**

Since this is a double-blind randomised placebo-controlled clinical trial, the treatment allocation will be blinded to the investigators, sponsor clinical trial management team, clinical staff, laboratory staff and the patient. Placebo capsules will be identical in appearance, weight, and all other obvious characteristics to the course of IMT, and will be handled by pharmacy identically; this will help in maintaining blinding. Trial randomisation will occur as soon as possible after satisfactory review and confirmation of patient eligibility at screening.

## 2.6. Outcomes:

The Schedule/ Summary of visits is shown in **Table 1.**

The primary outcome of the trial is the ability of the capsulised IMT given pre-HCT to increase and maintain intestinal microbiota diversity post-HCT. This will be assessed via measurement

of the difference between the change in alpha diversity (calculated using inverse Simpson index) 28 +/-3 days post-HCT from baseline for patients in the capsulised IMT groups *versus* the capsulised placebo group. The secondary objectives of the study relate to feasibility/ tolerability of the capsule, and impact of the IMT upon a range of clinically- and translationally-pertinent outcomes. These include: quality of life; microbiological/ infective outcomes; need for Intensive Care; and haematological outcomes, ranging from relapse, to GvHD, to impact upon engraftment and immune reconstitution. Microbiological/ infective outcomes will be assessed via conventional clinical microbiology techniques, as well as via gut microbiome diversity and taxonomic characterisation. These secondary outcomes are summarised in **Table 2.** In addition, the study has a range of discovery phase/ exploratory endpoints, including investigating the impact of IMT upon: markers of gut barrier function; metabolomic profiles in different biofluids; circulating cytokines; functionality of circulating monocytes and T cells.

The study flow chart/ participant timeline is shown in **Figure 1.** Of note, regardless of whether the patient is randomised to capsulised IMT or placebo, they will continue with their scheduled standard of care treatments/ assessments while also receiving study follow-up assessments at planned intervals, as shown in **Figure 1.** Other pre- and post-HCT care will be in accordance with the participating centres' policies. As such, patients are allowed to receive prophylactic antibiotics (such as ciprofloxacin) but should not receive broad-spectrum antibiotics after the trial treatment has taken place and prior to the start of HCT. It is recognised that this may not be always possible, as neutropenic fever may sometimes develop during the conditioning therapy. If this happens, patients will not be excluded from the trial, but the broad-spectrum antibiotic use and its duration must be documented at response assessments.

## 2.7. Data collection and management:

### 2.7.1. General approach:

CRFs for the study will be in English, using generic names for concomitant medications wherever possible. All written material to be used by participants must use vocabulary that is clearly understood and be in the language appropriate for the study site. The electronic case report form (eCRF) database will be in OpenClinica. The Investigator (or delegated

member of the site study team) will record all data relating to protocol assessments and procedures, laboratory, safety and efficacy data in the eCRF. All trial documentation, including that held at participating sites and the trial coordinating centre, will be archived for a minimum of 10 years following the end of the study.

**2.7.2. Confidentiality:**

The investigator will ensure that the participant's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator. The investigator shall permit direct access to participants' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and RECs.

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing and disclosure of personal information, and will uphold the Act's core principles.

**2.7.3. Oversight and monitoring:**

A Trial Steering Committee (TSC) will be convened, including as a minimum an independent Chair, independent clinician, the Chief Investigator, Independent Statistician, Trial Manager and PPI Representative. The role of the TSC is to provide overall supervision of trial conduct and progress. A Trial Management Group (TMG) will also be convened, including the Chief Investigator, co-investigators and key collaborators, trial statistician and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Furthermore, an Independent Data Monitoring Committee (IDMC) will be convened to include as a minimum an independent oncologist chair, an independent oncologist and an independent statistician. The role of the IDMC is advisory to the TSC, to ensure the highest standard of patient safety and data integrity.

The IDMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable, or if any issues are identified which may compromise patient

safety. In the case of early discontinuation of the study, response assessments will be completed for each participant, as far as possible.

The study will be monitored periodically by trial monitors to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy, and consistency of the data. Monitoring will be conducted centrally/remotely from the coordination centre and on-site. Monitoring procedures and requirements will be documented in a Monitoring Plan, developed in accordance with the risk assessment.

Quality Control will be performed according to Imperial College Trials Unit (ICTU) internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

The study may be participant to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

### 2.8. Statistical considerations:

#### 2.8.1. Sample size and powering:

Currently published data on alpha diversity change in patients undergoing HCT and/or IMT span different study types, vary in quality/granularity, and use different alpha diversity indices. The evidence available suggests larger decreases in alpha diversity at approximately one month post-HCT compared to baseline in patients who do not receive IMT relative to those that do[2 6 8 18 19]. [2] Fitting a mixed-effects model (with fixed effects for arm, time (day), their corresponding interaction and a random per-patient intercept effect) with quadratic splines at 5 degrees-of-freedom (3 internal knots) to longitudinal change in alpha diversity data (measured with inverse Simpson's index) from baseline[2], IMT patients had an expected change in baseline alpha diversity at day 28 post-HCT 3.46 (pooled SE = 2.19) units more than placebo (IMT mean change = -4.70, SE = 1.44, n = 14; placebo mean change = -8.16, SE = 1.66, n = 11). We have used these results on IMT post-HCT to design our study of IMT pre-HCT vs. placebo.

Our null hypothesis is there is no difference between the change in day 28 ± 3 days alpha diversity (inverse Simpson's) post-HCT from baseline in patients receiving pre-HCT IMT compared to patients receiving placebo capsules (i.e., difference in between-arm changes from baseline is zero). Using a two-sample t-test to compare IMT-arm change to placebo-arm change with two-sided alpha controlled at 20%[20 21], we need 46 patients randomised 1:1 between IMT and placebo (23 per arm) to have ≥80% power to detect a between-arm difference of 3.46 units (with pooled standard deviation of 5.45 estimated at day 28 post-HCT from mixed-effects model). To account for dropouts at a rate of up to 8% across both arms, we will recruit 50 patients in total. Modelling and sample size calculations have been performed using R v3.6.1.

**2.8.2. Statistical analysis:**

**2.8.2.1. Overall approach:**

Statistical analyses will be formally documented within a detailed Statistical Analysis Plan (SAP) and structured using the estimand framework (as described in the ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials[21 22]). Any deviations from the SAP will be documented and signed off by the statisticians and CI, and filed in the Trial Master File (TMF).

**2.8.2.2. Primary Estimand:**

*Population:* Adults with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), acute leukaemia (AL) of ambiguous lineage, high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML), and chronic myeloid leukaemia (CML) in blast phase, considered suitable/fit for allogeneic haematopoietic stem cell transplantation HCT.

*Treatment:* Capsulised IMT vs matched capsulised placebo.

*Variable:* Stool microbiota diversity post-HCT defined as the change in alpha diversity (measured as inverse Simpson's index) between IMT administration (at 14 +/- 3 days prior to HCT) and 28 +/-3 days after HCT.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at day 28.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy will be used to handle patient all defined intercurrent events, any patient response-assessment data collected post-randomisation will still be used in the analysis model if day 28 data is unavailable.

**2.8.2.3. Secondary Estimands:**

1) A complete case analysis will be undertaken of the primary outcome (using those with data recorded at all response-assessment timepoints).

*Population:* As per primary estimand.
*Treatment:* As per primary estimand.
*Variable:* As per primary estimand.
*Population-level summary:* As per primary estimand.
*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.
*Strategy to handle intercurrent events:* Treatment policy will be used in the event of adverse events or rescue medication. In the event of death, withdrawal or loss-to-follow-up a principal stratum strategy is to be followed such that only those that complete the assessment period are to be included for analysis.

2) Assessment of changes in inverse Simpson's index and other measures of gut microbiome diversity across all timepoints assessed, including alpha diversity and richness (i.e., as measured via Chao-1, Shannon, Faith's PD), and beta-diversity (Aitchinson's distance).

*Population:* As per primary estimand.
*Variable(s):* As per primary, plus Chao-1, Shannon, Faith's PD, Aitchinson's distance.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at days 7 & 14. Interaction effect for follow-up visit timepoints day 100, 200 and 365 will also be assessed.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use.

*Strategy to handle intercurrent events:* Treatment policy as primary estimand.

3) Haematological outcomes across all timepoints measured, including: non-relapse mortality, relapse incidence; occurrence and severity of graft-versus-host disease (GvHD), overall and GvHD-free relapse-free survival, and quality of life.

*Population:* As per primary estimand.

*Variable(s):* Overall and GvHD-free relapse-free survival.

*Population-level summary:* Log-rank test statistic, hazard-ratio (with 95% CI).

*Intercurrent Events:* Death unrelated to patient comorbidity (relapse-free survival only), death related to patient comorbidity (relapse-free survival only), loss-to-follow-up/patient withdrawal, adverse/serious adverse events, rescue therapy outside of antibiotic use.

*Strategy to handle intercurrent events:* Death unrelated to patient comorbidity (relapse-free survival only) will be censored at recorded time-of-death as part of a hypothetical strategy. Where death is potentially related, a composite strategy is to be considered where time-of-death will be taken as time-of-relapse. Loss-to-follow-up/patient withdrawal will be censored at time of last contact as part of a hypothetical strategy. Treatment policy will be used upon use of rescue therapy or under any adverse/serious adverse event which does not result in the withdrawal of the patient.

**2.8.2.4. Analysis of primary estimand:**

The primary outcome of between-arm difference in alpha diversity change from baseline at day 28 (± 3 days) will be analysed using a mixed-effects model, with change in alpha diversity from baseline as outcome, with treatment arm, time, treatment-by-time interactions and stratification variables used in randomisation included as fixed effects and also a per-patient intercept included as a random effect. The subsequent model estimate for the treatment-by-time interaction term at day 28 will be the effect of interest as per **Section 2.8.2.2.**

**2.8.2.5. Analysis of secondary estimands:**

The complete-case analysis will follow the same model as defined in **Section 2.8.2.4** (using only patients attending all visits as per estimand). Mixed-effect models incorporating a per-patient random effect alongside effects for time of assessment, and an interaction term of time-by-arm assessing changes in alpha diversity (inverse Simpson's index, Chao-1, Shannon index, Faith's PD) and β-diversity will provide treatment effects and 80% confidence intervals at Response assessments 1-5 and Follow-up assessments 1-3 (see **Figure 1**). Similar approaches will be used to assess changes in gut microbiome taxonomic composition based on shallow shotgun sequencing.

Overall survival (time from randomisation to death/date last seen alive) will be analysed using Kaplan-Meier methods and log-rank testing utilising the same stratification variables as per primary analysis model defined in **Section 2.8.2.4.** Additional survival analysis will include non-relapse mortality and GvHD-free relapse-free survival.

**2.8.2.6. Descriptive analysis of secondary outcome measures:**

Tolerability and acceptability of IMT/placebo will be descriptive, using patient perspective questionnaires. Additional descriptive analyses of clinical outcomes will include: number of days spent in Intensive Care; presence and severity of mucositis and length of time requiring parenteral nutrition; days of fever post-HCT corrected for length of admission; days of antibiotics including carbapenem; number and length of bloodstream infections; colonisation with multi-drug resistant bacteria, including extended-spectrum β-lactamases, vancomycin-resistant *Enterococci*, and carbapenemase-producing *Enterobacteriaceae*; and incidence of GvHD; and relapse incidence.

**2.8.2.7. Safety analysis:**

Additional safety outcomes - including AEs, ARs, SAE and SUSARs - will be reported as frequencies, unadjusted participant proportions and/or rates where appropriate. Differences between arms with 95% confidence intervals using exact methods will be produced where appropriate.

*BMJ Open – Protocol:*

## 2.9. Publication and Dissemination:

All publications will be in concordance with Consort guidelines/ checklists. Study participants will be notified of the outcome of the trial prior to any publications; the format of the dissemination will be based on guidance from the PPI Group at the time. A Clinical Study Report summarising the study results will be prepared and submitted to the REC within a year of the end of study. The results will also be submitted to the EudraCT results database in accordance with regulatory requirements.

## 3. Discussion:

The increasing recognition of the contribution of the gut microbiome in patients with haematological malignancies undergoing cellular therapies, coupled with emergent data supporting IMT as a strategy to alter the microbiome, necessitates robust placebo-controlled IMT trials. Primarily, phase 2a trials such as MAST aim to fully evaluate the specific contribution that IMT have as part of patient treatment and provide the launchpad for future phase 3 trials. We hope that associated microbiome, metabolomic and immune analyses will improve understanding of the mechanistic contribution of the gut microbiome to the clinical outcomes seen, potentially setting the stage for future novel targeted 'microbiome therapeutics' that avoid the drawbacks associated with IMT.

A growing body of non-randomised studies has described positive clinical signals when IMT was used in patients with haematological malignancies undergoing HCT[8]. However, it was also noteworthy that a recent phase II randomised double-blind placebo-controlled trial[23], administering capsulised IMT or placebo after HCT for AML, timing this for after neutrophil recovery, failed to achieve its primary outcome, showing no statistical difference in the infection rate by four months post-HCT in the IMT arm compared to placebo. One fundamental difference in design between that study and our study is that in our trial, the IMT is targeted at the pre- (rather than post-) HCT period. There were several reasons for us considering that this aspect of timing is particularly important. Most importantly, the published data related to the dynamics of the gut microbiome with HCT particularly demonstrate the close association between reduced gut microbiome diversity pre-HCT and future morbidity and mortality, as well as the emergence of *Enterococcus* domination within the gut microbiome within three weeks post-allogeneic HCT as influencing poor outcome[6 7].

Additionally, aberrant intestinal microbiome diversity is known to be associated with increased inflammatory response[24] and biomarkers of inflammation measured pre HCT were shown to be independent predictors of HCT outcomes[25].

Therefore, we concluded that the clearest window for intervention is pre-HCT, aiming to increase the pre-HCT gut microbiota diversity, and mitigate the risk of pathobiont overgrowth is prior to start of HCT conditioning (**Figure 2)**. The concept of targeting IMT prior to intervention has also been used successfully in oncology, with a phase 1 study evaluating IMT use prior to immune checkpoint inhibition in 20 patients with advanced melanoma demonstrating an objective response rate of 65% (*n*=13/20; including 4/20 complete responses)[26].

The use of IMT in the context of immunosuppressed patients requires certain considerations above and beyond those of, for instance, conventional use of IMT in treating recurrent CDI[27]. The use of a capsulised preparation is clearly more acceptable to this patient cohort than conventional IMT slurry, and may be safer avoiding potential aspiration of slurry. The donor screening protocol used donors is in full accordance with UK recommendations[9]; while the risk of CMV transmission via IMT appears extremely low[28], CMV negative donors are being used out of an abundance of caution. The window for IMT administration aims to be long enough after prior chemotherapy to allow full cell count recovery, but early enough before HCT to permit sufficient microbiota engraftment. This is important, since degree of microbiota engraftment has been associated with level of clinical improvement after IMT[29]. Our experience to date is that IMT mitigates the risk of invasive infections related to MDROs rather than decolonises them from the gut[15], but there is still uncertainty regarding this; one recent trial of IMT in a renal transplant population suggested certain ESBL-colonising strains being displaced by non-ESBL strains by strain competition[30]. The serial clinical assessment and collection of shotgun metagenomic data from study participants in a placebo-controlled fashion will allow much more granular assessment of the impact of IMT on MDROs than has been described previously.

In conclusion, the MAST trial aspires to give new clinical and translational insights into the role of gut microbiome manipulation in patients with haematological malignancy receiving

allogeneic HCT, with particular focus on the potential role of IMT on haematological and infective outcomes. The study aims to run recruitment for 24 months post-authorisation, and close in March 2027.

## 4. Ethics statement:

The institutional review board (North East - Tyne & Wear South, England, Ref: 23/NE/0105) and the national regulatory authorities, Medicines & Healthcare products Regulatory Agency (MHRA, Ref: CTA 19174/0441/001-0001) issued approval on the 3rd October 2023.

## 5. Author contributions:

BHM, AJI, RG, FJD, SA-B, NAJ, JRM, JP all contributed to initial drafting of the manuscript, with all authors reviewing and approving the final submitted manuscript. JP conceptualised the trial protocol and is responsible for its clinical aspects. BHM and JRM provided expertise in IMT provision and microbiome/ metabolome analysis for the study. AJI and JP were responsible for analysis of all haematological aspects of the study, both clinical and translational, and contributed to set-up of recruiting centres. RG and FJD provided expertise regarding microbiological/ infection-related aspects of outcome and analysis in the study. LAR provided input into patient-facing materials for the trial and oversees translational and exploratory outcomes. SA-B and LW both contributed to all aspects of trial approval and administrative/ logistical set-up. GW and NAJ oversaw all aspects of statistical analysis within the trial. PF, ABK, FK, PK (Kottaridis), PK (Krishnamurthy), EN and RP are all site principal investigators for the studies

## 6. Acknowledgements:

The authors are grateful to Dr James McIlroy and Dr Michael Smyth (both from EnteroBiotix Ltd) for review of this manuscript.

## 7. Data Statement:

The full version of the current protocol (v1.2, 17th January 2024) is available from the corresponding author on reasonable request. The trial website is available at: https://www.imperial.ac.uk/metabolism-digestion-reproduction/research/digestive-diseases/hepatology--gastroenterology/mast-study/ .

## 8. Figure legends:

**Figure 1: Study flow chart participant time line.**

**Figure 2: 'Prehabilitation' of the gut microbiome in MAST.** Dynamics of the gut microbiota conventionally through the peri-HCT period shown in black (as defined previously[6]); the red line is our predicted higher starting point and nadir for patients receiving a pre-HCT IMT in the MAST trial.

*BMJ Open – Protocol:*

## 9. References:

1. Stoma I, Littmann ER, Peled JU, et al. Compositional Flux Within the Intestinal Microbiota and Risk for Bloodstream Infection With Gram-negative Bacteria. *Clin Infect Dis* 2021;73(11):e4627-e35. doi: 10.1093/cid/ciaa068
2. Taur Y, Coyte K, Schluter J, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. *Sci Transl Med* 2018;10(460) doi: 10.1126/scitranslmed.aap9489
3. Tacconelli E, De Angelis G, Cataldo MA, et al. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. *Antimicrob Agents Chemother* 2009;53(10):4264-9. doi: 10.1128/AAC.00431-09 [published Online First: 20090810]
4. Danielsen AS, Franconeri L, Page S, et al. Clinical outcomes of antimicrobial resistance in cancer patients: a systematic review of multivariable models. *BMC Infect Dis* 2023;23(1):247. doi: 10.1186/s12879-023-08182-3 [published Online First: 20230418]
5. Shono Y, Docampo MD, Peled JU, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Sci Transl Med* 2016;8(339):339ra71. doi: 10.1126/scitranslmed.aaf2311
6. Peled JU, Gomes ALC, Devlin SM, et al. Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. *N Engl J Med* 2020;382(9):822-34. doi: 10.1056/NEJMoa1900623
7. Stein-Thoeringer CK, Nichols KB, Lazrak A, et al. Lactose drives Enterococcus expansion to promote graft-versus-host disease. *Science* 2019;366(6469):1143-49. doi: 10.1126/science.aax3760
8. Malard F, Gaugler B, Mohty M. Faecal microbiota transplantation in patients with haematological malignancies undergoing cellular therapies: from translational research to routine clinical practice. *Lancet Haematol* 2022;9(10):e776-e85. doi: 10.1016/S2352-3026(22)00223-X
9. Mullish BH, Merrick B, Quraishi MN, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridioides difficile infection and other potential indications: second edition of joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut* 2024;73(7):1052-75. doi: 10.1136/gutjnl-2023-331550 [published Online First: 20240606]
10. Allegretti JR, Khanna S, Mullish BH, et al. The Progression of Microbiome Therapeutics for the Management of Gastrointestinal Diseases and Beyond. *Gastroenterology* 2024 doi: 10.1053/j.gastro.2024.05.004 [published Online First: 20240514]
11. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *N Engl J Med* 2019;381(21):2043-50. doi: 10.1056/NEJMoa1910437 [published Online First: 20191030]

12. Bilinski J, Grzesiowski P, Sorensen N, et al. Fecal Microbiota Transplantation in Patients With Blood Disorders Inhibits Gut Colonization With Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clin Infect Dis* 2017;65(3):364-70. doi: 10.1093/cid/cix252
13. Millan B, Park H, Hotte N, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. *Clin Infect Dis* 2016;62(12):1479-86. doi: 10.1093/cid/ciw185 [published Online First: 20160329]
14. Innes AJ, Mullish BH, Fernando F, et al. Faecal microbiota transplant: a novel biological approach to extensively drug-resistant organism-related non-relapse mortality. *Bone Marrow Transplant* 2017;52(10):1452-54. doi: 10.1038/bmt.2017.151 [published Online First: 20170717]
15. Ghani R, Mullish BH, McDonald JAK, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. *Clin Infect Dis* 2021;72(8):1444-47. doi: 10.1093/cid/ciaa948
16. Innes AJ, Mullish BH, Ghani R, et al. Fecal Microbiota Transplant Mitigates Adverse Outcomes Seen in Patients Colonized With Multidrug-Resistant Organisms Undergoing Allogeneic Hematopoietic Cell Transplantation. *Front Cell Infect Microbiol* 2021;11:684659. doi: 10.3389/fcimb.2021.684659 [published Online First: 20210827]
17. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586. doi: 10.1136/bmj.e7586 [published Online First: 20130108]
18. van Lier YF, Davids M, Haverkate NJE, et al. Donor fecal microbiota transplantation ameliorates intestinal graft-versus-host disease in allogeneic hematopoietic cell transplant recipients. *Sci Transl Med* 2020;12(556) doi: 10.1126/scitranslmed.aaz8926
19. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368(5):407-15. doi: 10.1056/NEJMoa1205037 [published Online First: 20130116]
20. Rubinstein LV, Korn EL, Freidlin B, et al. Design issues of randomized phase II trials and a proposal for phase II screening trials. *J Clin Oncol* 2005;23(28):7199-206. doi: 10.1200/JCO.2005.01.149
21. Sargent DJ, Taylor JM. Current issues in oncology drug development, with a focus on Phase II trials. *J Biopharm Stat* 2009;19(3):556-62. doi: 10.1080/10543400902802474
22. E9 (R1) Step 5 addendum on estimands and Sensitivity Analysis in Clinical Trials to the guideline on statistical principles for clinical trials.
23. Rashidi A, Ebadi M, Rehman TU, et al. Randomized Double-Blind Phase II Trial of Fecal Microbiota Transplantation Versus Placebo in Allogeneic Hematopoietic Cell Transplantation and AML. *J Clin Oncol* 2023;41(34):5306-19. doi: 10.1200/JCO.22.02366 [published Online First: 20230526]
24. Tilg H, Zmora N, Adolph TE, et al. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* 2020;20(1):40-54. doi: 10.1038/s41577-019-0198-4 [published Online First: 20190806]
25. Patel A, Szydlo RM, Auner HW, et al. C-reactive protein prior to myeloablative allogeneic haematopoietic cell transplantation identifies patients at risk of early- and long-term mortality. *Br J Haematol* 2018;180(6):889-92. doi: 10.1111/bjh.14454 [published Online First: 20161129]

26. Routy B, Lenehan JG, Miller WH, Jr., et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. *Nat Med* 2023;29(8):2121-32. doi: 10.1038/s41591-023-02453-x [published Online First: 20230706]
27. Ghani R, Mullish BH, Davies FJ, et al. How to adapt an intestinal microbiota transplantation programme to reduce the risk of invasive multidrug-resistant infection. *Clin Microbiol Infect* 2022;28(4):502-12. doi: 10.1016/j.cmi.2021.11.006 [published Online First: 20211123]
28. Galperine T, Engelmann I, Hantz S, et al. Cytomegalovirus in donors for fecal microbiota transplantation, the phantom menace? *PLoS One* 2023;18(6):e0287847. doi: 10.1371/journal.pone.0287847 [published Online First: 20230629]
29. Ianiro G, Puncochar M, Karcher N, et al. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. *Nat Med* 2022;28(9):1913-23. doi: 10.1038/s41591-022-01964-3 [published Online First: 20220915]
30. Woodworth MH, Conrad RE, Haldopoulos M, et al. Fecal microbiota transplantation promotes reduction of antimicrobial resistance by strain replacement. *Sci Transl Med* 2023;15(720):eabo2750. doi: 10.1126/scitranslmed.abo2750 [published Online First: 20231101]

**Table 1: Schedule/ Summary of Visits:** *Continued overleaf*

| | Screening | Treatment | Response Assessment: | | | | | Follow-up Assessment: | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 |
| **Visit** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **Day of HCT** | From -42 | -14 (± 2 days) | -7 (± 2) | 0 (± 1) | +7 (± 3) | +14 (± 3) | +21 (± 3) | +100 (± 7) | +200 (± 7) | +365 (± 14) |
| Informed Consent | X | | | | | | | | | |
| Inclusion & Exclusion Criteria | X | | | | | | | | | |
| Baseline data collection/Comorbidity Index | X | | | | | | | | | |
| Review of demographics, medical/disease | X | | | | | | | | | |
| Pregnancy test[1] | X | | | | | | | | | |
| EORTC-QLQ-C30 and EQ-5D-5L Questionnaires | X | | | | | | X | X | X | X |
| Bone marrow assessment[2] | X | | | | | | | X | | X |
| Physical Examination/Vital Signs (ECG) | <<All Assessments (According to standard care practices)>> | | | | | | | | | |
| Lineage specific chimaerism[3] | | | | | | | | X | X | X |
| Lymphocyte subsets & IG levels[3] | | | | | | | | X | X | X |
| Stool Sample | X | | X | X | X | X | X | X | X | X |
| Urine Sample | X | | X | X | X | X | X | X | X | X |

*BMJ Open – Protocol:*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Blood Sample | X | X | X | X | X | X | X | X | X | X |
| Clinical data collection[5] | | X | X | X | X | X | X | X | X | X |
| Adverse event assessment[4] | << continuous assessment >> | | | | | | | | | |
| Assessment of GvHD | | | | | << continuous assessment >> | | | | | |
| Cell infusion (HCT)[6] | | | | X | | | | | | |

# 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 and sample collections may be arranged within the ranges as indicated above without need to report as protocol deviation.

1. Pregnancy test for women of childbearing potential: serum/urine (investigator's discretion) pregnancy test (sensitivity of at least 25 mIU/mL) within 72 hours prior to starting study therapy. This applies even if the patient practices complete abstinence from heterosexual contact.
2. The results of bone marrow morphological, immunophenotypic, cytogenetic, and molecular characterisation performed according to local practice within the time points above should be reported within the time points above.
3. Chimaerism tests should be performed in local laboratories on day +30, +60, +90, +120, +200, and +365, lymphocyte subsets and immunoglobulin levels should be performed in local laboratories on days +100, +200, +365.
4. All AEs to be collected from written to consent to the first day of transplantation conditioning. After initiation of transplantation conditioning only AEs that are equal to or greater than Grade 3 of the CTCAE version 5.0 will be reported (unless the event meets the definition of an SAE) and abnormal laboratory findings will be reported only if they are judged to be of significant clinical importance. Reporting will stop at day +28 of transplantation. SAEs that are judged to be at least possibly related to the IMP(s) and are unexpected must still be reported in an expedited manner irrespective of how long after IMP administration the reaction occurred.
5. Collection of clinical data (see **Supplementary Material 3, Appendix 4** for summary of Assessments),

The following data will be collected at all study Visits (1-10)– Vital Signs, Physical Examination, Full Blood Count, Coagulation, Biochemistry, and Virology, Nutrition, Completed dietary questionnaire and ITU Admission.

The following data below will be collected in addition to the repeating assessments,

- Recent, Microbiology Colonisation History, Fever, Infection and Treatment History (Visits 1-10)
- Haemopoietic Cell transplant details (Visit 5 only).
- VOD, and Relapse, Engraftment, Acute GvHD, GvHD Prophylaxis and Therapy assessments (Visits 5-10).
- Post-transplant intervention assessment (Visit 10 only).

6. Haematopoietic stem cell transplant is not a study procedure and will take place as planned by the multidisciplinary team before the patient enters the study following local standard of care procedures.

*BMJ Open – Protocol:*

**Table 2: Secondary objectives/ outcomes from the MAST study:**

| Objectives: | Outcome: |
|---|---|
| Determine the feasibility and tolerability of capsule IMT prior to HCT in a multi-centre setting. | Tolerability and acceptability of IMT/placebo (as assessed via patient perspective questionnaires, i.e. EQ-5D-5L and EORTC QLQ-C30 questionnaires). |
| Evaluate microbiological/ infective, haematological, and quality of life-related clinical outcomes of administering IMT prior to HCT. | Gut microbiome endpoints:<br>• Assessment of changes in inverse Simpson's index and other measures of gut microbiome diversity across all timepoints assessed, including alpha diversity and richness (i.e., as measured via Chao-1, Shannon, Faith's PD), and beta-diversity (Aitchinson's distance)<br>• Assessment of changes in gut microbiome taxonomic composition across all timepoints assessed (using shallow shotgun sequencing). |

*BMJ Open – Protocol:*

| | |
|---|---|
| | Clinical endpoints:<br>• Markers of general health across all timepoints measured, including: days on the Intensive Treatment Unit (ITU); presence and severity of mucositis; use of (and length of time that requiring) parenteral nutrition; severe acute kidney injury and severe liver dysfunction.<br>• Infective/ microbiological outcomes across all timepoints measured, including: days of fever post-HCT (corrected for length of admission); days on antibiotics (including use of carbapenem specifically); number and length of bloodstream infections; urinary tract infections; colonisation with multi-drug resistant bacteria (MDROs; including extended-spectrum beta-lactamases (ESBL), vancomycin-resistant enterococci (VRE), and carbapenemase-producing Enterobacteriales (CPE)), and use of antibiotics.<br>• Haematological outcomes across all timepoints measured, including: non-relapse mortality, relapse incidence; occurrence and severity of graft-versus=host disease (GvHD), overall and GvHD-free relapse-free survival, and quality of life. |
| Explore the potential for pre-HCT IMT to impact on HCT engraftment and immune reconstitution. | Neutrophil and platelet engraftment data as defined by EBMT will be routinely collected. Recovery of T-cell chimaerisms, T-cell count assessed by the lymphocyte subset analysis and immunoglobulin levels will be recorded at follow-up assessments. |

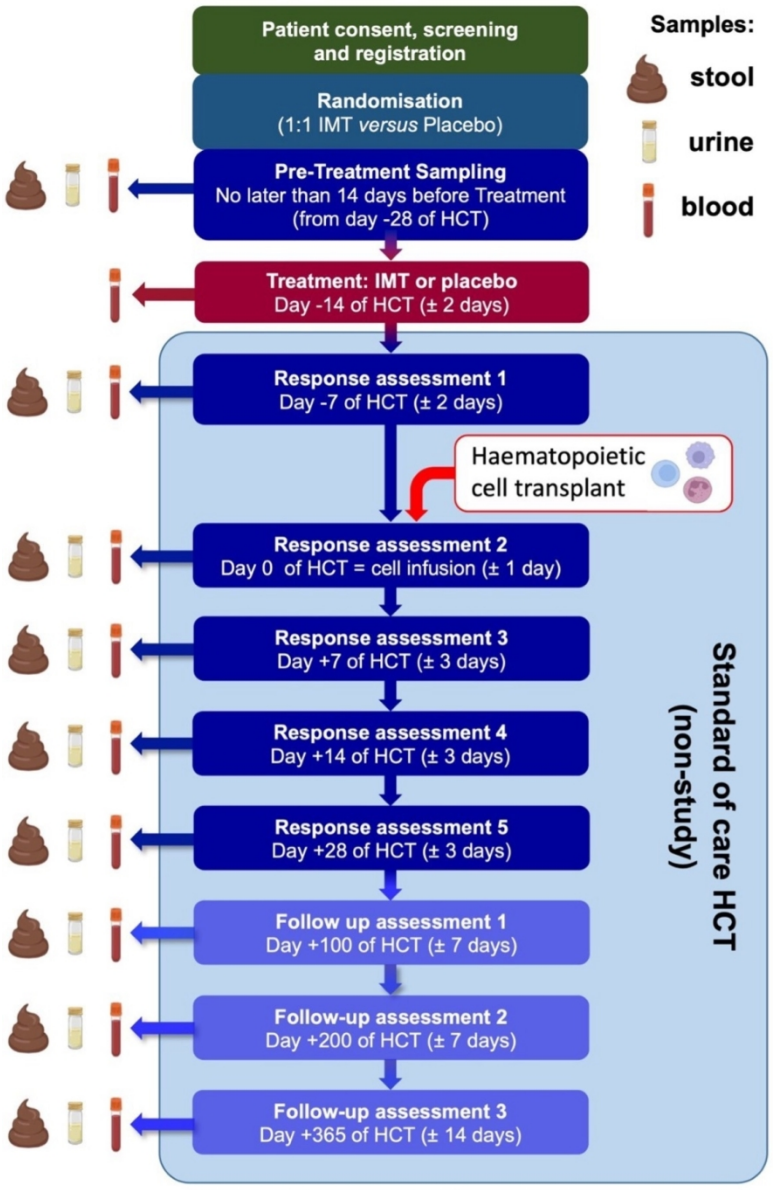



Figure 1

136x209mm (220 x 220 DPI)

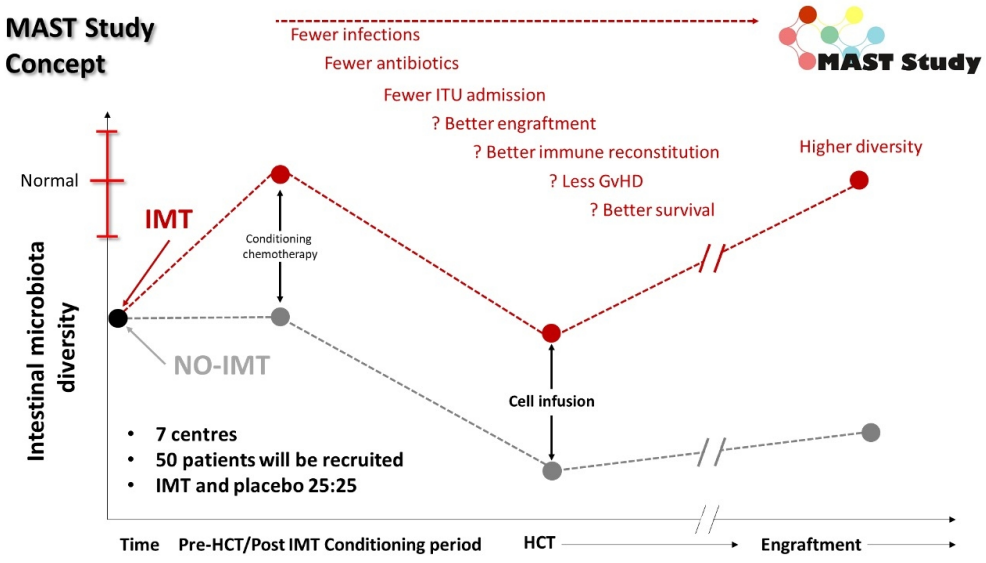



Figure 2

216x123mm (150 x 150 DPI)

[Insert Site Logo]

# MAST PATIENT INFORMATION SHEET

| | |
|---|---|
| **Study Title** | Microbiota Transplant Prior to Allogeneic Stem Cell Transplantation (MAST) trial |
| **IRAS Project ID** | 1006971 |

## Introduction:

You are being invited to take part in a research study. Before you decide whether you wish to take part, it is important for you to understand why the research is being done and what it will involve. Someone from our team will go through the information sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with friends, relatives, your General Practitioner (GP) and other doctors involved in your clinical care if you wish.

**Part 1** tells you the purpose of this study and what will happen to you if you take part.

**Part 2** gives you more detailed information about the conduct of the study

Please, ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

**Thank you for taking the time to read this information sheet.**

[Insert Site Logo]

## Glossary of Terms

| Term | Explanation |
|---|---|
| CFU | Colony forming units – Is the term used to describe the number of viable microorganisms, e.g., bacteria, there are in the capsule |
| Haematopoietic cell transplant | The clinical name for a bone marrow or blood stem cell transplant to treat blood cancer such as leukaemia. |
| Intestinal microbiota transplant | Taking stool material from a healthy donor and processing it into a capsule form for oral use. |
| Investigator | A researcher involved in a clinical study. |
| Microbiota | A collection of microorganisms that live in and on human body. |
| Microorganisms | Small organisms such as bacteria, virus particles and other single cell organisms. |
| Organism | A form of life considered as an entity, such as an animal, plant, fungus or bacterium. |
| Placebo | A substance that has no therapeutic effect, used as a control in testing new drugs |
| Plasma | The liquid part of blood that is left after all blood cells have been removed and only a clotting protein (called Fibrin) remains. |
| Phlebotomy | The procedure of drawing blood from the vein with the use of sterile material by trained and qualified healthcare personnel. |
| Sample | A small part of a substance or material obtained for testing such as blood, urine and stool/faecal material. |
| Serum | The liquid part of blood, after all, blood cells and the clotting protein (Fibrin) have been removed. |

[Insert Site Logo]

MAST Study

# PART 1

## 1. What is the purpose of this study?

Doctors and scientists have realised recently that there are billions of 'beneficial' bacteria and other microbes living in the human gut. These microbes do not cause us harm, but actually perform many roles in helping to keep us healthy, such as through their effects on how we process food or energy, stopping us getting infections from gut bacteria, and in how our immune system works.

When antibiotics are given to patients with blood cancers, they have a side effect of reducing the numbers of 'beneficial bacteria' in the gut, limiting its supportive role for the immune system. The number of 'beneficial' gut bacteria are important to maintain in patients who receive treatment that further impacts the immune system, such as bone marrow transplant (haematopoietic cell transplant).

The MAST clinical trial will test a way of restoration of the normal balance and diversity (range) of microbes that live in the gut prior to starting bone marrow or blood stem cell transplant (haematopoietic cell transplant). The study will also examine how this treatment affects the many complications involved in bone marrow transplantation, such as fevers (high temperatures) and infections during the transplant period. The treatment is called intestinal microbiota transplantation and involves taking bacteria from healthy people's gut, then processing it and putting it into a capsule which when swallowed, releases the microbes into the recipient.

[Insert Site Logo]

## 2. Why have I been chosen?

You are being invited to take part in the research study because you will be undergoing a haematopoietic cell transplant, as part of the normal treatment for blood cancer, and because of your previous treatment (chemotherapy), you are predicted to have a lower number, and smaller range (diversity) of bacteria (microbiota) in your gut (intestines). We are looking to recruit 50 participants in total to this study. These 50 people will have acute leukaemia (AML or ALL), advanced myelodysplastic syndromes, chronic myelomonocytic leukaemia (CMML), or chronic myeloid leukaemia (CML) in blast phase and will be undergoing standard treatment (bone marrow or blood stem cell transplant) for their disease. Please read this information carefully before you decide whether to participate and ask your doctor for an explanation of anything that is not clear to you.

## 3. Do I have to take part?

It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive from your doctor or the hospital.

## 4. What will happen to me if I take part?

You will be approached about entering the study before you are scheduled to have bone marrow transplantation. If you take part in this study, you will be asked to follow the study treatment plan, tests and hospital appointments for 14months). You should consider how these tests and visits will affect your work and family life and decide if you are able to commit to them.

Sometimes because we do not know which way of treating patients is best, we need to make comparisons. People will be put into groups and then compared.

The groups are selected by a computer which has no information about the individual – i.e. by chance. Subjects in each group then have a different treatment and these are compared'.

This means you have a 1 in 2 (50%) chance of receiving the treatment. Neither you nor your doctor will know which treatment group you are in (although, if your doctor needs to find out he/she can do so).

MAST Study

[Insert Site Logo]

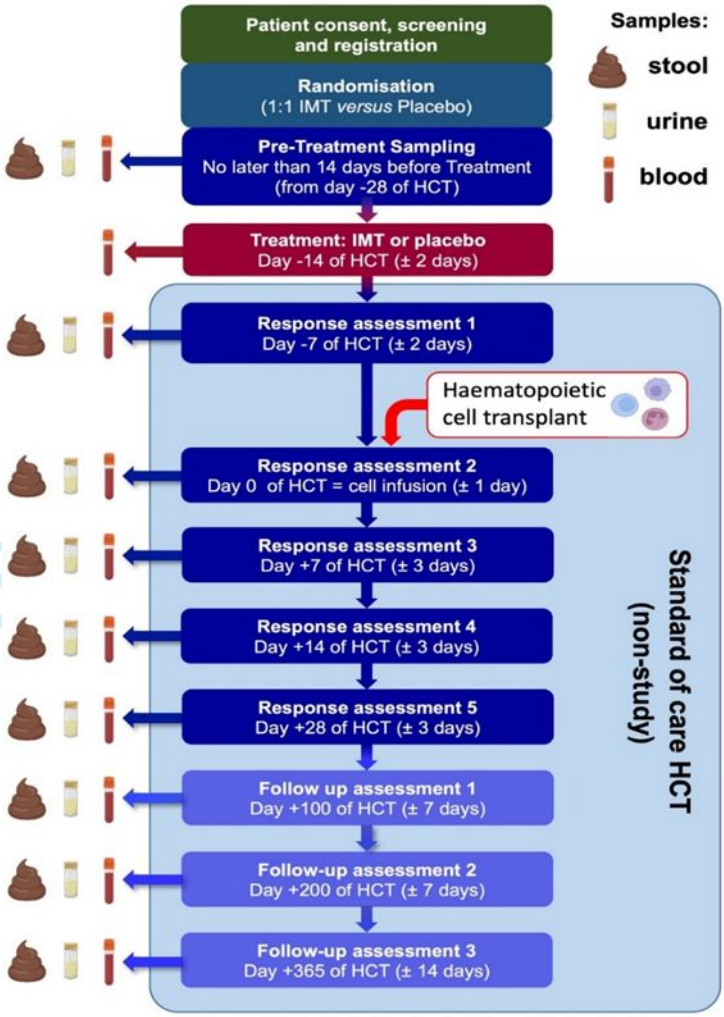
**The study will undertake the following:**

1. Recruit 50 people with blood cancers who had treatment with chemotherapy and are about to undergo a bone marrow transplant.

2. Take 25 randomly from this group and give them the intestinal microbiota transplant using an orally taken capsule and compare them to the remaining 25 patients who will be given a placebo capsules. The capsules will be taken prior to the bone marrow transplant

3. Collect stool, blood and urine from both groups for analysis over the period of their treatment.

4. Undertake health and quality of life assessments for up to a year after their intestinal microbiota transplant and bone marrow transplant (see the schedule to the right).




## 4.1. What will happen before I enter the trial?

**Initial Study Consultation**- An initial consultation will take place to discuss participation in the study on the phone or on site with a member of our team. We will ask you some questions to see if you would be suitable to join the study, which will last approximately **15** minutes.

If we decide from the initial assessment that you are not eligible to take part in the study from the initial visit, you will unfortunately not be able to take part in the study and will continue with your planned standard of care treatment.

MAST Study

[Insert Site Logo]

If we decide from the initial assessment that you may be eligible to take part in the study, we will invite you to attend a full screening visit **(Visit 1)**. We will also explain that we wish to collect a stool sample from you ahead of the next visit. We will provide you with a stool collection kit and instructions, to collect a sample at home 24 hours before or on the day of the next visit. Should you need support a family member or friend at home can help you with this or a nurse at your next study visit can help you with providing a sample.

## 4.2. What happens once you are confirmed suitable to take part?

**Visit 1: Consent and Screening (within 14 days before randomisation)** – After the Initial Study Consultation, you will be invited to attend a screening visit. If you are interested in joining the study, you will be asked to sign and date the study consent form. We will perform several tests to check you are eligible for the study. All the screening tests will be explained below.

**Screening Assessments (30-60 minutes):**

A review of your medical history and any medications you are taking or have recently taken (e.g., anti-cancer treatments, over-the-counter treatments including herbal or dietary supplements, prescription medications, and/or illegal drugs)

- **A physical examination** including height and weight.
- **An assessment of your vital signs** (tests to see how well your body is functioning) including blood pressure and pulse.
- **Collect dietary information** If you have been able to give a stool sample at this visit, we will ask you to complete a dietary questionnaire to report what you have eaten 24 hours before the collection of your stool sample.
- **Quality of life Assessment** – We will ask you to complete a questionnaire to collect this information.

At this visit, we will also collect the following samples from you:

- **Blood** – A blood sample for research purposes (2 tablespoons which is 30ml) will be collected in addition to your routine blood tests.
- **Urine** – A sample kit will be given to you to provide a sample.
- **Stool** – If you are not able to provide a stool sample at this visit you will be given a self-sample kit to collect a stool sample for the next study visit.

**After we complete all the screening assessments above,**

If you are **confirmed not to be eligible** to take part in the study, you will unfortunately not be able to take part in the study and will continue with the planned standard of care treatment.

MAST Study

[Insert Site Logo]

If you are **confirmed to be eligible** to take part in the study, you will randomly be assigned by a computer to one of the two treatment groups below before (within 2 weeks of Visit 2) your next scheduled study visit.

**Treatment Groups:**

**Group 1**–This is the 'treatment arm' of the study**:**
You will receive 10 Intestinal Microbiota Transplant(IMT) $1x10^6$ x $1x10^9$ CFU/g of viable microorganisms per oral capsule). The number of colony forming units (CFU) in each capsule may differ because the CFU of the original stool material used to make the capsules also differs. Oral capsules will be made from bacteria obtained from a healthy screened person's stool sample; there are an extensive array of screening procedures in place to ensure the capsules are safe to take. Whilst this is not a typical 'licensed' medication, it has been manufactured in line with very strict approval procedures from the UK regulatory body for medicines, and this sort of treatment has already been used safely in thousands of people around the world. These capsules will be taken orally with water.

Please note – while no animal products are used in the manufacturing process, there is a possibility that the material within the capsules may contain traces of certain non-digestible dietary/ food components (for example, prawn shells); any dietary concerns you may have will be discussed with the nurse or study doctor before starting treatment.

**Group 2:**
You will receive 10 dummy oral capsules (placebo) the capsule will look the exact same as the capsule given in group one, but will contain no medicine or active ingredients. These capsules will also be taken orally with water.

**Capsule Description:**

Each capsule will be size 0 (see picture below) the capsules will be coated so that they will be able to pass through your stomach without dissolving.

MAST Study

[Insert Site Logo]

**Visit 2 (Treatment)** – Prior to this visit you will be told to avoid eating food 30 minutes before the start of this visit. You will be given either the IMT oral capsules, or the placebo oral capsules and we will also take a research blood sample and if not collected at screening urine and stool samples from you at this visit. You will receive a diary card to report daily any symptoms listed in the booklet until the next patient visit. The nurse or doctor at your next visit will go through the booklet with you should you need any support with completing the diary before the next visit, there will be a number in the booklet to use to contact a member of the study team.

After the Screening and Treatment visits have been completed, you will follow the standard of care that your clinician has discussed with you for treating the blood cancer. We will ask for you to attend **8 more visits** over the following year. These visits will occur along with your monitoring visits that you will be making as part of your treatment described in the table below.

**Study Assessments (Visit 3-10) 30-45 minutes:**

The following schedule outlines the questionnaires and samples that will be collected from you at each study visit.

| Type of questionnaire | What is the questionnaire for? | When are they done? |
|---|---|---|
| **Health assessment questionnaire (EQ-5D-5L)** | To evaluate your general quality of life. | All Visits |
| **Quality of Life for Cancer patients (EORTC QLQ-C30)** | To evaluate your general quality of life as a cancer patient. | All Visits |
| **Dietary Questionnaire** | To understand before the stool sample was obtained, if any specific foods e.g., liquorice or fish may have been eaten. As eating certain foods can adjust the results observed during the analysis of the samples collected. | All Visits |

| Sample type | What is the procedure for? | When are they done? | How will they be done? |
|---|---|---|---|
| **Urine** | For analysis of chemicals that we think may change from before and after the | All Visits | At each visit, you will be given a labelled clean container to pass urine |

MAST Study

[Insert Site Logo]

| | | | |
|---|---|---|---|
| | intestinal microbiota transplant | | ideally when you wake up first thing in the morning. Guidance will be provided by the examiner or qualified member of the study team. |
| **Blood** | For analysis of chemicals that we think may change from before and after the intestinal microbiota transplant | All Visits | Bloods will be collected by a qualified member of the study team |
| **Faecal** | Analysis of the microorganisms in the faecal material before and after intestinal microbiota transplantation | All Visits | We will ask you, to collect a stool sample for each visit. You can collect the sample:<br>• 24 hours before your next study visit a family or friend may help you with this<br>• On the morning of each study or at the start of each visit if you would like support from a nurse.<br>You will be provided with a self-sampling kit that will contain:<br>• Clear instructions of the collection process, storage and its return<br>• Ice pack<br>• Bag to transport samples in |

[Insert Site Logo]

MAST Study

## 5. What do I have to do?

If you decide to take part, you will need to attend your local research centre for the assessments. If you normally require transport, we will help arrange this for you. Tests, sample collections and hospital appointments are explained in the table above, you must inform your study doctor of any medications you are currently taking or intend to take once you have entered the study.

During your participation in the MAST study, you should continue with your regular medication, and you will continue to be under the care of your regular medical team.

## 6. How will we assess whether the treatment is working and its effect on your quality of life?

You will also be asked to complete questionnaires on paper regarding your general health and cancer usually at the same time as your scans. The questionnaires should take approximately 15-20 minutes in total to complete. If you feel uncomfortable answering any of the questions, please talk to your study doctor or nurse. You can leave blank any questions you do not want to answer. This information will help us to understand how the treatment may affect your quality of life.

## 7. Pregnancy, contraception, and breastfeeding

If you would like to participate in this study and are a woman of childbearing potential, you must:

- Tell your study doctor immediately if you become pregnant during this study, your study doctor will advise you of the possible risks to your unborn child and discuss options for managing the pregnancy with you. If pregnancy occurs during the study, The study treatment will not be given if pregnancy occurs before the treatment visit, and you will be withdrawn from the study. If pregnancy occurs after the treatment visit you will continue attending the remaining study visits and the pregnancy will be followed until the conclusion if you give consent for this.
- Use (if you are sexually active with a male partner who has not been sterilised), one highly effective method of birth control and one additional effective barrier method of contraception at the same time. This should be done from the time of signing the informed consent form until study completion. Please discuss effective methods of contraception with your study doctor or nurse.

MAST Study

[Insert Site Logo]

## 8. What are the side effects, possible disadvantages and risks when taking part?

Being involved in a research study, such as a clinical trial, requires a degree of commitment to regular hospital visits and additional tests and surveys, and you may consider this to be a disadvantage.

The only risks associated with the study are related to some of the procedures. For example, there is minimal risk associated with blood tests, they can cause brief discomfort, bruising, or an infection in some cases, which might last for several days, and will, therefore, be performed by experienced members of the healthcare team.

Urine and stool self-collection procedures carry a minimum risk of contamination with stool and urine material; however, the risk has been minimised by the provision of an instruction manual for collection and hygiene.

There are very low risks associated with the intestinal microbiota transplantation itself and these include, fever, nausea, vomiting, bloating and constipation these should normally resolve in 1 to 2 days https://tinyurl.com/4zpf5kchThere is also a low risk of infection from intestinal microbiota transplantation (IMT) itself. This risk is managed by EBX carrying out extensive testing on donors and their stool under supervision of a medical doctor, including blood and stool tests to detect pathogenic infectious agents, over and above as recommended by the UK experts in this field (https://tinyurl.com/4zpf5kch). Every stool donated, as well as every batch of IMT capsules manufactured is tested for the presence of pathogenic infectious agents and is only released for use if these are not detected." There will be a contact number in the symptom diary card should there be any symptoms you would like to discuss the clinical team.

## 9. What are the possible benefits of taking part?

We cannot promise the study will definitely help, however, in a small study before this larger one, we have shown that a similar treatment reduced the number of admissions to the intensive care unit, the numbers of blood infections and days of fever (high temperature) in the early days after the patients had had their bone marrow transplant. This study also showed that intestinal microbiota transplant (the treatment/method) was safe in patients undergoing bone marrow transplant, and there were no major side-effects.

The information we collect on how treatment affects the complications related to bone marrow transplantation, such as fevers (high temperatures) and infections during the transplant period may help to improve treatment and the recovery of people with blood cancers who are undergoing bone marrow transplant.

[Insert Site Logo]

## 10. What if I feel unhappy about continuing in the study?

If you have concerns about continuing, please discuss these with your study doctor and team. You do not have to give a reason, and your study team can explain your options to you about any data or samples collected from you as part of the study. Please see section 2 in Part 2 of the information sheet for more details of what will happen if you stop the study while on treatment.

1. What if something goes wrong?

Your study doctor will be there to answer any questions you might have regarding the cancer, its treatment, and your participation in the study. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during this study, then there will be several options available to you. Full details are included in Part 2 of this information sheet.

## 11. Will I be compensated for taking part?

You will not be paid for taking part in the study. However, for every study visit you attend you will be able to claim back some of your expenses. You will be reimbursed up to the value of £50 (maximum £200 in total) for travel expenses per visit.

**If the information in Part 1 has interested you and you are considering taking part in the study, please read the additional information in Part 2 before making your decision.**

[Insert Site Logo]

# 12. PART 2

## 0. What if new information becomes available?

Sometimes during a research project, new information or incidental findings becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form.

## 1. What happens when the research study stops?

Once your participation is over, you will carry on with your standard treatment and medical care as usual.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained including any research samples unless you specifically withdraw your consent for this. At your last study visit a nurse or clinician will ask if you would like to know what treatment group you were assigned to which will be shared with you if requested by your chosen method of contact once the trial has ended.

To safeguard your rights, we will use the minimum personally identifiable information possible.

## 2. What if there is a problem?

Imperial College London holds insurance policies which apply to this study. If you experience harm or injury because of taking part in this study, you will be eligible to claim compensation without having to prove that Imperial College London is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, you may have grounds for legal action.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during this study you should immediately inform the Investigator.

The normal National Health Service complaints routes are also available to you, details can be obtained from your study doctor or nurse.

If you are still not satisfied with the response, you may contact the Imperial College, Research Governance, and Integrity Team.

[Insert Site Logo]

**Complaint's statement**

If you wish to complain about any aspect of the way in which you have been approached or treated during this study, you should contact the study team (contact details at the end of this document) or you may contact the patient advice and liaison services (PALS) in the trust you are receiving treatment in *<Insert trust name, PALS tel.no and email>*.

## 3. How will we use information about you?

Imperial College London is the sponsor for this study and will act as the data controller with Imperial Clinical Trials Unit – Cancer (ICTU-Ca) for this study. This means that we are responsible for looking after your information and using it appropriately. Imperial College London will keep your personal data for:

- 10 years after the study has finished in relation to data subject consent forms.
- 10 years after the study has been completed in relation to primary research data.

This study is expected to end 08/2026

We will need to use information from your medical records for this research project. This information will include your, initials, month and year of birth, gender, and ethnicity.

People within the College and study team will use this information to do the research or to check your records to make sure that the research is being done properly and the information held is accurate.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a unique code number (study ID) instead, and this code will also be used to label tissue and blood samples.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no one can work out that you took part in the study.

MAST Study

[Insert Site Logo]

## LEGAL BASIS

As a university, we use personally-identifiable information to conduct research to improve health, care and services. As a publicly funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in the research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Our legal basis for using your information under the General Data Protection Regulation (GDPR) and the Data Protection Act 2018, is as follows:

Imperial College London - "performance of a task carried out in the public interest"); Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research

## INTERNATIONAL TRANSFERS

There may be a requirement to transfer information to countries outside the United Kingdom (for example, to a research partner), either within the European Economic Area (EEA) or to other countries outside the EEA. Where this information contains your personal data, Imperial College London will ensure that it is transferred in accordance with data protection legislation. If the data is transferred in accordance with data protection legislation. If the data is transferred to a country which is not subject to a UK adequacy decision in respect of its data protection standards , Imperial College London will enter into a data sharing agreement with the recipient research partner that incorporates UK approved standard contractual clauses or utilise another transfer mechanism that safeguards how your personal data is processed .

You will not be able to be identified when sharing this data, but it may include demographic information such as the month and year of your birth as well as your study ID.

## SHARING YOUR INFORMATION WITH OTHERS

We will only share your personal data with certain third parties for the purposes referred to in this participant information sheet and by relying on the legal basis for processing your data as set out below.

- Other College employees, agents, contractors and service providers (for example, suppliers of printing and mailing services, email communication services or web services, or suppliers who help us carry out any of the activities described above). Our third-party service providers are required to enter into data processing agreements with us. We only permit them to process your personal data for specified purposes and in accordance with our policies.
- EnteroBiotix Ltd., provide the capsule IMT and placebo. The following data is shared with them in this capacity as part of an agreement with Imperial College London, as

well as ensuring appropriate oversight of any serious side effects that you and other study participants may experience:

- Results data used to write reports from the study, specifically on how effective and safe the study treatment is
- Data about serious side effect/s, including whether they got better
- Data about medications taken and whether these were to treat the serious side effect/s or were other medications that were being taken at the time the serious side effect/s occurred.

- The Medical Research Council (MRC) who fund the study, the following data is shared with them in this capacity as part of an agreement with Imperial College London, as well as ensuring appropriate oversight of any serious side effects that you and other study participants may experience:
  - Results data used to write reports from the study, specifically on how effective and safe the study treatment is
  - Data about serious side effect/s, including whether they got better
- UK ethics and regulatory authorities who are required by law to approve and oversee research. The following data is shared with them to ensure appropriate oversight of any serious side effects that you and other study participants may experience:
  - Data about serious side effect/s, including whether they got better
  - Data about medications taken and whether these were to treat the serious side effect/s or were other medications that were being taken at the time the serious side effect/s occurred.

## POTENTIAL USE OF STUDY DATA FOR FUTURE RESEARCH

When you agree to take part in a research study, the information collected either as part of the study or in preparation for the study (such as contact details) may, if you consent, be provided to researchers running other research studies at Imperial College London and in other organisations which may be universities or organisations involved in research in this country or abroad. Your information will only be used to conduct research in accordance with legislation including the GDPR and the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you, used against you or used to make decisions about you.

MAST Study

[Insert Site Logo]

## COMMERCIALISATION

Samples/data from the study may also be provided to organisations not named in this participant information sheet, e.g., commercial organisations or non-commercial organisations for the purposes of undertaking the current study, future research studies or commercial purposes such as development by a company of a new test, product or treatment. We will ensure your name and any identifying details will NOT be given to these third parties, instead you will be identified by a unique study number with any sample / data analysis having the potential to generate 'personal data'.

Aggregated (combined) or anonymised data sets (all identifying information is removed) may also be created using your data (in a way which does not identify you individually) and be used for such research or commercial purposes where the purposes align to relevant legislation (including the GDPR) and wider aims of the study. Your data will not be shared with a commercial organisation for marketing purposes.

## WHAT ARE YOUR CHOICES ABOUT HOW YOUR INFORMATION IS USED?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have, because some research using your data may have already taken place and this cannot be undone.

- If you choose to stop taking part in the study, we would like to continue collecting information about your health from your hospital. If you do not want this to happen, tell us and we will stop. This will not affect any healthcare or support you may be receiving separately
- We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you, if this could affect the wider study or the accuracy of data collected.
- If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study.

## WHERE CAN YOU FIND OUT MORE ABOUT HOW YOUR INFORMATION IS USED

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- by asking one of the research team
- by sending an email to mast-trial@imperial.ac.uk

- **COMPLAINT**

If you wish to raise a complaint on how we have handled your personal data, please contact Imperial College London's Data Protection Officer via email at dpo@imperial.ac.uk, via telephone on 020 7594 3502 and/or via post at Imperial College London, Data Protection Officer, Faculty Building Level 4, London SW7 2AZ.

If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO) www.ico.org.uk. The ICO does recommend that you seek to resolve matters with the data controller (us) first before involving the regulator.

## 4. Involvement of the General Practitioner/ family doctor (GP)

With your permission, your GP and other doctors involved in your clinical care will be informed that you are taking part in this study, but otherwise all information about you and your treatment will remain strictly confidential.

## 5. What will happen to any samples that I give?

**Blood:**

Routine blood samples will be taken and tested by your hospital as part of standard practice and destroyed immediately after testing.

Research blood samples will be sent an HTA authorised Imperial College university lab located in St Mary's Hospital for long-term storage for future use in ethically approved studies with your permission. The blood samples will be analysed to see what chemicals they contain. Any samples left over from the process will be destroyed.

**Stool**

Stool samples will be sent to an HTA authorised Imperial College university lab located in St Mary's Hospital for storage. Stool samples collected at your screening visit and 7th visit (around 28 days after the bone marrow transplant) may be sent abroad for testing (i.e., Bacterial DNA sequencing lab in Germany) your agreement to this is required to take part in the study. The samples will be analysed to see what different types of bacteria are present in your stools. Any samples left over from the process will be destroyed.

**Urine**

The urine samples collected will be sent an HTA authorised Imperial College university lab located in St Mary's Hospital for long-term storage for future use in ethically approved studies with your permission. They will also be analysed to see what chemicals they contain.

MAST Study

[Insert Site Logo]

## 6. What will happen to the results of the research study?

The results of the MAST study will be analysed by the MAST study research team. Results will be presented at Cancer meetings and will be published in associated journals for the wider research community to reference. No identifiable information is included in publications or presentations; therefore, you will not be identified in any report or publication. Your confidentiality is maintained throughout.

If you contact the researchers in the future, you can obtain a copy of the results.

Research data and all identifiable data will be stored by the sponsor for 10 years following the end of trial.

## 7. Optional consent for future use of samples

If you consent, your leftover samples will be stored at our HTA authorised Imperial College Bio Bank your samples will be pseudo-anonymised and may be used for further academic and/or commercial studies by the Principal Investigator. Any such tests will have an appropriate ethical review. Upon your request at any time, your remaining samples will be destroyed.

## 8. Who is organising and funding the research?

Imperial College London is the legal sponsor of this study and is organising the study through the Imperial Clinical Trials Unit – Cancer (ICTU-Ca). The study is funded by Medical Research Council (MRC) who will receive a study report declaring the results, but no individual research participant identifiable data will be shared. The study is organised by a research team at the Imperial Cancer Clinical Trials Unit.

The sponsor of this study will pay your hospital for including you in this study, but your doctor will not receive any personal financial payment if you take part.

## 9. Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by <Insert name of Ethics Committee>.

[Insert Site Logo]

MAST Study

# 10. Contact for Further Information

If you have additional questions during this study about the research or your rights as a research patient, you may address them to the study doctor(s) <insert name of doctor and tel.no> or the study staff <insert name and tel. no.>. Out of office hours < insert name and tel.no> please contact the study doctor in the event of the following occurring:

a) If you suffer an illness or a possible study-related injury
b) If you feel different in any way
c) If you are admitted to the hospital for any reason
d) If you are seen at a casualty (accident/emergency department) for any reason

To speak with a member of the MAST investigator team please contact the Study Manager,

Telephone: 02075943767

Email: mast-trial@imperial.ac.uk

**Thank you for reading this information sheet. If you are interested in taking part in the study, please contact the study team to arrange a screening appointment.**

**A copy of this written information and signed Informed Consent form will be given to you.**

MAST Study Imperial College London

**Centre Number:** _____

**Patient Study Identification Number:**_____

## CONSENT FORM: MAST Study

**Study title: M**icrobiota Transplant Prior to **A**llogeneic **S**tem Cell **T**ransplantation **(MAST)** study
**Short Title**: MAST

**Principal Investigator:** <Insert Name>

**IRAS Project ID:** 1006971

| | Consenting information | Please Initial each box |
|---|---|---|
| 1. | I confirm that I have read and understand the Patient Information Sheet, **Version**_____, dated _____ for the above study. I have spoken to_____ and had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | |
| 2. | I understand that my participation is voluntary and that I am free to withdraw at any time, without providing a reason. I know that my medical care and legal rights are not affected. | |
| 3. | I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Sponsor (Imperial College London), from the NHS organisations, Medical Research Council, Enterobiotix or regulatory/other authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | |
| 4. | I give consent for information collected about me to be used to support other ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |
| 5. | If Applicable, I agree to use effective contraception whilst taking part in the study, should I become pregnant after taking the study drug, I give/do not give permission for access to any of my medical notes and information collected about my pregnancy. | |

MAST Study Imperial College London [Please Insert Site Logo]

| | | |
|---|---|---|
| 6. | I give consent to the taking of blood **equivalent to two tablespoons** and providing urine samples for chemical analysis in this study. | |
| 7. | I understand the stool collection procedure and agree to comply with these instructions. | |
| 8. | I give permission for my stool samples to be sent outside of the UK for bacterial genetic analysis for this study. | |
| 9. | I agree that my GP, and / or other doctors involved in my clinical care, may be notified of my participation in this study. | |
| 10. | I understand that blood, urine and stool samples and / or data collected from me are a gift donated to Imperial College and that I will not personally benefit financially if this research leads to an invention and/or the successful development of a new test, medication treatment, product or service. | |
| 11. | I agree to take part in the **Microbiota** Transplant Prior to **A**llogeneic **S**tem Cell **T**ransplantation **(MAST)** study | |

| **Optional** | | **Initials** |
|---|---|---|
| 12. | I give/do not give consent for my pseudo-anonymised stool, blood and urine sample to be stored during and at the end of the study at the University (Imperial College London) bio bank to support future ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |
| 13. | I give permission for any pseudo-anonymised blood and urine samples to be sent outside of the UK for analysis to support future ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |

Participant Name Date Signature

Name of person taking consent Date Signature

**When completed. Take 2 Copies. One to be given to the participant, one copy should be filed in the medical notes and the original stored in the Investigator Site File.**

**Supplementary Material 3: Appendices:**

**Intestinal *M*icrobiota Transplant Prior to *A*llogeneic *S*tem Cell *T*ransplant (MAST) trial: Study Protocol for a Phase IIa Randomised Controlled Trial**

**Appendix 1 - Neutropenia inducing regimens:**

Daunorubicin and cytarabine (DA) (with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Liposomal cytarabine and daunorubicin (Vyxeos) (with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

High dose cytarabine (>1000mg/m2) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Azacitine/decitabine (including oral forms) and venetoclax

Fludarabine / Cytarabine / GCSF / Idarubicin (FLAG-Ida) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, venetoclax, dasatinib or ponatinib)

Clofarabine / Cytarabine / GCSF / Idarubicin (CLAG-Ida) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Mitozantrone / Etoposide / Cytarabine (MEC) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

UK-ALL 14 phase 1 induction (off trial) or similar (with or without additional venetoclax)

UK-ALL 14 phase 2 induction (off trial) or similar (with or without additional venetoclax)

UK-ALL14 intensification (High dose methotrexate) or similar (with or without additional venetoclax)

Cyclophosphamide / Dexamethasone / Doxorubicin / Vincristine / Cytarabine alternating with methotrexate / cytarabine (Hyper-CVAD / MA) (with or without additional venetoclax)

**Appendix 2 – Disease and response criteria:**

***Acute lymphoblastic leukaemia (ALL) classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias)) and response criteria (modified Center for International Blood and Marrow Transplant Research criteria)***

| **B-ALL** |
|---|
| B-ALL with recurrent genetic abnormalities |
| B-ALL with t(9;22)(q34.1;q11.2)/*BCR::ABL1* |
| with lymphoid only involvement |
| with multilineage involvement |
| B-ALL with t(v;11q23.3)/*KMT2A* rearranged |
| B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1* |
| B-ALL, hyperdiploid |
| B-ALL, low hypodiploid |
| B-ALL, near haploid |
| B-ALL with t(5;14)(q31.1;q32.3)/*IL3::*IGH |
| B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1* |
| B-ALL, *BCR*::*ABL1*–like, ABL-1 class rearranged |
| B-ALL, *BCR*::*ABL1*–like, JAK-STAT activated |
| B-ALL, *BCR*::*ABL1*–like, NOS |
| B-ALL with iAMP21 |
| B-ALL with *MYC* rearrangement |
| B-ALL with *DUX4* rearrangement |
| B-ALL with *MEF2D* rearrangement |
| B-ALL with *ZNF384(362)* rearrangement |
| B-ALL with *NUTM1* rearrangement |
| B-ALL with *HLF* rearrangement |
| B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* (“CDX2/UBTF”) |
| B-ALL with mutated *IKZF1* N159Y |
| B-ALL with mutated *PAX5* P80R |
| Provisional entity: B-ALL, *ETV6::RUNX1*-like |

| |
|---|
| Provisional entity: B-ALL, with *PAX5* alteration |
| Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/IGH::*CEBPE* |
| Provisional entity: B-ALL, *ZNF384* rearranged-like |
| Provisional entity: B-ALL, *KMT2A* rearranged-like |
| B-ALL, NOS |
| **T-ALL** |
| Early T-cell precursor ALLwith *BCL11B* rearrangement |
| Early T-cell precursor ALL, NOS |
| T-ALL, NOS |
| Provisional entities (see supplemental Table 7) |
| **Provisional entity: natural killer cell ALL** |

Complete Remission (CR)

Hematologic complete remission is defined as meeting **all** of the following response criteria for at least four weeks.

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- ANC (absolute neutrophil count) ≥ 1.0 x $10^9$/L
- Platelets ≥ 100 x $10^9$/L
- Transfusion independent

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment; in this case, CR should still be reported as the status at transplant, since it represents the "best assessment" prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks. The pre-transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

Include recipients who are MRD positive or where the MRD status is unknown. MRD assessments include cytogenetic, flow cytometry, and molecular methods.

Include recipients meeting the above CR criteria regardless of how many courses of therapy were required to achieve CR.

The number of this complete remission can be determined by using the following guidelines:

- 1st CR: no prior relapse
- 2nd CR: one prior relapse
- 3rd or higher: two or more prior relapses

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria for at least four weeks:

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Primary Induction Failure (PIF)

The patient received treatment for ALL but **never achieved CR or CRi at anytime**. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have never been in CR or CRi.

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting at least one of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

The number of this relapse can be determined by using the following guidelines:

- 1st relapse: one prior CR
- 2nd relapse: two prior CRs
- 3rd or higher: three or more CRs

Do not include a partial response (PR) when determining number of relapse. Recipients who achieve a PR to treatment should be classified as either PIF or relapse; PR in ALL is generally of short duration and is unlikely to predict clinical benefit.

***Acute myeloid leukaemia (AML) classification (international Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria (modified Center for International Blood and Marrow Transplant***

*BMJ Open – Protocol – Supplementary Material*

| |
|---|
| ***Research criteria)***Acute promyelocytic leukaemia (APL) with t(15;17)(q24.1;q21.2)/*PML*::*RARA* ≥ 10% |
| APL with other *RARA* rearrangements* ≥ 10% |
| AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* ≥ 10% |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11* ≥ 10% |
| AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A* ≥ 10% |
| AML with other *KMT2A* rearrangements† ≥ 10% |
| AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214* ≥ 10% |
| AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*; *MECOM*(*EVI1*) ≥ 10% |
| AML with other *MECOM* rearrangements‡ ≥ 10% |
| AML with other rare recurring translocations (see supplemental Table 5) ≥ 10% |
| AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*§ ≥ 20% |
| AML with mutated *NPM1* ≥ 10% |
| AML with in-frame bZIP *CEBPA* mutations ≥ 10% |
| AML and MDS/AML with mutated *TP53*† 10-19% (MDS/AML) and ≥ 20% (AML) |
| AML and MDS/AML with myelodysplasia-related gene mutations 10-19% (MDS/AML) and ≥ 20% (AML) |
| Defined by mutations in *ASXL1*, *BCOR*, *EZH2, RUNX1*, *SF3B1, SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2* |
| AML with myelodysplasia-related cytogenetic abnormalities 10-19% (MDS/AML) and ≥ 20% (AML) |
| Defined by detecting a complex karyotype (≥ 3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurring genetic abnormalities), del(5q)/t(5q)/add(5q), −7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), −17/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities |
| AML not otherwise specified (NOS) 10-19% (MDS/AML) and ≥ 20% (AML) |
| Myeloid sarcoma |

*

Includes AMLs with t(1;17)(q42.3;q21.2)/*IRF2BP2*::*RARA*; t(5;17)(q35.1;q21.2)/*NPM1*::*RARA*; t(11;17)(q23.2;q21.2)/*ZBTB16*::*RARA*; cryptic inv(17q) or del(17) (q21.2q21.2)/*STAT5B*::*RARA*, *STAT3*::*RARA*; Other genes rarely rearranged with *RARA:TBL1XR1* (3q26.3), *FIP1L1* (4q12), *BCOR*(Xp11.4).

†

Includes AMLs with t(4;11)(q21.3;q23.3)/*AFF1*::*KMT2A*[#]; t(6;11)(q27;q23.3)/*AFDN*::*KMT2A*; t(10;11)(p12.3;q23.3)/*MLLT10*::*KMT2A;* t(10;11)(q21.3;q23.3)/*TET1*::*KMT2A;* t(11;19)(q23.3;p13.1)/*KMT2A*::*ELL;* t(11;19)(q23.3;p13.3)/*KMT2A*::*MLLT1* (occurs predominantly in infants and children).

‡

Includes AMLs with t(2;3)(p11~23;q26.2)/*MECOM*::*?;* t(3;8)(q26.2;q24.2)/*MYC*, *MECOM*; t(3;12)(q26.2;p13.2)/*ETV6*::*MECOM*; t(3;21)(q26.2;q22.1)/*MECOM*::*RUNX1.*

§

The category of MDS/AML will not be used for AML with *BCR*::*ABL1* due to its overlap with progression of CML, *BCR*::*ABL1*-positive.

Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- No extramedullary disease (e.g., CNS, soft tissue disease)
- Neutrophils ≥ 1.0 x $10^9$/L
- Platelets ≥ 100 x $10^9$/L
- Transfusion independent

Include recipients who are MRD positive or where the MRD status is unknown. MRD assessments include cytogenetic, flow cytometry, and molecular methods.

Include recipients meeting the above CR criteria regardless of how many courses of therapy were required to achieve CR.

The number of this complete remission can be determined by using the following guidelines:

- 1st CR: no prior relapse
- 2nd CR: one prior relapse
- 3rd or higher: two or more prior relapses

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- No extramedullary disease (e.g., CNS, soft tissue disease)
-

Primary Induction Failure (PIF)

The patient received treatment for AML but **never achieved CR or CRi at anytime**. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have *never been in CR or CRi*.

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting one or more of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

The number of this relapse can be determined by using the following guidelines:

- 1st relapse: one prior CR
- 2nd relapse: two prior CRs
- 3rd or higher: three or more CRs

Do not include a partial response (PR) when determining number of relapse. Recipients who achieve a PR to treatment should be classified as either PIF or relapse; PR in AML is generally of short duration and is unlikely to predict clinical benefit.

***Myelodysplastic syndromes (MDS) classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria (modified Center for International Blood and Marrow Transplant Research criteria).***

| Myelodysplastic syndrome with mutated *SF3B1* |
|---|
| Myelodysplastic syndrome with del(5q) |

| |
|---|
| Myelodysplastic syndrome with mutated *TP53* |
| Myelodysplastic syndrome, not otherwise specified (MDS, NOS) |
| MDS, NOS without dysplasia |
| MDS, NOS with single lineage dysplasia |
| MDS, NOS with multilineage dysplasia |
| Myelodysplastic syndrome with excess blasts |
| Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) |
| MDS/AML with mutated *TP53* |
| MDS/AML with myelodysplasia-related gene mutations |
| MDS/AML with myelodysplasia-related cytogenetic abnormalities |
| MDS/AML, not otherwise specified |

Complete Remission (CR)

Requires all of the following maintained for a minimum of four weeks. When reporting the CR achievement date, report the first date when CR was achieved (not the four week date in which CR was maintained).

Bone marrow evaluation:

- < 5% myeloblasts with normal maturation of all cell lines

Blood evaluation

- Haemoglobin ≥ 110 g/L untransfused without erythropoietic support
- Absolute neutrophil count ≥ 1.0 x $10^9$/L without myeloid growth factor support
- Platelets ≥ 100 x $10^9$/L without thrombopoietic support
- 0% blasts in blood

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment. In this case, CR should still be reported as the status at transplant since it represents the “best assessment” prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks; the pre-transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

***Chronic myeloid leukaemia with blast transformation classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria***

Philadelphia positive or BCR:ABL1 CML in blast phase defined by the presence of blast ≥ 20% in blood or bone marrow.

Second chronic phase (CP2)

Requires all of the following

**Bone marrow evaluation:**

- < 5% myeloblasts with normal maturation of all cell lines

**Blood evaluation**

- Absolute neutrophil count ≥ 1.0 x $10^9$/L without myeloid growth factor support
- Platelets ≥ 100 x $10^9$/L without thrombopoietic support
- < 5% blasts in blood

**Appendix 3 – Karnofsky performance status score**

- 100 Normal; no complaints; no evidence of disease.
- 90 Able to carry on normal activity; minor signs or symptoms of disease.
- 80 Normal activity with effort; some signs or symptoms of disease.
- 70 Cares for self; unable to carry on normal activity or to do active work.
- 60 Requires occasional assistance but is able to care for most of their personal needs.
- 50 Requires considerable assistance and frequent medical care.
- 40 Disabled; requires special care and assistance.
- 30 Severely disabled; hospital admission is indicated although death not imminent.
- 20 Very sick; hospital admission necessary; active supportive treatment necessary.
- 10 Moribund; fatal processes progressing rapidly.
- 0 Dead

*BMJ Open – Protocol – Supplementary Material*

**Appendix 4 - Summary of Assessments:**

| | Visit | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Assessment Forms** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Consent** | X | | | | | | | | | |
| **Demographics** | X | | | | | | | | | |
| **Eligibility** | X | | | | | | | | | |
| **Significant Medical History** | X | | | | | | | | | |
| **Chemotherapy History** | X | | | | | | | | | |
| **Infection and Treatment History** | X | | | | | | | | | |
| **Microbiology Colonisation History** | X | | | | | | | | | |
| **Antibiotic History** | X | | | | | | | | | |
| **Transplant Donor Characteristics** | X | | | | | | | | | |
| **Comorbidity Index Score** | X | | | | | | | | | |
| **Vital Signs** | X | X | X | X | X | X | X | X | X | X |
| **Physical Examination** | X | X | X | X | X | X | X | X | X | X |
| **Randomisation** | X | | | | | | | | | |
| **Current Medication** | X | | | | | | | | | |
| **Dietary Questionnaire** | X | X | X | X | X | X | X | X | X | X |
| **Full Blood Count, Coagulation, Biochemistry, and Virology** | X | X | X | X | X | X | X | X | X | X |
| **Bone Marrow Assessment Results** | X | | | | | | | X | X | X |
| **EQ-5D-5L Questionnaire** | X | | | | | | X | X | X | X |
| **EOTRC QLQ-C30 Questionnaire** | X | | | | | | X | X | X | X |
| **Sample Collection Form** | | | X | X | X | X | X | X | X | X |
| **Nutrition** | | X | X | X | X | X | X | X | X | X |
| **Recent Microbiology History** | | | X | X | X | X | X | X | X | X |
| **Recent fever, Infection and Treatment History** | | X | X | X | X | X | X | X | X | X |

*BMJ Open – Protocol – Supplementary Material*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Recent Microbial Colonisation History** | | X | X | X | X | X | X | X | X | X |
| **IMP Administration** | | X | | | | | | | | |
| **IMP Symptom Report form** | | | X | X | X | X | X | X | X | X |
| **Adverse Event** | | X | X | X | X | X | X | | | |
| **Haematopoietic Cell Transplant Details** | | | | | X | | | | | |
| **VOD, and Relapse** | | | | | X | X | X | X | X | X |
| **Acute GvHD** | | | | | X | X | X | X | X | X |
| **GvHD Prophylaxis and Therapy** | | | | | X | X | X | X | X | X |
| **Engraftment** | | | | | | | X | X | X | X |
| **Lymphocyte Subsets and Immunoglobulin Levels - Blood** | | | | | | | | X | X | X |
| **Linage Specific Chimaerisms - Blood** | | | | | | | | X | X | X |
| **Chronic GVHD Assessment** | | | | | | | | X | X | X |
| **Post Transplant Intervention** | | | | | | | | | | X |
| **ITU Admissions Review** | | | | | | | | | | X |

BMJ Open

# Intestinal Microbiota Transplant Prior to Allogeneic Stem Cell Transplant - (MAST) trial: Study Protocol for a Multi-Centre, Double-Blinded, Placebo-Controlled, Phase IIa Trial



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2024-093120.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 15-Nov-2024 |





| | |
|---|---|
| Complete List of Authors: | Mullish, Benjamin; Imperial College London Faculty of Medicine, Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction<br>Innes, Andrew J; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Roberts, Lauren A; Imperial College London, Department of Metabolism, Digestion and Reproduction<br>Anim-Burton, Shian; Imperial College London, Department of Surgery and Cancer<br>Webber, Lee; Imperial College London, Department of Surgery and Cancer<br>Johnson, Nicholas; Imperial College London School of Public Health, Department of Public Health<br>Ghani, Rohma; Imperial College London, Department of Metabolism, Digestion and Reproduction; Imperial College Healthcare NHS Trust, Department of Infectious Diseases<br>Farshi, Pakhshan; Manchester Royal Infirmary, Department of Haematology<br>Khan, Anjum B; Leeds Teaching Hospitals NHS Trust, Department of Haematology<br>Kinsella, Francesca; University Hospitals Birmingham NHS Foundation Trust, Department of Haematology<br>Kottaridis, Panagiotis; University College London Hospitals NHS Foundation Trust, Department of Haematology<br>Krishnamurthy, Pramila; King's College Hospital NHS Foundation Trust, Department of Haematology<br>Nicholson, Emma; The Royal Marsden Hospital, Department of Haematology<br>Palanicawandar, Renuka; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Wheeler, Graham; Imperial College London, Imperial Clinical Trials Unit<br>Davies, Frances; Imperial College London, Department of Infectious Diseases<br>Marchesi, Julian R.; Imperial College London, Department of Metabolism, Digestion and Reproduction<br>Pavlů, Jiří; Imperial College London, Department of immunology and |

| | |
|---|---|
| | inflammation; Imperial College Healthcare NHS Trust, Department of Haematology |
| Primary Subject Heading: | Haematology (incl blood transfusion) |
| Secondary Subject Heading: | Gastroenterology and hepatology |
| Keywords: | Bone marrow transplantation < HAEMATOLOGY, Leukaemia < HAEMATOLOGY, Transplant medicine < INTERNAL MEDICINE, Microbiota |
| | |

*BMJ Open – Protocol:*

# Intestinal *M*icrobiota Transplant Prior to *A*llogeneic *S*tem Cell *T*ransplant - (MAST) trial: Study Protocol for a Multi-Centre, Double-Blinded, Placebo-Controlled, Phase IIa Trial

Benjamin H Mullish[1,2], Andrew J Innes[3,4], Lauren A Roberts[1], Shian Anim-Burton[5], Lee Webber[5], Nicholas A Johnson[6], Rohma Ghani[1,7], Pakhshan Farshi[8], Anjum B Khan[9], Francesca Kinsella[10], Panagiotis Kottaridis[11], Pramila Krishnamurthy[12], Emma Nicholson[13], Renuka Palanicawandar[3,4], Graham M Wheeler[6,14], Frances J Davies[7], Julian R Marchesi[1]*, Jiří Pavlů[3,4,]*

*Affiliations:*

[1]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, United Kingdom

[2]Department of Hepatology, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

[3]Centre for Haematology, Department of immunology and inflammation, Faculty of Medicine, Imperial College London, London, United Kingdom

[4]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

[5]Cancer Research UK Imperial Centre, Clinical Trials Section, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, United Kingdom

[6]Imperial Clinical Trials Unit, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom

[7]Department of Infectious Diseases, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom.

[8]Department of Haematology, Manchester Royal Infirmary, Manchester, United Kingdom

[9]Department of Haematology, Leeds Teaching Hospitals, Leeds, United Kingdom

[10]Department of Haematology, University Hospital Birmingham, Birmingham, United Kingdom

[11]Department of Haematology, University College London Hospital, London, United Kingdom

[12]Department of Haematology, King's College Hospital, London, United Kingdom

[13]Department of Haematology, The Royal Marsden Hospital, London, United Kingdom

[14]Statistics and Data Science Innovation Hub, GSK, London, United Kingdom

*Joint senior authors and trial co-chief investigators

Correspondence to: Prof Julian R Marchesi

Postal address: Department of Metabolism, Digestion and Reproduction
St Mary's Hospital Campus, Imperial College London
10th Floor, QEQM Wing
South Wharf Road
Paddington, London
W2 1NY
United Kingdom

Email: j.marchesi@imperial.ac.uk

Word count: 6793

*BMJ Open – Protocol:*

**Abstract:**

**Introduction:** Lower diversity of the gut microbiome pre-allogeneic haematopoietic cell transplantation (HCT) correlates with reduced survival after the intervention. Most patients undergoing HCT for a haematological malignancy have previously received intensive chemotherapy, resulting in prolonged neutropenic episodes requiring broad-spectrum antibiotics; use of these has been linked to reduced microbiome diversity. Intestinal microbiota transplant (IMT) is a novel treatment approach that restores this diversity. We hypothesised that IMT performed prior to initiation of HCT conditioning restores microbiome diversity during the early stages of HCT, leading to decreased frequency of complications and improved outcomes of HCT.

**Methods and analysis:** Fifty adult patients receiving allogeneic HCT will be recruited into this phase 2a trial and randomised 1:1 to receive capsulised IMT or matched placebo shortly prior to initiation of HCT conditioning and followed for up to twelve months. The primary outcome will be to assess the increase in alpha diversity between pre-IMT and that measured at ~42 days after the IMT administration (day +28 of HCT), comparing the difference between patients receiving IMT compared to placebo. Secondary outcomes will include tolerability, and the dynamics of gut microbiome diversity metrics and taxonomy over all timepoints assessed, as well as clinical outcomes (including burden of invasive infections, days of fever, admission to intensive care, development of graft-vs-host disease, and mortality).

**Ethics and dissemination:** This study was approved by a UK Research Ethics Committee (REC reference: 23/NE/0105). Dissemination of results will be in concert with patient and public involvement (PPI) group input and is expected to be primarily via abstract presentation at conferences and manuscripts in peer-reviewed journals.

**Trial registration number:** ClinicalTrials.gov ID: NCT 6355583; ISRCTN: https://doi.org/10.1186/ISRCTN13241761; EudraCT: 2022-003617-10

**Keywords:** Bone marrow transplantation; Leukaemia; Transplant medicine; Gut microbiome; Faecal microbiota transplant.

**Strengths and limitations of this study:**

- MAST is a multi-centre, randomised, placebo-controlled trial that will explore the impact of intestinal microbiota transplant (IMT) administered prior to haematopoietic stem cell transplantation (HCT) upon gut microbiome dynamics and clinical outcomes.
- This study will use a novel multi-donor capsule IMT preparation (as opposed to conventional IMT slurry), which was the patient preference during our patient and public involvement group feedback.
- However, we have no prior 'head to head' testing of IMT slurry compared to the novel capsule preparation in terms of efficacy.
- The timing of IMT pre-HCT (to 'prehabilitate' the gut microbiome) is a distinctive aspect of this study, as other studies have given this afterwards.
- Together with recording clinical outcomes post-IMT and HCT, we will collect patient samples for immunological and multi-omic profiling (including microbiome and metabolome analysis), to better understand the mechanisms of action of IMT in this setting.

## 1 Introduction:

Allogeneic haematopoietic cell transplantation (HCT) is a powerful therapeutic modality for patients with acute leukaemia and certain other haematological malignancies. Furthermore, with the advent of reduced intensity protocols, and approaches that permit safer use of mismatched donors, its frequency is increasing. In preparation for transplant, patients receive a conditioning regimen of high-dose chemotherapy and/or total-body irradiation, followed by infusion of compatible haematopoietic cells; the engraftment of these cells restores the recipient's haematopoesis, and exerts long-term remission from the graft-versus-leukaemia effect of the donor immune effector cells. However, this process is associated with marked perturbation of the gut microbiome, including reduced gut barrier integrity, loss of gut microbiome diversity, and microbiome enrichment in pathobiont bacteria[1] [2]. The immunosuppressive nature of both the underlying haematological malignancy, together with the treatments used, collectively result in a markedly increased risk of infections in these patients. More specifically, the increase in susceptibility to infection leads to an increase in antibiotic exposure, driving the dominance of pathobionts, and a further selection pressure for overgrowth of antimicrobial resistance (AMR) genes in the gut[3]. In this setting, the impact of antibiotics, and multidrug-resistant organism (MDRO)-associated infection, is associated with poorer clinical outcomes in patients[4]; for example, use of imipenem-cilastatin or piperacillin-tazobactam use to treat neutropenic fever has been associated with increased graft-versus-host disease (GvHD) mortality up to even five years post-HCT[5].

A large observational study of more than 1,300 patients from four centres observed that patients with patterns of microbiota disruption characterised by loss of diversity had a higher risk of transplantation-related death, and death attributable to GvHD[6]. Baseline samples obtained before HCT already showed evidence of microbiome disruption, and lower diversity before transplantation was closely associated with poor survival. Specific gut taxonomic features have also been linked with allo-HCT outcome; specifically, expansion of *Enterococcus* (particularly *Enterococcus faecium*) was observed across the period of having allo-HCT.

*Enterococcus* was found to associate closely with GvHD and mortality, with presence of the disaccharide lactose identified as a factor that promoted *Enterococcus* expansion[7].

These data support the hypothesis that a microbiome-based intervention, performed prior to initiation of HCT conditioning, may improve microbiome diversity during transplant, and has the potential to impact upon clinical outcomes. A number of approaches have been considered in this setting[8], including dietary/prebiotic interventions, probiotics, and non-absorbable antibiotics (such as rifaximin). In this study we have opted for a biological approach that attempts to restore the whole gut ecosystem, using intestinal microbiota transplant (IMT; also known as 'faecal microbiota transplant'). IMT consists of transferring minimally processed stool, from a healthy screened donor, into the gut of an recipient. This approach was pioneered in patients with recurrent *Clostridioides difficile* infection (rCDI), in which the major risk factor is recurrent antibiotic exposure[9]. The success in this setting had led to exploration of IMT in other conditions which the gut microbiome appears contributory to their aetiopathogenesis[10], with promising early data. Despite initial concerns about safety of IMT in immunocompromised patients - driven in part by descriptions of pathogen transmission via IMT in such patients[11] - adherence to strict screening protocols results in a safety profile comparable to that in immunocompetent recipients[8 9 12].

After previous reports that the use of IMT for rCDI was also associated with reduced antibiotic resistance genes within the gut microbiome[13], and our own observation of a clinical case where IMT seemed to show clinical benefit when used prior to HCT in a patient colonised with MDROs[14], we completed a cohort study of IMT performed prior to initiation of HCT conditioning in patients colonised and/or previous infected with MDROs. While we observed that rates of decolonisation of intestinal MDROs were comparable to that observed spontaneously, we saw a significant reduction in rates of bloodstream infection (including MDRO-related), length of stay, and days of carbapenem use, compared to a matched historical control arm[15]. With longer follow-up, these benefits translated to improvement in overall survival, such that the poor outcome associated with MDRO colonisation could be negated with IMT[16].

*BMJ Open – Protocol:*

In this clinical trial, we will investigate the broader role of IMT in HCT, both with and without MDRO colonisation. By randomising patients to receive IMT or placebo prior to HCT conditioning, and measuring microbiota diversity, in stool, as a surrogate for its impact upon gut ecology, we will determine the capacity of capsule IMT to restore a normal microbiome, and track the impact during the course of HCT. Using multi-omic profiling of stool, urine and blood, we will investigate the wider impact of IMT in HCT patients, while clinical parameters will explore the potential to overall outcome.

## 2 Methods and analysis:

### 2.1 Design and objectives:

The MAST study is a multi-centre, randomised, phase IIa double-blind placebo-controlled trial**.** The major objective of this trial is to determine the ability of capsulised IMT given prior to allogeneic HCT to increase and maintain stool microbiota diversity after HCT from baseline. Secondary objectives include determination of clinical tolerability, effects of capsule IMT upon clinical outcomes, and to explore microbiome and immune dynamics related to IMT use. The primary outcome is the change in alpha diversity (measured as inverse Simpson's index) after IMT administration measured at immediately prior to IMT (at 14 +/- 3 days prior to HCT) and 28 +/-3 days after HCT, comparing the change between patients receiving capsulised IMT *versus* placebo. The study is sponsored by Imperial College London. This is an investigator-led study; while funding for the study was only awarded after peer review, the funder, sponsor and industrial partner have had no direct role in any aspect of study design (although the funder arranged external peer review as part of the process to the award of funding, which did impact upon study design). We have used the SPIRIT checklist in writing this report[17].

The study start date is April 2024, with primary completion estimated as August 2026 and study completion estimated as March 2027.

### 2.2 Recruitment Strategy:

#### 2.2.1. Site Selection:

We have partnered with several of the largest haematology centres in the UK as recruitment sites to support adequate participant enrolment. These centres are: the Royal Marsden

Hospital, University College London Hospital, King's College London Hospital, University Hospital Birmingham, Leeds Teaching Hospital, Hammersmith Hospital, and Manchester University Hospital. Such centres are well-positioned to provide access to a high volume of eligible patients, due to their expertise and patient population in haematology and transplant services.

**2.2.2. Engagement with Patient Advocacy Groups:**

From its inception, the MAST trial was co-developed with the patient and public involvement group, itself based around the NCRI AML Supportive care group. The group refined the protocol and participant-facing documents and provided input into the design to improve the communication and reach of the study to potential participants.

**2.2.3. Regular Communication and Updates:**

Our dedicated trials unit maintains regular communication with participating sites to support recruitment efforts. This includes helping to support barriers to enrolment and providing ongoing assistance to sustain recruitment momentum, ensuring that sites have the resources and support needed to meet target enrolment goals.

**2.2.4. Patient Support and Accessibility Measures:**

We have developed study several supportive resources to improve participant understanding, engagement and accessibility to help boost retention. Examples include informational videos to guide participants providing samples, study-specific standard operation procedures to streamline processes across sites and maintain consistency, and translated versions of the participant information sheets to accommodate diverse language needs. Additionally, funding for transportation costs is available to reduce transportation barriers to make participation more accessible.

**2.3 Study setting and participants:**

This trial will be performed across seven Haematology Units in the United Kingdom which regularly undertake HCT. The study will recruit adults with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), acute leukaemia (AL) of ambiguous lineage, high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML), and chronic

*BMJ Open – Protocol:*

myeloid leukaemia (CML) in blast phase, considered suitable/fit for allogeneic haematopoietic stem cell transplantation HCT. Patients will be eligible to enter the study if they achieved complete remission (defined as < 5% blasts), have received a minimum of two cycles of intensive chemotherapy (**Supplementary Material 1, Appendix 1**), and have received broad-spectrum antibiotics within three months of HCT**.** Inclusion and exclusion criteria are summarised below:

2.3.1 **Inclusion criteria:**

1. Patients aged 18 years and over with a morphological documented diagnosis of ALL, AML, AL of ambiguous lineage, MDS, CMML, and CML in blast phase (**Supplementary Material 1, Appendix 2**) who are deemed fit for allogenic HCT with one of the following disease characteristics:

   *ALL, AML, AL of ambiguous lineage*

   - Patients in first complete remission (CR1) or second complete remission (CR2) including complete remission with incomplete blood count recovery with < 5% blasts (**Supplementary Material 1, Appendix 2**)
   - Secondary leukaemia (defined as previous history of MDS, antecedent haematological disease or chemotherapy exposure) in CR1 or CR2 defined as < 5% blasts (**Supplementary Material 1, Appendix 2**)

   *MDS and CMML*

   - Patients with advanced or high risk MDS with an IPSS-M moderate high or higher including intermediate or high risk CMML who have < 5% blasts at the time of randomisation (**Supplementary Material 1, Appendix 2**)

   *CML in blast phase*

   - Patients with Philadelphia or BCR:ABL1 positive chronic myeloid leukaemia (CML) in blast phase defined by the presence of ≥ 20% blasts in blood or bone marrow who have achieved second chronic phase with < 5% blasts (**Supplementary Material 1, Appendix 2**).
2. Patients must have completed minimum of two cycles of intensive chemotherapy prior to trial enrolment (**Supplementary Material 1, Appendix 1**).
3. Patients must have received broad-spectrum antibiotics within 3 months prior to trial enrolment

*BMJ Open – Protocol:*

4. Patients must be considered suitable/fit to undergo allogeneic HCT, as clinically judged by the Local investigator
5. Patients with a Karnofsky performance status score 60 or above (**Supplementary Material 1, Appendix 3**).
6. Females of and male patients of reproductive potential (i.e., not post-menopausal or surgically sterilised) must use appropriate, highly effective, contraception from the point of commencing therapy until 6 months after treatment
7. Patients have given written informed consent
8. Patients willing and able to comply with scheduled study visits and laboratory tests

**2.3.2 Exclusion criteria:**

1. Patients with contraindications to receiving allogeneic HCT.
2. Female patients who are pregnant or breastfeeding. All women of childbearing potential must have a negative pregnancy test before commencing treatment.
3. Adults of reproductive potential not willing to use appropriate, highly effective, contraception during the specified period.
4. Patients with renal or hepatic impairment as clinically judged by the Local Investigator.
5. Patients with active infection, HIV-positive or chronic active HBV or HCV.
6. Patients with a concurrent active malignancy or a prior malignancy, except lobular breast carcinoma *in situ*, fully resected basal cell or squamous cell carcinoma of skin or treated cervical carcinoma *in situ*, incidental histologic finding of prostate cancer (T1a or T1b using the tumour, node, metastasis (TNM) clinical staging system), previous MDS, CMML, MPN resulting in secondary AML. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed.
7. Swallowing difficulties that may preclude safe use of IMT capsules.
8. Administration of IMT within 3 months prior to enrolment (probiotic administration prior to enrolment is allowed but should be recorded at screening).
9. Patients taking probiotics after enrolment to the trial.
10. Gastrointestinal disorders and diseases, including delayed gastric emptying, coeliac disease, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, chronic diarrhoea, and colonic perforation or fistula.

11. Any autoimmune disease requiring, or that may require, systemic treatment with steroids and/or other immunosuppressants/immunomodulators.

12. Significant bleeding disorder (ALL, AML, AL of ambiguous lineage, MDS, CMML, and CML satisfying inclusion criteria are not excluded).

13. Anaphylactic food allergy.

14. Requirement for vasopressors.

15. Valvular heart disease or known structural defects of the heart.

16. Known severe allergy to capsule components.

## 2.4 Interventions:

### 2.4.1 Allocation:

50 adult patients will be allocated 1:1 between two groups:

- Capsulised IMT – as a single oral dose of 10 capsules of EBX-102-02. EBX-102-02 is encased within an intrinsically enteric-resistant capsule containing pooled, dried, full-spectrum microbial ecosystems obtained from rigorously screened donors. EBX-102-02 is characterised by the absence of pathogens, a minimum viable count of anaerobic microorganisms, and the presence of particular identified genera (such as *Faecalibacterium*), all measured by proprietary nucleic acid-based assays and other technologies. EBX-102-02 will be administered within two weeks of the initial study screening visit. Given the immunosuppressed nature of the recipients, out of an abundance of caution, EBX-102-02 will be prepared from CMV-negative donors, as per suggestions from current guidelines[9].
- Matched capsulised placebo – containing microcrystalline cellulose and magnesium stearate; administered at the same point as capsulised IMT.

Treatment with either IMT or placebo will take place at 14 (+/- 2) days prior to haematopoietic cell infusion in a hospital setting. Both IMT and placebo will be stored in a refrigerator (at 2 – 8$^{o}$C) until administration, with temperature monitoring of the investigational medicinal product prior to administration. Study participants will be nil by mouth for at least 30 minutes prior to – and one hour after – each course of IMT/ placebo capsule administration. They will be asked to take each capsule with sips of water, and will be monitored for at least 15 minutes after capsule administration for complications (e.g. nausea).

*BMJ Open – Protocol:*

Randomisation will be performed centrally by the Imperial College Trials Unit – Cancer using OpenClinica electronic data capture system (EDC). The system applies stratified randomisation to reduce relevant imbalances and increase statistical power, randomisation will be stratified by disease history (either: 1. patients known to have intestinal colonisation or blood-borne infection with multidrug-resistant organisms during previous therapy; or: 2. patients without this history) to ensure there is a balanced distribution across treatment arms. To reduce predictability in the randomisation sequence, blocks of multiple sizes have been used during sequence generation.

The allocation sequence will be generated using a computerised algorithm on the Sealed Envelope system designed to maintain allocation concealment and integrity. The study uses kit codes which are pre-generated by the drug manufacturer, the kit code is linked to the treatment allocation sequence but to do not reveal treatment assignment (capsule IMT or placebo). The kit codes are randomly assigned to participants through the Sealed Envelope system when randomisation is initiated in OpenClinica.

The system ensures that the allocation sequence remains concealed until the end of the study. User restrictions are in place to maintain the blinding; only personnel with distributor access have the ability to view the unblinded code lists. Study Investigators and those enrolling participants cannot access these lists to preserve the double blinded nature of the study. This ensures that the treatment assignments are hidden to both participants and investigators until the end of the study.

### 2.4.2 Blinding:

Since this is a double-blind randomised placebo-controlled clinical trial, the treatment allocation will be blinded to the investigators, sponsor clinical trial management team, clinical staff, laboratory staff and the patient. Placebo capsules will be identical in appearance, weight, and all other obvious characteristics to the course of IMT, and will be handled by pharmacy identically; this will help in maintaining blinding. Trial randomisation will occur as soon as possible after satisfactory review and confirmation of patient eligibility at screening.

*BMJ Open – Protocol:*

Unblinding will only be considered in cases where the identity of the drug assignment is absolutely necessary for the safety of the patient. This will be possible 24 hours a day and 365 days of the year, but with strong recommendation that the chief investigator and/or sponsor is contacted prior to the unblinding of the patient to discuss the reasons for unblinding. Where unblinding is required, sites will use the unique login provided by the sponsor to access the treatment assignment; if the database cannot be accessed, there will be a manual unblinding procedure in place using unblinding cards located in the pharmacy folder.

### 2.5 Outcomes:

The Schedule/ Summary of visits is shown in **Supplementary Material 1, Appendix 4.**

The primary outcome of the trial is the ability of the capsulised IMT given pre-HCT to increase and maintain intestinal microbiota diversity post-HCT. This will be assessed via measurement of the difference between the change in alpha diversity (calculated using inverse Simpson index) 28 +/-3 days post-HCT from baseline for patients in the capsulised IMT groups *versus* the capsulised placebo group. The secondary objectives of the study relate to feasibility/ tolerability of the capsule, and impact of the IMT upon a range of clinically- and translationally-pertinent outcomes. These include: quality of life; microbiological/ infective outcomes; need for Intensive Care; and haematological outcomes, ranging from relapse, to GvHD, to impact upon engraftment and immune reconstitution. Microbiological/ infective outcomes will be assessed via conventional clinical microbiology techniques, as well as via gut microbiome diversity and taxonomic characterisation. These secondary outcomes are summarised in **Table 1.** In addition, the study has a range of discovery phase/ exploratory endpoints, including investigating the impact of IMT upon: markers of gut barrier function; metabolomic profiles in different biofluids; circulating cytokines; functionality of circulating monocytes and T cells. In addition to lymphocyte subset characterisation, collection of peripheral blood mononuclear cell (PBMC) at day 100 (visit 8), and day 365 (visit 10) will allow further exploration of the impact of IMT on immune reconstitution, and in particular, T-cell repertoire.

*BMJ Open – Protocol:*

The study flow chart/ participant timeline is shown in **Figure 1.** Of note, regardless of whether the patient is randomised to capsulised IMT or placebo, they will continue with their scheduled standard of care treatments/ assessments while also receiving study follow-up assessments at planned intervals, as shown in **Figure 1.** Other pre- and post-HCT care will be in accordance with the participating centres' policies. As such, patients are allowed to receive prophylactic antibiotics (such as ciprofloxacin) but should not receive broad-spectrum antibiotics after the trial treatment has taken place and prior to the start of HCT. It is recognised that this may not be always possible, as neutropenic fever may sometimes develop during the conditioning therapy. If this happens, patients will not be excluded from the trial, but the broad-spectrum antibiotic use and its duration must be documented at response assessments.

### 2.6 Data collection and management:

#### 2.6.1 General approach:

CRFs for the study will be in English, using generic names for concomitant medications wherever possible. All written material to be used by participants must use vocabulary that is clearly understood and be in the language appropriate for the study site. The electronic case report form (eCRF) database will be in OpenClinica. The Investigator (or delegated member of the site study team) will record all data relating to protocol assessments and procedures, laboratory, safety and efficacy data in the eCRF. All trial documentation, including that held at participating sites and the trial coordinating centre, will be archived for a minimum of 20 years following the end of the study.

#### 2.6.2 Confidentiality:

The investigator will ensure that the participant's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator. The investigator shall permit direct access to participants' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and RECs.

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing and disclosure of personal information, and will uphold the Act's core principles.

### 2.6.3 Oversight and monitoring:

A Trial Steering Committee (TSC) will be convened, including as a minimum an independent Chair, independent clinician, the Chief Investigator, Independent Statistician, Trial Manager and PPI Representative. The role of the TSC is to provide overall supervision of trial conduct and progress. A Trial Management Group (TMG) will also be convened, including the Chief Investigator, co-investigators and key collaborators, trial statistician and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Furthermore, an Independent Data Monitoring Committee (IDMC) will be convened to include as a minimum an independent oncologist chair, an independent oncologist and an independent statistician. The role of the IDMC is advisory to the TSC, to ensure the highest standard of patient safety and data integrity.

The IDMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable, or if any issues are identified which may compromise patient safety. In the case of early discontinuation of the study, response assessments will be completed for each participant, as far as possible.

The study will be monitored periodically by trial monitors to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy, and consistency of the data. Monitoring will be conducted centrally/remotely from the coordination centre and on-site. Monitoring procedures and requirements will be documented in a Monitoring Plan, developed in accordance with the risk assessment.

Quality Control will be performed according to Imperial College Trials Unit (ICTU) internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

*BMJ Open – Protocol:*

The study may be participant to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

## 2.7 Statistical considerations:

### 2.7.1 Sample size and powering:

Currently published data on alpha diversity change in patients undergoing HCT and/or IMT span different study types, vary in quality/granularity, and use different alpha diversity indices. The evidence available suggests larger decreases in alpha diversity at approximately one month post-HCT compared to baseline in patients who do not receive IMT relative to those that do[2 6 8 18 19]. Fitting a mixed-effects model (with fixed effects for arm, time (day), their corresponding interaction and a random per-patient intercept effect) with quadratic splines at 5 degrees-of-freedom (3 internal knots) to longitudinal change in alpha diversity data (measured with inverse Simpson's index) from baseline[2], IMT patients had an expected change in baseline alpha diversity at day 28 post-HCT 3.46 (pooled SE = 2.19) units more than placebo (IMT mean change = -4.70, SE = 1.44, n = 14; placebo mean change = -8.16, SE = 1.66, n = 11). We have used these results on IMT post-HCT to design our study of IMT pre-HCT vs. placebo.

Our null hypothesis is there is no difference between the change in day 28 ± 3 days alpha diversity (inverse Simpson's) post-HCT from baseline in patients receiving pre-HCT IMT compared to patients receiving placebo capsules (i.e., difference in between-arm changes from baseline is zero). Using a two-sample t-test to compare IMT-arm change to placebo-arm change with two-sided alpha controlled at 20%[20 21], we need 46 patients randomised 1:1 between IMT and placebo (23 per arm) to have ≥80% power to detect a between-arm difference of 3.46 units (with pooled standard deviation of 5.45 estimated at day 28 post-HCT from mixed-effects model). To account for dropouts at a rate of up to 8% across both arms, we will recruit 50 patients in total. Modelling and sample size calculations have been performed using R v3.6.1.

### 2.7.2 Statistical analysis:

*BMJ Open – Protocol:*

#### 2.7.2.1 Overall approach:

Statistical analyses will be formally documented within a detailed Statistical Analysis Plan (SAP) and structured using the estimand framework (as described in the ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials[21 22]) with intercurrent events and subsequent analysis strategies defined accordingly. Protocol non-adherence and other non-defined intercurrent events will be incorporated into analysis via a treatment policy where the patient is assessed based on their randomised arm regardless of the event in question. Any deviations from the SAP will be documented and signed off by the statisticians and CI, and filed in the Trial Master File (TMF).

#### 2.7.2.2 Primary Estimand:

*Population:* Adults with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), acute leukaemia (AL) of ambiguous lineage, high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML), and chronic myeloid leukaemia (CML) in blast phase, considered suitable/fit for allogeneic haematopoietic stem cell transplantation HCT.

*Treatment:* Capsulised IMT vs matched capsulised placebo.

*Variable:* Stool microbiota diversity post-HCT defined as the change in alpha diversity (measured as inverse Simpson's index) between IMT administration (at 14 +/- 3 days prior to HCT) and 28 +/-3 days after HCT.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at day 28.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy will be used to handle patient all defined intercurrent events, any patient response-assessment data collected post-randomisation will still be used in the analysis model if day 28 data is unavailable.

#### 2.7.2.2.1 Secondary Estimand (of Primary Outcome):

A complete-case approach will be undertaken on the primary outcome (taking patients that provide primary outcome data across all response-assessment timepoints)

*Population:* As primary estimand.

*Treatment:* As primary estimand.

*Variable:* As primary estimand.

*Population-level summary:* As per primary estimand.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy will be used in the event of adverse events or rescue medication. In the event of death, withdrawal or loss-to-follow-up a principal stratum strategy is to be followed such that only those that complete the assessment period are to be included for analysis.

#### 2.7.2.3 Secondary Estimands:

1) Assessment of tolerability and acceptability of treatment versus placebo through the Functional Assessment of Cancer Therapy measured using health-related quality of life and patient perspective questionnaires.

*Population:* As per primary estimand.

*Treatment:* As per primary estimand.

*Variable:* Scores arising from health-related quality of life (EQ-5D-5L) and EORTC patient perspective (EORTC_QLQ-C30) questionnaires.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at day 28, plus at follow-up timepoints day 100, day 200 and day 365.

*BMJ Open – Protocol:*

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy as primary estimand

2) Assessment of changes in inverse Simpson's index and other measures of gut microbiome diversity across all timepoints assessed, including alpha diversity and richness (i.e., as measured via Chao-1, Shannon, Faith's PD), and beta-diversity (Aitchinson's distance) as well as changes in gut microbiome taxonomic composition.

*Population:* As per primary estimand.

*Variable(s):* As per primary, plus Chao-1, Shannon, Faith's PD, Aitchinson's distance and taxonomic composition.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at days 7 & 14. Interaction effect for follow-up visit timepoints day 100, 200 and 365 will also be assessed.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use.

*Strategy to handle intercurrent events:* Treatment policy as primary estimand.

3) Clinical endpoints including markers of general health, infective/microbiological and haematological outcomes across all timepoints measured, including: admission to intensive care unit, survival, non-relapse mortality, relapse incidence; occurrence and severity of graft-versus-host disease (GvHD), overall and GvHD-free relapse-free survival, and quality of life.

*Population:* As per primary estimand.

*Variable(s):* Overall and GvHD-free relapse-free survival.

*Population-level summary:* Log-rank test statistic, hazard-ratio (with 95% CI).

*Intercurrent Events:* Death unrelated to patient comorbidity (relapse-free survival only), death related to patient comorbidity (relapse-free survival only), loss-to-follow-up/patient withdrawal, adverse/serious adverse events, rescue therapy outside of antibiotic use.

*BMJ Open – Protocol:*

*Strategy to handle intercurrent events:* Death unrelated to patient comorbidity (relapse-free survival only) will be censored at recorded time-of-death as part of a hypothetical strategy. Where death is potentially related, a composite strategy is to be considered where time-of-death will be taken as time-of-relapse. Loss-to-follow-up/patient withdrawal will be censored at time of last contact as part of a hypothetical strategy. Treatment policy will be used upon use of rescue therapy or under any adverse/serious adverse event which does not result in the withdrawal of the patient.

Estimands for additional variables covered in secondary outcome #3 will be provided within the Statistical Analysis Plan (SAP). These include; Markers of general health (ITU Admission, Severity of Mucositis, Occurrence of Severe Acute Kidney Injury (AKI), Occurrence of Severe liver dysfunction, Use of Parenteral Nutrition), Infective Haematological Outcomes (Fever Occurrence, Fever CTCAE Grade, Infection, Multi drug Resistant Bacterial Colonisation (MDROs), Antibiotic Use), Neutrophil and platelet engraftment data, Recovery of T-cell Chimaerisms, Haematological Outcomes (Non-relapsed mortality, Occurrence Graft vs Host Disease, Severity of graft vs Host Disease)

#### 2.7.2.4 Analysis of primary estimand:

The primary outcome of between-arm difference in alpha diversity change from baseline at day 28 (± 3 days) will be analysed using a mixed-effects model, with change in alpha diversity from baseline as outcome, with treatment arm, time, treatment-by-time interactions and stratification variables used in randomisation included as fixed effects and also a per-patient intercept included as a random effect. The subsequent model estimate for the treatment-by-time interaction term at day 28 will be the effect of interest as per **Section 2.7.2.2.**

#### 2.7.2.5 Analysis of secondary estimands:

The complete-case analysis will follow the same model as defined in **Section 2.7.2.4** (using only patients attending all visits as per estimand). Mixed-effect models incorporating a per-patient random effect alongside effects for time of assessment, and an interaction term of time-by-arm assessing changes in alpha diversity (inverse Simpson's index, Chao-1, Shannon index, Faith's PD) and β-diversity will provide treatment effects and 80% confidence intervals

*BMJ Open – Protocol:*

at Response assessments 1-5 and Follow-up assessments 1-3 (see **Figure 1**). Similar approaches will be used to assess changes in gut microbiome taxonomic composition based on shallow shotgun sequencing.

Overall survival (time from randomisation to death/date last seen alive) will be analysed using Kaplan-Meier methods and log-rank testing utilising the same stratification variables as per primary analysis model defined in **Section 2.7.2.4.** Additional survival analysis will include non-relapse mortality and GvHD-free relapse-free survival.

**2.7.2.6 Analysis of secondary outcome measures:**

Additional analyses of clinical outcomes will include: number of days spent in Intensive Care; presence and severity of mucositis and length of time requiring parenteral nutrition; days of fever post-HCT corrected for length of admission; days of antibiotics including carbapenem; number and length of bloodstream infections; colonisation with multi-drug resistant bacteria, including extended-spectrum β-lactamases, vancomycin-resistant *Enterococci*, and carbapenemase-producing *Enterobacteriaceae*; and incidence of GvHD; and relapse incidence. Neutrophil and platelet engraftment data, Recovery of T-cell Chimaerisms, Haematological Outcomes (Non-relapsed mortality, Occurrence Graft vs Host Disease, Severity of graft vs Host Disease)

Analysis will be completed via presentation of descriptive statistics or summary tables. Continuous outcomes will be assessed via the same mixed-model approach as per primary estimand. Frequency outcomes will utilise a negative binomial approach, adjusting for the same covariates as the primary estimand analysis model. In the event where data fails to satisfy model assumptions and transformation is not suitable, an appropriate non-parametric approach may be used in replacement.

Full details of analysis methodology to be provided in the SAP.

#### 2.7.2.7 Safety analysis:

Additional safety outcomes - including AEs, ARs, SAE and SUSARs - will be reported as frequencies, unadjusted participant proportions and/or rates where appropriate. Differences between arms with 95% confidence intervals using exact methods will be produced where appropriate.

## 3 Discussion:

The increasing recognition of the contribution of the gut microbiome in patients with haematological malignancies undergoing cellular therapies, coupled with emergent data supporting IMT as a strategy to alter the microbiome, necessitates robust placebo-controlled IMT trials. Primarily, phase IIa trials such as MAST aim to fully evaluate the specific contribution that IMT have as part of patient treatment and provide the launchpad for future phase 3 trials. We hope that associated microbiome, metabolomic and immune analyses will improve understanding of the mechanistic contribution of the gut microbiome to the clinical outcomes seen, potentially setting the stage for future novel targeted 'microbiome therapeutics' that avoid the drawbacks associated with IMT. Whilst we envisage that most of our analyses will involve comparison of the dynamics of clinical and biological variables between the IMT and placebo arms, there may also be within-group exploratory analyses which provide further relevant insight as well (e.g. comparison of 'responders' and 'non-responders' to FMT within the treatment arm only, looking for the impact of baseline host gut microbiome diversity and/or specific taxonomic features upon the likelihood of response).

A growing body of non-randomised studies has described positive clinical signals when IMT was used in patients with haematological malignancies undergoing HCT[8]. However, it was also noteworthy that a recent phase II randomised double-blind placebo-controlled trial[23], administering capsulised IMT or placebo after HCT for AML, timing this for after neutrophil recovery, failed to achieve its primary outcome, showing no statistical difference in the infection rate by four months post-HCT in the IMT arm compared to placebo. One fundamental difference in design between that study and our study is that in our trial, the IMT is targeted at the pre- (rather than post-) HCT period. There were several reasons for us considering that this aspect of timing is particularly important. Most importantly, the published data related to the dynamics of the gut microbiome with HCT particularly

demonstrate the close association between reduced gut microbiome diversity pre-HCT and future morbidity and mortality, as well as the emergence of *Enterococcus* domination within the gut microbiome within three weeks post-allogeneic HCT as influencing poor outcome[6 7]. Additionally, aberrant intestinal microbiome diversity is known to be associated with increased inflammatory response[24] and biomarkers of inflammation measured pre HCT were shown to be independent predictors of HCT outcomes[25].

Therefore, we concluded that the clearest window for intervention is pre-HCT, aiming to increase the pre-HCT gut microbiota diversity, and mitigate the risk of pathobiont overgrowth is prior to start of HCT conditioning (**Figure 2**). The concept of targeting IMT prior to intervention has also been used successfully in oncology, with a phase 1 study evaluating IMT use prior to immune checkpoint inhibition in 20 patients with advanced melanoma demonstrating an objective response rate of 65% (*n*=13/20; including 4/20 complete responses)[26].

The use of IMT in the context of immunosuppressed patients requires certain considerations above and beyond those of, for instance, conventional use of IMT in treating recurrent CDI[27]. The use of a capsulised preparation is clearly more acceptable to this patient cohort than conventional IMT slurry, and may be safer avoiding potential aspiration of slurry. The donor screening protocol used donors is in full accordance with UK recommendations[9]; while the risk of CMV transmission via IMT appears extremely low[28], CMV negative donors are being used out of an abundance of caution. The window for IMT administration aims to be long enough after prior chemotherapy to allow full cell count recovery, but early enough before HCT to permit sufficient microbiota engraftment. This is important, since degree of microbiota engraftment has been associated with level of clinical improvement after IMT[29]. Our experience to date is that IMT mitigates the risk of invasive infections related to MDROs rather than decolonises them from the gut[15], but there is still uncertainty regarding this; one recent trial of IMT in a renal transplant population suggested certain ESBL-colonising strains being displaced by non-ESBL strains by strain competition[30]. The serial clinical assessment and collection of shotgun metagenomic data from study participants in a placebo-controlled fashion will allow much more granular assessment of the impact of IMT on MDROs than has been described previously.

In conclusion, the MAST trial aspires to give new clinical and translational insights into the role of gut microbiome manipulation in patients with haematological malignancy receiving allogeneic HCT, with particular focus on the potential role of IMT on haematological and infective outcomes. The study aims to run recruitment for 24 months post-authorisation, and close in May 2027.

## 4 Ethics and Dissemination:

### 4.1 Research Ethics Approval:

The institutional review board (North East - Tyne & Wear South, England, Ref: 23/NE/0105) and the national regulatory authorities, Medicines & Healthcare products Regulatory Agency (MHRA, Ref: CTA 19174/0441/001-0001) issued approval on the 3rd October 2023.

#### 4.1.1 Other ethical considerations:

##### 4.1.1.1 Consent:

Patients will be identified as per site established processes and invited to participate by their primary haematology team. Eligible patients will be provided with the patient information sheet (PIS) (**Supplementary Material 2**), and given sufficient time to consider the study, with opportunities to discuss and ask questions. Investigators will ensure that they adequately explain the study, including the aims, trial treatment, anticipated benefits and potential risks of participation. The right of the patient to refuse participation in the trial, or withdraw at any point, without giving explanation will be respected. Informed consent will be requested from the patient by the investigator who has been delegated the responsibility on the delegation log. Consent will be obtained no earlier than 24 hours after receiving the PIS to give them time to read and understand what their participation in the study entails (consent form provided as **Supplementary Material 3**). With patient consent, it is the investigator's responsibility to inform the patient's General Practitioner regarding study participation.

##### 4.1.1.2. Study Conduct and Safety Measures:

The study prioritises the highest safety and ethical standards, ensuring full compliance with the 1964 Declaration of Helsinki and International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines). Rigorous pharmacovigilance measures are in place

to monitor and reporting of serious or non-serious adverse events/reactions to enable a prompt and appropriate clinical response. There are stringent protocols in place for the reporting of causality, expectedness, and severity assessments; every reported event undergoes thorough evaluation by clinical professionals. These safety measures are essential for maintaining participant welfare and upholding study integrity. Additionally, robust donor screenings and contraception requirements minimise potential risks associated infection transmission and unanticipated pregnancy outcomes respectively, to reinforce a comprehensive approach to participant safety.

#### 4.1.1.3. Dissemination:

The dissemination of results from this study will at all times be performed with input from our PPI group. This will be foremost via abstract presentations at conferences and manuscripts in peer-reviewed journals. All such publications will be circulated to all authors prior to submission for their review and approval. Publications will be made in concordance with Consort guidelines/ checklists. Study participants will be notified of the outcome of the trial prior to any publications. A Clinical Study Report summarising the study results will be prepared and submitted to the Research Ethics Committee within a year of the end of study. The results will also be submitted to the EudraCT results database in accordance with regulatory requirements.

## 5. References:


1. Stoma I, Littmann ER, Peled JU, et al. Compositional Flux Within the Intestinal Microbiota and Risk for Bloodstream Infection With Gram-negative Bacteria. *Clin Infect Dis* 2021;73(11):e4627-e35. doi: 10.1093/cid/ciaa068
2. Taur Y, Coyte K, Schluter J, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. *Sci Transl Med* 2018;10(460) doi: 10.1126/scitranslmed.aap9489
3. Tacconelli E, De Angelis G, Cataldo MA, et al. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. *Antimicrob Agents Chemother* 2009;53(10):4264-9. doi: 10.1128/AAC.00431-09 [published Online First: 20090810]
4. Danielsen AS, Franconeri L, Page S, et al. Clinical outcomes of antimicrobial resistance in cancer patients: a systematic review of multivariable models. *BMC Infect Dis* 2023;23(1):247. doi: 10.1186/s12879-023-08182-3 [published Online First: 20230418]
5. Shono Y, Docampo MD, Peled JU, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Sci Transl Med* 2016;8(339):339ra71. doi: 10.1126/scitranslmed.aaf2311
6. Peled JU, Gomes ALC, Devlin SM, et al. Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. *N Engl J Med* 2020;382(9):822-34. doi: 10.1056/NEJMoa1900623
7. Stein-Thoeringer CK, Nichols KB, Lazrak A, et al. Lactose drives Enterococcus expansion to promote graft-versus-host disease. *Science* 2019;366(6469):1143-49. doi: 10.1126/science.aax3760
8. Malard F, Gaugler B, Mohty M. Faecal microbiota transplantation in patients with haematological malignancies undergoing cellular therapies: from translational research to routine clinical practice. *Lancet Haematol* 2022;9(10):e776-e85. doi: 10.1016/S2352-3026(22)00223-X
9. Mullish BH, Merrick B, Quraishi MN, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridioides difficile infection and other potential indications: second edition of joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut* 2024;73(7):1052-75. doi: 10.1136/gutjnl-2023-331550 [published Online First: 20240606]

*BMJ Open – Protocol:*

10. Allegretti JR, Khanna S, Mullish BH, et al. The Progression of Microbiome Therapeutics for the Management of Gastrointestinal Diseases and Beyond. *Gastroenterology* 2024 doi: 10.1053/j.gastro.2024.05.004 [published Online First: 20240514]
11. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *N Engl J Med* 2019;381(21):2043-50. doi: 10.1056/NEJMoa1910437 [published Online First: 20191030]
12. Bilinski J, Grzesiowski P, Sorensen N, et al. Fecal Microbiota Transplantation in Patients With Blood Disorders Inhibits Gut Colonization With Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clin Infect Dis* 2017;65(3):364-70. doi: 10.1093/cid/cix252
13. Millan B, Park H, Hotte N, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. *Clin Infect Dis* 2016;62(12):1479-86. doi: 10.1093/cid/ciw185 [published Online First: 20160329]
14. Innes AJ, Mullish BH, Fernando F, et al. Faecal microbiota transplant: a novel biological approach to extensively drug-resistant organism-related non-relapse mortality. *Bone Marrow Transplant* 2017;52(10):1452-54. doi: 10.1038/bmt.2017.151 [published Online First: 20170717]
15. Ghani R, Mullish BH, McDonald JAK, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. *Clin Infect Dis* 2021;72(8):1444-47. doi: 10.1093/cid/ciaa948
16. Innes AJ, Mullish BH, Ghani R, et al. Fecal Microbiota Transplant Mitigates Adverse Outcomes Seen in Patients Colonized With Multidrug-Resistant Organisms Undergoing Allogeneic Hematopoietic Cell Transplantation. *Front Cell Infect Microbiol* 2021;11:684659. doi: 10.3389/fcimb.2021.684659 [published Online First: 20210827]
17. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586. doi: 10.1136/bmj.e7586 [published Online First: 20130108]
18. van Lier YF, Davids M, Haverkate NJE, et al. Donor fecal microbiota transplantation ameliorates intestinal graft-versus-host disease in allogeneic hematopoietic cell transplant recipients. *Sci Transl Med* 2020;12(556) doi: 10.1126/scitranslmed.aaz8926
19. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368(5):407-15. doi: 10.1056/NEJMoa1205037 [published Online First: 20130116]
20. Rubinstein LV, Korn EL, Freidlin B, et al. Design issues of randomized phase II trials and a proposal for phase II screening trials. *J Clin Oncol* 2005;23(28):7199-206. doi: 10.1200/JCO.2005.01.149
21. Sargent DJ, Taylor JM. Current issues in oncology drug development, with a focus on Phase II trials. *J Biopharm Stat* 2009;19(3):556-62. doi: 10.1080/10543400902802474
22. E9 (R1) Step 5 addendum on estimands and Sensitivity Analysis in Clinical Trials to the guideline on statistical principles for clinical trials.
23. Rashidi A, Ebadi M, Rehman TU, et al. Randomized Double-Blind Phase II Trial of Fecal Microbiota Transplantation Versus Placebo in Allogeneic Hematopoietic Cell Transplantation and AML. *J Clin Oncol* 2023;41(34):5306-19. doi: 10.1200/JCO.22.02366 [published Online First: 20230526]

24. Tilg H, Zmora N, Adolph TE, et al. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* 2020;20(1):40-54. doi: 10.1038/s41577-019-0198-4 [published Online First: 20190806]
25. Patel A, Szydlo RM, Auner HW, et al. C-reactive protein prior to myeloablative allogeneic haematopoietic cell transplantation identifies patients at risk of early- and long-term mortality. *Br J Haematol* 2018;180(6):889-92. doi: 10.1111/bjh.14454 [published Online First: 20161129]
26. Routy B, Lenehan JG, Miller WH, Jr., et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. *Nat Med* 2023;29(8):2121-32. doi: 10.1038/s41591-023-02453-x [published Online First: 20230706]
27. Ghani R, Mullish BH, Davies FJ, et al. How to adapt an intestinal microbiota transplantation programme to reduce the risk of invasive multidrug-resistant infection. *Clin Microbiol Infect* 2022;28(4):502-12. doi: 10.1016/j.cmi.2021.11.006 [published Online First: 20211123]
28. Galperine T, Engelmann I, Hantz S, et al. Cytomegalovirus in donors for fecal microbiota transplantation, the phantom menace? *PLoS One* 2023;18(6):e0287847. doi: 10.1371/journal.pone.0287847 [published Online First: 20230629]
29. Ianiro G, Puncochar M, Karcher N, et al. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. *Nat Med* 2022;28(9):1913-23. doi: 10.1038/s41591-022-01964-3 [published Online First: 20220915]
30. Woodworth MH, Conrad RE, Haldopoulos M, et al. Fecal microbiota transplantation promotes reduction of antimicrobial resistance by strain replacement. *Sci Transl Med* 2023;15(720):eabo2750. doi: 10.1126/scitranslmed.abo2750 [published Online First: 20231101]
31. Salvador Castell G, Serra-Majem L, Ribas-Barba L. What and how much do we eat? 24-hour dietary recall method. *Nutr Hosp* 2015;31 Suppl 3:46-8. doi: 10.3305/nh.2015.31.sup3.8750 [published Online First: 20150226]

## 6. Author contributions:

BHM, AJI, RG, FJD, SA-B, NAJ, JRM, JP all contributed to initial drafting of the manuscript, with all authors reviewing and approving the final submitted manuscript. JP conceptualised the trial protocol and is responsible for its clinical aspects. BHM and JRM provided expertise in IMT provision and microbiome/ metabolome analysis for the study. AJI and JP were responsible for analysis of all haematological aspects of the study, both clinical and translational, and contributed to set-up of recruiting centres. RG and FJD provided expertise regarding microbiological/ infection-related aspects of outcome and analysis in the study. LAR provided input into patient-facing materials for the trial and oversees translational and exploratory outcomes. SA-B and LW both contributed to all aspects of trial approval and administrative/ logistical set-up. GW and NAJ oversaw all aspects of statistical analysis within the trial. PF, ABK, FK, PK (Kottaridis), PK (Krishnamurthy), EN and RP are all site principal investigators for the studies. BHM is the guarantor.

## 7. Acknowledgements:

The authors are grateful to Dr James McIlroy and Dr Michael Smyth (both from EnteroBiotix Ltd) for review of this manuscript.

## 8. Data Statement:

The full version of the current protocol (v1.2, 17th January 2024) is available from the corresponding author on reasonable request. The trial website is available at: https://www.imperial.ac.uk/metabolism-digestion-reproduction/research/digestive-diseases/hepatology--gastroenterology/mast-study/ .

Information regarding the handling of clinical data for this study is provided in **Section 2.6.** Regarding biological data – all such data (including sequencing, immunological and metabolomic data) will be stored locally at Imperial College London in password-controlled areas of OneDrive, SharePoint or Research Data Store as appropriate. All metagenomic sequencing data generated in this trial will be deposited at the EBI's ENA repository for public use, with all metabolomic data uploaded to the EMBL EBI MetaboLights repository; all such data will be made open access. The biological data is stored in perpetuity at the EBI's site, whilst all other trial data will be stored for 20 years after the trial has closed.

## 9. Funding statement:

This study is funded via the Medical Research Council (MRC) Developmental Pathway Funding Scheme (DPFS) (grant number: MR/X004996/1). The Division of Digestive Diseases and Centre for Haematology receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre (BRC) based at Imperial College London and Imperial College Healthcare NHS Trust. BHM is the recipient of a Medical Research Council (MRC) Clinician Scientist award (MR/Z504002/1) and was the recipient of an NIHR Academic Clinical Lectureship (CL-2019-21-002).

## 10. Competing interests statement:

GMW is an employee of and holds share in GSK. All other authors have nothing to report.

**11. Figure legends:**

**Figure 1: Study flow chart participant time line.**

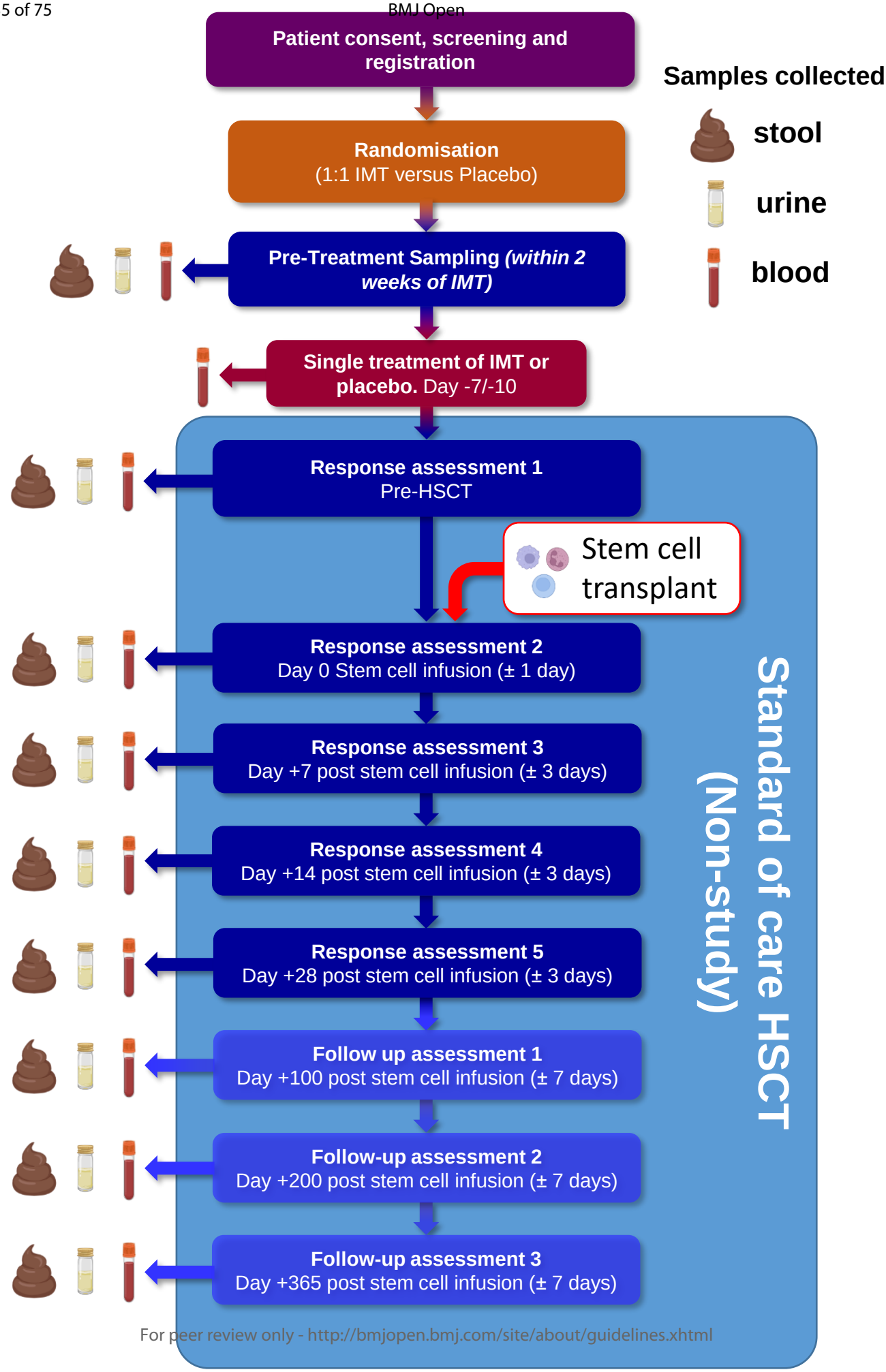
**Figure 2: 'Prehabilitation' of the gut microbiome in MAST.** Dynamics of the gut microbiota conventionally through the peri-HCT period shown in black (as defined previously[6]); the red line is our predicted higher starting point and nadir for patients receiving a pre-HCT IMT in the MAST trial.

**Table 1: Secondary objectives/ outcomes from the MAST study:**

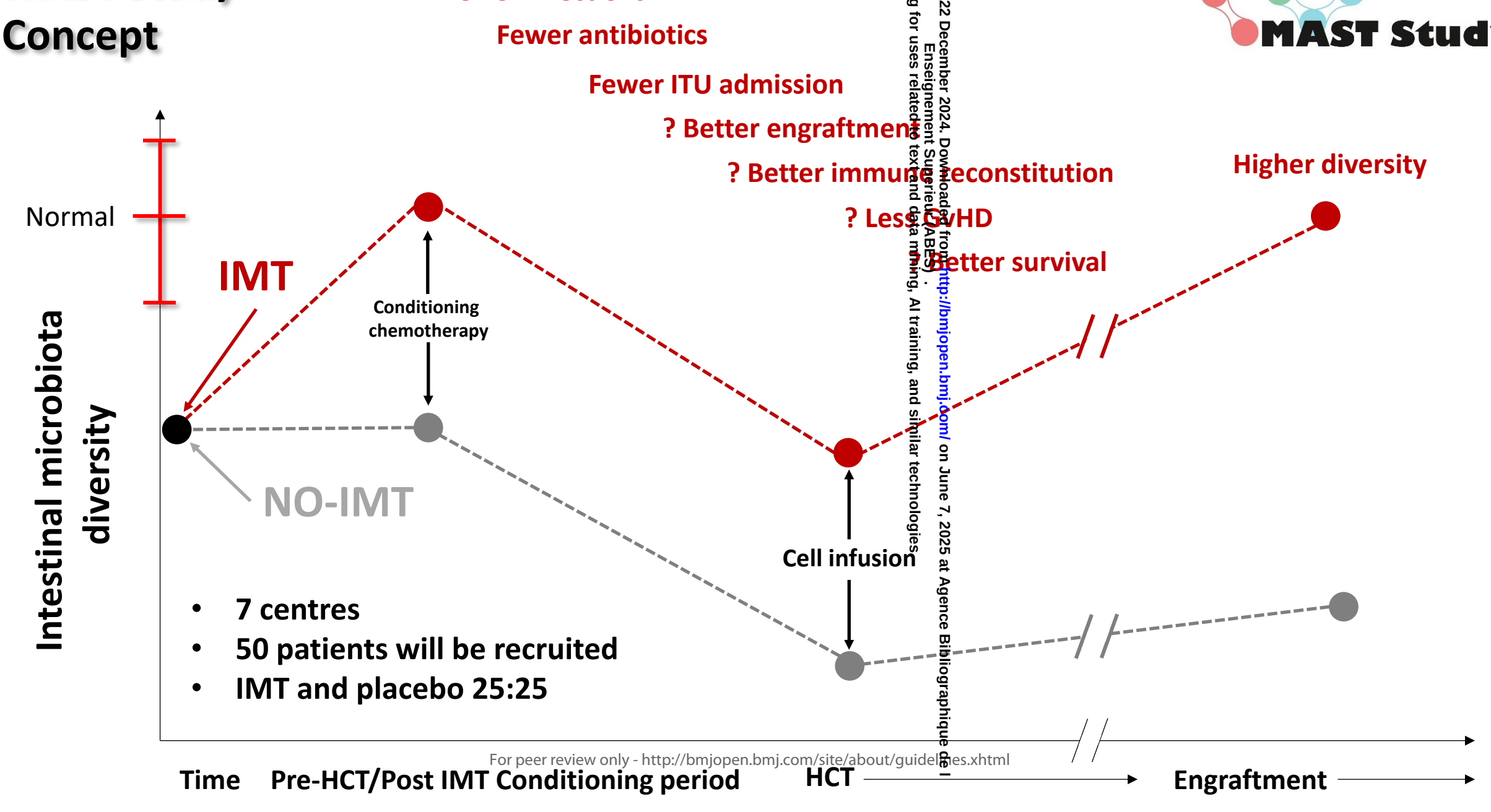
| Objectives: | Outcome: |
|---|---|
| Determine the feasibility and tolerability of capsule IMT prior to HCT in a multi-centre setting. | Tolerability and acceptability of IMT/placebo (as assessed via patient perspective questionnaires, i.e. EQ-5D-5L and EORTC QLQ-C30 questionnaires). |
| Evaluate microbiological/ infective, haematological, and quality of life-related clinical outcomes of administering IMT prior to HCT. | Gut microbiome endpoints:<br>• Assessment of changes in inverse Simpson's index and other measures of gut microbiome diversity across all timepoints assessed, including alpha diversity and richness (i.e., as measured via Chao-1, Shannon, Faith's PD), and beta-diversity (Aitchinson's distance)<br>• Assessment of changes in gut microbiome taxonomic composition across all timepoints assessed (using shallow shotgun sequencing). |

*BMJ Open – Protocol:*

| | |
|---|---|
| | Clinical endpoints:<br>• Markers of general health across all timepoints measured, including: days on the Intensive Treatment Unit (ITU); presence and severity of mucositis; use of (and length of time that requiring) parenteral nutrition; severe acute kidney injury and severe liver dysfunction.<br>• Infective/ microbiological outcomes across all timepoints measured, including: days of fever post-HCT (corrected for length of admission); days on antibiotics (including use of carbapenem specifically); number and length of bloodstream infections; urinary tract infections; colonisation with multi-drug resistant bacteria (MDROs; including extended-spectrum beta-lactamases (ESBL), vancomycin-resistant enterococci (VRE), and carbapenemase-producing Enterobacteriales (CPE)), and use of antibiotics.<br>• Haematological outcomes across all timepoints measured, including: non-relapse mortality, relapse incidence; occurrence and severity of graft-versus=host disease (GvHD), overall and GvHD-free relapse-free survival, and quality of life. |
| Explore the potential for pre-HCT IMT to impact on HCT engraftment and immune reconstitution. | Neutrophil and platelet engraftment data as defined by EBMT will be routinely collected. Recovery of T-cell chimaerisms, T-cell count assessed by the lymphocyte subset analysis and immunoglobulin levels will be recorded at follow-up assessments. |



# MAST Study Concept



*BMJ Open – Protocol – Supplementary Material*

**Supplementary Material 1: Appendices:**

**Intestinal *M*icrobiota Transplant Prior to *A*llogeneic *S*tem Cell *T*ransplant (MAST) trial:**

**Study Protocol for a Phase IIa Randomised Controlled Trial**

**Appendix 1 - Neutropenia inducing regimens:**

Daunorubicin and cytarabine (DA) (with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Liposomal cytarabine and daunorubicin (Vyxeos) (with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

High dose cytarabine (>1000mg/m2) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Azacitine/decitabine (including oral forms) and venetoclax

Fludarabine / Cytarabine / GCSF / Idarubicin (FLAG-Ida) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, venetoclax, dasatinib or ponatinib)

Clofarabine / Cytarabine / GCSF / Idarubicin (CLAG-Ida) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Mitozantrone / Etoposide / Cytarabine (MEC) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

UK-ALL 14 phase 1 induction (off trial) or similar (with or without additional venetoclax)

UK-ALL 14 phase 2 induction (off trial) or similar (with or without additional venetoclax)

UK-ALL14 intensification (High dose methotrexate) or similar (with or without additional venetoclax)

Cyclophosphamide / Dexamethasone / Doxorubicin / Vincristine / Cytarabine alternating with methotrexate / cytarabine (Hyper-CVAD / MA) (with or without additional venetoclax)

*BMJ Open – Protocol – Supplementary Material*

**Appendix 2 – Disease and response criteria:**

***Acute lymphoblastic leukaemia (ALL) classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias)) and response criteria (modified Center for International Blood and Marrow Transplant Research criteria)***

| **B-ALL** |
|---|
| B-ALL with recurrent genetic abnormalities |
| B-ALL with t(9;22)(q34.1;q11.2)/*BCR::ABL1* |
| with lymphoid only involvement |
| with multilineage involvement |
| B-ALL with t(v;11q23.3)/*KMT2A* rearranged |
| B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1* |
| B-ALL, hyperdiploid |
| B-ALL, low hypodiploid |
| B-ALL, near haploid |
| B-ALL with t(5;14)(q31.1;q32.3)/*IL3::*IGH |
| B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1* |
| B-ALL, *BCR*::*ABL1*–like, ABL-1 class rearranged |
| B-ALL, *BCR*::*ABL1*–like, JAK-STAT activated |
| B-ALL, *BCR*::*ABL1*–like, NOS |
| B-ALL with iAMP21 |
| B-ALL with *MYC* rearrangement |
| B-ALL with *DUX4* rearrangement |
| B-ALL with *MEF2D* rearrangement |
| B-ALL with *ZNF384(362)* rearrangement |
| B-ALL with *NUTM1* rearrangement |
| B-ALL with *HLF* rearrangement |
| B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* (“CDX2/UBTF”) |
| B-ALL with mutated *IKZF1* N159Y |
| B-ALL with mutated *PAX5* P80R |
| Provisional entity: B-ALL, *ETV6::RUNX1*-like |

| |
|---|
| Provisional entity: B-ALL, with *PAX5* alteration |
| Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/IGH::*CEBPE* |
| Provisional entity: B-ALL, *ZNF384* rearranged-like |
| Provisional entity: B-ALL, *KMT2A* rearranged-like |
| B-ALL, NOS |
| **T-ALL** |
| Early T-cell precursor ALLwith *BCL11B* rearrangement |
| Early T-cell precursor ALL, NOS |
| T-ALL, NOS |
| Provisional entities (see supplemental Table 7) |
| **Provisional entity: natural killer cell ALL** |

Complete Remission (CR)

Hematologic complete remission is defined as meeting **all** of the following response criteria for at least four weeks.

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- ANC (absolute neutrophil count) ≥ 1.0 x $10^9$/L
- Platelets ≥ 100 x $10^9$/L
- Transfusion independent

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment; in this case, CR should still be reported as the status at transplant, since it represents the “best assessment” prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks. The pre-transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

Include recipients who are MRD positive or where the MRD status is unknown. MRD assessments include cytogenetic, flow cytometry, and molecular methods.

Include recipients meeting the above CR criteria regardless of how many courses of therapy were required to achieve CR.

The number of this complete remission can be determined by using the following guidelines:

- 1st CR: no prior relapse
- 2nd CR: one prior relapse
- 3rd or higher: two or more prior relapses

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria for at least four weeks:

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Primary Induction Failure (PIF)

The patient received treatment for ALL but **never achieved CR or CRi at anytime**. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have never been in CR or CRi.

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting at least one of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

The number of this relapse can be determined by using the following guidelines:

- 1st relapse: one prior CR
- 2nd relapse: two prior CRs
- 3rd or higher: three or more CRs

Do not include a partial response (PR) when determining number of relapse. Recipients who achieve a PR to treatment should be classified as either PIF or relapse; PR in ALL is generally of short duration and is unlikely to predict clinical benefit.

***Acute myeloid leukaemia (AML) classification (international Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria (modified Center for International Blood and Marrow Transplant***

| |
|---|
| ***Research criteria)***Acute promyelocytic leukaemia (APL) with t(15;17)(q24.1;q21.2)/*PML*::*RARA* ≥ 10% |
| APL with other *RARA* rearrangements* ≥ 10% |
| AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* ≥ 10% |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11* ≥ 10% |
| AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A* ≥ 10% |
| AML with other *KMT2A* rearrangements† ≥ 10% |
| AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214* ≥ 10% |
| AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*; *MECOM*(*EVI1*) ≥ 10% |
| AML with other *MECOM* rearrangements‡ ≥ 10% |
| AML with other rare recurring translocations (see supplemental Table 5) ≥ 10% |
| AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*§ ≥ 20% |
| AML with mutated *NPM1* ≥ 10% |
| AML with in-frame bZIP *CEBPA* mutations ≥ 10% |
| AML and MDS/AML with mutated *TP53*† 10-19% (MDS/AML) and ≥ 20% (AML) |
| AML and MDS/AML with myelodysplasia-related gene mutations 10-19% (MDS/AML) and ≥ 20% (AML) |
| Defined by mutations in *ASXL1*, *BCOR*, *EZH2, RUNX1*, *SF3B1, SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2* |
| AML with myelodysplasia-related cytogenetic abnormalities 10-19% (MDS/AML) and ≥ 20% (AML) |
| Defined by detecting a complex karyotype (≥ 3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurring genetic abnormalities), del(5q)/t(5q)/add(5q), −7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), −17/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities |
| AML not otherwise specified (NOS) 10-19% (MDS/AML) and ≥ 20% (AML) |
| Myeloid sarcoma |

*

Includes AMLs with t(1;17)(q42.3;q21.2)/*IRF2BP2*::*RARA*; t(5;17)(q35.1;q21.2)/*NPM1*::*RARA*; t(11;17)(q23.2;q21.2)/*ZBTB16*::*RARA*; cryptic inv(17q) or del(17) (q21.2q21.2)/*STAT5B*::*RARA*, *STAT3*::*RARA*; Other genes rarely rearranged with *RARA:TBL1XR1* (3q26.3), *FIP1L1* (4q12), *BCOR*(Xp11.4).

†

Includes AMLs with t(4;11)(q21.3;q23.3)/*AFF1*::*KMT2A*[#]; t(6;11)(q27;q23.3)/*AFDN*::*KMT2A*; t(10;11)(p12.3;q23.3)/*MLLT10*::*KMT2A;* t(10;11)(q21.3;q23.3)/*TET1*::*KMT2A;* t(11;19)(q23.3;p13.1)/*KMT2A*::*ELL;* t(11;19)(q23.3;p13.3)/*KMT2A*::*MLLT1* (occurs predominantly in infants and children).

‡

Includes AMLs with t(2;3)(p11~23;q26.2)/*MECOM*::*?;* t(3;8)(q26.2;q24.2)/*MYC*, *MECOM*; t(3;12)(q26.2;p13.2)/*ETV6*::*MECOM*; t(3;21)(q26.2;q22.1)/*MECOM*::*RUNX1.*

§

The category of MDS/AML will not be used for AML with *BCR*::*ABL1* due to its overlap with progression of CML, *BCR*::*ABL1*-positive.

Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- No extramedullary disease (e.g., CNS, soft tissue disease)
- Neutrophils ≥ 1.0 x $10^9$/L
- Platelets ≥ 100 x $10^9$/L
- Transfusion independent

Include recipients who are MRD positive or where the MRD status is unknown. MRD assessments include cytogenetic, flow cytometry, and molecular methods.

Include recipients meeting the above CR criteria regardless of how many courses of therapy were required to achieve CR.

The number of this complete remission can be determined by using the following guidelines:

- 1st CR: no prior relapse
- 2nd CR: one prior relapse
- 3rd or higher: two or more prior relapses

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- No extramedullary disease (e.g., CNS, soft tissue disease)
-

Primary Induction Failure (PIF)

The patient received treatment for AML but **never achieved CR or CRi at anytime**. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have *never been in CR or CRi*.

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting one or more of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

The number of this relapse can be determined by using the following guidelines:

- 1st relapse: one prior CR
- 2nd relapse: two prior CRs
- 3rd or higher: three or more CRs

Do not include a partial response (PR) when determining number of relapse. Recipients who achieve a PR to treatment should be classified as either PIF or relapse; PR in AML is generally of short duration and is unlikely to predict clinical benefit.

***Myelodysplastic syndromes (MDS) classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria (modified Center for International Blood and Marrow Transplant Research criteria).***

| |
|---|
| Myelodysplastic syndrome with mutated *SF3B1* |
| Myelodysplastic syndrome with del(5q) |

| |
|---|
| Myelodysplastic syndrome with mutated *TP53* |
| Myelodysplastic syndrome, not otherwise specified (MDS, NOS) |
| MDS, NOS without dysplasia |
| MDS, NOS with single lineage dysplasia |
| MDS, NOS with multilineage dysplasia |
| Myelodysplastic syndrome with excess blasts |
| Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) |
| MDS/AML with mutated *TP53* |
| MDS/AML with myelodysplasia-related gene mutations |
| MDS/AML with myelodysplasia-related cytogenetic abnormalities |
| MDS/AML, not otherwise specified |

Complete Remission (CR)

Requires all of the following maintained for a minimum of four weeks. When reporting the CR achievement date, report the first date when CR was achieved (not the four week date in which CR was maintained).

Bone marrow evaluation:

- < 5% myeloblasts with normal maturation of all cell lines

Blood evaluation

- Haemoglobin ≥ 110 g/L untransfused without erythropoietic support
- Absolute neutrophil count ≥ 1.0 x $10^9$/L without myeloid growth factor support
- Platelets ≥ 100 x $10^9$/L without thrombopoietic support
- 0% blasts in blood

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment. In this case, CR should still be reported as the status at transplant since it represents the “best assessment” prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks; the pre-transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

***Chronic myeloid leukaemia with blast transformation classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria***

Philadelphia positive or BCR:ABL1 CML in blast phase defined by the presence of blast ≥ 20% in blood or bone marrow.

Second chronic phase (CP2)

Requires all of the following

**Bone marrow evaluation:**

- < 5% myeloblasts with normal maturation of all cell lines

**Blood evaluation**

- Absolute neutrophil count ≥ 1.0 x $10^9$/L without myeloid growth factor support
- Platelets ≥ 100 x $10^9$/L without thrombopoietic support
- < 5% blasts in blood

**Appendix 3 – Karnofsky performance status score**

- 100 Normal; no complaints; no evidence of disease.
- 90 Able to carry on normal activity; minor signs or symptoms of disease.
- 80 Normal activity with effort; some signs or symptoms of disease.
- 70 Cares for self; unable to carry on normal activity or to do active work.
- 60 Requires occasional assistance but is able to care for most of their personal needs.
- 50 Requires considerable assistance and frequent medical care.
- 40 Disabled; requires special care and assistance.
- 30 Severely disabled; hospital admission is indicated although death not imminent.
- 20 Very sick; hospital admission necessary; active supportive treatment necessary.
- 10 Moribund; fatal processes progressing rapidly.
- 0 Dead

**Appendix 4: Schedule/ Summary of Visits:** *Continued overleaf*

| | Screening | Treatment | Response Assessment: | | | | | Follow-up Assessment: | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 |
| **Visit** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **Day of HCT** | From -42 | -14 (± 2 days) | -7 (± 2) | 0 (± 1) | +7 (± 3) | +14 (± 3) | +28 (± 3) | +100 (± 7) | +200 (± 7) | +365 (± 14) |
| Informed Consent | X | | | | | | | | | |
| Inclusion & Exclusion Criteria | X | | | | | | | | | |
| Baseline data collection/Comorbidity Index | X | | | | | | | | | |
| Review of demographics, medical/disease | X | | | | | | | | | |
| Pregnancy test[1] | X | | | | | | | | | |
| EORTC-QLQ-C30 and EQ-5D-5L Questionnaires | X | | | | | | X | X | X | X |
| Bone marrow assessment[2] | X | | | | | | | X | | X |
| Physical Examination/Vital Signs (ECG) | <<All assessments (According to standard care practices)>> | | | | | | | | | |
| Lineage specific chimaerism[3] | | | | | | | | X | X | X |
| Lymphocyte subsets & IG levels[3] | | | | | | | | X | X | X |
| Stool Sample[7] | X | | X | X | X | X | X | X | X | X |
| Urine Sample[7] | X | | X | X | X | X | X | X | X | X |
| Blood Sample[7] | X | X | X | X | X | X | X | X | X | X |
| Clinical data collection[5] | | X | X | X | X | X | X | X | X | X |
| Adverse event assessment[4] | << Continuous assessment >> | | | | | | | | | |
| Assessment of GvHD | | | | | << Continuous assessment >> | | | | | |
| Cell infusion (HCT)[6] | | | | X | | | | | | |

# 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 and sample collections may be arranged within the ranges as indicated above without need to report as protocol deviation.

1. Pregnancy test for women of childbearing potential: serum/urine (investigator's discretion) pregnancy test (sensitivity of at least 25 mIU/mL) within 72 hours prior to starting study therapy. This applies even if the patient practices complete abstinence from heterosexual contact.
2. The results of bone marrow morphological, immunophenotypic, cytogenetic, and molecular characterisation performed according to local practice within the time points above should be reported within the time points above.
3. Chimaerism tests should be performed in local laboratories on day +30, +60, +90, +120, +200, and +365, lymphocytes subsets and immunoglobulin levels should be performed in local laboratories on days +100, +200, +365.
4. All AEs to be collected from written to consent to the first day of transplantation conditioning. After initiation of transplantation conditioning only AEs that are equal to or greater than Grade 3 of the CTCAE version 5.0 will be reported (unless the event meets the definition of an SAE) and abnormal laboratory findings will be reported only if they are judged to be of significant clinical importance. Reporting will stop at day +28 of transplantation. SAEs that are judged to be at least possibly related to the IMP(s) and are unexpected must still be reported in an expedited manner irrespective of how long after IMP administration the reaction occurred.
5. Collection of clinical data (see **Supplementary Material 1, Appendix 5** for summary of Assessments),

The following data will be collected at all study Visits (1-10)– Vital Signs, Physical Examination, Full Blood Count, Coagulation, Biochemistry, and Virology, Nutrition, Completed dietary questionnaire and ITU Admission. The dietary questionnaire comprises 24 hour dietary recall, a widely-used tool in studies with a nutritional component[1]; these dietary data will serve as useful metadata for the analysis of microbiome and metabolome data generated within the trial. The following data below will be collected in addition to the repeating assessments,

- Recent, Microbiology Colonisation History, Fever, Infection and Treatment History (Visits 2-10)
- Haemopoietic Cell transplant details (Visit 5 only).
- VOD, and Relapse, Engraftment, Acute GvHD, GvHD Prophylaxis and Therapy assessments (Visits 5-10).
- Post-transplant intervention assessment (Visit 10 only).

6. Haematopoietic stem cell transplant is not a study procedure and will take place as planned by the multi-disciplinary team before the patient enters the study following local standard of care procedures.
7. A summary of details for biosample collection, storage, and processing is given in **Supplementary Material 1, Appendix 6.**

*BMJ Open – Protocol – Supplementary Material*

**Appendix 5 - Summary of Assessments:**

| | Visit | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Assessment Forms** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Consent** | X | | | | | | | | | |
| **Demographics** | X | | | | | | | | | |
| **Eligibility** | X | | | | | | | | | |
| **Significant Medical History** | X | | | | | | | | | |
| **Chemotherapy History** | X | | | | | | | | | |
| **Infection and Treatment History** | X | | | | | | | | | |
| **Microbiology Colonisation History** | X | | | | | | | | | |
| **Antibiotic History** | X | | | | | | | | | |
| **Transplant Donor Characteristics** | X | | | | | | | | | |
| **Comorbidity Index Score** | X | | | | | | | | | |
| **Vital Signs** | X | X | X | X | X | X | X | X | X | X |
| **Physical Examination** | X | X | X | X | X | X | X | X | X | X |
| **Randomisation** | X | | | | | | | | | |
| **Current Medication** | X | | | | | | | | | |
| **Dietary Questionnaire** | X | X | X | X | X | X | X | X | X | X |
| **Full Blood Count, Coagulation, Biochemistry, and Virology** | X | X | X | X | X | X | X | X | X | X |
| **Bone Marrow Assessment Results** | X | | | | | | | X | X | X |
| **EQ-5D-5L Questionnaire** | X | | | | | | X | X | X | X |
| **EOTRC QLQ-C30 Questionnaire** | X | | | | | | X | X | X | X |
| **Sample Collection Form** | | | X | X | X | X | X | X | X | X |
| **Nutrition** | | X | X | X | X | X | X | X | X | X |
| **Recent Microbiology History** | | | X | X | X | X | X | X | X | X |
| **Recent fever, Infection and Treatment History** | | X | X | X | X | X | X | X | X | X |

*BMJ Open – Protocol – Supplementary Material*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Recent Microbial Colonisation History** | | X | X | X | X | X | X | X | X | X |
| **IMP Administration** | | X | | | | | | | | |
| **IMP Symptom Report form** | | | X | X | X | X | X | X | X | X |
| **Adverse Event** | | X | X | X | X | X | X | | | |
| **Haematopoietic Cell Transplant Details** | | | | | X | | | | | |
| **VOD, and Relapse** | | | | | X | X | X | X | X | X |
| **Acute GvHD** | | | | | X | X | X | X | X | X |
| **GvHD Prophylaxis and Therapy** | | | | | X | X | X | X | X | X |
| **Engraftment** | | | | | | | X | X | X | X |
| **Lymphocyte Subsets and Immunoglobulin Levels - Blood** | | | | | | | | X | X | X |
| **Linage Specific Chimaerisms - Blood** | | | | | | | | X | X | X |
| **Chronic GVHD Assessment** | | | | | | | | X | X | X |
| **Post Transplant Intervention** | | | | | | | | | | X |
| **ITU Admissions Review** | | | | | | | | | | X |

*BMJ Open – Protocol – Supplementary Material*

**Appendix 6 – Biosample collection, storage, and processing**

| Sample | Collection | Timepoints | Processing | Storage | Planned analysis |
|---|---|---|---|---|---|
| Stool | A faeces collection kit will be given to the patient to provide a stool sample. The sample will be placed into two tubes, an empty faeces tube and a tube containing a preservative. | All visits, except visit 2 | None | Stored at -80°C | Shotgun sequencing, metabolomics, metaproteomics, potential future culturing. |
| Urine | Sample will be collected in a urine pot (50 ml). Urine should be mid-stream of the first. Fasted urine of the day. | All visits, except visit 2 | None | Stored at -80°C | Metabolomics. |
| Blood – plasma | Sample will be collected using 4x 3 ml lithium heparin blood tubes. | All visits | Blood tubes will be centrifuged to pellet blood cells. Plasma will be aliquoted into 2 ml cryovials. Blood will be processed within 2 hours, or within 24 hours stored at 4°C. | Stored at -80°C | Metabolomics, analysis for markers of gut barrier integrity. |

*BMJ Open – Protocol – Supplementary Material*

| | | | | | |
|---|---|---|---|---|---|
| Blood - PBMC | Sample will be collected using 2x 10 ml EDTA blood tubes. | Visit 8 and 10 | PBMCs will be separated from blood by density gradient centrifugation using Ficoll. PBMCs will be washed, and a platelet removal centrifugation step will be carried out. Cells will be enumerated using a cell counter and resuspended in freezing media at 10 million cells per ml. Blood will be processed within 24 hours. | Stored in liquid nitrogen (-196°C). | Immune reconstitution assessment by lymphocyte subsets and T-cell repertoire characterisation. |
| Blood – PaxGene RNA | Sample will be collected using 1x 2.5 ml PaxGene RNA blood tube. | Visit 8 and 10 | None. Blood will be frozen within 6 hours from collection. | Stored at -80°C | |

**References:**

1. Salvador Castell G, Serra-Majem L, Ribas-Barba L. What and how much do we eat? 24-hour dietary recall method. *Nutr Hosp* 2015;31 Suppl 3:46-8. doi: 10.3305/nh.2015.31.sup3.8750 [published Online First: 20150226]

[Insert Site Logo]

# MAST PATIENT INFORMATION SHEET

| | |
|---|---|
| **Study Title** | Microbiota Transplant Prior to Allogeneic Stem Cell Transplantation (MAST) trial |
| **IRAS Project ID** | 1006971 |

## Introduction:

You are being invited to take part in a research study. Before you decide whether you wish to take part, it is important for you to understand why the research is being done and what it will involve. Someone from our team will go through the information sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with friends, relatives, your General Practitioner (GP) and other doctors involved in your clinical care if you wish.

**Part 1** tells you the purpose of this study and what will happen to you if you take part.

**Part 2** gives you more detailed information about the conduct of the study

Please, ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

**Thank you for taking the time to read this information sheet.**

MAST Study

[Insert Site Logo]

## Glossary of Terms

| Term | Explanation |
|---|---|
| CFU | Colony forming units – Is the term used to describe the number of viable microorganisms, e.g., bacteria, there are in the capsule |
| Haematopoietic cell transplant | The clinical name for a bone marrow or blood stem cell transplant to treat blood cancer such as leukaemia. |
| Intestinal microbiota transplant | Taking stool material from a healthy donor and processing it into a capsule form for oral use. |
| Investigator | A researcher involved in a clinical study. |
| Microbiota | A collection of microorganisms that live in and on human body. |
| Microorganisms | Small organisms such as bacteria, virus particles and other single cell organisms. |
| Organism | A form of life considered as an entity, such as an animal, plant, fungus or bacterium. |
| Placebo | A substance that has no therapeutic effect, used as a control in testing new drugs |
| Plasma | The liquid part of blood that is left after all blood cells have been removed and only a clotting protein (called Fibrin) remains. |
| Phlebotomy | The procedure of drawing blood from the vein with the use of sterile material by trained and qualified healthcare personnel. |
| Sample | A small part of a substance or material obtained for testing such as blood, urine and stool/faecal material. |
| Serum | The liquid part of blood, after all, blood cells and the clotting protein (Fibrin) have been removed. |

[Insert Site Logo]

MAST Study

# PART 1

## 1. What is the purpose of this study?

Doctors and scientists have realised recently that there are billions of 'beneficial' bacteria and other microbes living in the human gut. These microbes do not cause us harm, but actually perform many roles in helping to keep us healthy, such as through their effects on how we process food or energy, stopping us getting infections from gut bacteria, and in how our immune system works.

When antibiotics are given to patients with blood cancers, they have a side effect of reducing the numbers of 'beneficial bacteria' in the gut, limiting its supportive role for the immune system. The number of 'beneficial' gut bacteria are important to maintain in patients who receive treatment that further impacts the immune system, such as bone marrow transplant (haematopoietic cell transplant).

The MAST clinical trial will test a way of restoration of the normal balance and diversity (range) of microbes that live in the gut prior to starting bone marrow or blood stem cell transplant (haematopoietic cell transplant). The study will also examine how this treatment affects the many complications involved in bone marrow transplantation, such as fevers (high temperatures) and infections during the transplant period. The treatment is called intestinal microbiota transplantation and involves taking bacteria from healthy people's gut, then processing it and putting it into a capsule which when swallowed, releases the microbes into the recipient.

[Insert Site Logo]

## 2. Why have I been chosen?

You are being invited to take part in the research study because you will be undergoing a haematopoietic cell transplant, as part of the normal treatment for blood cancer, and because of your previous treatment (chemotherapy), you are predicted to have a lower number, and smaller range (diversity) of bacteria (microbiota) in your gut (intestines). We are looking to recruit 50 participants in total to this study. These 50 people will have acute leukaemia (AML or ALL), advanced myelodysplastic syndromes, chronic myelomonocytic leukaemia (CMML), or chronic myeloid leukaemia (CML) in blast phase and will be undergoing standard treatment (bone marrow or blood stem cell transplant) for their disease. Please read this information carefully before you decide whether to participate and ask your doctor for an explanation of anything that is not clear to you.

## 3. Do I have to take part?

It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive from your doctor or the hospital.

## 4. What will happen to me if I take part?

You will be approached about entering the study before you are scheduled to have bone marrow transplantation. If you take part in this study, you will be asked to follow the study treatment plan, tests and hospital appointments for 14months). You should consider how these tests and visits will affect your work and family life and decide if you are able to commit to them.

Sometimes because we do not know which way of treating patients is best, we need to make comparisons. People will be put into groups and then compared.

The groups are selected by a computer which has no information about the individual – i.e. by chance. Subjects in each group then have a different treatment and these are compared'.

This means you have a 1 in 2 (50%) chance of receiving the treatment. Neither you nor your doctor will know which treatment group you are in (although, if your doctor needs to find out he/she can do so).

MAST Study

[Insert Site Logo]

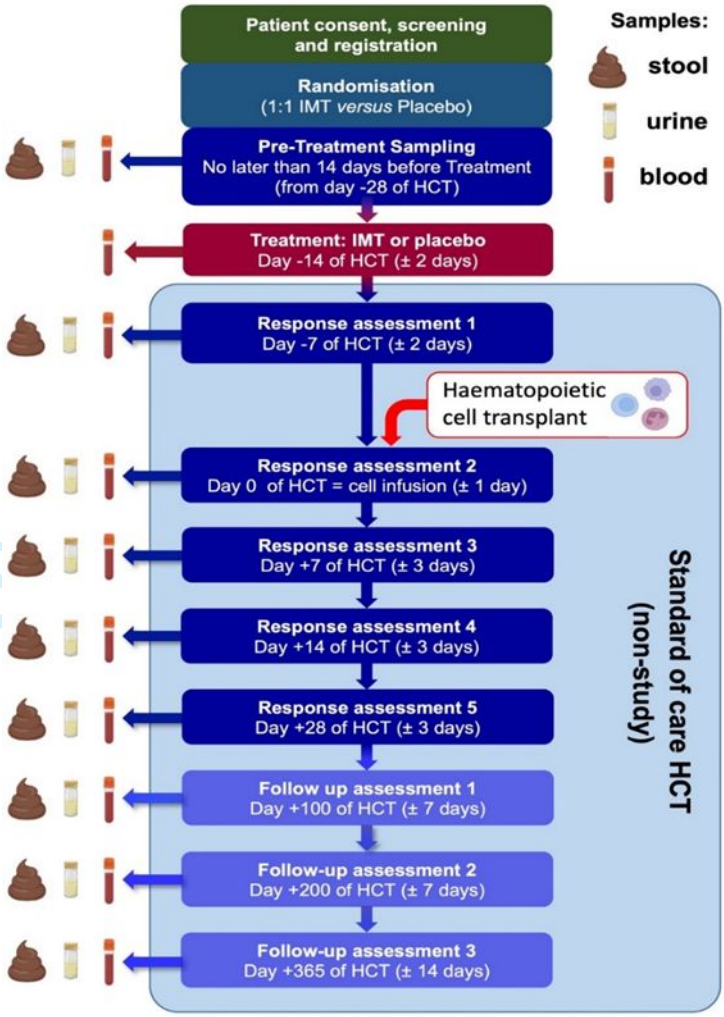
**The study will undertake the following:**

1. Recruit 50 people with blood cancers who had treatment with chemotherapy and are about to undergo a bone marrow transplant.

2. Take 25 randomly from this group and give them the intestinal microbiota transplant using an orally taken capsule and compare them to the remaining 25 patients who will be given a placebo capsules. The capsules will be taken prior to the bone marrow transplant

3. Collect stool, blood and urine from both groups for analysis over the period of their treatment.

4. Undertake health and quality of life assessments for up to a year after their intestinal microbiota transplant and bone marrow transplant (see the schedule to the right).




## 4.1. What will happen before I enter the trial?

**Initial Study Consultation**- An initial consultation will take place to discuss participation in the study on the phone or on site with a member of our team. We will ask you some questions to see if you would be suitable to join the study, which will last approximately **15** minutes.

If we decide from the initial assessment that you are not eligible to take part in the study from the initial visit, you will unfortunately not be able to take part in the study and will continue with your planned standard of care treatment.

[Insert Site Logo]

If we decide from the initial assessment that you may be eligible to take part in the study, we will invite you to attend a full screening visit **(Visit 1)**. We will also explain that we wish to collect a stool sample from you ahead of the next visit. We will provide you with a stool collection kit and instructions, to collect a sample at home 24 hours before or on the day of the next visit. Should you need support a family member or friend at home can help you with this or a nurse at your next study visit can help you with providing a sample.

## 4.2. What happens once you are confirmed suitable to take part?

**Visit 1: Consent and Screening (within 14 days before randomisation)** – After the Initial Study Consultation, you will be invited to attend a screening visit. If you are interested in joining the study, you will be asked to sign and date the study consent form. We will perform several tests to check you are eligible for the study. All the screening tests will be explained below.

**Screening Assessments (30-60 minutes):**

A review of your medical history and any medications you are taking or have recently taken (e.g., anti-cancer treatments, over-the-counter treatments including herbal or dietary supplements, prescription medications, and/or illegal drugs)

- **A physical examination** including height and weight.
- **An assessment of your vital signs** (tests to see how well your body is functioning) including blood pressure and pulse.
- **Collect dietary information** If you have been able to give a stool sample at this visit, we will ask you to complete a dietary questionnaire to report what you have eaten 24 hours before the collection of your stool sample.
- **Quality of life Assessment** – We will ask you to complete a questionnaire to collect this information.

At this visit, we will also collect the following samples from you:

- **Blood** – A blood sample for research purposes (2 tablespoons which is 30ml) will be collected in addition to your routine blood tests.
- **Urine** – A sample kit will be given to you to provide a sample.
- **Stool** – If you are not able to provide a stool sample at this visit you will be given a self-sample kit to collect a stool sample for the next study visit.

**After we complete all the screening assessments above,**

If you are **confirmed not to be eligible** to take part in the study, you will unfortunately not be able to take part in the study and will continue with the planned standard of care treatment.

MAST Study

[Insert Site Logo]

If you are **confirmed to be eligible** to take part in the study, you will randomly be assigned by a computer to one of the two treatment groups below before (within 2 weeks of Visit 2) your next scheduled study visit.

**Treatment Groups:**

**Group 1**–This is the 'treatment arm' of the study**:**
You will receive 10 Intestinal Microbiota Transplant(IMT) $1x10^6$ x $1x10^9$ CFU/g of viable microorganisms per oral capsule). The number of colony forming units (CFU) in each capsule may differ because the CFU of the original stool material used to make the capsules also differs. Oral capsules will be made from bacteria obtained from a healthy screened person's stool sample; there are an extensive array of screening procedures in place to ensure the capsules are safe to take. Whilst this is not a typical 'licensed' medication, it has been manufactured in line with very strict approval procedures from the UK regulatory body for medicines, and this sort of treatment has already been used safely in thousands of people around the world. These capsules will be taken orally with water.

Please note – while no animal products are used in the manufacturing process, there is a possibility that the material within the capsules may contain traces of certain non-digestible dietary/ food components (for example, prawn shells); any dietary concerns you may have will be discussed with the nurse or study doctor before starting treatment.

**Group 2:**
You will receive 10 dummy oral capsules (placebo) the capsule will look the exact same as the capsule given in group one, but will contain no medicine or active ingredients. These capsules will also be taken orally with water.

**Capsule Description:**

Each capsule will be size 0 (see picture below) the capsules will be coated so that they will be able to pass through your stomach without dissolving.

MAST Study

[Insert Site Logo]

**Visit 2 (Treatment)** – Prior to this visit you will be told to avoid eating food 30 minutes before the start of this visit. You will be given either the IMT oral capsules, or the placebo oral capsules and we will also take a research blood sample and if not collected at screening urine and stool samples from you at this visit. You will receive a diary card to report daily any symptoms listed in the booklet until the next patient visit. The nurse or doctor at your next visit will go through the booklet with you should you need any support with completing the diary before the next visit, there will be a number in the booklet to use to contact a member of the study team.

After the Screening and Treatment visits have been completed, you will follow the standard of care that your clinician has discussed with you for treating the blood cancer. We will ask for you to attend **8 more visits** over the following year. These visits will occur along with your monitoring visits that you will be making as part of your treatment described in the table below.

**Study Assessments (Visit 3-10) 30-45 minutes:**

The following schedule outlines the questionnaires and samples that will be collected from you at each study visit.

| Type of questionnaire | What is the questionnaire for? | When are they done? |
|---|---|---|
| **Health assessment questionnaire (EQ-5D-5L)** | To evaluate your general quality of life. | All Visits |
| **Quality of Life for Cancer patients (EORTC QLQ-C30)** | To evaluate your general quality of life as a cancer patient. | All Visits |
| **Dietary Questionnaire** | To understand before the stool sample was obtained, if any specific foods e.g., liquorice or fish may have been eaten. As eating certain foods can adjust the results observed during the analysis of the samples collected. | All Visits |

| Sample type | What is the procedure for? | When are they done? | How will they be done? |
|---|---|---|---|
| **Urine** | For analysis of chemicals that we think may change from before and after the | All Visits | At each visit, you will be given a labelled clean container to pass urine |

MAST Study

[Insert Site Logo]

| | | | |
|---|---|---|---|
| | intestinal microbiota transplant | | ideally when you wake up first thing in the morning. Guidance will be provided by the examiner or qualified member of the study team. |
| **Blood** | For analysis of chemicals that we think may change from before and after the intestinal microbiota transplant | All Visits | Bloods will be collected by a qualified member of the study team |
| **Faecal** | Analysis of the microorganisms in the faecal material before and after intestinal microbiota transplantation | All Visits | We will ask you, to collect a stool sample for each visit. You can collect the sample:<br>• 24 hours before your next study visit a family or friend may help you with this<br>• On the morning of each study or at the start of each visit if you would like support from a nurse.<br>You will be provided with a self-sampling kit that will contain:<br>• Clear instructions of the collection process, storage and its return<br>• Ice pack<br>• Bag to transport samples in |

[Insert Site Logo]

## 5. What do I have to do?

If you decide to take part, you will need to attend your local research centre for the assessments. If you normally require transport, we will help arrange this for you. Tests, sample collections and hospital appointments are explained in the table above, you must inform your study doctor of any medications you are currently taking or intend to take once you have entered the study.

During your participation in the MAST study, you should continue with your regular medication, and you will continue to be under the care of your regular medical team.

## 6. How will we assess whether the treatment is working and its effect on your quality of life?

You will also be asked to complete questionnaires on paper regarding your general health and cancer usually at the same time as your scans. The questionnaires should take approximately 15-20 minutes in total to complete. If you feel uncomfortable answering any of the questions, please talk to your study doctor or nurse. You can leave blank any questions you do not want to answer. This information will help us to understand how the treatment may affect your quality of life.

## 7. Pregnancy, contraception, and breastfeeding

If you would like to participate in this study and are a woman of childbearing potential, you must:

- Tell your study doctor immediately if you become pregnant during this study, your study doctor will advise you of the possible risks to your unborn child and discuss options for managing the pregnancy with you. If pregnancy occurs during the study, The study treatment will not be given if pregnancy occurs before the treatment visit, and you will be withdrawn from the study. If pregnancy occurs after the treatment visit you will continue attending the remaining study visits and the pregnancy will be followed until the conclusion if you give consent for this.
- Use (if you are sexually active with a male partner who has not been sterilised), one highly effective method of birth control and one additional effective barrier method of contraception at the same time. This should be done from the time of signing the informed consent form until study completion. Please discuss effective methods of contraception with your study doctor or nurse.

MAST Study

[Insert Site Logo]

## 8. What are the side effects, possible disadvantages and risks when taking part?

Being involved in a research study, such as a clinical trial, requires a degree of commitment to regular hospital visits and additional tests and surveys, and you may consider this to be a disadvantage.

The only risks associated with the study are related to some of the procedures. For example, there is minimal risk associated with blood tests, they can cause brief discomfort, bruising, or an infection in some cases, which might last for several days, and will, therefore, be performed by experienced members of the healthcare team.

Urine and stool self-collection procedures carry a minimum risk of contamination with stool and urine material; however, the risk has been minimised by the provision of an instruction manual for collection and hygiene.

There are very low risks associated with the intestinal microbiota transplantation itself and these include, fever, nausea, vomiting, bloating and constipation these should normally resolve in 1 to 2 days https://tinyurl.com/4zpf5kchThere is also a low risk of infection from intestinal microbiota transplantation (IMT) itself. This risk is managed by EBX carrying out extensive testing on donors and their stool under supervision of a medical doctor, including blood and stool tests to detect pathogenic infectious agents, over and above as recommended by the UK experts in this field (https://tinyurl.com/4zpf5kch). Every stool donated, as well as every batch of IMT capsules manufactured is tested for the presence of pathogenic infectious agents and is only released for use if these are not detected." There will be a contact number in the symptom diary card should there be any symptoms you would like to discuss the clinical team.

## 9. What are the possible benefits of taking part?

We cannot promise the study will definitely help, however, in a small study before this larger one, we have shown that a similar treatment reduced the number of admissions to the intensive care unit, the numbers of blood infections and days of fever (high temperature) in the early days after the patients had had their bone marrow transplant. This study also showed that intestinal microbiota transplant (the treatment/method) was safe in patients undergoing bone marrow transplant, and there were no major side-effects.

The information we collect on how treatment affects the complications related to bone marrow transplantation, such as fevers (high temperatures) and infections during the transplant period may help to improve treatment and the recovery of people with blood cancers who are undergoing bone marrow transplant.

[Insert Site Logo]

## 10. What if I feel unhappy about continuing in the study?

If you have concerns about continuing, please discuss these with your study doctor and team. You do not have to give a reason, and your study team can explain your options to you about any data or samples collected from you as part of the study. Please see section 2 in Part 2 of the information sheet for more details of what will happen if you stop the study while on treatment.

1. What if something goes wrong?

Your study doctor will be there to answer any questions you might have regarding the cancer, its treatment, and your participation in the study. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during this study, then there will be several options available to you. Full details are included in Part 2 of this information sheet.

## 11. Will I be compensated for taking part?

You will not be paid for taking part in the study. However, for every study visit you attend you will be able to claim back some of your expenses. You will be reimbursed up to the value of £50 (maximum £200 in total) for travel expenses per visit.

**If the information in Part 1 has interested you and you are considering taking part in the study, please read the additional information in Part 2 before making your decision.**

[Insert Site Logo]

MAST Study

# 12. PART 2

## 0. What if new information becomes available?

Sometimes during a research project, new information or incidental findings becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form.

## 1. What happens when the research study stops?

Once your participation is over, you will carry on with your standard treatment and medical care as usual.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained including any research samples unless you specifically withdraw your consent for this. At your last study visit a nurse or clinician will ask if you would like to know what treatment group you were assigned to which will be shared with you if requested by your chosen method of contact once the trial has ended.

To safeguard your rights, we will use the minimum personally identifiable information possible.

## 2. What if there is a problem?

Imperial College London holds insurance policies which apply to this study. If you experience harm or injury because of taking part in this study, you will be eligible to claim compensation without having to prove that Imperial College London is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, you may have grounds for legal action.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during this study you should immediately inform the Investigator.

The normal National Health Service complaints routes are also available to you, details can be obtained from your study doctor or nurse.

If you are still not satisfied with the response, you may contact the Imperial College, Research Governance, and Integrity Team.

**Complaint's statement**

If you wish to complain about any aspect of the way in which you have been approached or treated during this study, you should contact the study team (contact details at the end of this document) or you may contact the patient advice and liaison services (PALS) in the trust you are receiving treatment in *<Insert trust name, PALS tel.no and email>*.

## 3. How will we use information about you?

Imperial College London is the sponsor for this study and will act as the data controller with Imperial Clinical Trials Unit – Cancer (ICTU-Ca) for this study. This means that we are responsible for looking after your information and using it appropriately. Imperial College London will keep your personal data for:

- 10 years after the study has finished in relation to data subject consent forms.
- 10 years after the study has been completed in relation to primary research data.

This study is expected to end 08/2026

We will need to use information from your medical records for this research project. This information will include your, initials, month and year of birth, gender, and ethnicity.

People within the College and study team will use this information to do the research or to check your records to make sure that the research is being done properly and the information held is accurate.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a unique code number (study ID) instead, and this code will also be used to label tissue and blood samples.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no one can work out that you took part in the study.

MAST Study

[Insert Site Logo]

## LEGAL BASIS

As a university, we use personally-identifiable information to conduct research to improve health, care and services. As a publicly funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in the research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Our legal basis for using your information under the General Data Protection Regulation (GDPR) and the Data Protection Act 2018, is as follows:

Imperial College London - "performance of a task carried out in the public interest"); Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research

## INTERNATIONAL TRANSFERS

There may be a requirement to transfer information to countries outside the United Kingdom (for example, to a research partner), either within the European Economic Area (EEA) or to other countries outside the EEA. Where this information contains your personal data, Imperial College London will ensure that it is transferred in accordance with data protection legislation. If the data is transferred in accordance with data protection legislation. If the data is transferred to a country which is not subject to a UK adequacy decision in respect of its data protection standards , Imperial College London will enter into a data sharing agreement with the recipient research partner that incorporates UK approved standard contractual clauses or utilise another transfer mechanism that safeguards how your personal data is processed .

You will not be able to be identified when sharing this data, but it may include demographic information such as the month and year of your birth as well as your study ID.

## SHARING YOUR INFORMATION WITH OTHERS

We will only share your personal data with certain third parties for the purposes referred to in this participant information sheet and by relying on the legal basis for processing your data as set out below.

- Other College employees, agents, contractors and service providers (for example, suppliers of printing and mailing services, email communication services or web services, or suppliers who help us carry out any of the activities described above). Our third-party service providers are required to enter into data processing agreements with us. We only permit them to process your personal data for specified purposes and in accordance with our policies.
- EnteroBiotix Ltd., provide the capsule IMT and placebo. The following data is shared with them in this capacity as part of an agreement with Imperial College London, as

well as ensuring appropriate oversight of any serious side effects that you and other study participants may experience:

- Results data used to write reports from the study, specifically on how effective and safe the study treatment is
- Data about serious side effect/s, including whether they got better
- Data about medications taken and whether these were to treat the serious side effect/s or were other medications that were being taken at the time the serious side effect/s occurred.

- The Medical Research Council (MRC) who fund the study, the following data is shared with them in this capacity as part of an agreement with Imperial College London, as well as ensuring appropriate oversight of any serious side effects that you and other study participants may experience:
  - Results data used to write reports from the study, specifically on how effective and safe the study treatment is
  - Data about serious side effect/s, including whether they got better
- UK ethics and regulatory authorities who are required by law to approve and oversee research. The following data is shared with them to ensure appropriate oversight of any serious side effects that you and other study participants may experience:
  - Data about serious side effect/s, including whether they got better
  - Data about medications taken and whether these were to treat the serious side effect/s or were other medications that were being taken at the time the serious side effect/s occurred.

## POTENTIAL USE OF STUDY DATA FOR FUTURE RESEARCH

When you agree to take part in a research study, the information collected either as part of the study or in preparation for the study (such as contact details) may, if you consent, be provided to researchers running other research studies at Imperial College London and in other organisations which may be universities or organisations involved in research in this country or abroad. Your information will only be used to conduct research in accordance with legislation including the GDPR and the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you, used against you or used to make decisions about you.

MAST Study

[Insert Site Logo]

## COMMERCIALISATION

Samples/data from the study may also be provided to organisations not named in this participant information sheet, e.g., commercial organisations or non-commercial organisations for the purposes of undertaking the current study, future research studies or commercial purposes such as development by a company of a new test, product or treatment. We will ensure your name and any identifying details will NOT be given to these third parties, instead you will be identified by a unique study number with any sample / data analysis having the potential to generate 'personal data'.

Aggregated (combined) or anonymised data sets (all identifying information is removed) may also be created using your data (in a way which does not identify you individually) and be used for such research or commercial purposes where the purposes align to relevant legislation (including the GDPR) and wider aims of the study. Your data will not be shared with a commercial organisation for marketing purposes.

## WHAT ARE YOUR CHOICES ABOUT HOW YOUR INFORMATION IS USED?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have, because some research using your data may have already taken place and this cannot be undone.

- If you choose to stop taking part in the study, we would like to continue collecting information about your health from your hospital. If you do not want this to happen, tell us and we will stop. This will not affect any healthcare or support you may be receiving separately
- We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you, if this could affect the wider study or the accuracy of data collected.
- If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study.

## WHERE CAN YOU FIND OUT MORE ABOUT HOW YOUR INFORMATION IS USED

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- by asking one of the research team
- by sending an email to mast-trial@imperial.ac.uk

- **COMPLAINT**

If you wish to raise a complaint on how we have handled your personal data, please contact Imperial College London's Data Protection Officer via email at dpo@imperial.ac.uk, via telephone on 020 7594 3502 and/or via post at Imperial College London, Data Protection Officer, Faculty Building Level 4, London SW7 2AZ.

If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO) www.ico.org.uk. The ICO does recommend that you seek to resolve matters with the data controller (us) first before involving the regulator.

## 4. Involvement of the General Practitioner/ family doctor (GP)

With your permission, your GP and other doctors involved in your clinical care will be informed that you are taking part in this study, but otherwise all information about you and your treatment will remain strictly confidential.

## 5. What will happen to any samples that I give?

**Blood:**

Routine blood samples will be taken and tested by your hospital as part of standard practice and destroyed immediately after testing.

Research blood samples will be sent an HTA authorised Imperial College university lab located in St Mary's Hospital for long-term storage for future use in ethically approved studies with your permission. The blood samples will be analysed to see what chemicals they contain. Any samples left over from the process will be destroyed.

**Stool**

Stool samples will be sent to an HTA authorised Imperial College university lab located in St Mary's Hospital for storage. Stool samples collected at your screening visit and 7th visit (around 28 days after the bone marrow transplant) may be sent abroad for testing (i.e., Bacterial DNA sequencing lab in Germany) your agreement to this is required to take part in the study. The samples will be analysed to see what different types of bacteria are present in your stools. Any samples left over from the process will be destroyed.

**Urine**

The urine samples collected will be sent an HTA authorised Imperial College university lab located in St Mary's Hospital for long-term storage for future use in ethically approved studies with your permission. They will also be analysed to see what chemicals they contain.

MAST Study

[Insert Site Logo]

## 6. What will happen to the results of the research study?

The results of the MAST study will be analysed by the MAST study research team. Results will be presented at Cancer meetings and will be published in associated journals for the wider research community to reference. No identifiable information is included in publications or presentations; therefore, you will not be identified in any report or publication. Your confidentiality is maintained throughout.

If you contact the researchers in the future, you can obtain a copy of the results.

Research data and all identifiable data will be stored by the sponsor for 10 years following the end of trial.

## 7. Optional consent for future use of samples

If you consent, your leftover samples will be stored at our HTA authorised Imperial College Bio Bank your samples will be pseudo-anonymised and may be used for further academic and/or commercial studies by the Principal Investigator. Any such tests will have an appropriate ethical review. Upon your request at any time, your remaining samples will be destroyed.

## 8. Who is organising and funding the research?

Imperial College London is the legal sponsor of this study and is organising the study through the Imperial Clinical Trials Unit – Cancer (ICTU-Ca). The study is funded by Medical Research Council (MRC) who will receive a study report declaring the results, but no individual research participant identifiable data will be shared. The study is organised by a research team at the Imperial Cancer Clinical Trials Unit.

The sponsor of this study will pay your hospital for including you in this study, but your doctor will not receive any personal financial payment if you take part.

## 9. Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by <Insert name of Ethics Committee>.

[Insert Site Logo]

# 10. Contact for Further Information

If you have additional questions during this study about the research or your rights as a research patient, you may address them to the study doctor(s) <insert name of doctor and tel.no> or the study staff <insert name and tel. no.>. Out of office hours < insert name and tel.no> please contact the study doctor in the event of the following occurring:

a) If you suffer an illness or a possible study-related injury
b) If you feel different in any way
c) If you are admitted to the hospital for any reason
d) If you are seen at a casualty (accident/emergency department) for any reason

To speak with a member of the MAST investigator team please contact the Study Manager,

Telephone: 02075943767

Email: mast-trial@imperial.ac.uk

**Thank you for reading this information sheet. If you are interested in taking part in the study, please contact the study team to arrange a screening appointment.**

**A copy of this written information and signed Informed Consent form will be given to you.**

MAST Study Imperial College London

**Centre Number:** _____

**Patient Study Identification Number:**_____

## CONSENT FORM: MAST Study

**Study title: M**icrobiota Transplant Prior to **A**llogeneic **S**tem Cell **T**ransplantation **(MAST)** study
**Short Title**: MAST

**Principal Investigator:** <Insert Name>

**IRAS Project ID:** 1006971

| | Consenting information | Please Initial each box |
|---|---|---|
| 1. | I confirm that I have read and understand the Patient Information Sheet, **Version**_____, dated _____ for the above study. I have spoken to_____ and had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | |
| 2. | I understand that my participation is voluntary and that I am free to withdraw at any time, without providing a reason. I know that my medical care and legal rights are not affected. | |
| 3. | I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Sponsor (Imperial College London), from the NHS organisations, Medical Research Council, Enterobiotix or regulatory/other authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | |
| 4. | I give consent for information collected about me to be used to support other ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |
| 5. | If Applicable, I agree to use effective contraception whilst taking part in the study, should I become pregnant after taking the study drug, I give/do not give permission for access to any of my medical notes and information collected about my pregnancy. | |

| | | |
|---|---|---|
| 6. | I give consent to the taking of blood **equivalent to two tablespoons** and providing urine samples for chemical analysis in this study. | |
| 7. | I understand the stool collection procedure and agree to comply with these instructions. | |
| 8. | I give permission for my stool samples to be sent outside of the UK for bacterial genetic analysis for this study. | |
| 9. | I agree that my GP, and / or other doctors involved in my clinical care, may be notified of my participation in this study. | |
| 10. | I understand that blood, urine and stool samples and / or data collected from me are a gift donated to Imperial College and that I will not personally benefit financially if this research leads to an invention and/or the successful development of a new test, medication treatment, product or service. | |
| 11. | I agree to take part in the **Microbiota** Transplant Prior to **A**llogeneic **S**tem Cell **T**ransplantation **(MAST)** study | |

| **Optional** | | **Initials** |
|---|---|---|
| 12. | I give/do not give consent for my pseudo-anonymised stool, blood and urine sample to be stored during and at the end of the study at the University (Imperial College London) bio bank to support future ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |
| 13. | I give permission for any pseudo-anonymised blood and urine samples to be sent outside of the UK for analysis to support future ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |

Participant Name Date Signature

Name of person taking consent Date Signature

**When completed. Take 2 Copies. One to be given to the participant, one copy should be filed in the medical notes and the original stored in the Investigator Site File.**

BMJ Open

# Intestinal Microbiota Transplant Prior to Allogeneic Stem Cell Transplant - (MAST) trial: Study Protocol for a Multi-Centre, Double-Blinded, Placebo-Controlled, Phase IIa Trial



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2024-093120.R2 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 22-Nov-2024 |




| | |
|---|---|
| Complete List of Authors: | Mullish, Benjamin; Imperial College London Faculty of Medicine, Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction<br>Innes, Andrew J; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Roberts, Lauren A; Imperial College London, Department of Metabolism, Digestion and Reproduction<br>Anim-Burton, Shian; Imperial College London, Department of Surgery and Cancer<br>Webber, Lee; Imperial College London, Department of Surgery and Cancer<br>Johnson, Nicholas; Imperial College London School of Public Health, Department of Public Health<br>Ghani, Rohma; Imperial College London, Department of Metabolism, Digestion and Reproduction; Imperial College Healthcare NHS Trust, Department of Infectious Diseases<br>Farshi, Pakhshan; Manchester Royal Infirmary, Department of Haematology<br>Khan, Anjum B; Leeds Teaching Hospitals NHS Trust, Department of Haematology<br>Kinsella, Francesca; University Hospitals Birmingham NHS Foundation Trust, Department of Haematology<br>Kottaridis, Panagiotis; University College London Hospitals NHS Foundation Trust, Department of Haematology<br>Krishnamurthy, Pramila; King's College Hospital NHS Foundation Trust, Department of Haematology<br>Nicholson, Emma; The Royal Marsden Hospital, Department of Haematology<br>Palanicawandar, Renuka; Imperial College London, Department of immunology and inflammation; Imperial College Healthcare NHS Trust, Department of Haematology<br>Wheeler, Graham; Imperial College London, Imperial Clinical Trials Unit<br>Davies, Frances; Imperial College London, Department of Infectious Diseases<br>Marchesi, Julian R.; Imperial College London, Department of Metabolism, Digestion and Reproduction<br>Pavlů, Jiří; Imperial College London, Department of immunology and |

| | |
|---|---|
| | inflammation; Imperial College Healthcare NHS Trust, Department of Haematology |
| Primary Subject Heading: | Haematology (incl blood transfusion) |
| Secondary Subject Heading: | Gastroenterology and hepatology, Infectious diseases |
| Keywords: | Bone marrow transplantation < HAEMATOLOGY, Leukaemia < HAEMATOLOGY, Transplant medicine < INTERNAL MEDICINE, Microbiota |
| | |

*BMJ Open – Protocol:*

# Intestinal *M*icrobiota Transplant Prior to *A*llogeneic *S*tem Cell *T*ransplant - (MAST) trial: Study Protocol for a Multi-Centre, Double-Blinded, Placebo-Controlled, Phase IIa Trial

Benjamin H Mullish[1,2], Andrew J Innes[3,4], Lauren A Roberts[1], Shian Anim-Burton[5], Lee Webber[5], Nicholas A Johnson[6], Rohma Ghani[1,7], Pakhshan Farshi[8], Anjum B Khan[9], Francesca Kinsella[10], Panagiotis Kottaridis[11], Pramila Krishnamurthy[12], Emma Nicholson[13], Renuka Palanicawandar[3,4], Graham M Wheeler[6,14], Frances J Davies[7], Julian R Marchesi[1]*, Jiří Pavlů[3,4,]*

*Affiliations:*

[1]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, United Kingdom

[2]Department of Hepatology, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

[3]Centre for Haematology, Department of immunology and inflammation, Faculty of Medicine, Imperial College London, London, United Kingdom

[4]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

[5]Cancer Research UK Imperial Centre, Clinical Trials Section, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, United Kingdom

[6]Imperial Clinical Trials Unit, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom

[7]Department of Infectious Diseases, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom.

[8]Department of Haematology, Manchester Royal Infirmary, Manchester, United Kingdom

[9]Department of Haematology, Leeds Teaching Hospitals, Leeds, United Kingdom

[10]Department of Haematology, University Hospital Birmingham, Birmingham, United Kingdom

[11]Department of Haematology, University College London Hospital, London, United Kingdom

[12]Department of Haematology, King's College Hospital, London, United Kingdom

*BMJ Open – Protocol:*

[13]Department of Haematology, The Royal Marsden Hospital, London, United Kingdom

[14]Statistics and Data Science Innovation Hub, GSK, London, United Kingdom

*Joint senior authors and trial co-chief investigators

Correspondence to: Prof Julian R Marchesi

Postal address: Department of Metabolism, Digestion and Reproduction
St Mary's Hospital Campus, Imperial College London
10th Floor, QEQM Wing
South Wharf Road
Paddington, London
W2 1NY
United Kingdom

Email: j.marchesi@imperial.ac.uk

Word count: 7286

*BMJ Open – Protocol:*

**Abstract:**

**Introduction:** Lower diversity of the gut microbiome pre-allogeneic haematopoietic cell transplantation (HCT) correlates with reduced survival after the intervention. Most patients undergoing HCT for a haematological malignancy have previously received intensive chemotherapy, resulting in prolonged neutropenic episodes requiring broad-spectrum antibiotics; use of these has been linked to reduced microbiome diversity. Intestinal microbiota transplant (IMT) is a novel treatment approach that restores this diversity. We hypothesised that IMT performed prior to initiation of HCT conditioning restores microbiome diversity during the early stages of HCT, leading to decreased frequency of complications and improved outcomes of HCT.

**Methods and analysis:** Fifty adult patients receiving allogeneic HCT will be recruited into this phase 2a trial and randomised 1:1 to receive capsulised IMT or matched placebo shortly prior to initiation of HCT conditioning and followed for up to twelve months. The primary outcome will be to assess the increase in alpha diversity between pre-IMT and that measured at ~42 days after the IMT administration (day +28 of HCT), comparing the difference between patients receiving IMT compared to placebo. Secondary outcomes will include tolerability, and the dynamics of gut microbiome diversity metrics and taxonomy over all timepoints assessed, as well as clinical outcomes (including burden of invasive infections, days of fever, admission to intensive care, development of graft-vs-host disease, and mortality).

**Ethics and dissemination:** This study was approved by a UK Research Ethics Committee (REC reference: 23/NE/0105). Dissemination of results will be in concert with patient and public involvement (PPI) group input and is expected to be primarily via abstract presentation at conferences and manuscripts in peer-reviewed journals.

**Trial registration number:** ClinicalTrials.gov ID: NCT 6355583; ISRCTN: https://doi.org/10.1186/ISRCTN13241761; EudraCT: 2022-003617-10

**Keywords:** Bone marrow transplantation; Leukaemia; Transplant medicine; Gut microbiome; Faecal microbiota transplant.

**Strengths and limitations of this study:**

- While our prior observational study of intestinal microbiota transplant (IMT) administered prior to haematopoietic stem cell transplantation (HCT) suggested clinical benefits, the multi-centre, randomised, placebo-controlled nature of the MAST trial will enable exploration of this observation in a more robust setting.
- This study will use a multi-donor capsule IMT preparation (as opposed to conventional IMT slurry), which was the patient preference during our patient and public involvement group feedback, primarily due to its less invasive nature.
- However, we have no prior 'head to head' testing of IMT slurry compared to this capsule preparation in terms of efficacy.
- The timing of IMT pre-HCT (to 'prehabilitate' the gut microbiome) is a distinctive novel aspect of this study – there is a biological rationale to support this choice, but there are certain drawbacks of this approach too.
- Together with recording clinical outcomes post-IMT and HCT, we will collect patient samples for immunological and multi-omic profiling (including microbiome and metabolome analysis), to better understand the mechanisms of action of IMT in this setting.

## 1 Introduction:

Allogeneic haematopoietic cell transplantation (HCT) is a powerful therapeutic modality for patients with acute leukaemia and certain other haematological malignancies. Furthermore, with the advent of reduced intensity protocols, and approaches that permit safer use of mismatched donors, its frequency is increasing. In preparation for transplant, patients receive a conditioning regimen of high-dose chemotherapy and/or total-body irradiation, followed by infusion of compatible haematopoietic cells; the engraftment of these cells restores the recipient's haematopoesis, and exerts long-term remission from the graft-versus-leukaemia effect of the donor immune effector cells. However, this process is associated with marked perturbation of the gut microbiome, including reduced gut barrier integrity, loss of gut microbiome diversity, and microbiome enrichment in pathobiont bacteria[1] [2]. The immunosuppressive nature of both the underlying haematological malignancy, together with the treatments used, collectively result in a markedly increased risk of infections in these patients. More specifically, the increase in susceptibility to infection leads to an increase in antibiotic exposure, driving the dominance of pathobionts, and a further selection pressure for overgrowth of antimicrobial resistance (AMR) genes in the gut[3]. In this setting, the impact of antibiotics, and multidrug-resistant organism (MDRO)-associated infection, is associated with poorer clinical outcomes in patients[4]; for example, use of imipenem-cilastatin or piperacillin-tazobactam use to treat neutropenic fever has been associated with increased graft-versus-host disease (GvHD) mortality up to even five years post-HCT[5].

A large observational study of more than 1,300 patients from four centres observed that patients with patterns of microbiota disruption characterised by loss of diversity had a higher risk of transplantation-related death, and death attributable to GvHD[6]. Baseline samples obtained before HCT already showed evidence of microbiome disruption, and lower diversity before transplantation was closely associated with poor survival. Specific gut taxonomic features have also been linked with allo-HCT outcome; specifically, expansion of *Enterococcus* (particularly *Enterococcus faecium*) was observed across the period of having allo-HCT.

*Enterococcus* was found to associate closely with GvHD and mortality, with presence of the disaccharide lactose identified as a factor that promoted *Enterococcus* expansion[7].

These data support the hypothesis that a microbiome-based intervention, performed prior to initiation of HCT conditioning, may improve microbiome diversity during transplant, and has the potential to impact upon clinical outcomes. Several approaches have been considered in this setting[8], including dietary/prebiotic interventions, probiotics, and non-absorbable antibiotics (such as rifaximin). In this study we have opted for a biological approach that attempts to restore the whole gut ecosystem, using intestinal microbiota transplant (IMT; also known as 'faecal microbiota transplant'). IMT consists of transferring minimally processed stool, from a healthy screened donor, into the gut of a recipient. This approach was pioneered in patients with recurrent *Clostridioides difficile* infection (rCDI), in which the major risk factor is recurrent antibiotic exposure[9]. The success in this setting had led to exploration of IMT in other conditions which the gut microbiome appears contributory to their aetiopathogenesis[10], with promising early data. Despite initial concerns about safety of IMT in immunocompromised patients - driven in part by descriptions of pathogen transmission via IMT in such patients[11] - adherence to strict screening protocols results in a safety profile comparable to that in immunocompetent recipients[8 9 12].

After previous reports that the use of IMT for rCDI was also associated with reduced antibiotic resistance genes within the gut microbiome[13], and our own observation of a clinical case where IMT seemed to show clinical benefit when used prior to HCT in a patient colonised with MDROs[14], we completed a cohort study of IMT performed prior to initiation of HCT conditioning in patients colonised and/or previous infected with MDROs. While we observed that rates of decolonisation of intestinal MDROs were comparable to that observed spontaneously, we saw a significant reduction in rates of bloodstream infection (including MDRO-related), length of stay, and days of carbapenem use, compared to a matched historical control arm[15]. With longer follow-up, these benefits translated to improvement in overall survival, such that the poor outcome associated with MDRO colonisation could be negated with IMT[16].

*BMJ Open – Protocol:*

In this clinical trial, we will investigate the broader role of IMT in HCT, both with and without MDRO colonisation. By randomising patients to receive IMT or placebo prior to HCT conditioning, and measuring microbiota diversity, in stool, as a surrogate for its impact upon gut ecology, we will determine the capacity of capsule IMT to restore a normal microbiome, and track the impact during HCT. Using multi-omic profiling of stool, urine and blood, we will investigate the wider impact of IMT in HCT patients, while clinical parameters will explore the potential to overall outcome.

## 2 Methods and analysis:

### 2.1 Design and objectives:

The MAST study is a multi-centre, randomised, phase IIa double-blind placebo-controlled trial**.** The major objective of this trial is to determine the ability of capsulised IMT given prior to allogeneic HCT to increase and maintain stool microbiota diversity after HCT from baseline. Secondary objectives include determination of clinical tolerability, effects of capsule IMT upon clinical outcomes, and to explore microbiome and immune dynamics related to IMT use. The primary outcome is the change in alpha diversity (measured as inverse Simpson's index) after IMT administration measured at immediately prior to IMT (at 14 +/- 3 days prior to HCT) and 28 +/-3 days after HCT, comparing the change between patients receiving capsulised IMT *versus* placebo. The study is sponsored by Imperial College London. This is an investigator-led study; while funding for the study was only awarded after peer review, the funder, sponsor and industrial partner have had no direct role in any aspect of study design (although the funder arranged external peer review as part of the process to the award of funding, which did impact upon study design). We have used the SPIRIT checklist in writing this report[17].

The study start date is April 2024, with primary completion estimated as August 2026 and study completion estimated as March 2027.

### 2.2 Recruitment Strategy:

#### 2.2.1. Site Selection:

We have partnered with several of the largest haematology centres in the UK as recruitment sites to support adequate participant enrolment. These centres are: the Royal Marsden

Hospital, University College London Hospital, King's College London Hospital, University Hospital Birmingham, Leeds Teaching Hospital, Hammersmith Hospital, and Manchester University Hospital. Such centres are well-positioned to provide access to a high volume of eligible patients, due to their expertise and patient population in haematology and transplant services.

**2.2.2. Engagement with Patient Advocacy Groups:**

From its inception, the MAST trial was co-developed with the patient and public involvement group, itself based around the NCRI AML Supportive care group. The group refined the protocol and participant-facing documents and provided input into the design to improve the communication and reach of the study to potential participants.

**2.2.3. Regular Communication and Updates:**

Our dedicated trials unit maintains regular communication with participating sites to support recruitment efforts. This includes helping to support barriers to enrolment and providing ongoing assistance to sustain recruitment momentum, ensuring that sites have the resources and support needed to meet target enrolment goals.

**2.2.4. Patient Support and Accessibility Measures:**

We have developed study several supportive resources to improve participant understanding, engagement and accessibility to help boost retention. Examples include informational videos to guide participants providing samples, study-specific standard operation procedures to streamline processes across sites and maintain consistency, and translated versions of the participant information sheets to accommodate diverse language needs. Additionally, funding for transportation costs is available to reduce transportation barriers to make participation more accessible.

**2.3 Study setting and participants:**

This trial will be performed across seven Haematology Units in the United Kingdom which regularly undertake HCT. The study will recruit adults with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), acute leukaemia (AL) of ambiguous lineage, high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML), and chronic

myeloid leukaemia (CML) in blast phase, considered suitable/fit for allogeneic haematopoietic stem cell transplantation HCT. Patients will be eligible to enter the study if they achieved complete remission (defined as < 5% blasts), have received a minimum of two cycles of intensive chemotherapy (**Supplementary Material 1, Appendix 1**), and have received broad-spectrum antibiotics within three months of HCT**.** Inclusion and exclusion criteria are summarised below:

2.3.1 **Inclusion criteria:**

1. Patients aged 18 years and over with a morphological documented diagnosis of ALL, AML, AL of ambiguous lineage, MDS, CMML, and CML in blast phase (**Supplementary Material 1, Appendix 2**) who are deemed fit for allogenic HCT with one of the following disease characteristics:

   *ALL, AML, AL of ambiguous lineage*

   - Patients in first complete remission (CR1) or second complete remission (CR2) including complete remission with incomplete blood count recovery with < 5% blasts (**Supplementary Material 1, Appendix 2**)
   - Secondary leukaemia (defined as previous history of MDS, antecedent haematological disease or chemotherapy exposure) in CR1 or CR2 defined as < 5% blasts (**Supplementary Material 1, Appendix 2**)

   *MDS and CMML*

   - Patients with advanced or high risk MDS with an IPSS-M moderate high or higher including intermediate or high risk CMML who have < 5% blasts at the time of randomisation (**Supplementary Material 1, Appendix 2**)

   *CML in blast phase*

   - Patients with Philadelphia or BCR:ABL1 positive chronic myeloid leukaemia (CML) in blast phase defined by the presence of ≥ 20% blasts in blood or bone marrow who have achieved second chronic phase with < 5% blasts (**Supplementary Material 1, Appendix 2**).
2. Patients must have completed minimum of two cycles of intensive chemotherapy prior to trial enrolment (**Supplementary Material 1, Appendix 1**).
3. Patients must have received broad-spectrum antibiotics within 3 months prior to trial enrolment

4. Patients must be considered suitable/fit to undergo allogeneic HCT, as clinically judged by the Local investigator
5. Patients with a Karnofsky performance status score 60 or above (**Supplementary Material 1, Appendix 3**).
6. Females of and male patients of reproductive potential (i.e., not post-menopausal or surgically sterilised) must use appropriate, highly effective, contraception from the point of commencing therapy until 6 months after treatment
7. Patients have given written informed consent
8. Patients willing and able to comply with scheduled study visits and laboratory tests

**2.3.2 Exclusion criteria:**

1. Patients with contraindications to receiving allogeneic HCT.
2. Female patients who are pregnant or breastfeeding. All women of childbearing potential must have a negative pregnancy test before commencing treatment.
3. Adults of reproductive potential not willing to use appropriate, highly effective, contraception during the specified period.
4. Patients with renal or hepatic impairment as clinically judged by the Local Investigator.
5. Patients with active infection, HIV-positive or chronic active HBV or HCV.
6. Patients with a concurrent active malignancy or a prior malignancy, except lobular breast carcinoma *in situ*, fully resected basal cell or squamous cell carcinoma of skin or treated cervical carcinoma *in situ*, incidental histologic finding of prostate cancer (T1a or T1b using the tumour, node, metastasis (TNM) clinical staging system), previous MDS, CMML, MPN resulting in secondary AML. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed.
7. Swallowing difficulties that may preclude safe use of IMT capsules.
8. Administration of IMT within 3 months prior to enrolment (probiotic administration prior to enrolment is allowed but should be recorded at screening).
9. Patients taking probiotics after enrolment to the trial.
10. Gastrointestinal disorders and diseases, including delayed gastric emptying, coeliac disease, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, chronic diarrhoea, and colonic perforation or fistula.

*BMJ Open – Protocol:*

11. Any autoimmune disease requiring, or that may require, systemic treatment with steroids and/or other immunosuppressants/immunomodulators.
12. Significant bleeding disorder (ALL, AML, AL of ambiguous lineage, MDS, CMML, and CML satisfying inclusion criteria are not excluded).
13. Anaphylactic food allergy.
14. Requirement for vasopressors.
15. Valvular heart disease or known structural defects of the heart.
16. Known severe allergy to capsule components.

## 2.4 Interventions:

### 2.4.1 Allocation:

50 adult patients will be allocated 1:1 between two groups:

- Capsulised IMT – as a single oral dose of 10 capsules of EBX-102-02. EBX-102-02 is encased within an intrinsically enteric-resistant capsule containing pooled, dried, full-spectrum microbial ecosystems obtained from rigorously screened donors. EBX-102-02 is characterised by the absence of pathogens, a minimum viable count of anaerobic microorganisms, and the presence of pre-identified genera (such as *Faecalibacterium*), all measured by proprietary nucleic acid-based assays and other technologies. EBX-102-02 will be administered within two weeks of the initial study screening visit. Given the immunosuppressed nature of the recipients, out of an abundance of caution, EBX-102-02 will be prepared from CMV-negative donors, as per suggestions from current guidelines[9].
- Matched capsulised placebo – containing microcrystalline cellulose and magnesium stearate; administered at the same point as capsulised IMT.

Treatment with either IMT or placebo will take place at 14 (+/- 2) days prior to haematopoietic cell infusion in a hospital setting. Both IMT and placebo will be stored in a refrigerator (at 2 – 8$^{o}$C) until administration, with temperature monitoring of the investigational medicinal product prior to administration. Study participants will be nil by mouth for at least 30 minutes prior to – and one hour after – each course of IMT/ placebo capsule administration. They will be asked to take each capsule with sips of water, and will be monitored for at least 15 minutes after capsule administration for complications (e.g. nausea).

Randomisation will be performed centrally by the Imperial College Trials Unit – Cancer using OpenClinica electronic data capture system (EDC). The system applies stratified randomisation to reduce relevant imbalances and increase statistical power, randomisation will be stratified by disease history (either: 1. patients known to have intestinal colonisation or blood-borne infection with multidrug-resistant organisms during previous therapy; or: 2. patients without this history) to ensure there is a balanced distribution across treatment arms. To reduce predictability in the randomisation sequence, blocks of multiple sizes have been used during sequence generation.

The allocation sequence will be generated using a computerised algorithm on the Sealed Envelope system designed to maintain allocation concealment and integrity. The study uses kit codes which are pre-generated by the drug manufacturer, the kit code is linked to the treatment allocation sequence but to do not reveal treatment assignment (capsule IMT or placebo). The kit codes are randomly assigned to participants through the Sealed Envelope system when randomisation is initiated in OpenClinica.

The system ensures that the allocation sequence remains concealed until the end of the study. User restrictions are in place to maintain the blinding; only personnel with distributor access can view the unblinded code lists. Study Investigators and those enrolling participants cannot access these lists to preserve the double blinded nature of the study. This ensures that the treatment assignments are hidden to both participants and investigators until the end of the study.

### 2.4.2 Blinding:

Since this is a double-blind randomised placebo-controlled clinical trial, the treatment allocation will be blinded to the investigators, sponsor clinical trial management team, clinical staff, laboratory staff and the patient. Placebo capsules will be identical in appearance, weight, and all other obvious characteristics to the course of IMT, and will be handled by pharmacy identically; this will help in maintaining blinding. Trial randomisation will occur as soon as possible after satisfactory review and confirmation of patient eligibility at screening.

Unblinding will only be considered in cases where the identity of the drug assignment is necessary for the safety of the patient. This will be possible 24 hours a day and 365 days of the year, but with strong recommendation that the chief investigator and/or sponsor is contacted prior to the unblinding of the patient to discuss the reasons for unblinding. Where unblinding is required, sites will use the unique login provided by the sponsor to access the treatment assignment; if the database cannot be accessed, there will be a manual unblinding procedure in place using unblinding cards located in the pharmacy folder.

### 2.5 Outcomes:

The Schedule/ Summary of visits is shown in **Supplementary Material 1, Appendix 4,** with a summary of assessments to be undertaken given in **Supplementary Material 1, Appendix 5.**

The primary outcome of the trial is the ability of the capsulised IMT given pre-HCT to increase and maintain intestinal microbiota diversity post-HCT. This will be assessed via measurement of the difference between the change in alpha diversity (calculated using inverse Simpson index) 28 +/-3 days post-HCT from baseline for patients in the capsulised IMT groups *versus* the capsulised placebo group. The secondary objectives of the study relate to feasibility/ tolerability of the capsule, and impact of the IMT upon a range of clinically- and translationally pertinent outcomes. These include: quality of life; microbiological/ infective outcomes; need for Intensive Care; and haematological outcomes, ranging from relapse, to GvHD, to impact upon engraftment and immune reconstitution. Microbiological/ infective outcomes will be assessed via conventional clinical microbiology techniques, as well as via gut microbiome diversity and taxonomic characterisation. These secondary outcomes are summarised in **Table 1.** In addition, the study has a range of discovery phase/ exploratory endpoints, including investigating the impact of IMT upon: markers of gut barrier function; metabolomic profiles in different biofluids (namely stool, urine and plasma); circulating cytokines; and functionality of circulating monocytes and T cells. In addition to lymphocyte subset characterisation, collection of peripheral blood mononuclear cell (PBMC) at day 100 (visit 8), and day 365 (visit 10) will allow further exploration of the impact of IMT on immune reconstitution, and in particular, T-cell repertoire. Further details about biosample collection, storage and processing are given in **Supplementary Material 1, Appendix 6.**

The study flow chart/ participant timeline is shown in **Figure 1.** Of note, regardless of whether the patient is randomised to capsulised IMT or placebo, they will continue with their scheduled standard of care treatments/ assessments while also receiving study follow-up assessments at planned intervals, as shown in **Figure 1.** Other pre- and post-HCT care will be in accordance with the participating centres' policies. As such, patients are allowed to receive prophylactic antibiotics (such as ciprofloxacin) but should not receive broad-spectrum antibiotics after the trial treatment has taken place and prior to the start of HCT. It is recognised that this may not be always possible, as neutropenic fever may sometimes develop during the conditioning therapy. If this happens, patients will not be excluded from the trial, but the broad-spectrum antibiotic use and its duration must be documented at response assessments.

## 2.6 Data collection and management:

### 2.6.1 General approach:

CRFs for the study will be in English, using generic names for concomitant medications wherever possible. All written material to be used by participants must use vocabulary that is clearly understood and be in the language appropriate for the study site. The electronic case report form (eCRF) database will be in OpenClinica. The Investigator (or delegated member of the site study team) will record all data relating to protocol assessments and procedures, laboratory, safety and efficacy data in the eCRF. All trial documentation, including that held at participating sites and the trial coordinating centre, will be archived for a minimum of 20 years following the end of the study.

### 2.6.2 Confidentiality:

The investigator will ensure that the participant's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, participants will be identified by a participant ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator. The investigator shall permit direct access to participants' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and RECs.

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing and disclosure of personal information, and will uphold the Act's core principles.

### 2.6.3 Oversight and monitoring:

A Trial Steering Committee (TSC) will be convened, including as a minimum an independent Chair, independent clinician, the Chief Investigator, Independent Statistician, Trial Manager and PPI Representative. The role of the TSC is to provide overall supervision of trial conduct and progress. A Trial Management Group (TMG) will also be convened, including the Chief Investigator, co-investigators and key collaborators, trial statistician and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Furthermore, an Independent Data Monitoring Committee (IDMC) will be convened to include as a minimum an independent oncologist chair, an independent oncologist and an independent statistician. The role of the IDMC is advisory to the TSC, to ensure the highest standard of patient safety and data integrity.

The IDMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable, or if any issues are identified which may compromise patient safety. In the case of early discontinuation of the study, response assessments will be completed for each participant, as far as possible.

The study will be monitored periodically by trial monitors to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy, and consistency of the data. Monitoring will be conducted centrally/remotely from the coordination centre and on-site. Monitoring procedures and requirements will be documented in a Monitoring Plan, developed in accordance with the risk assessment.

Quality Control will be performed according to Imperial College Trials Unit (ICTU) internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

The study may be participant to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

## 2.7 Statistical considerations:

### 2.7.1 Sample size and powering:

Currently published data on alpha diversity change in patients undergoing HCT and/or IMT span different study types, vary in quality/granularity, and use different alpha diversity indices. The evidence available suggests larger decreases in alpha diversity at approximately one-month post-HCT compared to baseline in patients who do not receive IMT relative to those that do[2 6 8 18 19]. Fitting a mixed-effects model (with fixed effects for arm, time (day), their corresponding interaction and a random per-patient intercept effect) with quadratic splines at 5 degrees-of-freedom (3 internal knots) to longitudinal change in alpha diversity data (measured with inverse Simpson's index) from baseline[2], IMT patients had an expected change in baseline alpha diversity at day 28 post-HCT 3.46 (pooled SE = 2.19) units more than placebo (IMT mean change = -4.70, SE = 1.44, n = 14; placebo mean change = -8.16, SE = 1.66, n = 11). We have used these results on IMT post-HCT to design our study of IMT pre-HCT vs. placebo.

Our null hypothesis is there is no difference between the change in day 28 ± 3 days alpha diversity (inverse Simpson's) post-HCT from baseline in patients receiving pre-HCT IMT compared to patients receiving placebo capsules (i.e., difference in between-arm changes from baseline is zero). Using a two-sample t-test to compare IMT-arm change to placebo-arm change with two-sided alpha controlled at 20%[20 21], we need 46 patients randomised 1:1 between IMT and placebo (23 per arm) to have ≥80% power to detect a between-arm difference of 3.46 units (with pooled standard deviation of 5.45 estimated at day 28 post-HCT from mixed-effects model). To account for dropouts at a rate of up to 8% across both arms, we will recruit 50 patients in total. Modelling and sample size calculations have been performed using R v3.6.1.

### 2.7.2 Statistical analysis:

#### 2.7.2.1 Overall approach:

Statistical analyses will be formally documented within a detailed Statistical Analysis Plan (SAP) and structured using the estimand framework (as described in the ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials[21 22]) with intercurrent events and subsequent analysis strategies defined accordingly. Protocol non-adherence and other non-defined intercurrent events will be incorporated into analysis via a treatment policy where the patient is assessed based on their randomised arm regardless of the event in question. Any deviations from the SAP will be documented and signed off by the statisticians and CI, and filed in the Trial Master File (TMF).

#### 2.7.2.2 Primary Estimand:

*Population:* Adults with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), acute leukaemia (AL) of ambiguous lineage, high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML), and chronic myeloid leukaemia (CML) in blast phase, considered suitable/fit for allogeneic haematopoietic stem cell transplantation HCT.

*Treatment:* Capsulised IMT vs matched capsulised placebo.

*Variable:* Stool microbiota diversity post-HCT defined as the change in alpha diversity (measured as inverse Simpson's index) between IMT administration (at 14 +/- 3 days prior to HCT) and 28 +/-3 days after HCT.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at day 28.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy will be used to handle patient all defined intercurrent events, any patient response-assessment data collected post-randomisation will still be used in the analysis model if day 28 data is unavailable.

*BMJ Open – Protocol:*

**2.7.2.2.1 Secondary Estimand (of Primary Outcome):**

A complete-case approach will be undertaken on the primary outcome (taking patients that provide primary outcome data across all response-assessment timepoints)

*Population:* As primary estimand.

*Treatment:* As primary estimand.

*Variable:* As primary estimand.

*Population-level summary:* As per primary estimand.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy will be used in the event of adverse events or rescue medication. In the event of death, withdrawal or loss-to-follow-up a principal stratum strategy is to be followed such that only those that complete the assessment period are to be included for analysis.

**2.7.2.3 Secondary Estimands:**

1) Assessment of tolerability and acceptability of treatment versus placebo through the Functional Assessment of Cancer Therapy measured using health-related quality of life and patient perspective questionnaires.

*Population:* As per primary estimand.

*Treatment:* As per primary estimand.

*Variable:* Scores arising from health-related quality of life (EQ-5D-5L) and EORTC patient perspective (EORTC_QLQ-C30) questionnaires.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at day 28, plus at follow-up timepoints day 100, day 200 and day 365.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use, loss-to-follow-up/withdrawal.

*Strategy to handle intercurrent events:* Treatment policy as primary estimand

2) Assessment of changes in inverse Simpson's index and other measures of gut microbiome diversity across all timepoints assessed, including alpha diversity and richness (i.e., as measured via Chao-1, Shannon, Faith's PD), and beta-diversity (Aitchinson's distance) as well as changes in gut microbiome taxonomic composition.

*Population:* As per primary estimand.

*Variable(s):* As per primary, plus Chao-1, Shannon, Faith's PD, Aitchinson's distance and taxonomic composition.

*Population-level summary:* The model-produced estimate for the treatment * time interaction effect at days 7 & 14. Interaction effect for follow-up visit timepoints day 100, 200 and 365 will also be assessed.

*Intercurrent Events:* Death, adverse/serious adverse events, rescue therapy outside of antibiotic use.

*Strategy to handle intercurrent events:* Treatment policy as primary estimand.

3) Clinical endpoints including markers of general health, infective/microbiological and haematological outcomes across all timepoints measured, including: admission to intensive care unit, survival, non-relapse mortality, relapse incidence; occurrence and severity of graft-versus-host disease (GvHD), overall and GvHD-free relapse-free survival, and quality of life.

*Population:* As per primary estimand.

*Variable(s):* Overall and GvHD-free relapse-free survival.

*Population-level summary:* Log-rank test statistic, hazard-ratio (with 95% CI).

*Intercurrent Events:* Death unrelated to patient comorbidity (relapse-free survival only), death related to patient comorbidity (relapse-free survival only), loss-to-follow-up/patient withdrawal, adverse/serious adverse events, rescue therapy outside of antibiotic use.

*BMJ Open – Protocol:*

*Strategy to handle intercurrent events:* Death unrelated to patient comorbidity (relapse-free survival only) will be censored at recorded time-of-death as part of a hypothetical strategy. Where death is potentially related, a composite strategy is to be considered where time-of-death will be taken as time-of-relapse. Loss-to-follow-up/patient withdrawal will be censored at time of last contact as part of a hypothetical strategy. Treatment policy will be used upon use of rescue therapy or under any adverse/serious adverse event which does not result in the withdrawal of the patient.

Estimands for additional variables covered in secondary outcome #3 will be provided within the Statistical Analysis Plan (SAP). These include; Markers of general health (ITU Admission, Severity of Mucositis, Occurrence of Severe Acute Kidney Injury (AKI), Occurrence of Severe liver dysfunction, Use of Parenteral Nutrition), Infective Haematological Outcomes (Fever Occurrence, Fever CTCAE Grade, Infection, Multi drug Resistant Bacterial Colonisation (MDROs), Antibiotic Use), Neutrophil and platelet engraftment data, Recovery of T-cell Chimaerisms, Haematological Outcomes (Non-relapsed mortality, Occurrence Graft vs Host Disease, Severity of graft vs Host Disease)

#### 2.7.2.4 Analysis of primary estimand:

The primary outcome of between-arm difference in alpha diversity change from baseline at day 28 (± 3 days) will be analysed using a mixed-effects model, with change in alpha diversity from baseline as outcome, with treatment arm, time, treatment-by-time interactions and stratification variables used in randomisation included as fixed effects and also a per-patient intercept included as a random effect. The subsequent model estimate for the treatment-by-time interaction term at day 28 will be the effect of interest as per **Section 2.7.2.2.**

#### 2.7.2.5 Analysis of secondary estimands:

The complete-case analysis will follow the same model as defined in **Section 2.7.2.4** (using only patients attending all visits as per estimand). Mixed-effect models incorporating a per-patient random effect alongside effects for time of assessment, and an interaction term of time-by-arm assessing changes in alpha diversity (inverse Simpson's index, Chao-1, Shannon index, Faith's PD) and β-diversity will provide treatment effects and 80% confidence intervals

at Response assessments 1-5 and Follow-up assessments 1-3 (see **Figure 1**). Similar approaches will be used to assess changes in gut microbiome taxonomic composition based on shallow shotgun sequencing.

Overall survival (time from randomisation to death/date last seen alive) will be analysed using Kaplan-Meier methods and log-rank testing utilising the same stratification variables as per primary analysis model defined in **Section 2.7.2.4.** Additional survival analysis will include non-relapse mortality and GvHD-free relapse-free survival.

**2.7.2.6 Analysis of secondary outcome measures:**

Additional analyses of clinical outcomes will include: number of days spent in Intensive Care; presence and severity of mucositis and length of time requiring parenteral nutrition; days of fever post-HCT corrected for length of admission; days of antibiotics including carbapenem; number and length of bloodstream infections; colonisation with multi-drug resistant bacteria, including extended-spectrum β-lactamases, vancomycin-resistant *Enterococci*, and carbapenemase-producing *Enterobacteriaceae*; and incidence of GvHD; and relapse incidence. Neutrophil and platelet engraftment data, Recovery of T-cell Chimaerisms, Haematological Outcomes (Non-relapsed mortality, Occurrence Graft vs Host Disease, Severity of graft vs Host Disease)

Analysis will be completed via presentation of descriptive statistics or summary tables. Continuous outcomes will be assessed via the same mixed-model approach as per primary estimand. Frequency outcomes will utilise a negative binomial approach, adjusting for the same covariates as the primary estimand analysis model. In the event where data fails to satisfy model assumptions and transformation is not suitable, an appropriate non-parametric approach may be used in replacement.

Full details of analysis methodology to be provided in the SAP.

#### 2.7.2.7 Safety analysis:

Additional safety outcomes - including AEs, ARs, SAE and SUSARs - will be reported as frequencies, unadjusted participant proportions and/or rates where appropriate. Differences between arms with 95% confidence intervals using exact methods will be produced where appropriate.

## 3 Discussion:

The increasing recognition of the contribution of the gut microbiome in patients with haematological malignancies undergoing cellular therapies, coupled with emergent data supporting IMT as a strategy to alter the microbiome, necessitates robust placebo-controlled IMT trials. Primarily, phase IIa trials such as MAST aim to fully evaluate the specific contribution that IMT have as part of patient treatment and provide the launchpad for future phase 3 trials. We hope that associated microbiome, metabolomic and immune analyses will improve understanding of the mechanistic contribution of the gut microbiome to the clinical outcomes seen, potentially setting the stage for future novel targeted 'microbiome therapeutics' that avoid the drawbacks associated with IMT. Whilst we envisage that most of our analyses will involve comparison of the dynamics of clinical and biological variables between the IMT and placebo arms, there may also be within-group exploratory analyses which provide further relevant insight as well (e.g. comparison of 'responders' and 'non-responders' to FMT within the treatment arm only, looking for the impact of baseline host gut microbiome diversity and/or specific taxonomic features upon the likelihood of response).

A growing body of non-randomised studies has described positive clinical signals when IMT was used in patients with haematological malignancies undergoing HCT[8]. However, it was also noteworthy that a recent phase II randomised double-blind placebo-controlled trial[23], administering capsulised IMT or placebo after HCT for AML, timing this for after neutrophil recovery, failed to achieve its primary outcome, showing no statistical difference in the infection rate by four months post-HCT in the IMT arm compared to placebo. One fundamental difference in design between that study and our study is that in our trial, the IMT is targeted at the pre- (rather than post-) HCT period. There were several reasons for us considering that this aspect of timing is particularly important, a factor that has also been introduced elsewhere[24]. Most importantly, the published data related to the dynamics of the

gut microbiome with HCT particularly demonstrate the close association between reduced gut microbiome diversity pre-HCT and future morbidity and mortality, as well as the emergence of *Enterococcus* domination within the gut microbiome within three weeks post-allogeneic HCT as influencing poor outcome[6 7]. Additionally, aberrant intestinal microbiome diversity is known to be associated with increased inflammatory response[25] and biomarkers of inflammation measured pre HCT were shown to be independent predictors of HCT outcomes[26].

Therefore, we concluded that the clearest window for intervention is pre-HCT, aiming to increase the pre-HCT gut microbiota diversity, and mitigate the risk of pathobiont overgrowth is prior to start of HCT conditioning (**Figure 2**). The concept of targeting IMT prior to intervention has also been used successfully in oncology, with a phase 1 study evaluating IMT use prior to immune checkpoint inhibition in 20 patients with advanced melanoma demonstrating an objective response rate of 65% (*n*=13/20; including 4/20 complete responses)[27].

The use of IMT in the context of immunosuppressed patients requires certain considerations above and beyond those of, for instance, conventional use of IMT in treating recurrent CDI[28]. The use of a capsulised preparation is clearly more acceptable to this patient cohort than conventional IMT slurry, and may be safer avoiding potential aspiration of slurry. The donor screening protocol used donors is in full accordance with UK recommendations[9]; while the risk of CMV transmission via IMT appears extremely low[29], CMV negative donors are being used out of an abundance of caution. The window for IMT administration aims to be long enough after prior chemotherapy to allow full cell count recovery, but early enough before HCT to permit sufficient microbiota engraftment. This is important, since degree of microbiota engraftment has been associated with level of clinical improvement after IMT[30]. Our experience to date is that IMT mitigates the risk of invasive infections related to MDROs rather than decolonises them from the gut[15], but there is still uncertainty regarding this; one recent trial of IMT in a renal transplant population suggested certain ESBL-colonising strains being displaced by non-ESBL strains by strain competition[31]. The serial clinical assessment and collection of shotgun metagenomic data from study participants in a placebo-controlled

fashion will allow much more granular assessment of the impact of IMT on MDROs than has been described previously.

In conclusion, the MAST trial aspires to give new clinical and translational insights into the role of gut microbiome manipulation in patients with haematological malignancy receiving allogeneic HCT, with particular focus on the potential role of IMT on haematological and infective outcomes. The study aims to run recruitment for 24 months post-authorisation, and close in May 2027.

## 4 Ethics and Dissemination:

### 4.1 Research Ethics Approval:

The institutional review board (North East - Tyne & Wear South, England, Ref: 23/NE/0105) and the national regulatory authorities, Medicines & Healthcare products Regulatory Agency (MHRA, Ref: CTA 19174/0441/001-0001) issued approval on the 3rd October 2023.

#### 4.1.1 Other ethical considerations:

##### 4.1.1.1 Consent:

Patients will be identified as per site established processes and invited to participate by their primary haematology team. Eligible patients will be provided with the patient information sheet (PIS) (**Supplementary Material 2**), and given sufficient time to consider the study, with opportunities to discuss and ask questions. Investigators will ensure that they adequately explain the study, including the aims, trial treatment, anticipated benefits and potential risks of participation. The right of the patient to refuse participation in the trial, or withdraw at any point, without giving explanation will be respected. Informed consent will be requested from the patient by the investigator who has been delegated the responsibility on the delegation log. Consent will be obtained no earlier than 24 hours after receiving the PIS to give them time to read and understand what their participation in the study entails (consent form provided as **Supplementary Material 3**). With patient consent, it is the investigator's responsibility to inform the patient's General Practitioner regarding study participation.

##### 4.1.1.2. Study Conduct and Safety Measures:

The study prioritises the highest safety and ethical standards, ensuring full compliance with the 1964 Declaration of Helsinki and International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines). Rigorous pharmacovigilance measures are in place to monitor and reporting of serious or non-serious adverse events/reactions to enable a prompt and appropriate clinical response. There are stringent protocols in place for the reporting of causality, expectedness, and severity assessments; every reported event undergoes thorough evaluation by clinical professionals. These safety measures are essential for maintaining participant welfare and upholding study integrity. Additionally, robust donor screenings and contraception requirements minimise potential risks associated infection transmission and unanticipated pregnancy outcomes respectively, to reinforce a comprehensive approach to participant safety.

**4.1.1.3. Dissemination:**

The dissemination of results from this study will always be performed with input from our PPI group. This will be foremost via abstract presentations at conferences and manuscripts in peer-reviewed journals. All such publications will be circulated to all authors prior to submission for their review and approval. Publications will be made in concordance with Consort guidelines/ checklists. Study participants will be notified of the outcome of the trial prior to any publications. A Clinical Study Report summarising the study results will be prepared and submitted to the Research Ethics Committee within a year of the end of study. The results will also be submitted to the EudraCT results database in accordance with regulatory requirements.

*BMJ Open – Protocol:*

## 5. References:


1. Stoma I, Littmann ER, Peled JU, et al. Compositional Flux Within the Intestinal Microbiota and Risk for Bloodstream Infection With Gram-negative Bacteria. *Clin Infect Dis* 2021;73(11):e4627-e35. doi: 10.1093/cid/ciaa068
2. Taur Y, Coyte K, Schluter J, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. *Sci Transl Med* 2018;10(460) doi: 10.1126/scitranslmed.aap9489
3. Tacconelli E, De Angelis G, Cataldo MA, et al. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. *Antimicrob Agents Chemother* 2009;53(10):4264-9. doi: 10.1128/AAC.00431-09 [published Online First: 20090810]
4. Danielsen AS, Franconeri L, Page S, et al. Clinical outcomes of antimicrobial resistance in cancer patients: a systematic review of multivariable models. *BMC Infect Dis* 2023;23(1):247. doi: 10.1186/s12879-023-08182-3 [published Online First: 20230418]
5. Shono Y, Docampo MD, Peled JU, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Sci Transl Med* 2016;8(339):339ra71. doi: 10.1126/scitranslmed.aaf2311
6. Peled JU, Gomes ALC, Devlin SM, et al. Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. *N Engl J Med* 2020;382(9):822-34. doi: 10.1056/NEJMoa1900623
7. Stein-Thoeringer CK, Nichols KB, Lazrak A, et al. Lactose drives Enterococcus expansion to promote graft-versus-host disease. *Science* 2019;366(6469):1143-49. doi: 10.1126/science.aax3760
8. Malard F, Gaugler B, Mohty M. Faecal microbiota transplantation in patients with haematological malignancies undergoing cellular therapies: from translational research to routine clinical practice. *Lancet Haematol* 2022;9(10):e776-e85. doi: 10.1016/S2352-3026(22)00223-X
9. Mullish BH, Merrick B, Quraishi MN, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory Clostridioides difficile infection and other potential indications: second edition of joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut* 2024;73(7):1052-75. doi: 10.1136/gutjnl-2023-331550 [published Online First: 20240606]
10. Allegretti JR, Khanna S, Mullish BH, et al. The Progression of Microbiome Therapeutics for the Management of Gastrointestinal Diseases and Beyond. *Gastroenterology* 2024 doi: 10.1053/j.gastro.2024.05.004 [published Online First: 20240514]
11. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. *N Engl J Med* 2019;381(21):2043-50. doi: 10.1056/NEJMoa1910437 [published Online First: 20191030]
12. Bilinski J, Grzesiowski P, Sorensen N, et al. Fecal Microbiota Transplantation in Patients With Blood Disorders Inhibits Gut Colonization With Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clin Infect Dis* 2017;65(3):364-70. doi: 10.1093/cid/cix252
13. Millan B, Park H, Hotte N, et al. Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients With Recurrent Clostridium difficile Infection. *Clin Infect Dis* 2016;62(12):1479-86. doi: 10.1093/cid/ciw185 [published Online First: 20160329]

14. Innes AJ, Mullish BH, Fernando F, et al. Faecal microbiota transplant: a novel biological approach to extensively drug-resistant organism-related non-relapse mortality. *Bone Marrow Transplant* 2017;52(10):1452-54. doi: 10.1038/bmt.2017.151 [published Online First: 20170717]
15. Ghani R, Mullish BH, McDonald JAK, et al. Disease Prevention Not Decolonization: A Model for Fecal Microbiota Transplantation in Patients Colonized With Multidrug-resistant Organisms. *Clin Infect Dis* 2021;72(8):1444-47. doi: 10.1093/cid/ciaa948
16. Innes AJ, Mullish BH, Ghani R, et al. Fecal Microbiota Transplant Mitigates Adverse Outcomes Seen in Patients Colonized With Multidrug-Resistant Organisms Undergoing Allogeneic Hematopoietic Cell Transplantation. *Front Cell Infect Microbiol* 2021;11:684659. doi: 10.3389/fcimb.2021.684659 [published Online First: 20210827]
17. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586. doi: 10.1136/bmj.e7586 [published Online First: 20130108]
18. van Lier YF, Davids M, Haverkate NJE, et al. Donor fecal microbiota transplantation ameliorates intestinal graft-versus-host disease in allogeneic hematopoietic cell transplant recipients. *Sci Transl Med* 2020;12(556) doi: 10.1126/scitranslmed.aaz8926
19. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368(5):407-15. doi: 10.1056/NEJMoa1205037 [published Online First: 20130116]
20. Rubinstein LV, Korn EL, Freidlin B, et al. Design issues of randomized phase II trials and a proposal for phase II screening trials. *J Clin Oncol* 2005;23(28):7199-206. doi: 10.1200/JCO.2005.01.149
21. Sargent DJ, Taylor JM. Current issues in oncology drug development, with a focus on Phase II trials. *J Biopharm Stat* 2009;19(3):556-62. doi: 10.1080/10543400902802474
22. E9 (R1) Step 5 addendum on estimands and Sensitivity Analysis in Clinical Trials to the guideline on statistical principles for clinical trials.
23. Rashidi A, Ebadi M, Rehman TU, et al. Randomized Double-Blind Phase II Trial of Fecal Microbiota Transplantation Versus Placebo in Allogeneic Hematopoietic Cell Transplantation and AML. *J Clin Oncol* 2023;41(34):5306-19. doi: 10.1200/JCO.22.02366 [published Online First: 20230526]
24. Ghani R, Chrysostomou D, Roberts LA, et al. Faecal (or intestinal) microbiota transplant: a tool for repairing the gut microbiome. *Gut Microbes* 2024;16(1):2423026. doi: 10.1080/19490976.2024.2423026 [published Online First: 20241105]
25. Tilg H, Zmora N, Adolph TE, et al. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* 2020;20(1):40-54. doi: 10.1038/s41577-019-0198-4 [published Online First: 20190806]
26. Patel A, Szydlo RM, Auner HW, et al. C-reactive protein prior to myeloablative allogeneic haematopoietic cell transplantation identifies patients at risk of early- and long-term mortality. *Br J Haematol* 2018;180(6):889-92. doi: 10.1111/bjh.14454 [published Online First: 20161129]
27. Routy B, Lenehan JG, Miller WH, Jr., et al. Fecal microbiota transplantation plus anti-PD-1 immunotherapy in advanced melanoma: a phase I trial. *Nat Med* 2023;29(8):2121-32. doi: 10.1038/s41591-023-02453-x [published Online First: 20230706]

28. Ghani R, Mullish BH, Davies FJ, et al. How to adapt an intestinal microbiota transplantation programme to reduce the risk of invasive multidrug-resistant infection. *Clin Microbiol Infect* 2022;28(4):502-12. doi: 10.1016/j.cmi.2021.11.006 [published Online First: 20211123]
29. Galperine T, Engelmann I, Hantz S, et al. Cytomegalovirus in donors for fecal microbiota transplantation, the phantom menace? *PLoS One* 2023;18(6):e0287847. doi: 10.1371/journal.pone.0287847 [published Online First: 20230629]
30. Ianiro G, Puncochar M, Karcher N, et al. Variability of strain engraftment and predictability of microbiome composition after fecal microbiota transplantation across different diseases. *Nat Med* 2022;28(9):1913-23. doi: 10.1038/s41591-022-01964-3 [published Online First: 20220915]
31. Woodworth MH, Conrad RE, Haldopoulos M, et al. Fecal microbiota transplantation promotes reduction of antimicrobial resistance by strain replacement. *Sci Transl Med* 2023;15(720):eabo2750. doi: 10.1126/scitranslmed.abo2750 [published Online First: 20231101]

*BMJ Open – Protocol:*

## 6. Author contributions:

BHM, AJI, RG, FJD, SA-B, NAJ, JRM, JP all contributed to initial drafting of the manuscript, with all authors reviewing and approving the final submitted manuscript. JP conceptualised the trial protocol and is responsible for its clinical aspects. BHM and JRM provided expertise in IMT provision and microbiome/ metabolome analysis for the study. AJI and JP were responsible for analysis of all haematological aspects of the study, both clinical and translational, and contributed to set-up of recruiting centres. RG and FJD provided expertise regarding microbiological/ infection-related aspects of outcome and analysis in the study. LAR provided input into patient-facing materials for the trial and oversees translational and exploratory outcomes. SA-B and LW both contributed to all aspects of trial approval and administrative/ logistical set-up. GW and NAJ oversaw all aspects of statistical analysis within the trial. PF, ABK, FK, PK (Kottaridis), PK (Krishnamurthy), EN and RP are all site principal investigators for the studies. BHM is the guarantor.

## 7. Acknowledgements:

The authors are grateful to Dr James McIlroy and Dr Michael Smyth (both from EnteroBiotix Ltd) for review of this manuscript.

## 8. Data Statement:

The full version of the current protocol (v1.2, 17th January 2024) is available from the corresponding author on reasonable request. The trial website is available at: https://www.imperial.ac.uk/metabolism-digestion-reproduction/research/digestive-diseases/hepatology--gastroenterology/mast-study/ .

Information regarding the handling of clinical data for this study is provided in **Section 2.6.** Regarding biological data – all such data (including sequencing, immunological and metabolomic data) will be stored locally at Imperial College London in password-controlled areas of OneDrive, SharePoint or Research Data Store as appropriate. All metagenomic sequencing data generated in this trial will be deposited at the EBI's ENA repository for public use, with all metabolomic data uploaded to the EMBL EBI MetaboLights repository; all such data will be made open access. The biological data is stored in perpetuity at the EBI's site, whilst all other trial data will be stored for 20 years after the trial has closed.

**9. Funding statement:**

This study is funded via the Medical Research Council (MRC) Developmental Pathway Funding Scheme (DPFS) (grant number: MR/X004996/1). The Division of Digestive Diseases and Centre for Haematology receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre (BRC) based at Imperial College London and Imperial College Healthcare NHS Trust. BHM is the recipient of a Medical Research Council (MRC) Clinician Scientist award (MR/Z504002/1) and was the recipient of an NIHR Academic Clinical Lectureship (CL-2019-21-002).

**10. Competing interests statement:**

GMW is an employee of and holds share in GSK. All other authors have nothing to report.

*BMJ Open – Protocol:*

**11. Figure legends:**

**Figure 1: Study flow chart participant time line.**

**Figure 2: 'Prehabilitation' of the gut microbiome in MAST.** Dynamics of the gut microbiota conventionally through the peri-HCT period shown in black (as defined previously[6]); the red line is our predicted higher starting point and nadir for patients receiving a pre-HCT IMT in the MAST trial.

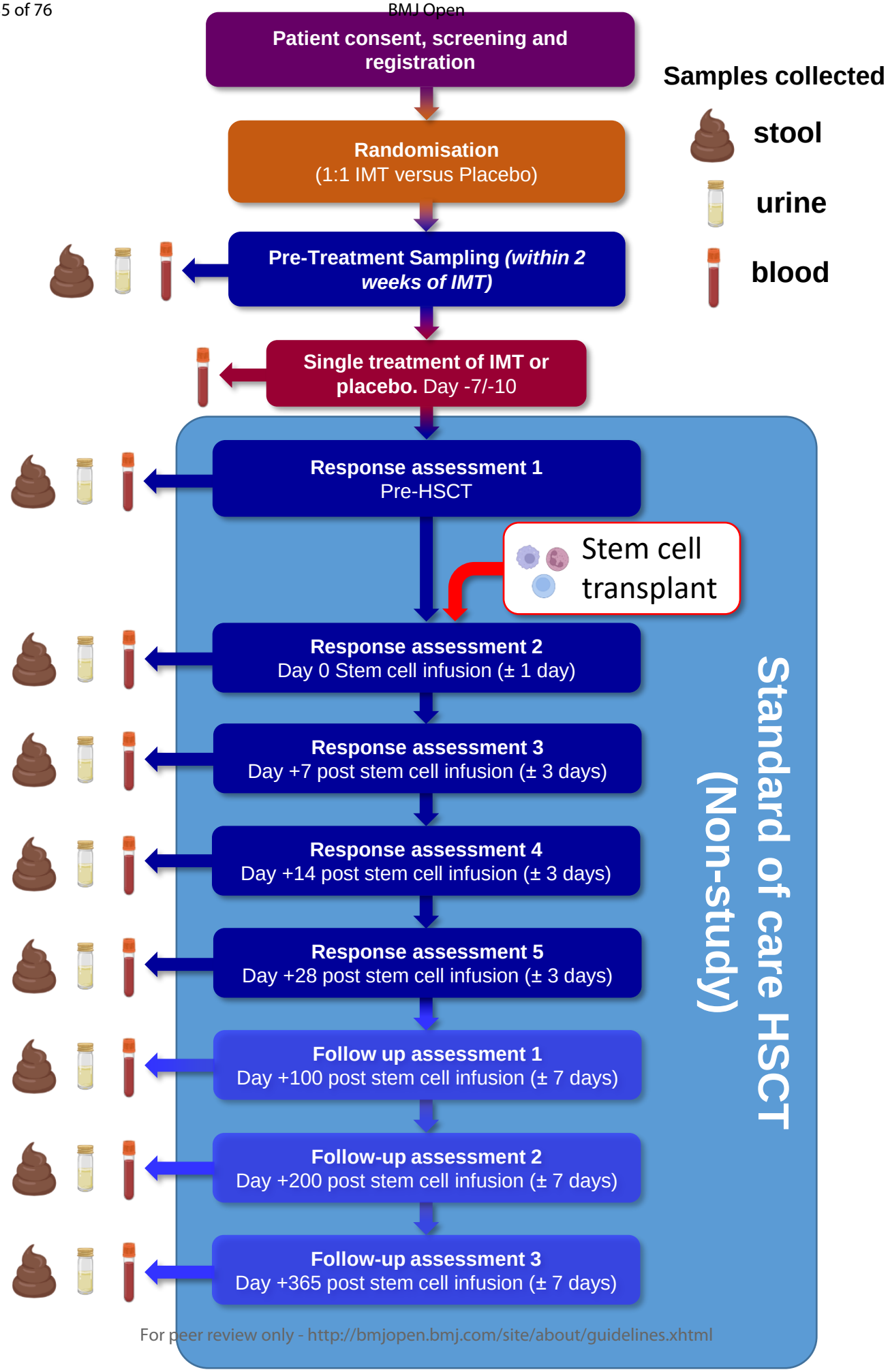
*BMJ Open – Protocol:*

**Table 1: Secondary objectives/ outcomes from the MAST study:**

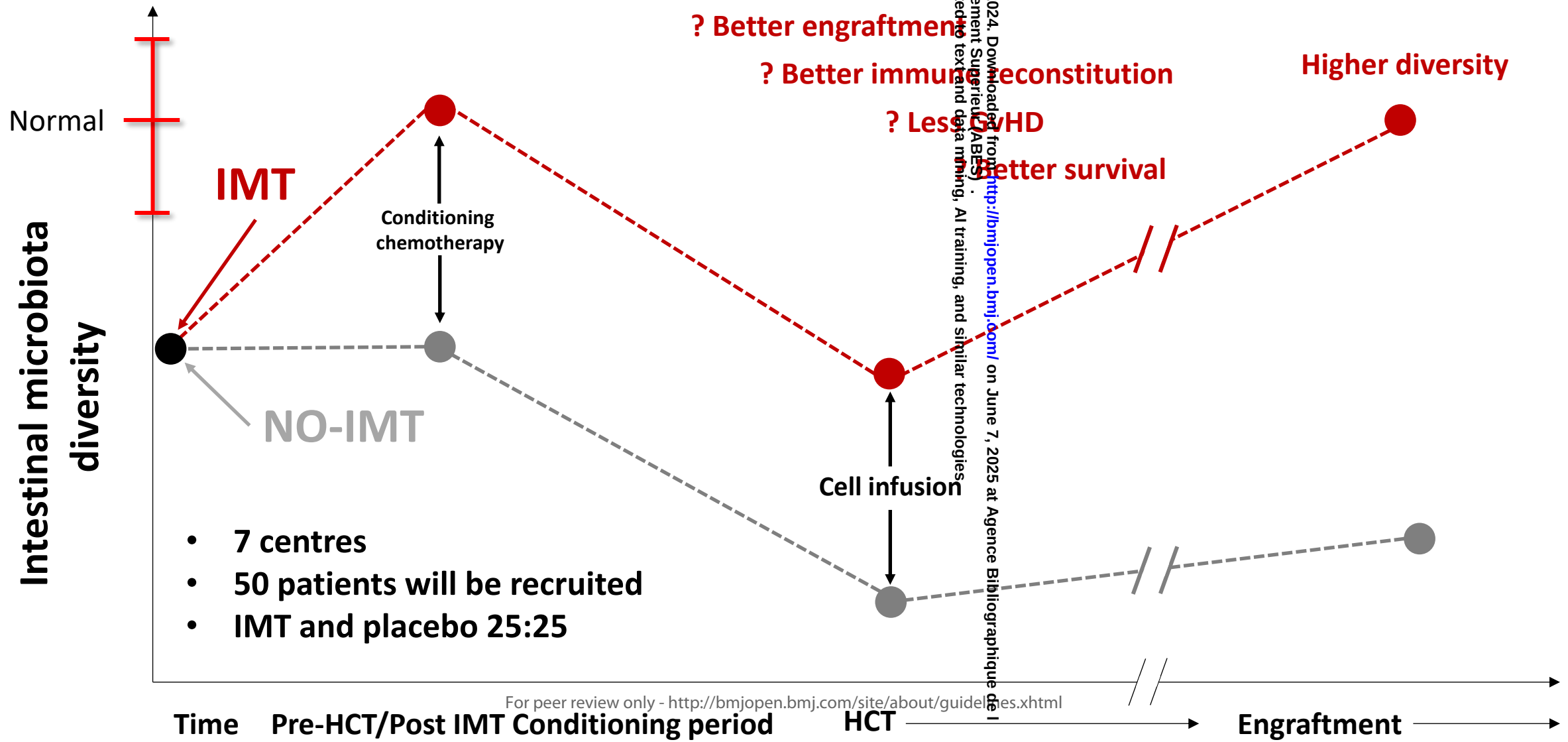
| Objectives: | Outcome: |
|---|---|
| Determine the feasibility and tolerability of capsule IMT prior to HCT in a multi-centre setting. | Tolerability and acceptability of IMT/placebo (as assessed via patient perspective questionnaires, i.e. EQ-5D-5L and EORTC QLQ-C30 questionnaires). |
| Evaluate microbiological/ infective, haematological, and quality of life-related clinical outcomes of administering IMT prior to HCT. | Gut microbiome endpoints:<br>Assessment of changes in inverse Simpson's index and other measures of gut microbiome diversity across all timepoints assessed, including alpha diversity and richness (i.e., as measured via Chao-1, Shannon, Faith's PD), and beta-diversity (Aitchinson's distance)<br><br>Assessment of changes in gut microbiome taxonomic composition across all timepoints assessed (using shallow shotgun sequencing). |

*BMJ Open – Protocol:*

| | |
|---|---|
| | Clinical endpoints:<br>Markers of general health across all timepoints measured, including: days on the Intensive Treatment Unit (ITU); presence and severity of mucositis; use of (and length of time that requiring) parenteral nutrition; severe acute kidney injury and severe liver dysfunction.<br>Infective/ microbiological outcomes across all timepoints measured, including: days of fever post-HCT (corrected for length of admission); days on antibiotics (including use of carbapenem specifically); number and length of bloodstream infections; urinary tract infections; colonisation with multi-drug resistant bacteria (MDROs; including extended-spectrum beta-lactamases (ESBL), vancomycin-resistant enterococci (VRE), and carbapenemase-producing Enterobacteriales (CPE)), and use of antibiotics.<br>Haematological outcomes across all timepoints measured, including: non-relapse mortality, relapse incidence; occurrence and severity of graft-versus=host disease (GvHD), overall and GvHD-free relapse-free survival, and quality of life. |
| Explore the potential for pre-HCT IMT to impact on HCT engraftment and immune reconstitution. | Neutrophil and platelet engraftment data as defined by EBMT will be routinely collected. Recovery of T-cell chimaerisms, T-cell count assessed by the lymphocyte subset analysis and immunoglobulin levels will be recorded at follow-up assessments. |



# MAST Study Concept



*BMJ Open – Protocol – Supplementary Material*

**Supplementary Material 1: Appendices:**

**Intestinal *M*icrobiota Transplant Prior to *A*llogeneic *S*tem Cell *T*ransplant (MAST) trial:**

**Study Protocol for a Phase IIa Randomised Controlled Trial**

**Appendix 1 - Neutropenia inducing regimens:**

Daunorubicin and cytarabine (DA) (with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Liposomal cytarabine and daunorubicin (Vyxeos) (with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

High dose cytarabine (>1000mg/m2) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Azacitine/decitabine (including oral forms) and venetoclax

Fludarabine / Cytarabine / GCSF / Idarubicin (FLAG-Ida) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, venetoclax, dasatinib or ponatinib)

Clofarabine / Cytarabine / GCSF / Idarubicin (CLAG-Ida) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

Mitozantrone / Etoposide / Cytarabine (MEC) (with or with or without a FLT-3 inhibitor, gemtuzumab ozogamicin, or venetoclax)

UK-ALL 14 phase 1 induction (off trial) or similar (with or without additional venetoclax)

UK-ALL 14 phase 2 induction (off trial) or similar (with or without additional venetoclax)

UK-ALL14 intensification (High dose methotrexate) or similar (with or without additional venetoclax)

Cyclophosphamide / Dexamethasone / Doxorubicin / Vincristine / Cytarabine alternating with methotrexate / cytarabine (Hyper-CVAD / MA) (with or without additional venetoclax)

*BMJ Open – Protocol – Supplementary Material*

**Appendix 2 – Disease and response criteria:**

***Acute lymphoblastic leukaemia (ALL) classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias)) and response criteria (modified Center for International Blood and Marrow Transplant Research criteria)***

| **B-ALL** |
|---|
| B-ALL with recurrent genetic abnormalities |
| B-ALL with t(9;22)(q34.1;q11.2)/*BCR::ABL1* |
| with lymphoid only involvement |
| with multilineage involvement |
| B-ALL with t(v;11q23.3)/*KMT2A* rearranged |
| B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1* |
| B-ALL, hyperdiploid |
| B-ALL, low hypodiploid |
| B-ALL, near haploid |
| B-ALL with t(5;14)(q31.1;q32.3)/*IL3::*IGH |
| B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1* |
| B-ALL, *BCR*::*ABL1*–like, ABL-1 class rearranged |
| B-ALL, *BCR*::*ABL1*–like, JAK-STAT activated |
| B-ALL, *BCR*::*ABL1*–like, NOS |
| B-ALL with iAMP21 |
| B-ALL with *MYC* rearrangement |
| B-ALL with *DUX4* rearrangement |
| B-ALL with *MEF2D* rearrangement |
| B-ALL with *ZNF384(362)* rearrangement |
| B-ALL with *NUTM1* rearrangement |
| B-ALL with *HLF* rearrangement |
| B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* (“CDX2/UBTF”) |
| B-ALL with mutated *IKZF1* N159Y |
| B-ALL with mutated *PAX5* P80R |
| Provisional entity: B-ALL, *ETV6::RUNX1*-like |

| |
|---|
| Provisional entity: B-ALL, with *PAX5* alteration |
| Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/IGH::*CEBPE* |
| Provisional entity: B-ALL, *ZNF384* rearranged-like |
| Provisional entity: B-ALL, *KMT2A* rearranged-like |
| B-ALL, NOS |
| **T-ALL** |
| Early T-cell precursor ALLwith *BCL11B* rearrangement |
| Early T-cell precursor ALL, NOS |
| T-ALL, NOS |
| Provisional entities (see supplemental Table 7) |
| **Provisional entity: natural killer cell ALL** |

Complete Remission (CR)

Hematologic complete remission is defined as meeting **all** of the following response criteria for at least four weeks.

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- ANC (absolute neutrophil count) ≥ 1.0 x $10^9$/L
- Platelets ≥ 100 x $10^9$/L
- Transfusion independent

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment; in this case, CR should still be reported as the status at transplant, since it represents the “best assessment” prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks. The pre-transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

Include recipients who are MRD positive or where the MRD status is unknown. MRD assessments include cytogenetic, flow cytometry, and molecular methods.

Include recipients meeting the above CR criteria regardless of how many courses of therapy were required to achieve CR.

The number of this complete remission can be determined by using the following guidelines:

- 1st CR: no prior relapse
- 2nd CR: one prior relapse
- 3rd or higher: two or more prior relapses

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria for at least four weeks:

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

Primary Induction Failure (PIF)

The patient received treatment for ALL but **never achieved CR or CRi at anytime**. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have never been in CR or CRi.

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting at least one of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

The number of this relapse can be determined by using the following guidelines:

- 1st relapse: one prior CR
- 2nd relapse: two prior CRs
- 3rd or higher: three or more CRs

Do not include a partial response (PR) when determining number of relapse. Recipients who achieve a PR to treatment should be classified as either PIF or relapse; PR in ALL is generally of short duration and is unlikely to predict clinical benefit.

***Acute myeloid leukaemia (AML) classification (international Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria (modified Center for International Blood and Marrow Transplant***

*BMJ Open – Protocol – Supplementary Material*

| |
|---|
| ***Research criteria)***Acute promyelocytic leukaemia (APL) with t(15;17)(q24.1;q21.2)/*PML*::*RARA* ≥ 10% |
| APL with other *RARA* rearrangements* ≥ 10% |
| AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* ≥ 10% |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11* ≥ 10% |
| AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A* ≥ 10% |
| AML with other *KMT2A* rearrangements† ≥ 10% |
| AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214* ≥ 10% |
| AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*; *MECOM*(*EVI1*) ≥ 10% |
| AML with other *MECOM* rearrangements‡ ≥ 10% |
| AML with other rare recurring translocations (see supplemental Table 5) ≥ 10% |
| AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*§ ≥ 20% |
| AML with mutated *NPM1* ≥ 10% |
| AML with in-frame bZIP *CEBPA* mutations ≥ 10% |
| AML and MDS/AML with mutated *TP53*† 10-19% (MDS/AML) and ≥ 20% (AML) |
| AML and MDS/AML with myelodysplasia-related gene mutations 10-19% (MDS/AML) and ≥ 20% (AML) |
| Defined by mutations in *ASXL1*, *BCOR*, *EZH2, RUNX1*, *SF3B1, SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2* |
| AML with myelodysplasia-related cytogenetic abnormalities 10-19% (MDS/AML) and ≥ 20% (AML) |
| Defined by detecting a complex karyotype (≥ 3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurring genetic abnormalities), del(5q)/t(5q)/add(5q), −7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), −17/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities |
| AML not otherwise specified (NOS) 10-19% (MDS/AML) and ≥ 20% (AML) |
| Myeloid sarcoma |

*

Includes AMLs with t(1;17)(q42.3;q21.2)/*IRF2BP2*::*RARA*; t(5;17)(q35.1;q21.2)/*NPM1*::*RARA*; t(11;17)(q23.2;q21.2)/*ZBTB16*::*RARA*; cryptic inv(17q) or del(17) (q21.2q21.2)/*STAT5B*::*RARA*, *STAT3*::*RARA*; Other genes rarely rearranged with *RARA:TBL1XR1* (3q26.3), *FIP1L1* (4q12), *BCOR*(Xp11.4).

†

Includes AMLs with t(4;11)(q21.3;q23.3)/*AFF1*::*KMT2A*#; t(6;11)(q27;q23.3)/*AFDN*::*KMT2A*; t(10;11)(p12.3;q23.3)/*MLLT10*::*KMT2A;* t(10;11)(q21.3;q23.3)/*TET1*::*KMT2A;* t(11;19)(q23.3;p13.1)/*KMT2A*::*ELL;* t(11;19)(q23.3;p13.3)/*KMT2A*::*MLLT1* (occurs predominantly in infants and children).

‡

Includes AMLs with t(2;3)(p11~23;q26.2)/*MECOM*::*?;* t(3;8)(q26.2;q24.2)/*MYC*, *MECOM*; t(3;12)(q26.2;p13.2)/*ETV6*::*MECOM*; t(3;21)(q26.2;q22.1)/*MECOM*::*RUNX1.*

§

The category of MDS/AML will not be used for AML with *BCR*::*ABL1* due to its overlap with progression of CML, *BCR*::*ABL1*-positive.

Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- No extramedullary disease (e.g., CNS, soft tissue disease)
- Neutrophils ≥ 1.0 x $10^9$/L
- Platelets ≥ 100 x $10^9$/L
- Transfusion independent

Include recipients who are MRD positive or where the MRD status is unknown. MRD assessments include cytogenetic, flow cytometry, and molecular methods.

Include recipients meeting the above CR criteria regardless of how many courses of therapy were required to achieve CR.

The number of this complete remission can be determined by using the following guidelines:

- 1st CR: no prior relapse
- 2nd CR: one prior relapse
- 3rd or higher: two or more prior relapses

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- No extramedullary disease (e.g., CNS, soft tissue disease)
-

Primary Induction Failure (PIF)

The patient received treatment for AML but **never achieved CR or CRi at anytime**. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have *never been in CR or CRi*.

Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting one or more of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

The number of this relapse can be determined by using the following guidelines:

- 1st relapse: one prior CR
- 2nd relapse: two prior CRs
- 3rd or higher: three or more CRs

Do not include a partial response (PR) when determining number of relapse. Recipients who achieve a PR to treatment should be classified as either PIF or relapse; PR in AML is generally of short duration and is unlikely to predict clinical benefit.

***Myelodysplastic syndromes (MDS) classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria (modified Center for International Blood and Marrow Transplant Research criteria).***

| |
|---|
| Myelodysplastic syndrome with mutated *SF3B1* |
| Myelodysplastic syndrome with del(5q) |

| |
|---|
| Myelodysplastic syndrome with mutated *TP53* |
| Myelodysplastic syndrome, not otherwise specified (MDS, NOS) |
| MDS, NOS without dysplasia |
| MDS, NOS with single lineage dysplasia |
| MDS, NOS with multilineage dysplasia |
| Myelodysplastic syndrome with excess blasts |
| Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) |
| MDS/AML with mutated *TP53* |
| MDS/AML with myelodysplasia-related gene mutations |
| MDS/AML with myelodysplasia-related cytogenetic abnormalities |
| MDS/AML, not otherwise specified |

Complete Remission (CR)

Requires all of the following maintained for a minimum of four weeks. When reporting the CR achievement date, report the first date when CR was achieved (not the four week date in which CR was maintained).

Bone marrow evaluation:

- < 5% myeloblasts with normal maturation of all cell lines

Blood evaluation

- Haemoglobin ≥ 110 g/L untransfused without erythropoietic support
- Absolute neutrophil count ≥ 1.0 x $10^9$/L without myeloid growth factor support
- Platelets ≥ 100 x $10^9$/L without thrombopoietic support
- 0% blasts in blood

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment. In this case, CR should still be reported as the status at transplant since it represents the “best assessment” prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks; the pre-transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria:

- < 5% blasts in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)

***Chronic myeloid leukaemia with blast transformation classification (International Consensus Classification of Myeloid Neoplasms and Acute Leukemias) and response criteria***

Philadelphia positive or BCR:ABL1 CML in blast phase defined by the presence of blast ≥ 20% in blood or bone marrow.

Second chronic phase (CP2)

Requires all of the following

**Bone marrow evaluation:**

- < 5% myeloblasts with normal maturation of all cell lines

**Blood evaluation**

- Absolute neutrophil count ≥ 1.0 x $10^9$/L without myeloid growth factor support
- Platelets ≥ 100 x $10^9$/L without thrombopoietic support
- < 5% blasts in blood

**Appendix 3 – Karnofsky performance status score**

- 100 Normal; no complaints; no evidence of disease.
- 90 Able to carry on normal activity; minor signs or symptoms of disease.
- 80 Normal activity with effort; some signs or symptoms of disease.
- 70 Cares for self; unable to carry on normal activity or to do active work.
- 60 Requires occasional assistance but is able to care for most of their personal needs.
- 50 Requires considerable assistance and frequent medical care.
- 40 Disabled; requires special care and assistance.
- 30 Severely disabled; hospital admission is indicated although death not imminent.
- 20 Very sick; hospital admission necessary; active supportive treatment necessary.
- 10 Moribund; fatal processes progressing rapidly.
- 0 Dead

**Appendix 4: Schedule/ Summary of Visits:** *Continued overleaf*

| | Screening | Treatment | Response Assessment: | | | | | Follow-up Assessment: | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 |
| **Visit** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **Day of HCT** | From -42 | -14 (± 2 days) | -7 (± 2) | 0 (± 1) | +7 (± 3) | +14 (± 3) | +28 (± 3) | +100 (± 7) | +200 (± 7) | +365 (± 14) |
| Informed Consent | X | | | | | | | | | |
| Inclusion & Exclusion Criteria | X | | | | | | | | | |
| Baseline data collection/Comorbidity Index | X | | | | | | | | | |
| Review of demographics, medical/disease | X | | | | | | | | | |
| Pregnancy test[1] | X | | | | | | | | | |
| EORTC-QLQ-C30 and EQ-5D-5L Questionnaires | X | | | | | | X | X | X | X |
| Bone marrow assessment[2] | X | | | | | | | X | | X |
| Physical Examination/Vital Signs (ECG) | <<All assessments (According to standard care practices)>> | | | | | | | | | |
| Lineage specific chimaerism[3] | | | | | | | | X | X | X |
| Lymphocyte subsets & IG levels[3] | | | | | | | | X | X | X |
| Stool Sample[7] | X | | X | X | X | X | X | X | X | X |
| Urine Sample[7] | X | | X | X | X | X | X | X | X | X |
| Blood Sample[7] | X | X | X | X | X | X | X | X | X | X |
| Clinical data collection[5] | | X | X | X | X | X | X | X | X | X |
| Adverse event assessment[4] | << Continuous assessment >> | | | | | | | | | |
| Assessment of GvHD | | | | | << Continuous assessment >> | | | | | |
| Cell infusion (HCT)[6] | | | | X | | | | | | |

# 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 and sample collections may be arranged within the ranges as indicated above without need to report as protocol deviation.

1. Pregnancy test for women of childbearing potential: serum/urine (investigator's discretion) pregnancy test (sensitivity of at least 25 mIU/mL) within 72 hours prior to starting study therapy. This applies even if the patient practices complete abstinence from heterosexual contact.
2. The results of bone marrow morphological, immunophenotypic, cytogenetic, and molecular characterisation performed according to local practice within the time points above should be reported within the time points above.
3. Chimaerism tests should be performed in local laboratories on day +30, +60, +90, +120, +200, and +365, lymphocyte subsets and immunoglobulin levels should be performed in local laboratories on days +100, +200, +365.
4. All AEs to be collected from written to consent to the first day of transplantation conditioning. After initiation of transplantation conditioning only AEs that are equal to or greater than Grade 3 of the CTCAE version 5.0 will be reported (unless the event meets the definition of an SAE) and abnormal laboratory findings will be reported only if they are judged to be of significant clinical importance. Reporting will stop at day +28 of transplantation. SAEs that are judged to be at least possibly related to the IMP(s) and are unexpected must still be reported in an expedited manner irrespective of how long after IMP administration the reaction occurred.
5. Collection of clinical data (see **Supplementary Material 1, Appendix 5** for summary of Assessments),

The following data will be collected at all study Visits (1-10)– Vital Signs, Physical Examination, Full Blood Count, Coagulation, Biochemistry, and Virology, Nutrition, Completed dietary questionnaire and ITU Admission. The dietary questionnaire comprises 24 hour dietary recall, a widely-used tool in studies with a nutritional component[1]; these dietary data will serve as useful metadata for the analysis of microbiome and metabolome data generated within the trial. The following data below will be collected in addition to the repeating assessments,

- Recent, Microbiology Colonisation History, Fever, Infection and Treatment History (Visits 2-10)
- Haemopoietic Cell transplant details (Visit 5 only).
- VOD, and Relapse, Engraftment, Acute GvHD, GvHD Prophylaxis and Therapy assessments (Visits 5-10).
- Post-transplant intervention assessment (Visit 10 only).

6. Haematopoietic stem cell transplant is not a study procedure and will take place as planned by the multi-disciplinary team before the patient enters the study following local standard of care procedures.
7. A summary of details for biosample collection, storage, and processing is given in **Supplementary Material 1, Appendix 6.**

**Appendix 5 - Summary of Assessments:**

| | Visit | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Assessment Forms** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **Consent** | X | | | | | | | | | |
| **Demographics** | X | | | | | | | | | |
| **Eligibility** | X | | | | | | | | | |
| **Significant Medical History** | X | | | | | | | | | |
| **Chemotherapy History** | X | | | | | | | | | |
| **Infection and Treatment History** | X | | | | | | | | | |
| **Microbiology Colonisation History** | X | | | | | | | | | |
| **Antibiotic History** | X | | | | | | | | | |
| **Transplant Donor Characteristics** | X | | | | | | | | | |
| **Comorbidity Index Score** | X | | | | | | | | | |
| **Vital Signs** | X | X | X | X | X | X | X | X | X | X |
| **Physical Examination** | X | X | X | X | X | X | X | X | X | X |
| **Randomisation** | X | | | | | | | | | |
| **Current Medication** | X | | | | | | | | | |
| **Dietary Questionnaire** | X | X | X | X | X | X | X | X | X | X |
| **Full Blood Count, Coagulation, Biochemistry, and Virology** | X | X | X | X | X | X | X | X | X | X |
| **Bone Marrow Assessment Results** | X | | | | | | | X | X | X |
| **EQ-5D-5L Questionnaire** | X | | | | | | X | X | X | X |
| **EOTRC QLQ-C30 Questionnaire** | X | | | | | | X | X | X | X |
| **Sample Collection Form** | | | X | X | X | X | X | X | X | X |
| **Nutrition** | | X | X | X | X | X | X | X | X | X |
| **Recent Microbiology History** | | | X | X | X | X | X | X | X | X |
| **Recent fever, Infection and Treatment History** | | X | X | X | X | X | X | X | X | X |

*BMJ Open – Protocol – Supplementary Material*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Recent Microbial Colonisation History** | | X | X | X | X | X | X | X | X | X |
| **IMP Administration** | | X | | | | | | | | |
| **IMP Symptom Report form** | | | X | X | X | X | X | X | X | X |
| **Adverse Event** | | X | X | X | X | X | X | | | |
| **Haematopoietic Cell Transplant Details** | | | | | X | | | | | |
| **VOD, and Relapse** | | | | | X | X | X | X | X | X |
| **Acute GvHD** | | | | | X | X | X | X | X | X |
| **GvHD Prophylaxis and Therapy** | | | | | X | X | X | X | X | X |
| **Engraftment** | | | | | | | X | X | X | X |
| **Lymphocyte Subsets and Immunoglobulin Levels - Blood** | | | | | | | | X | X | X |
| **Linage Specific Chimaerisms - Blood** | | | | | | | | X | X | X |
| **Chronic GVHD Assessment** | | | | | | | | X | X | X |
| **Post Transplant Intervention** | | | | | | | | | | X |
| **ITU Admissions Review** | | | | | | | | | | X |

*BMJ Open – Protocol – Supplementary Material*

**Appendix 6 – Biosample collection, storage, and processing**

| Sample | Collection | Timepoints | Processing | Storage | Planned analysis |
|---|---|---|---|---|---|
| Stool | A faeces collection kit will be given to the study participant to provide a stool sample, which will be collected using a 'Faeces Catcher'. | All visits, except visit 2 | The study participant will scoop up the collected faecal sample and transfer this into two tubes. These are an empty faeces tube (Starstedt faeces tube), and a tube containing a preservative (Zymo DNA/ RNA Shield Fecal Collection tube). Study participant instructions for faecal collection are available at: https://youtu.be/hG0wI5p4NKw?si=9tNMmSm-iClcot1C . | Study participants will be given an ice pack and insulated back to transfer the collected stool in tubes from their home to the medical facility. Samples will be stored at -20°C | Shotgun sequencing, metabolomics, metaproteomics, potential future culturing. |
| Urine | Sample will be collected in a urine pot (50 ml; to collect the whole urine sample) at the medical facility. | All visits, except visit 2 | None | Stored at -20°C | Metabolomics. |

| | | | | | |
|---|---|---|---|---|---|
| | Urine should be mid-stream of the first fasted urine of the day. | | | | |
| Blood – plasma | Sample will be collected using 4x 3 ml lithium heparin blood tubes (i.e. 12ml of blood collected) at the medical facility. | All visits | Blood tubes will be centrifuged to pellet blood cells. Plasma will be aliquoted into 2 ml cryovials. Blood will be processed within 2 hours, or within 24 hours stored at 4°C. | Stored at -80°C | Metabolomics, analysis for markers of gut barrier integrity. |
| Blood - PBMC | Sample will be collected using 2x 10 ml EDTA blood tubes, i.e. 20ml of blood collected) at the medical facility. | Visit 8 and 10 | PBMCs will be separated from blood by density gradient centrifugation using Ficoll. PBMCs will be washed, and a platelet removal centrifugation step will be carried out. Cells will be enumerated using a cell counter and resuspended in freezing media at 10 million cells per ml. | Stored in liquid nitrogen (-196°C). | Immune reconstitution assessment by lymphocyte subsets and T-cell repertoire characterisation. |

*BMJ Open – Protocol – Supplementary Material*

| | | | | | |
|---|---|---|---|---|---|
| | | | Blood will be processed within 24 hours. | | |
| Blood – PaxGene RNA | Sample will be collected using 1x 2.5 ml PaxGene RNA blood tube (i.e. 2.5ml of blood collected) at the medical facility. | Visit 8 and 10 | None. Blood will be frozen within 6 hours from collection. | Stored at -20°C | |

**References:**

1. Salvador Castell G, Serra-Majem L, Ribas-Barba L. What and how much do we eat? 24-hour dietary recall method. *Nutr Hosp* 2015;31 Suppl 3:46-8. doi: 10.3305/nh.2015.31.sup3.8750 [published Online First: 20150226]

[Insert Site Logo]

# MAST PATIENT INFORMATION SHEET

| | |
|---|---|
| **Study Title** | Microbiota Transplant Prior to Allogeneic Stem Cell Transplantation (MAST) trial |
| **IRAS Project ID** | 1006971 |

## Introduction:

You are being invited to take part in a research study. Before you decide whether you wish to take part, it is important for you to understand why the research is being done and what it will involve. Someone from our team will go through the information sheet with you and answer any questions you have. Please take time to read the following information carefully and discuss it with friends, relatives, your General Practitioner (GP) and other doctors involved in your clinical care if you wish.

**Part 1** tells you the purpose of this study and what will happen to you if you take part.

**Part 2** gives you more detailed information about the conduct of the study

Please, ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

**Thank you for taking the time to read this information sheet.**

MAST Study

[Insert Site Logo]

## Glossary of Terms

| Term | Explanation |
|---|---|
| CFU | Colony forming units – Is the term used to describe the number of viable microorganisms, e.g., bacteria, there are in the capsule |
| Haematopoietic cell transplant | The clinical name for a bone marrow or blood stem cell transplant to treat blood cancer such as leukaemia. |
| Intestinal microbiota transplant | Taking stool material from a healthy donor and processing it into a capsule form for oral use. |
| Investigator | A researcher involved in a clinical study. |
| Microbiota | A collection of microorganisms that live in and on human body. |
| Microorganisms | Small organisms such as bacteria, virus particles and other single cell organisms. |
| Organism | A form of life considered as an entity, such as an animal, plant, fungus or bacterium. |
| Placebo | A substance that has no therapeutic effect, used as a control in testing new drugs |
| Plasma | The liquid part of blood that is left after all blood cells have been removed and only a clotting protein (called Fibrin) remains. |
| Phlebotomy | The procedure of drawing blood from the vein with the use of sterile material by trained and qualified healthcare personnel. |
| Sample | A small part of a substance or material obtained for testing such as blood, urine and stool/faecal material. |
| Serum | The liquid part of blood, after all, blood cells and the clotting protein (Fibrin) have been removed. |

[Insert Site Logo]

# PART 1

## 1. What is the purpose of this study?

Doctors and scientists have realised recently that there are billions of 'beneficial' bacteria and other microbes living in the human gut. These microbes do not cause us harm, but actually perform many roles in helping to keep us healthy, such as through their effects on how we process food or energy, stopping us getting infections from gut bacteria, and in how our immune system works.

When antibiotics are given to patients with blood cancers, they have a side effect of reducing the numbers of 'beneficial bacteria' in the gut, limiting its supportive role for the immune system. The number of 'beneficial' gut bacteria are important to maintain in patients who receive treatment that further impacts the immune system, such as bone marrow transplant (haematopoietic cell transplant).

The MAST clinical trial will test a way of restoration of the normal balance and diversity (range) of microbes that live in the gut prior to starting bone marrow or blood stem cell transplant (haematopoietic cell transplant). The study will also examine how this treatment affects the many complications involved in bone marrow transplantation, such as fevers (high temperatures) and infections during the transplant period. The treatment is called intestinal microbiota transplantation and involves taking bacteria from healthy people's gut, then processing it and putting it into a capsule which when swallowed, releases the microbes into the recipient.

MAST Study

[Insert Site Logo]

## 2. Why have I been chosen?

You are being invited to take part in the research study because you will be undergoing a haematopoietic cell transplant, as part of the normal treatment for blood cancer, and because of your previous treatment (chemotherapy), you are predicted to have a lower number, and smaller range (diversity) of bacteria (microbiota) in your gut (intestines). We are looking to recruit 50 participants in total to this study. These 50 people will have acute leukaemia (AML or ALL), advanced myelodysplastic syndromes, chronic myelomonocytic leukaemia (CMML), or chronic myeloid leukaemia (CML) in blast phase and will be undergoing standard treatment (bone marrow or blood stem cell transplant) for their disease. Please read this information carefully before you decide whether to participate and ask your doctor for an explanation of anything that is not clear to you.

## 3. Do I have to take part?

It is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive from your doctor or the hospital.

## 4. What will happen to me if I take part?

You will be approached about entering the study before you are scheduled to have bone marrow transplantation. If you take part in this study, you will be asked to follow the study treatment plan, tests and hospital appointments for 14months). You should consider how these tests and visits will affect your work and family life and decide if you are able to commit to them.

Sometimes because we do not know which way of treating patients is best, we need to make comparisons. People will be put into groups and then compared.

The groups are selected by a computer which has no information about the individual – i.e. by chance. Subjects in each group then have a different treatment and these are compared'.

This means you have a 1 in 2 (50%) chance of receiving the treatment. Neither you nor your doctor will know which treatment group you are in (although, if your doctor needs to find out he/she can do so).

MAST Study

[Insert Site Logo]

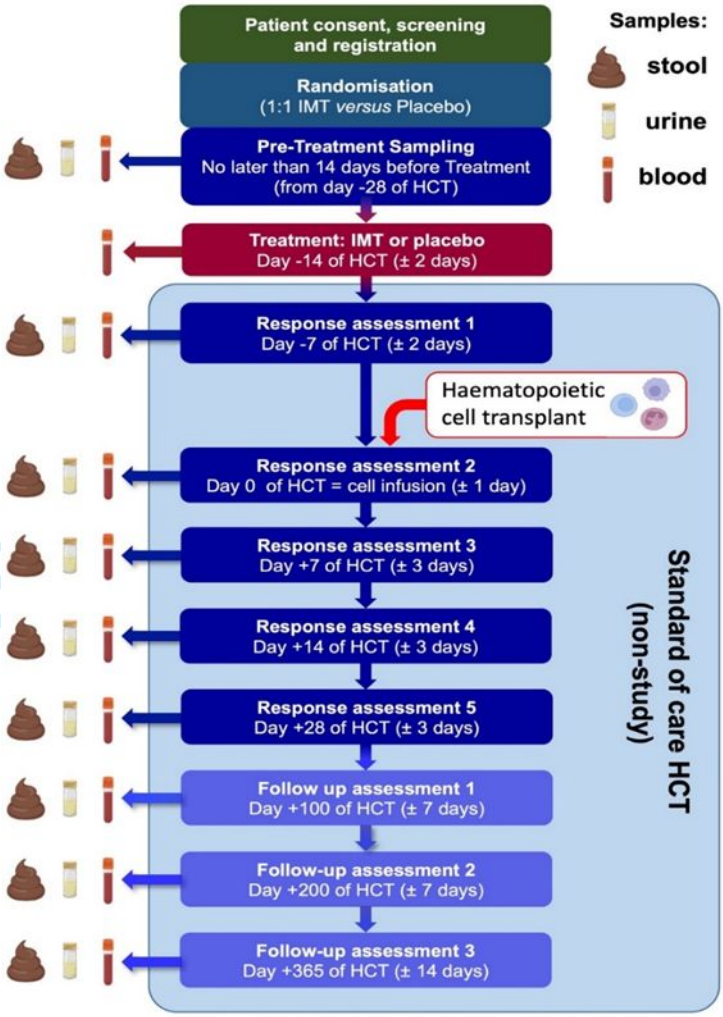
**The study will undertake the following:**

1. Recruit 50 people with blood cancers who had treatment with chemotherapy and are about to undergo a bone marrow transplant.

2. Take 25 randomly from this group and give them the intestinal microbiota transplant using an orally taken capsule and compare them to the remaining 25 patients who will be given a placebo capsules. The capsules will be taken prior to the bone marrow transplant

3. Collect stool, blood and urine from both groups for analysis over the period of their treatment.

4. Undertake health and quality of life assessments for up to a year after their intestinal microbiota transplant and bone marrow transplant (see the schedule to the right).




## 4.1. What will happen before I enter the trial?

**Initial Study Consultation**- An initial consultation will take place to discuss participation in the study on the phone or on site with a member of our team. We will ask you some questions to see if you would be suitable to join the study, which will last approximately **15** minutes.

If we decide from the initial assessment that you are not eligible to take part in the study from the initial visit, you will unfortunately not be able to take part in the study and will continue with your planned standard of care treatment.

MAST Study

[Insert Site Logo]

If we decide from the initial assessment that you may be eligible to take part in the study, we will invite you to attend a full screening visit **(Visit 1)**. We will also explain that we wish to collect a stool sample from you ahead of the next visit. We will provide you with a stool collection kit and instructions, to collect a sample at home 24 hours before or on the day of the next visit. Should you need support a family member or friend at home can help you with this or a nurse at your next study visit can help you with providing a sample.

## 4.2. What happens once you are confirmed suitable to take part?

**Visit 1: Consent and Screening (within 14 days before randomisation)** – After the Initial Study Consultation, you will be invited to attend a screening visit. If you are interested in joining the study, you will be asked to sign and date the study consent form. We will perform several tests to check you are eligible for the study. All the screening tests will be explained below.

**Screening Assessments (30-60 minutes):**

A review of your medical history and any medications you are taking or have recently taken (e.g., anti-cancer treatments, over-the-counter treatments including herbal or dietary supplements, prescription medications, and/or illegal drugs)

- **A physical examination** including height and weight.
- **An assessment of your vital signs** (tests to see how well your body is functioning) including blood pressure and pulse.
- **Collect dietary information** If you have been able to give a stool sample at this visit, we will ask you to complete a dietary questionnaire to report what you have eaten 24 hours before the collection of your stool sample.
- **Quality of life Assessment** – We will ask you to complete a questionnaire to collect this information.

At this visit, we will also collect the following samples from you:

- **Blood** – A blood sample for research purposes (2 tablespoons which is 30ml) will be collected in addition to your routine blood tests.
- **Urine** – A sample kit will be given to you to provide a sample.
- **Stool** – If you are not able to provide a stool sample at this visit you will be given a self-sample kit to collect a stool sample for the next study visit.

**After we complete all the screening assessments above,**

If you are **confirmed not to be eligible** to take part in the study, you will unfortunately not be able to take part in the study and will continue with the planned standard of care treatment.

[Insert Site Logo]

If you are **confirmed to be eligible** to take part in the study, you will randomly be assigned by a computer to one of the two treatment groups below before (within 2 weeks of Visit 2) your next scheduled study visit.

**Treatment Groups:**

**Group 1**–This is the 'treatment arm' of the study**:**
You will receive 10 Intestinal Microbiota Transplant(IMT) $1x10^6$ x $1x10^9$ CFU/g of viable microorganisms per oral capsule). The number of colony forming units (CFU) in each capsule may differ because the CFU of the original stool material used to make the capsules also differs. Oral capsules will be made from bacteria obtained from a healthy screened person's stool sample; there are an extensive array of screening procedures in place to ensure the capsules are safe to take. Whilst this is not a typical 'licensed' medication, it has been manufactured in line with very strict approval procedures from the UK regulatory body for medicines, and this sort of treatment has already been used safely in thousands of people around the world. These capsules will be taken orally with water.

Please note – while no animal products are used in the manufacturing process, there is a possibility that the material within the capsules may contain traces of certain non-digestible dietary/ food components (for example, prawn shells); any dietary concerns you may have will be discussed with the nurse or study doctor before starting treatment.

**Group 2:**
You will receive 10 dummy oral capsules (placebo) the capsule will look the exact same as the capsule given in group one, but will contain no medicine or active ingredients. These capsules will also be taken orally with water.

**Capsule Description:**

Each capsule will be size 0 (see picture below) the capsules will be coated so that they will be able to pass through your stomach without dissolving.

MAST Study

[Insert Site Logo]

**Visit 2 (Treatment)** – Prior to this visit you will be told to avoid eating food 30 minutes before the start of this visit. You will be given either the IMT oral capsules, or the placebo oral capsules and we will also take a research blood sample and if not collected at screening urine and stool samples from you at this visit. You will receive a diary card to report daily any symptoms listed in the booklet until the next patient visit. The nurse or doctor at your next visit will go through the booklet with you should you need any support with completing the diary before the next visit, there will be a number in the booklet to use to contact a member of the study team.

After the Screening and Treatment visits have been completed, you will follow the standard of care that your clinician has discussed with you for treating the blood cancer. We will ask for you to attend **8 more visits** over the following year. These visits will occur along with your monitoring visits that you will be making as part of your treatment described in the table below.

**Study Assessments (Visit 3-10) 30-45 minutes:**

The following schedule outlines the questionnaires and samples that will be collected from you at each study visit.

| Type of questionnaire | What is the questionnaire for? | When are they done? |
|---|---|---|
| **Health assessment questionnaire (EQ-5D-5L)** | To evaluate your general quality of life. | All Visits |
| **Quality of Life for Cancer patients (EORTC QLQ-C30)** | To evaluate your general quality of life as a cancer patient. | All Visits |
| **Dietary Questionnaire** | To understand before the stool sample was obtained, if any specific foods e.g., liquorice or fish may have been eaten. As eating certain foods can adjust the results observed during the analysis of the samples collected. | All Visits |

| Sample type | What is the procedure for? | When are they done? | How will they be done? |
|---|---|---|---|
| **Urine** | For analysis of chemicals that we think may change from before and after the | All Visits | At each visit, you will be given a labelled clean container to pass urine |

| | | | |
|---|---|---|---|
| | intestinal microbiota transplant | | ideally when you wake up first thing in the morning. Guidance will be provided by the examiner or qualified member of the study team. |
| **Blood** | For analysis of chemicals that we think may change from before and after the intestinal microbiota transplant | All Visits | Bloods will be collected by a qualified member of the study team |
| **Faecal** | Analysis of the microorganisms in the faecal material before and after intestinal microbiota transplantation | All Visits | We will ask you, to collect a stool sample for each visit. You can collect the sample:<br>• 24 hours before your next study visit a family or friend may help you with this<br>• On the morning of each study or at the start of each visit if you would like support from a nurse.<br>You will be provided with a self-sampling kit that will contain:<br>• Clear instructions of the collection process, storage and its return<br>• Ice pack<br>• Bag to transport samples in |

## 5. What do I have to do?

If you decide to take part, you will need to attend your local research centre for the assessments. If you normally require transport, we will help arrange this for you. Tests, sample collections and hospital appointments are explained in the table above, you must inform your study doctor of any medications you are currently taking or intend to take once you have entered the study.

During your participation in the MAST study, you should continue with your regular medication, and you will continue to be under the care of your regular medical team.

## 6. How will we assess whether the treatment is working and its effect on your quality of life?

You will also be asked to complete questionnaires on paper regarding your general health and cancer usually at the same time as your scans. The questionnaires should take approximately 15-20 minutes in total to complete. If you feel uncomfortable answering any of the questions, please talk to your study doctor or nurse. You can leave blank any questions you do not want to answer. This information will help us to understand how the treatment may affect your quality of life.

## 7. Pregnancy, contraception, and breastfeeding

If you would like to participate in this study and are a woman of childbearing potential, you must:

- Tell your study doctor immediately if you become pregnant during this study, your study doctor will advise you of the possible risks to your unborn child and discuss options for managing the pregnancy with you. If pregnancy occurs during the study, The study treatment will not be given if pregnancy occurs before the treatment visit, and you will be withdrawn from the study. If pregnancy occurs after the treatment visit you will continue attending the remaining study visits and the pregnancy will be followed until the conclusion if you give consent for this.
- Use (if you are sexually active with a male partner who has not been sterilised), one highly effective method of birth control and one additional effective barrier method of contraception at the same time. This should be done from the time of signing the informed consent form until study completion. Please discuss effective methods of contraception with your study doctor or nurse.

[Insert Site Logo]

## 8. What are the side effects, possible disadvantages and risks when taking part?

Being involved in a research study, such as a clinical trial, requires a degree of commitment to regular hospital visits and additional tests and surveys, and you may consider this to be a disadvantage.

The only risks associated with the study are related to some of the procedures. For example, there is minimal risk associated with blood tests, they can cause brief discomfort, bruising, or an infection in some cases, which might last for several days, and will, therefore, be performed by experienced members of the healthcare team.

Urine and stool self-collection procedures carry a minimum risk of contamination with stool and urine material; however, the risk has been minimised by the provision of an instruction manual for collection and hygiene.

There are very low risks associated with the intestinal microbiota transplantation itself and these include, fever, nausea, vomiting, bloating and constipation these should normally resolve in 1 to 2 days https://tinyurl.com/4zpf5kchThere is also a low risk of infection from intestinal microbiota transplantation (IMT) itself. This risk is managed by EBX carrying out extensive testing on donors and their stool under supervision of a medical doctor, including blood and stool tests to detect pathogenic infectious agents, over and above as recommended by the UK experts in this field (https://tinyurl.com/4zpf5kch). Every stool donated, as well as every batch of IMT capsules manufactured is tested for the presence of pathogenic infectious agents and is only released for use if these are not detected." There will be a contact number in the symptom diary card should there be any symptoms you would like to discuss the clinical team.

## 9. What are the possible benefits of taking part?

We cannot promise the study will definitely help, however, in a small study before this larger one, we have shown that a similar treatment reduced the number of admissions to the intensive care unit, the numbers of blood infections and days of fever (high temperature) in the early days after the patients had had their bone marrow transplant. This study also showed that intestinal microbiota transplant (the treatment/method) was safe in patients undergoing bone marrow transplant, and there were no major side-effects.

The information we collect on how treatment affects the complications related to bone marrow transplantation, such as fevers (high temperatures) and infections during the transplant period may help to improve treatment and the recovery of people with blood cancers who are undergoing bone marrow transplant.

MAST Study

[Insert Site Logo]

## 10. What if I feel unhappy about continuing in the study?

If you have concerns about continuing, please discuss these with your study doctor and team. You do not have to give a reason, and your study team can explain your options to you about any data or samples collected from you as part of the study. Please see section 2 in Part 2 of the information sheet for more details of what will happen if you stop the study while on treatment.

1. What if something goes wrong?

Your study doctor will be there to answer any questions you might have regarding the cancer, its treatment, and your participation in the study. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during this study, then there will be several options available to you. Full details are included in Part 2 of this information sheet.

## 11. Will I be compensated for taking part?

You will not be paid for taking part in the study. However, for every study visit you attend you will be able to claim back some of your expenses. You will be reimbursed up to the value of £50 (maximum £200 in total) for travel expenses per visit.

**If the information in Part 1 has interested you and you are considering taking part in the study, please read the additional information in Part 2 before making your decision.**

[Insert Site Logo]

# 12. PART 2

## 0. What if new information becomes available?

Sometimes during a research project, new information or incidental findings becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form.

## 1. What happens when the research study stops?

Once your participation is over, you will carry on with your standard treatment and medical care as usual.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained including any research samples unless you specifically withdraw your consent for this. At your last study visit a nurse or clinician will ask if you would like to know what treatment group you were assigned to which will be shared with you if requested by your chosen method of contact once the trial has ended.

To safeguard your rights, we will use the minimum personally identifiable information possible.

## 2. What if there is a problem?

Imperial College London holds insurance policies which apply to this study. If you experience harm or injury because of taking part in this study, you will be eligible to claim compensation without having to prove that Imperial College London is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, you may have grounds for legal action.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during this study you should immediately inform the Investigator.

The normal National Health Service complaints routes are also available to you, details can be obtained from your study doctor or nurse.

If you are still not satisfied with the response, you may contact the Imperial College, Research Governance, and Integrity Team.

**Complaint's statement**

If you wish to complain about any aspect of the way in which you have been approached or treated during this study, you should contact the study team (contact details at the end of this document) or you may contact the patient advice and liaison services (PALS) in the trust you are receiving treatment in *<Insert trust name, PALS tel.no and email>*.

## 3. How will we use information about you?

Imperial College London is the sponsor for this study and will act as the data controller with Imperial Clinical Trials Unit – Cancer (ICTU-Ca) for this study. This means that we are responsible for looking after your information and using it appropriately. Imperial College London will keep your personal data for:

- 10 years after the study has finished in relation to data subject consent forms.
- 10 years after the study has been completed in relation to primary research data.

This study is expected to end 08/2026

We will need to use information from your medical records for this research project. This information will include your, initials, month and year of birth, gender, and ethnicity.

People within the College and study team will use this information to do the research or to check your records to make sure that the research is being done properly and the information held is accurate.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a unique code number (study ID) instead, and this code will also be used to label tissue and blood samples.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no one can work out that you took part in the study.

## LEGAL BASIS

As a university, we use personally-identifiable information to conduct research to improve health, care and services. As a publicly funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in the research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Our legal basis for using your information under the General Data Protection Regulation (GDPR) and the Data Protection Act 2018, is as follows:

Imperial College London - "performance of a task carried out in the public interest"); Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research

## INTERNATIONAL TRANSFERS

There may be a requirement to transfer information to countries outside the United Kingdom (for example, to a research partner), either within the European Economic Area (EEA) or to other countries outside the EEA. Where this information contains your personal data, Imperial College London will ensure that it is transferred in accordance with data protection legislation. If the data is transferred in accordance with data protection legislation. If the data is transferred to a country which is not subject to a UK adequacy decision in respect of its data protection standards , Imperial College London will enter into a data sharing agreement with the recipient research partner that incorporates UK approved standard contractual clauses or utilise another transfer mechanism that safeguards how your personal data is processed .

You will not be able to be identified when sharing this data, but it may include demographic information such as the month and year of your birth as well as your study ID.

## SHARING YOUR INFORMATION WITH OTHERS

We will only share your personal data with certain third parties for the purposes referred to in this participant information sheet and by relying on the legal basis for processing your data as set out below.

- Other College employees, agents, contractors and service providers (for example, suppliers of printing and mailing services, email communication services or web services, or suppliers who help us carry out any of the activities described above). Our third-party service providers are required to enter into data processing agreements with us. We only permit them to process your personal data for specified purposes and in accordance with our policies.
- EnteroBiotix Ltd., provide the capsule IMT and placebo. The following data is shared with them in this capacity as part of an agreement with Imperial College London, as

well as ensuring appropriate oversight of any serious side effects that you and other study participants may experience:

- Results data used to write reports from the study, specifically on how effective and safe the study treatment is
- Data about serious side effect/s, including whether they got better
- Data about medications taken and whether these were to treat the serious side effect/s or were other medications that were being taken at the time the serious side effect/s occurred.

- The Medical Research Council (MRC) who fund the study, the following data is shared with them in this capacity as part of an agreement with Imperial College London, as well as ensuring appropriate oversight of any serious side effects that you and other study participants may experience:
  - Results data used to write reports from the study, specifically on how effective and safe the study treatment is
  - Data about serious side effect/s, including whether they got better
- UK ethics and regulatory authorities who are required by law to approve and oversee research. The following data is shared with them to ensure appropriate oversight of any serious side effects that you and other study participants may experience:
  - Data about serious side effect/s, including whether they got better
  - Data about medications taken and whether these were to treat the serious side effect/s or were other medications that were being taken at the time the serious side effect/s occurred.

## POTENTIAL USE OF STUDY DATA FOR FUTURE RESEARCH

When you agree to take part in a research study, the information collected either as part of the study or in preparation for the study (such as contact details) may, if you consent, be provided to researchers running other research studies at Imperial College London and in other organisations which may be universities or organisations involved in research in this country or abroad. Your information will only be used to conduct research in accordance with legislation including the GDPR and the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you, used against you or used to make decisions about you.

[Insert Site Logo]

## COMMERCIALISATION

Samples/data from the study may also be provided to organisations not named in this participant information sheet, e.g., commercial organisations or non-commercial organisations for the purposes of undertaking the current study, future research studies or commercial purposes such as development by a company of a new test, product or treatment. We will ensure your name and any identifying details will NOT be given to these third parties, instead you will be identified by a unique study number with any sample / data analysis having the potential to generate 'personal data'.

Aggregated (combined) or anonymised data sets (all identifying information is removed) may also be created using your data (in a way which does not identify you individually) and be used for such research or commercial purposes where the purposes align to relevant legislation (including the GDPR) and wider aims of the study. Your data will not be shared with a commercial organisation for marketing purposes.

## WHAT ARE YOUR CHOICES ABOUT HOW YOUR INFORMATION IS USED?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have, because some research using your data may have already taken place and this cannot be undone.

- If you choose to stop taking part in the study, we would like to continue collecting information about your health from your hospital. If you do not want this to happen, tell us and we will stop. This will not affect any healthcare or support you may be receiving separately
- We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you, if this could affect the wider study or the accuracy of data collected.
- If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study.

## WHERE CAN YOU FIND OUT MORE ABOUT HOW YOUR INFORMATION IS USED

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- by asking one of the research team
- by sending an email to mast-trial@imperial.ac.uk

- **COMPLAINT**

If you wish to raise a complaint on how we have handled your personal data, please contact Imperial College London's Data Protection Officer via email at dpo@imperial.ac.uk, via telephone on 020 7594 3502 and/or via post at Imperial College London, Data Protection Officer, Faculty Building Level 4, London SW7 2AZ.

If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO) www.ico.org.uk. The ICO does recommend that you seek to resolve matters with the data controller (us) first before involving the regulator.

## 4. Involvement of the General Practitioner/ family doctor (GP)

With your permission, your GP and other doctors involved in your clinical care will be informed that you are taking part in this study, but otherwise all information about you and your treatment will remain strictly confidential.

## 5. What will happen to any samples that I give?

**Blood:**

Routine blood samples will be taken and tested by your hospital as part of standard practice and destroyed immediately after testing.

Research blood samples will be sent an HTA authorised Imperial College university lab located in St Mary's Hospital for long-term storage for future use in ethically approved studies with your permission. The blood samples will be analysed to see what chemicals they contain. Any samples left over from the process will be destroyed.

**Stool**

Stool samples will be sent to an HTA authorised Imperial College university lab located in St Mary's Hospital for storage. Stool samples collected at your screening visit and 7th visit (around 28 days after the bone marrow transplant) may be sent abroad for testing (i.e., Bacterial DNA sequencing lab in Germany) your agreement to this is required to take part in the study. The samples will be analysed to see what different types of bacteria are present in your stools. Any samples left over from the process will be destroyed.

**Urine**

The urine samples collected will be sent an HTA authorised Imperial College university lab located in St Mary's Hospital for long-term storage for future use in ethically approved studies with your permission. They will also be analysed to see what chemicals they contain.

[Insert Site Logo]

## 6. What will happen to the results of the research study?

The results of the MAST study will be analysed by the MAST study research team. Results will be presented at Cancer meetings and will be published in associated journals for the wider research community to reference. No identifiable information is included in publications or presentations; therefore, you will not be identified in any report or publication. Your confidentiality is maintained throughout.

If you contact the researchers in the future, you can obtain a copy of the results.

Research data and all identifiable data will be stored by the sponsor for 10 years following the end of trial.

## 7. Optional consent for future use of samples

If you consent, your leftover samples will be stored at our HTA authorised Imperial College Bio Bank your samples will be pseudo-anonymised and may be used for further academic and/or commercial studies by the Principal Investigator. Any such tests will have an appropriate ethical review. Upon your request at any time, your remaining samples will be destroyed.

## 8. Who is organising and funding the research?

Imperial College London is the legal sponsor of this study and is organising the study through the Imperial Clinical Trials Unit – Cancer (ICTU-Ca). The study is funded by Medical Research Council (MRC) who will receive a study report declaring the results, but no individual research participant identifiable data will be shared. The study is organised by a research team at the Imperial Cancer Clinical Trials Unit.

The sponsor of this study will pay your hospital for including you in this study, but your doctor will not receive any personal financial payment if you take part.

## 9. Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by <Insert name of Ethics Committee>.

[Insert Site Logo]

MAST Study

## 10. Contact for Further Information

If you have additional questions during this study about the research or your rights as a research patient, you may address them to the study doctor(s) <insert name of doctor and tel.no> or the study staff <insert name and tel. no.>. Out of office hours < insert name and tel.no> please contact the study doctor in the event of the following occurring:

a) If you suffer an illness or a possible study-related injury
b) If you feel different in any way
c) If you are admitted to the hospital for any reason
d) If you are seen at a casualty (accident/emergency department) for any reason

To speak with a member of the MAST investigator team please contact the Study Manager,

Telephone: 02075943767

Email: mast-trial@imperial.ac.uk

**Thank you for reading this information sheet. If you are interested in taking part in the study, please contact the study team to arrange a screening appointment.**

**A copy of this written information and signed Informed Consent form will be given to you.**

MAST Study Imperial College London [Please Insert Site Logo]

**Centre Number:** _____

**Patient Study Identification Number:**_____

## CONSENT FORM: MAST Study

**Study title: M**icrobiota Transplant Prior to **A**llogeneic **S**tem Cell **T**ransplantation **(MAST)** study
**Short Title**: MAST

**Principal Investigator:** <Insert Name>

**IRAS Project ID:** 1006971

| | Consenting information | Please Initial each box |
|---|---|---|
| 1. | I confirm that I have read and understand the Patient Information Sheet, **Version**_____, dated _____ for the above study. I have spoken to_____ and had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | |
| 2. | I understand that my participation is voluntary and that I am free to withdraw at any time, without providing a reason. I know that my medical care and legal rights are not affected. | |
| 3. | I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Sponsor (Imperial College London), from the NHS organisations, Medical Research Council, Enterobiotix or regulatory/other authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | |
| 4. | I give consent for information collected about me to be used to support other ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |
| 5. | If Applicable, I agree to use effective contraception whilst taking part in the study, should I become pregnant after taking the study drug, I give/do not give permission for access to any of my medical notes and information collected about my pregnancy. | |

| | | |
|---|---|---|
| 6. | I give consent to the taking of blood **equivalent to two tablespoons** and providing urine samples for chemical analysis in this study. | |
| 7. | I understand the stool collection procedure and agree to comply with these instructions. | |
| 8. | I give permission for my stool samples to be sent outside of the UK for bacterial genetic analysis for this study. | |
| 9. | I agree that my GP, and / or other doctors involved in my clinical care, may be notified of my participation in this study. | |
| 10. | I understand that blood, urine and stool samples and / or data collected from me are a gift donated to Imperial College and that I will not personally benefit financially if this research leads to an invention and/or the successful development of a new test, medication treatment, product or service. | |
| 11. | I agree to take part in the **Microbiota** Transplant Prior to **A**llogeneic **S**tem Cell **T**ransplantation **(MAST)** study | |

| **Optional** | | **Initials** |
|---|---|---|
| 12. | I give/do not give consent for my pseudo-anonymised stool, blood and urine sample to be stored during and at the end of the study at the University (Imperial College London) bio bank to support future ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |
| 13. | I give permission for any pseudo-anonymised blood and urine samples to be sent outside of the UK for analysis to support future ethically approved research by an academic institution or commercial company in the future, including those outside of the United Kingdom (which Imperial has ensured will keep this information secure). | |

Participant Name Date Signature

Name of person taking consent Date Signature

MAST Study

Imperial College London

[Please Insert Site Logo]

**When completed. Take 2 Copies. One to be given to the participant, one copy should be filed in the medical notes and the original stored in the Investigator Site File.**