Faecal microbiota transplantation to prevent complications after allogeneic stem cell transplantation for haematological malignancies: a study protocol for a randomised controlled phase-II trial (the FMT-allo study)

Aurore Dougé, Aurélie Ravinet, Alexandrine Corriger, Aurélie Cabrespine, Mathieu Wasiak, Bruno Pereira, Harry Sokol, Stéphanie Nguyen, Jacques-Olivier Bay

BMJ Open

Faecal microbiota transplantation to prevent complications after allogeneic stem cell transplantation for haematological malignancies: a study protocol for a randomised controlled phase-II trial (the FMT-allo study)

Aurore Dougé,[1] Aurélie Ravinet,[2] Alexandrine Corriger ,[2] Aurélie Cabrespine,[3] Mathieu Wasiak,[4] Bruno Pereira,[3] Harry Sokol,[5] Stéphanie Nguyen,[6] Jacques-Olivier Bay[2]

To cite: Dougé A, Ravinet A, Corriger A, *et al*. Faecal microbiota transplantation to prevent complications after allogeneic stem cell transplantation for haematological malignancies: a study protocol for a randomised controlled phase-II trial (the FMT-allo study). *BMJ Open* 2023;**13**:e068480. doi:10.1136/bmjopen-2022-068480

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-068480).

Received 19 September 2022
Accepted 06 April 2023

For numbered affiliations see end of article.

Correspondence to
Dr Aurélie Ravinet;
aravinet@chu-clermontferrand.fr

ABSTRACT

Introduction Allogeneic haematopoietic stem-cell transplantation (allo-HSCT) is a major treatment for many haematological malignancies. The procedure has a good success rate but high transplant-related toxicity (TRM). TRM is mostly related to graft-versus-host disease (GvHD) and infectious complications. Alterations of the intestinal microbiota plays a major role in the development of allo-HSCT complications. The gut microbiota could be restored by faecal microbiota transplantation (FMT). However, there are no published randomised studies assessing the efficacy of FMT for GvHD prophylaxis.

Methods and analysis This prospective, open-label, multi-centre, parallel-group, randomised phase-II clinical trial has been designed to assess the effect of FMT on toxicity in patients treated with myeloablative allo-HSCT for haematological malignancy. Based on Fleming's single-stage sample size estimation procedure, the design plans to include 60 male and female patients aged 18 or over per arm, to be randomly assigned to two groups, one with and one without (control group) FMT. The primary endpoint is GvHD-free relapse-free survival rate at 1 year after allo-HSCT. Secondary endpoints are outcome measures of the impact of FMT on allo-HSCT-related morbidity and mortality (overall survival and progression-free survival at 1 and 2 years, haematological parameters, infectious complications, tolerance and safety of FMT). The primary endpoint will be evaluated according to assumptions of the single-stage Fleming design, compared between groups by a log-rank test and further investigated in a multivariate marginal structural Cox model taking into account centre effect. The proportional-hazard hypothesis will be verified using Schoenfeld's test and by plotting residuals.

Ethics and dissemination The local institutional review board (CPP Sud-Est II, France) issued approval on 27 January 2021. The French national authorities issued approval on 15 April 2021. The outcome of the study will be disseminated via peer-reviewed publications and at congresses.

Trial registration number NCT04935684.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Graft-versus-host disease (GvHD)-free/relapse-free survival (GRFS) is a composite endpoint of GvHD-free, relapse-free survival after allogeneic haematopoietic stem-cell transplantation (allo-HSCT), defined as first occurrence of grade III–IV acute GvHD or systemic therapy-requiring chronic GvHD, disease relapse, or death from any cause during the first 12 months after allo-HSCT, each of these GRFS components being clinically meaningful.

⇒ Some limitations concern the absence of placebo arm due to an ethical point of view, and the absence of blinding but we believe that there will be no impact on the validity of the findings since the primary outcome measure has a high degree of objectivity.

⇒ Another limitation concerns the use in the ancillary study of 16S analyses of stool samples, which does not allow to precisely determine strain engraftment, but based on funding availability, shotgun sequencing will be performed to better evaluate donor's microbiota engraftment.

INTRODUCTION

Allogeneic haematopoietic stem-cell transplantation (allo-HSCT) is a major treatment for many haematological malignancies. It requires the use of high-dose chemotherapy as a pre-allo-HSCT conditioning regimen which, together with artificial nutrition and broad-spectrum antibiotics, disrupts the diversity of the intestinal microbiota (IM).[1 2] These procedures can compromise the integrity of the intestinal mucus, thus promoting translocation of pro-inflammatory microbes and release of pro-inflammatory cytokines. This phenomenon may lead to an increased activation of the donor's immune

cells promoting graft-versus-host disease (GvHD) due to a cytokine storm.[2–5] GvHD is the leading toxic cause of post-allograft mortality, with acute GvHD (aGvHD) occurring in 40%–50% of cases and chronic GvHD (cGvHD) in 30%–70%.[6–8] GvHD can significantly erode the patient's quality of life and requires additional immunosuppressive drugs, thus increasing the risk of infectious diseases. GvHD therapies have improved, but the mortality rate remains high in corticosteroid-resistant patients.

IM alterations are associated with an increased risk of post-allogeneic relapse[9] and is involved in post-allo-HSCT mortality, as high-IM-diversity patients have better overall survival at 3 years.[10] IM alterations at the time of engraftment correlate with GvHD[11] and mortality.[12] Faecal microbiota transplantation (FMT) after allo-HSCT could (i) restore intestinal-tract bacterial diversity, (ii) reactivate the anti-inflammatory abilities of commensal bacteria, (iii) limit the risk of complications and (iv) improve overall patient survival. FMT involves implanting a stool from a healthy donor into the digestive tract of a recipient patient to rebalance the host's altered intestinal flora. The only indication for FMT validated to date is for multiple recurrent *Clostridoides difficile* infections. This procedure has an 80%–90% success rate and has not been associated with SAEs when performed carefully.[13 14] The results, in terms of efficacy and safety, are the same in immunocompromised patients, including allo-HSCT patients.[15 16]

There are many ongoing studies to evaluate the effectiveness of FMT for other indications such as inflammatory bowel disease and eradication of multidrug-resistant bacteria. Promising results are beginning to emerge with FMT in the field of allo-HSCT. Most of these studies have evaluated the efficacy or safety of FMT for GvHD treatment, but with small numbers of patients.[17–22] However, there are no published randomised studies assessing the efficacy FMT for GvHD prophylaxis.

The purpose of this trial is to investigate the safety and efficacy of FMT for the prevention of complications in allo-HSCT patients. The hypothesis is that allogeneic FMT will improve outcomes in these patients.

METHODS AND ANALYSIS

Design and objective

The main objective of this prospective, open-label, multicentre, parallel-group, randomised phase-II clinical trial is to assess the effect of allogeneic FMT versus no treatment on GvHD-free relapse-free survival (GRFS)[23] at 1 year, in adult patients treated by myeloablative allo-HSCT for haematological malignancy.

Study setting and participants

This study will take place in French centres participating in the Francophone Society for Bone Marrow Transplantation and Cellular Therapy (SFGM-TC) (online supplemental table 1). Patients will be screened by the investigating haematologist or oncologist during the pretransplantation consultation. After first checking the inclusion and non-inclusion criteria (box 1), the study will be proposed to the patient, who will be given a written information form (online supplemental file 1). Before admission to the hospital for the allo-HSCT, the patient will have the time to read the information form and give his/her consent to participate. Patients who have signed the informed consent form will be enrolled in the study on the first day of hospitalisation for allo-HSCT, pending a re-check of the eligibility criteria by the medical investigator.

Box 1 Inclusion and exclusion criteria of patients

Inclusion criteria

⇒ ≥18 years old.
⇒ Men or women.
⇒ Undergoing a myeloablative allo-HSCT (peripheral stem cells).
⇒ Controlled haematological malignant disease.
⇒ Signed and dated informed consent.

Exclusion criteria

⇒ Tumour progressive at the time of allo-HSCT.
⇒ Inability to understand the protocol (language barriers, cognitive difficulties).
⇒ Medical history of progressive or other cancer in the previous 3 years (excluding basal cell carcinoma).
⇒ Presence of a simultaneous serious and uncontrolled disease (severe cardiac, renal, hepatic or respiratory failure, severe sepsis).
⇒ Faecal incontinence.
⇒ Participation in another clinical trial studying an allograft procedure, including type of graft, type of immunosuppression, preventive or curative treatment of GvHD, or another clinical trial studying the effectiveness of FMT in another indication.
⇒ Pregnant women.
⇒ Patient under guardianship, curatorship or tutorship.

allo-HSCT, allogeneic haematopoietic stem-cell transplantation; FMT, faecal microbiota transplantation; GvHD, graft-versus-host disease.

Stool donors will be healthy unrelated volunteers recruited by the Clinical Investigation Platform/Clinical Investigation Center—Inserm 1405 (PIC/CIC) of Clermont-Ferrand University Hospital. The eligibility criteria will follow the French National Health Authority (ANSM) recommendations on the use of FMT in clinical trials (online supplemental table 2).

The consent form will inform patients and donors (online supplemental files 1 and 2) that a biological collection is scheduled as part of this study and will clearly state that included subjects are free to oppose this procedure at any time.

Interventions

Allocation

Patients will be randomised into the following two groups:

- The 'FMT' group of patients receiving an FMT by enema within 4 weeks after engraftment.
- The 'no FMT' group of patients who do not receive an FMT and therefore no enema or colonic preparation (comparator group).

Box 2 Exclusion criteria at the time of randomisation

Exclusion criteria (randomisation)

⇒ Hyperacute GvHD occurring prior to engraftment, as defined by the MD Anderson Cancer Center.
⇒ Chronic overlapping GvHD ('overlap syndrome') occurring prior to engraftment, as defined by National Institutes of Health (NIH) criteria.
⇒ Relapse or persistence of haematological malignancy requiring rapid withdrawal of immunosuppressive therapy.
⇒ Uncontrolled infection requiring antibiotic treatment.
⇒ Persistent severe mucositis or colitis, diarrhoea or painful haemorrhoids.
⇒ Absence of a neutrophil count >500/mm/mm³ for more than 3 consecutive days.
⇒ >4 weeks postengrafment.

GvHD, graft-versus-host disease.

Randomisation will be conducted by the electronic Case Report Form (eCRF) designed with Ennov Clinical (V.8.2). The randomisation sequence will be generated by minimisation and stratified by Disease Risk Index score, use of antithymocyte globulin during the conditioning regimen and centre to factor in the 'centre effect'.

The data needed for randomisation will be entered in the eCRF by the clinical research associate (CRA) or the local site investigator. A request will be sent to the Clermont-Ferrand clinical research unit to start the randomisation process. Patients will be informed of their randomisation group.

Randomisation will be done after haematopoietic stem-cell engraftment (defined as an absolute neutrophil count of $>0.5\times10^9$/L on the first three consecutive days), provided there are no exclusion criteria at that time (box 2). Randomisation may be delayed if necessary but has to be completed in time to ensure the FMT is done within the first 4 weeks following engraftment, to reduce as much as possible the risk of aGvHD development before the FMT. If FMT is performed after this delay, the concerned patients will be removed from the study. Allocated intervention can be discontinued by:

- Withdrawal of consent by the patient.
- Decision by the sponsor and/or independent monitoring committee if one of the groups shows excess mortality or toxicity.

Data collected up until the time of discontinuation will be used for the study.

The result of the randomisation will be sent by email to the local site investigator in charge of the patient, to the CRA, to the Clermont-Ferrand University Hospital pharmacy, and to the pharmacy of the centre concerned. To respect patient anonymity, a study number will be given to each patient.

Blinding

As this is an open-label study, neither investigator and medical team nor patient will be blinded. Members of the independent monitoring committee and the biostatistician will be blinded for the analyses.

Study treatment and comparator

Patients randomised to the 'FMT group' will receive FMT within 4 weeks following post-allo-HSCT engraftment. Antibiotics and antifungals have to be stopped at least 72 hours pre-FMT (based on the half-life of most antimicrobial drugs) and for 48 hours post-FMT to avoid any deleterious effect on the transplanted microbiota. In the event of active infection requiring antibiotics, FMT will be rescheduled (but still has to be performed within the month following haematopoietic stem-cell engraftment).

The stool transplant will be done by enema. The day before FMT, the patient will undergo bowel cleansing by ingestion of 2 L of polyethylene glycol solution. On the day of FMT, a colon cleansing enema will be performed and the FMT will be delivered around 2 hours later.

Frozen stool transplants (−80°C) will be prepared and stored at Clermont-Ferrand Hospital pharmacy. Frozen preparations will be sent (−20°C) to the hospital pharmacy of the investigating centre on the days before FMT. The distribution of donations will adhere to stool donor–recipient absence of serodiscordance for Epstein-Barr virus, cytomegalovirus and toxoplasma serologies. A biological screening on blood and stool, including a stool PCR test for SARS-CoV-2 will also be performed at the beginning and at the end of the stool donation period (online supplemental figures 1 and 2). Preparations will be thawed at room temperature by the recruiting centre pharmacy and sent to the haematology unit on the day of transplant.

The enema (50 g of stool sample diluted in 250 mL of 10% glycerol solution diluted with NaCl 0.9%) will be performed by a qualified member of the study team using a rectal cannula within 6 hours after thawing. The patient will need to keep the enema in for as long as possible (at least 30 min). To achieve this goal, the patient will remain supine during and after the FMT enema. Post-transplant monitoring will include abdominal examination and blood pressure, pulse and temperature monitoring every hour for the first 4 hours post-FMT. If the patient fails to keep the enema for at least 30 min, then FMT will not be repeated but the patient will continue the follow-up protocol. If the patient is discharged from hospital, the FMT can be done on an outpatient basis with the same procedure.

Allo-HSCT modalities are to only allow myeloablative conditioning. Granulocyte-colony stimulating factor (G-CSF)-mobilised peripheral blood will be collected by cytapheresis. Bone marrow source is not allowed. Any donor HSCT will be allowed except cord blood. All medications (conditioning regimen, GvHD prophylaxis, toxicity management post-transplant and/or for complications) will be given as per the local centre's routine practice. All patients, regardless of randomisation, will get standard-of-care follow-up at each centre, including the diet regimen.

Outcomes

Table 1 summarises the data collection schedule. The primary endpoint is GRFS rate at 1 year after allo-HSCT in both groups. GRFS is a composite endpoint in which events include grade III–IV aGvHD (Mount Sinai Acute GvHD International Consortium (MAGIC) - criteria[23]) or systemic therapy-requiring cGvHD (National Institutes of Health Consensus Criteria (NIHCC)[24]), disease relapse or death from any cause during the first 12 months after allo-HSCT.[25]

Secondary endpoints measure impact of FMT on allo-HSCT-related morbidity and mortality:

- Overall survival and progression-free survival assessed at 1 and 2 years post-allo-HSCT.
- Haematological parameters:
  - Haematopoietic reconstitution assessed by: neutrophil recovery time >0.50×10^9/L; spontaneous platelet recovery time >20×10^9/L; spontaneous platelet recovery time >50×10^9/L; number of red blood cell and platelet transfusions between D0 and D100.
  - Engraftment rates evaluated by a chimerism measure at M1, M2, M3, M6, 1 year and 2 years post-allo-HSCT.
  - Transplant-related mortality evaluated at M6, 1 year and 2 years post-allo-HSCT.
- Cumulative incidence of aGvHD (MAGIC criteria[23]) at 1 year post-allo-HSCT and cGvHD (NIHCC criteria[24]) assessed at 2 years post-allo-HSCT.
- Cumulative incidence of infectious complications evaluated at 1 year. Severe infections will be defined according to GREFIG score.[26] Impact of FMT on multidrug-resistant bacteria, extended-spectrum beta-lactamases and *C. difficile* infection will be assessed at 1 year by evaluating persistence or disappearance of these pathogenic bacteria after FMT.
- Tolerance and safety of FMT: each unexpected event that could be FMT-related will be notified (abdominal pain, diarrhoea, bacterial translocation or any enema-related adverse event). Cumulative incidence of diarrhoea will be assessed at M6 post-allo-HSCT.
- Microbiota composition of the recipient assessed by 16S rRNA gene sequencing before the conditioning regimen, between D7 and D14 after engraftment (and before the FMT), at M1, M3 and 1 year after engraftment.
- Quality-of-life will be self-evaluated by patients using a validated questionnaire (European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ-C30)[27] before the conditioning regimen, between D7 and D14 after engraftment (and before the FMT), at M1, M3, M6, 1 year and 2 years after engraftment.
- An ancillary study will be performed on stool and blood collections from patients and stool donors.

Participant timeline

The patient timeline is presented in figure 1. Recruitment of stool donors to set up a frozen stool bank will begin approximately 3 months prior to inclusion of the first patient in the study. Donations must be made within 8 weeks of the date of biological screening. Each donor will have to make at least two stool donations during this period, and the number of donations is not limited if the donations are made within 8 weeks following baseline biological screening. The PIC/CIC will send the faeces sample to Clermont-Ferrand Hospital pharmacy for preparation of the faecal transplants. At the end of each donation period, a repeat screening test will be carried out to confirm eligibility of the faeces.

Sample size

As this is a phase-II efficacy study, the estimated number of subjects required is based on a Fleming design, as recommended in the literature.[28 29] Sample size estimation is based on a single-stage Fleming design because interim evaluation cannot be planned due to the timeframe for the evaluation of the primary endpoint.

For GRFS at 1 year, we decided to reject a rate of <40% and to accept a rate of >60%, based on data from the SFGM-TC register between 2013 and 2016 and on the appropriate literature.[25] With a one-sided test significance level (α=0.05; β=0.10), the minimum number of evaluable patients to enrol is 60 per arm. With this design, allogeneic FMT will be considered as an acceptable treatment if 1 year GRFS is observed for 31 (out of 60) patients or more. Sample size was estimated with sampsi_fleming programme from Stata software.[28] To take into account included patients who will be ruled out from randomisation by exclusion criteria at the time of randomisation, the study will include 75 patients per arm, but the intention-to-treat analysis will only concern randomised patients. A per-protocol-analysis will include only patients who have kept the FMT enema during at least 30 min. If patient are lost to follow-up, data will be collected-up until the point of dropping out. Data from patients who do not complete the study until the end of the follow-up will be included in the intention-to-treat analyses.

Data collection and management

Assessment and collection of outcomes

The investigators and the medical team at each centre will be trained in the protocol to ensure all members know the trial procedures. The allo-HSCT procedure and allied follow-up are well-known by each medical team. The quality-of-life questionnaire is a validated self-report questionnaire (EORTC-QLQ-C30)[27] that has previously been used in several studies. All the data from clinical examinations, biological check-ups, questionnaires and type of artificial nutrition (if applicable) is to be recorded in the patient's medical file, which will constitute the source data for the protocol. This data will be collected directly in the eCRF.

Allo-HSCT patients benefit from a dense schedule of medical and biological follow-up that will serve to measure and monitor the evaluation criteria for the primary and secondary endpoints until 2 years. If

Table 1 Data collection schedule

Visit	Selection visit	Inclusion visit	Conditioning	Allo-HSCT	Aplasia	Engraftment	Randomisation	Follow-up
Date	D90 to D15	Day 1 of hospitalisation	D7 to D1	D0	D0 to D30 (or end of hospitalisation)		Within 4 weeks after engraftment	D30 to D720
Location	Pretransplant consultation	Haematology unit						Haematology unit or consultation
Verification of inclusion and exclusion criteria	X	X				X		
Informed consent	X	X						
Randomisation						X		
Experimental treatment							FMT or no FMT	
Physical examination, interview		X	Daily					Until D720
Disease status					X	X	X	Until D720
Haemogram, ionogram, renal and hepatic function		X	Daily					Until D720
Aspergillus antigenemia, PCR EBV, CMV, ±toxoplasmose, HHV6		X	Weekly					D30, D60, D90, D180, D720
MRB and ESBL swabbing		X						D30, D60, D90, D180, D360, D720
Chimerism								D30, D60, D90, D180, D360, D720
GvHD					X	X	X	Until D720
FMT toxicities							X	Until D180
Infections		X	X	X	X	X	X	Until D360
SAE		X	X	X	X	X	X	Until D720
Protocoled blood samples (cryostem bank)		X (before chemotherapy)					X (before FMT)	D30, D90, D360
Protocoled stool samples		X (before chemotherapy)					X (before FMT)	D30, D90, D360
Quality of life (EORTC QLQ C30)		X						D30, D90, D180, D360, D720

allo-HSCT, allogeneic haematopoietic stem-cell transplantation; CMV, cytomegalovirus; D, day; EBV, Epstein-Barr virus; EORTC QLQ C30, European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire; ESBL, extended-spectrum beta-lactamase; FMT, faecal microbiota transplantation; M, month(s); MRB, multidrug-resistant bacteria; SAE, serious adverse event.

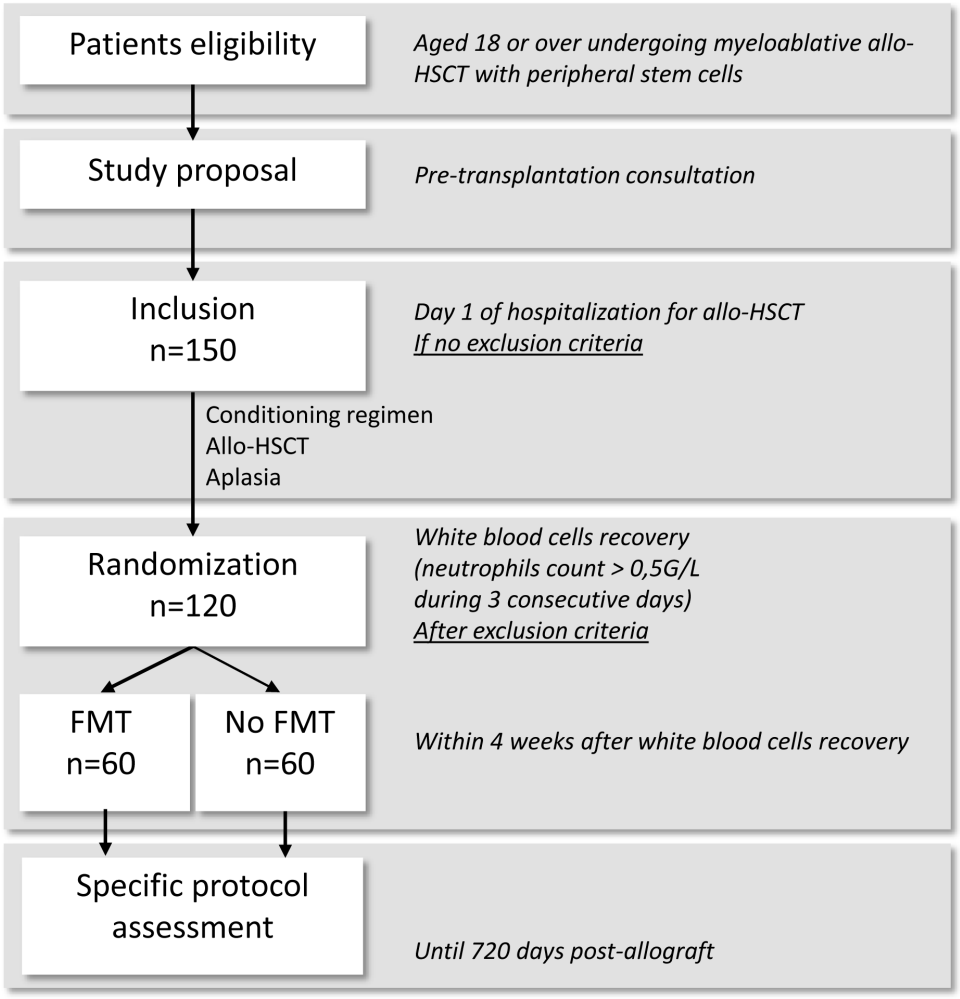

Figure 1 Patient timeline. Patients will be screened for eligibility during the pretransplantation consultation. The inclusion visit will take place during the first day of hospitalisation for eligible patients who have signed the consent form. The patient will have a complete clinical examination, the usual biological check-up, a stool sample for microbiota analysis and a blood sample for serum collection. The period D7 to D1 corresponds to completion of the myeloablative conditioning regimen followed by the reinjection of haematopoietic stem cells which, by convention, is called D0. During the entire hospitalisation period, the patient will be followed as per usual standard-of-care practice with daily clinical and biological monitoring. Randomisation will be done at the end of engraftment (and maximum 4 weeks after), provided the patient does not present exclusion criteria at that time. Both patient groups will have a stool sample for the microbiota analysis and blood samples for serum collection within these 4 weeks (before the FMT for patients randomised to the 'FMT group'). Follow-up will continue either in day hospital or in consultation at least once a month for a year. The evaluation criteria for the primary and secondary endpoints will be measured throughout routine clinical and biological follow-up until 2 years. A stool sample for microbiota analysis and a blood sample for serum collection will be collected at M1, M3 and 1 year after engraftment. allo-HSCT, allogeneic haematopoietic stem-cell transplantation; FMT, faecal microbiota transplantation.

patients are lost to follow-up, data will be collected up until point of drop-out. Data from patients who are lost to follow-up will be included in the intention-to-treat analysis.

Data management

In order to meet regulatory requirements (Guidance for Computerized systems Used in Clinical Trials, International Conference on Harmonisation, GCP 2001/20/CE), eCRF design, data monitoring and database extractions will be performed with the Ennov Clinical Suite software package.

Confidentiality

Persons with direct access to study data shall take all the necessary precautions to ensure the confidentiality of all information related to the nature of the experimental medicinal products, the trial, identity of participants, results obtained, etc. During the research or at its end, all data collected on the participants and transmitted to the sponsor by the investigators (or any other specialised contributors) will be anonymised. Subjects will be assigned a study-specific coded number indicating their order of inclusion.

Statistical methods

Primary and secondary outcomes

All statistical analyses will be performed using Stata software (V.15, StataCorp) before breaking the randomisation codes, as per International Conference on Harmonization–Good Clinical Practice (GCP) guidelines. Baseline description will be carried out per arm without inferential statistical tests, as per Consolidated Standards of Reporting Trials guidelines. Categorical and discrete data will be expressed as frequencies and percentages, and the continuous data will be expressed as mean and SD or median and IQR, according to statistical distribution. The Shapiro-Wilk test will be used to test for normality. Statistical analyses will be run on intention-to-treat and per-protocol samples. A description of all patients excluded and the reasons for all protocol deviations will be done.

The primary endpoint will be estimated by the Kaplan-Meier method. For analysis of the primary endpoint, GRFS at 1-year post-transplant will be compared using the 40% and 60% Fleming sample-size estimation thresholds, based on data from the SFGM-TC register between 2013 and 2016 and on the appropriate literature. The primary endpoint will then be compared between groups, as described by Holtan *et al*,[25] using the log-rank test, and further investigated in a multivariate marginal structural Cox model taking into account centre effect. Covariates will be determined according to univariate results (aforementioned log-rank test used to determine associated factors of the primary endpoint) and to clinical relevance (anticipated relationship with the primary outcome, including stratification parameters). A particular attention will be paid on multicollinearity. The proportional hazard hypothesis will be verified using Schoenfeld's test and by plotting residuals. Results will be expressed as HRs and 95% CIs. Other censored data will be analysed as described for the primary endpoint. Competitive risk analysis (Fine and Gray model) will be conducted if necessary.

A detailed descriptive analysis of the tolerance and safety of FMT after allo-HSCT will be presented. Then, continuous endpoints (such as number of red blood cell and platelet transfusions between D0 and D100) will be compared between randomisation groups using the unpaired t-test or the Mann-Whitney U test when appropriate. The assumption of homoscedasticity will be assessed using a Fisher-Snedecor test. The results will be expressed as effect-sizes and 95% CIs. In multivariate settings, multiple linear mixed models will be performed, with centre as random effect. The normality of residuals will be checked as mentioned above. If appropriate, a transformation will be proposed to achieve normality of the dependent variable. Categorical endpoints (such as neutrophil recovery time $>0.50\times10^9$/L, spontaneous platelet recovery time $>20\times10^9$/L, spontaneous platelet recovery time $>50\times10^9$/L, each unexpected event that could be FMT-related will be notified (abdominal pain, diarrhoea, bacterial translocation or any enema-related adverse event), cumulative incidence of aGVH at 1 year post-allo-HSCT and cGVH assessed at 2 years post-allo-HSCT) will be compared between groups by a χ^2 test or, if appropriate, Fisher's exact test. Generalised linear mixed models will be completed. Results will be expressed as relative risks and 95% CIs.

In order to compare the evolution of repeat-collected longitudinal data (such as microbiota composition, quality-of-life evaluated with EORTC-QLQ-C30, engraftment rates), the analyses will be carried out using suitable random-effects models to study arm, point-in-time evaluation and their interactions as fixed effects, taking into account between-patient and within-patient variability, in addition to the centre effect as random effect.

There are no interim analyses in this study.

Additional analyses

Faecal DNA extraction will be performed as previously described.[29] Gut microbiota composition and diversity will be determined using 16S sequencing. Following PCR (V3-V4 region, PCR1F_460: 5′ CTTTCCCTACACGACG CTCTTCCGATCTACGGRAGGCAGCAG 3′, PCR1R_460: 5′ GGAGTTCAGACGTGTGCTCTTCCGATCTTACC AGGGTATCTAATCCT 3′), amplicon quality will be verified by gel electrophoresis before sequencing on an Illumina MiSeq (Illumina, San Diego, California, USA). The dada2 software package in the R programming language will be used to perform quality control, read trimming and identification of amplicon sequence variants (ASVs). The Silva reference database (V.138 or later) will be used for taxonomic assignment. Prevalence filtering will exclude ASVs present in only one sample. The Shannon diversity index will be used to estimate alpha diversity, based on the number of unique ASVs and their evenness of distribution. Statistical significance for diversity will be tested using the Wilcoxon rank-sum test. Beta diversity will be calculated using the Bray-Curtis divergence on proportional (total sum scaled) data using the vegan package (V.2.5-6 or later), with Permutational Multivariate Analysis of Variance (PERMANOVA) performed using the adonis function (999 permutations). Data analysis will be performed through the phyloseq package (V.1.30.0 or later). Sequencing and analysis will be centralised in a single lab.

Missing data

To put significant results into perspective, a sensitivity analysis will be conducted to measure the impact of missing data. In intention-to-treat analyses, patients randomised to the FMT arm who did not keep the FMT enema for at least 30 min will be considered as a failure. If necessary, additional analyses will be performed according to the statistical nature of missing data (missing at random or not), including multiple imputation or the estimations proposed by Verbeke and Molenberghs (https://link.springer.com/book/10.1007/b98969) adapted specifically to repeated data.

Oversight and monitoring

Coordinating centre

The coordinating centre team is composed of a principal and coordinating investigator, subinvestigators, a coordinating CRA, pharmacists, a data manager and a biostatistician. This team will open the centres with a visit by the principal investigator and/or subinvestigators and the coordinating CRA, train the haematology nurses on the practical aspects of FMT, handle centralised randomisation of patients, and run all statistical, methodology and data management procedures. A sponsor-mandated CRA will ensure that the study is properly carried out in each centre and that the data generated in writing is collected, documented, recorded and reported in compliance with Clermont-Ferrand University Hospital Standard Operating Procedures, with GCP, and with all governing legal and regulatory provisions. Frequency of these visits will be defined according to number of inclusions at each centre.

Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) has been set up, and is composed of a transplant haematologist, a gastroenterologist practicing FMT, a pharmacist and a methodologist, none of whom will participate in the study. This DSMB will meet at the start of the study and then at any point deemed necessary during the study, on its own initiative or at the request of the sponsor, and in particular to analyse the safety data. Furthermore, as the main endpoint will be assessed at 1 year, the 3 month toxicity data will be reported to the independent DSMB annually. Inclusions will not be stopped during this safety assessment.

Adverse events

The investigator has an obligation to report any serious adverse event (SAE) to the sponsor and the pharmacovigilance authority. Any SAEs are to be promptly reported on the day the investigator becomes aware of them, whether during the active phase of the study or after the end of the trial. An SAE form will be completed, indicating the date of occurrence, intensity, relationship to the treatment (or the study) and follow-up. If an SAE persists at the end of the study, the investigator will follow the patient until the event is considered resolved. All SAEs will be recorded in the eCRF.

Frequency and plans for auditing trial conduct

The sponsor may carry out a functional trial audit of the participating centres to verify that the provisions described in the study procedures are fully adhered to.

Plans for communicating important protocol amendments to relevant parties

Any substantial modifications of the protocol will be submitted by the sponsor to the DSMB, and then to the IRB and the ANSM. Any other minor amendments will be sent to the IRB and the ANSM. Any amendments made will become effective after approval issued by the appropriate authorities. Participants will be informed of any change of the protocol and/or eligibility criteria, and will be given an updated information sheet and consent form.

Patient and public involvement statement

None.

DISCUSSION

Allo-HSCT is a major treatment for many haematological malignancies, with around 2000 allo-HSCTs performed every year in France. However, the toxicity associated with the procedure is high. GvHD is the main cause of toxic death after allo-HSCT. After years without a curative alternative to steroids, ongoing prospective trials are testing a number of emerging new drugs (JAK2 inhibitors, BCR inhibitors and others) in an attempt to improve outcomes in aGvHD or cGvHD patients.

The few relevant reports in the literature assume that FMT is safe and innovative in restoring gut microbiota diversity and might be an alternative cure for GvHD.[17–22] Retrospective data gives convincing arguments for a negative impact of gut microbiota alterations on post-allo-HSCT outcomes, but there might be some bias in interpreting the observations (patients who receive large, broad-spectrum antibiotics and thus an impaired IM, might also be more advanced patients). Despite strong evidence of the impact of IM alterations on GvHD, transplant-related mortality and overall survival[1–5 9–12 30–32] and the favourable effect of restoring a healthy IM by FMT, only two studies have set out to evaluate the feasibility/safety/effectiveness of allogeneic FMT after allo-HSCT (ClinicalTrials.gov NCT03720392 and NCT02733744). This randomised study will enrol a total of 120 patients. It can thus provide valuable insight into the effect of an early IM restoration with a healthy donor IM.

The main evaluation criterion of this study is 1-year GRFS, which is a strong marker of allo-HSCT success and has been widely used in allo-HSCT trials since The Blood and Marrow Transplant Clinical Trials Network recognised the potential utility of a composite endpoint.[33] Secondary endpoints will evaluate haematological and infectious outcomes, plus the impact of FMT on microbiota composition and diversity. Microbiota analysis will give us an important picture of the difference in diversity of bacterial communities between patients from the FMT group (before and after FMT) and the patients from the 'no FMT' group. Since we plan a 1-year post-FMT analysis, it will also give valuable data on the long-term impact of FMT in allo-HSCT patients. We hope to identify the microbial factors of donor microbiota that are associated with positive clinical outcomes as a starting point for standardising the faecal microbiota to select for GvHD prophylaxis.

We believe that the absence of blinding will not impact the validity of the findings since the primary outcome measure (1 year GRFS) has a high degree of objectivity.

We also chose to select only the myeloablative conditioning regimen and to use G-CSF-mobilised peripheral-blood HSC grafts (i) to homogenise the population and (ii) because these modalities are associated with a higher incidence of GvHD.

The donor selection criteria have been made extremely restrictive in order to ensure the safety of the FMT. Only a few donors will be selected and asked to repeat their donation several times to constitute the frozen stool bank.

Ethics and dissemination

The local institutional review board (CPP Sud-Est II, France) issued approval on 27 January 2021. The French national authorities (ANSM) issued approval on 15 April 2021. Informed consent will be obtained from all patients and healthy volunteers prior to participation in this trial.

Full protocol, participant-level data, statistical codes, trial datasets and analyses will be made available on request from the corresponding author after examining the request. Two participant information sheets and consent forms have been written: one for patients (online supplemental file 1) and the other for healthy volunteers (online supplemental file 2). The outcome of the study will be disseminated via peer-reviewed publications and at congresses.

Author affiliations
[1]Service d'Oncologie Médicale, Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont-Ferrand, France
[2]Service de Thérapie Cellulaire et d'Hématologie Clinique Adulte, Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont-Ferrand, France
[3]Secteur Biométrie et Médico-économie, Direction de la Recherche Clinique et de l'Innovation, Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont-Ferrand, France
[4]Pôle Pharmacie, Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont-Ferrand, France
[5]Faculté de médecine, Sorbonne Université, Paris, France
[6]Service d'hématologie clinique, Hôpital de la Pitié-Salpêtrière AP-HP, Paris, France

Acknowledgements The authors thank Dr Julien Scanzi (CHU de Clermont-Ferrand, Clermont-Ferrand, France) for his help on FMT modalities and Dr Laurence Bérard (Unité de Recherche Clinique, Hôpital Saint Antoine, Paris, France) for mobilising her clinical research expertise on stool donor recruitment.

Contributors J-OB is the main investigator and SN is the main associated investigator. Together, they developed the study concept with AD and AR. ACa and ACo helped develop the study design and lead coordination. MW contributed to the set-up of pharmaceutical procedures. HS advises on FMT modalities and biological analysis. BP advises on the methodology and will be in charge of randomisation and data analyses. ACa will be in charge of data management. All authors have read and approved this manuscript and will read and approve the final manuscript.

Funding This trial was supported by a grant from the French Ministry of Health via the National Hospital Clinical Research Programme (PHRC-K 2018 BAY), from 'Force Hémato' and from Clermont-Ferrand University Hospital (AOI 2020).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD
Alexandrine Corriger http://orcid.org/0000-0002-1170-8018

REFERENCES

1 Staffas A, Burgos da Silva M, van den Brink MRM. The intestinal microbiota in allogeneic hematopoietic cell transplant and graft-versus-host disease. *Blood* 2017;129:927–33.
2 Holler E, Butzhammer P, Schmid K, *et al*. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant* 2014;20:640–5.
3 Peled JU, Hanash AM, Jenq RR. Role of the intestinal mucosa in acute gastrointestinal GVHD. *Blood* 2016;128:2395–402.
4 Eriguchi Y, Takashima S, Oka H, *et al*. Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. *Blood* 2012;120:223–31.
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;8:339ra71.
6 Lee S-E, Cho B-S, Kim J-H, *et al*. Risk and prognostic factors for acute GVHD based on NIH consensus criteria. *Bone Marrow Transplant* 2013;48:587–92.
7 Jagasia M, Arora M, Flowers MED, *et al*. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood* 2012;119:296–307.
8 Jagasia MH, Greinix HT, Arora M, *et al*. National Institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. the 2014 diagnosis and staging Working Group report. *Biol Blood Marrow Transplant* 2015;21:389–401.
9 Peled JU, Devlin SM, Staffas A, *et al*. Intestinal microbiota and relapse after hematopoietic-cell transplantation. *J Clin Oncol* 2017;35:1650–9.
10 Taur Y, Jenq RR, Perales M-A, *et al*. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* 2014;124:1174–82.
11 Han L, Zhang H, Chen S, *et al*. Intestinal microbiota can predict acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2019;25:1944–55.
12 Peled JU, Gomes ALC, Devlin SM, *et al*. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2020;382:822–34.
13 Quraishi MN, Widlak M, Bhala N, *et al*. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Aliment Pharmacol Ther* 2017;46:479–93.
14 van Nood E, Vrieze A, Nieuwdorp M, *et al*. Duodenal infusion of donor feces for recurrent Clostridium difficile . *N Engl J Med* 2013;368:407–15.
15 Mandalia A, Ward A, Tauxe W, *et al*. Fecal transplant is as effective and safe in immunocompromised as non-immunocompromised patients for Clostridium difficile. *Int J Colorectal Dis* 2016;31:1059–60.
16 Webb BJ, Brunner A, Ford CD, *et al*. Fecal microbiota transplantation for recurrent Clostridium difficile infection in hematopoietic stem cell transplant recipients. *Transpl Infect Dis* 2016;18:628–33.
17 Kakihana K, Fujioka Y, Suda W, *et al*. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood* 2016;128:2083–8.
18 Spindelboeck W, Schulz E, Uhl B, *et al*. Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. *Haematologica* 2017;102:e210–3.

19 DeFilipp Z, Peled JU, Li S, *et al*. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. *Blood Adv* 2018;2:745–53.
20 Qi X, Li X, Zhao Y, *et al*. Treating steroid refractory intestinal acute graft-vs.-host disease with fecal microbiota transplantation: a pilot study. *Front Immunol* 2018;9:2195.
21 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:eaap9489.
22 Battipaglia G, Malard F, Rubio MT, *et al*. Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematologic malignancies carrying multidrug-resistance bacteria. *Haematologica* 2019;104:1682–8.
23 Harris AC, Young R, Devine S, *et al*. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai acute GVHD International Consortium. *Biol Blood Marrow Transplant* 2016;22:4–10.
24 Filipovich AH, Weisdorf D, Pavletic S, *et al*. National Institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005;11:945–56.
25 Holtan SG, DeFor TE, Lazaryan A, *et al*. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood* 2015;125:1333–8.
26 Cordonnier C, Maury S, Ribaud P, *et al*. A grading system based on severity of infection to predict mortality in allogeneic stem cell transplant recipients. *Transplantation* 2006;82:86–92.
27 Fayers P, Bottomley A. Quality of life research within the EORTC—the EORTC QLQ-C30. *Eur J Cancer* 2002;38:125–33.
28 Mander A. SAMPSI_FLEMING: stata module to compute exact sample size calculation for single-stage designs. 2014. Available: https://econpapers.repec.org/software/bocbocode/s457055.htm [Accessed 27 Feb 2023].
29 Lamas B, Richard ML, Leducq V, *et al*. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat Med* 2016;22:598–605.
30 Kusakabe S, Fukushima K, Yokota T, *et al*. Enterococcus: a predictor of ravaged microbiota and poor prognosis after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2020;26:1028–33.
31 Ubeda C, Taur Y, Jenq RR, *et al*. Vancomycin-Resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* 2010;120:4332–41.
32 Taur Y, Xavier JB, Lipuma L, *et al*. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2012;55:905–14.
33 Sankoh AJ, Li H, D'Agostino RB. Use of composite endpoints in clinical trials. *Stat Med* 2014;33:4709–14.