Design of the study:
Prospective, interventional, randomized, two-arm, multicentre phase II clinical trial

Title of the study:
Fecal microbiota transplantation for prophylaxis of infectious complications and graft versus host disease after allogeneic hematopoietic cell transplantation

Acronym:
FECALLO

Principal investigator:
Grzegorz W. Basak, Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw

Background & Rationale:
Fecal microbiota transplantation (FMT) has become a widely used procedure for treatment of resistant and refractory Clostridium difficile infection [1]. Its action is thought to rely on recovery of physiological diversity of gut flora thus increasing resistance to colonization or infection by pathological organisms. There is also evidence that diversity and content of gut flora has strong impact on transplant-related mortality after allogeneic hematopoietic cell transplantation [2], but also relapse rate [3]. It was observed that FMT used for treatment of C. difficile in patients with graft versus host disease (GvHD) may result in remission of this immune phenomenon [4]. It has been documented that diversity of gut flora falls rapidly after initiation of conditioning therapy [2]. Specific strains or families of gut bacteria were also linked to transplant outcomes [3, 5, 6] that was seen by correlation between abundance of certain bacteria, their metabolites and study end-points.
Recently, we have shown that gut colonization by antibiotic-resistant bacteria (especially those ESBL+, KPC+, NDM1+, VRE) is a very strong and independent prognostic factor for survival after transplantation and transplant-related mortality [7]. 48% of colonized patients experienced episodes of confirmed microbiologically bloodstream infections in the peritransplant period, compared to 24% of non-colonized (p=0.01). The frequency of infections with ARB was also higher (30% vs. 9%, p<0.01). Colonized patients more frequently developed grade 2-4 acute GvHD (42% vs. 23%, p<0.05), especially with gut involvement (33% vs. 13.5%, p=0.07). Altogether 2-year TRM in colonized vs. non-colonized patients was 42% vs. 11% (p=0.001) and OS was 34% vs. 74% (p<0.001). These results were confirmed by Zagreb group, which showed cumulative incidence of gut aGvHD in about 29% of patients colonized vs. about 12% non-colonized (p=0.02) [8].

Consequently, we performed a clinical trial, which attempted to reverse the gut colonization status in hematology patients by FMT [9]. We performed 25 FMTs in 20 patients (repeated procedures in cases of partial decolonization). Isolates of fecal material were administered as fresh preparations, via nasogastric tube in patients colonized by ARB. The overall efficacy of complete decolonization at 1 month after FMT (decolonization of all the colonizing bacteria) was 60%, but partial decolonization (decolonization from min. 1 bacteria) was achieved in 80% of cases. The antibiotic use within 7 days after FMT significantly decreased success rate (complete decolonization without antibiotics in 79% vs. 36% in those with antibiotics, p<0.05). The side effects were negligible and no serious adverse events were noted.

On the ground of these observations, we would like to propose a prospective clinical trial in which FMT will be used before alloHCT in patients colonized with ARB and with C. difficile with the goal to decolonize those patients before conditioning is administered, decrease rate of bloodstream infections and for prevention of aGvHD.

References:


Short description of the study:

This will be a prospective, interventional, randomised phase II study of FMT before alloHCT. Selected patients being candidates for alloHCT (please see inclusion and exclusion criteria) will be offered opportunity to enter clinical trial.

The patients will be randomised into two groups: A) treated with FMT and B) without intervention. FMT will be performed within 2 months-2 weeks before planned start of conditioning before alloHCT. Fecal transplant material will be given via nasoduodenal route on 2 subsequent days according to the unified procedure (Appendix A).

Fecal transplant material will be obtained from one source – Infection Prevention Institute (Dr Paweł Grzesiowski, Warsaw). The order should be made minimum 24 hours before administration by phone, it will be shipped by train. It will be a responsibility of treating team to collect the material from the train station. The material is obtained from carefully selected donors (please see attached
recruitment criteria, Appendix A) and isolated according to procedures as in Appendix B.

All the patients will be investigated for gut colonization just before FMT and at chosen time points by the same unified methods in all centers (Appendix C). The colonization status will be then checked before conditioning, on day 0, day 14, day 28 and day 100 (Appendix C). Patients will undergo alloHCT procedures as investigated and established in each center. They will carefully investigate all the infectious episodes by routine methods. Acute GvHD diagnosis and grading will be performed according to MAGIC criteria, with obligatory description of confidence level [Harris et al. Biology of blood and marrow transplantation 2016; 22(1): 4-10, Appendix D]. The colonization status as well as clinical findings will be collected in dedicated CRF forms (to be written).

**Primary objective:**

To decrease transplant-related mortality by reduced incidence of infections and grade 2-4 aGvHD.

**Secondary objectives:**

- To reduce incidence of gut GvHD
- To decrease the incidence of colonization and infections caused by ARB.

**Primary Endpoint:**

Proportion of patients decolonized before administration of conditioning (complete and partial decolonization)

**Secondary Endpoints:**

- TRM
- OS
- Cumulative incidence of aGvHD
- Cumulative incidence of grade 2-4 aGvHD
- Cumulative incidence of grade 3-4 aGvHD
- Cumulative incidence of gut aGvHD
- Incidence and cumulative incidence of septic episodes (as defined by 2016 surviving sepsis campaign)
- Incidence and cumulative incidence of septic shock episodes (as defined by 2016 surviving sepsis campaign)
- Incidence and cumulative incidence of overall bloodstream infections
- Incidence and cumulative incidence of bloodstream infections with ARB
- Median number of days with fever >38oC
- Proportion of patients decolonized on each of the time points

**Research design:**

Prospective, interventional, multicentre two-arm, randomized phase II clinical trial.

Arm A: FMT  Arm B: no intervention

**Number** of patients per group: Arm A: N=20; Arm B: N=20

CRO: required

Insurance: required

Sponsor: PALG

Bioethical committee: agreement needed – central, Medical University of Warsaw

The centers will be required to collect and store stool samples from patients obtained just before FMT, from FMT material, obtained just before starting conditioning, on day 0, 14, 28 and 100. The samples should be stored at -20oC and will be used for Next Generation Sequencing of gut microbiome. At the same time points, serum and urine samples (6 ml in three 2 ml aliquots) patients should be collected and frozen at -20 oC for subsequent metabolomic analysis.

**All adverse events will be reported to the Advisory Board of the study within 12 hours from its occurrence.**

Expected study period: 5/2018-5/2019

**Study Population:**

Subsequent patients qualified to allogeneic hematopoietic cell transplantation in centers participating in the study.

**Inclusion criteria:**
- aged >=18 years
- patients qualified to alloHCT to be performed within 2 months time
- colonization of the GI tract with antibiotic-resistant bacteria, particularly CPE (including those with genetic resistance mechanisms against carbapenem), extended-spectrum b-lactamase positive (ESBL+) Enterobacteriaceae, vancomycin-resistant Enterococcus faecalis and Enterococcus faecium (VRE), and other bacteria with documented resistance to at least two classes of antibiotics. These ARB must be documented by at least two positive cultures of material from rectal swabs taken <2 weeks before FMT. Colonization with ARB should be investigated according to Polish standards (www.korl.edu.pl).
- both myeloablative and RIC/NMA protocols
- MUD and MRD
- ATG used according to local criteria
- Karnosfksy score >=80% at alloHCT
- Comorbidity index <=3 pkt
- Not taking antibiotics or not scheduled to take antibiotics within 7 days after FMT
- Afebrile
- CRP<20
- Neutrophil count >0.5 G/L
- Plt count >50 G/L
- able to give informed consent

**Exclusion criteria:**
- not meeting inclusion criteria
- requiring antibiotic prophylaxis or therapy on and within 7 days after FMT
- mMUD, mMRD, haplo, UCB
- patients <18 years of age
- Lack of consent or lack of logical contact.
- An absolute neutrophil count on the day of FMT < 5 \times 10^8 neutrophils/L or an expected decrease in the count within the following 2 days.
- Incapacitation, conscription into the military, imprisonment, or dependent on business with or any other form of dependency on the researchers.
Expected number of patients: 40

**Data Collection & Statistical Analysis Plan:**

Variables to be collected:

- colonization status by ARB and fungi at FMT, before conditioning, on day 0, day 14, day 28 and day 100
- basic patients’ characteristics: age, sex, disease, disease status at alloHCT,
- alloHCT characteristics: donor, stem cell source, conditioning, immunosuppression,
- Outcomes: NEU and Plt regeneration, occurrence of aGvHD according to MAGIC criteria, grade and stage and diagnosis confidence level, date of its incidence, occurrence of bloodstream infection – date of episode, microorganism identified, mechanism of antibiotic resistance, ARB or not, sepsis – yes/no, when, septic shock – yes/no/when, number of episodes, occurrence of other infections, as above
- Results of gut microbiome sequencing and metabolomics analyses of serum and urine (separate research project)

**Study budget:**

*To be calculated*

The study will follow Authorship Guidelines for PALG Publications

**Study Advisory Board:**

Grzegorz W. Basak, Jarosław Biliński, Paweł Grzesiowski, Lidia Gil, Anna Czyż, Tomasz Czerw, Sebastian Giebel.

**Timeline**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Timeframe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparative work</td>
<td>12.2017-04.2018</td>
</tr>
<tr>
<td>Inclusion participants</td>
<td>05.2018-12.2018</td>
</tr>
<tr>
<td>Data collection</td>
<td>05.2018-05.2019</td>
</tr>
<tr>
<td>Data processing and analysis</td>
<td>05-06.2019</td>
</tr>
<tr>
<td>Report preparation</td>
<td>06-08.2019</td>
</tr>
<tr>
<td>Final manuscript</td>
<td>06-09.2019</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Total time needed</td>
<td>21 months</td>
</tr>
</tbody>
</table>
Appendix A.
Fecal microbiota transplantation – procedure

- The day before FMT bowel lavage will carried out using macrogols.
- Each participant will fast for at least 12h before transplantation and a proton pump inhibitor will be administered in the evening before FMT and twice daily on the day of transplantation to neutralize the gastric acid.
- Patient will receive antiemetic treatment at least 30 mins before the procedure (metoclopramide)
- The fecal microbiota suspension will be administered on day 1 and 2 (on each day: two syringes that each will contain 50 g feces in 100 ml sterile saline [100g/200ml in total]).
- The fecal microbiota suspension will be administered via an intraduodenal tube.
- After placement of the nasoduodenal tube, the recipient will be advised not to drink anything or take any medications for 1–2 h.
- In case of transplantation of frozen material, it will be thawed immediately at patient’s bedside to room temperature and administered as soon as possible.
Appendix B.
Donor recruitment

- Each donor will sign an informed written consent form that states that he or she agreed to undergo clinical checkups and to provide fecal samples for a particular recipient. The donors’ personal data will be unavailable to the recipient.

- Potential donors will be excluded from donating if the clinician who carried out the donor screening procedure obtained information that indicated that the donor had an increased risk of infectious diseases in the phase between screening and the donation of feces (such as a recent visit to a tropical area in the last three months, risky sexual behavior defined as a new sexual contact in the last six months, recent needle stick accident, receiving blood products, or getting a tattoo); any GI condition or symptoms (abdominal discomfort, frequent loose stools, or constipation); a family history of intestinal cancer or inflammatory bowel disease; any other condition that required the use of medication that could be excreted in feces and subsequently pose a potential risk to the participants.

The donors will undergo extensive testing as follows:

A. Blood tests:
   - Hepatitis A virus (HAV; anti-HAV IgM and IgG);
   - Hepatitis B virus (HBV; HBsAg, anti-hepatitis B core antigen [HBc], HBV DNA);
   - Hepatitis C virus (HCV; anti-HCV antibodies, HCV RNA);
   - Human immunodeficiency virus (HIV; anti-HIV antibodies, HIV RNA, HIV proviral DNA);
   - Syphilis (serology);
   - Cytomegalovirus (CMV; anti-CVM IgM and IgG);
   - Epstein–Barr virus (EBV; anti-EBV IgM and IgG).

B. Fecal sample tests:
   - Parasites (microscopic examination) + Lamblia antigen in stool;
   - *C. difficile* toxin A/B (ELISA or equivalent);
   - Enteropathogenic microflora (classical culture)
   - Carriage of antibiotic-resistant bacteria according to above listed standards

The donor will be required to meet additional requirements:
• six months without antibiotic treatment;
• generally good health, with no autoimmune or metabolic diseases (based on an interview and clinical examination);
• ordinary diet (excluded vegan, purely vegetarian etc);
• no alcohol intake within 3 days before donation
• no relationship (or any permanent contact) with the recipient.
**Appendix C:**

**Preparation of FMT product**

Each donor will donate a fecal sample up to 2 h prior to FMT or 1 h prior to the sample being processed and frozen:

- Each donor will place a 10-cm³ fecal sample into a sterile disposable container in sterile conditions (at home or in a medical facility) after being given precise instructions by medical personnel on personal hygiene before defecation and during the handling of the sample;
- The sample will be immediately forwarded to the Isolating Laboratory;
- Only samples of formed stool (not loose) will be processed further.

Aseptic procedures will be carried out at the Isolating Laboratory:

- to prepare each FMT product, 100 g feces will be suspended in 100 ml sterile saline in a sterile test tube;
- the suspension will be homogenized for 2–4 min;
- slow filtration through a sterile sieve, double gauze, or another suitable sterile filter will be carried out;
- the filtrate will be diluted with sterile saline to a volume of 100 ml in a sterile tube, homogenized slowly, and filtered as before;
- the filtrate will be diluted with sterile saline to a final volume of 200 ml and it will be divided into two 100 ml syringes (each syringe will contain 50 g feces diluted to a total volume of 100 ml with sterile saline – total 100g/200ml);
- FMT products will be then immediately transferred to the treatment room to administer them to the recipient.
Appendix D

MICROBIOLOGICAL ASSESSMENT

The potential participants will be routinely screened for gut colonization by the following ARB: methicillin-resistant *S. aureus* (MRSA), VRE, ESBL+ *Enterobacteriaceae* and CPE. Colonization status will be ascertained by the microbiological examination of rectal swabs cultured on one of the following types of chromogenic media: MRSA Agar (Graso, Gdańsk, Poland), VRE Agar (Graso), chromID® ESBL (bioMérieux S.A., Marcy l’Etoile, France), and CHROMAgar KPC (Graso). Pathogens will be inoculated on Columbia Agar plates that contained 5% sheep blood (Becton, Dickinson, Franklin Lakes, NJ, USA), and identified using matrix-assisted laser desorption/ionization (MALDI) (Microflex; Bruker Daltonik GmVH, Bremen, Germany). The presence of MRSA, VRE, ESBL+ *Enterobacteriaceae* and CPE will be verified using phenotypic methods, in accordance with the national recommendations in Poland [1-3].

Additionally, the ability of isolates to produce carbapenemases (*metallo-β-lactamase* [MBL], *K. pneumoniae carbapenemase* [KPC] and extended-spectrum oxacillinase-48 [OXA-48]) will be investigated using the Rapidec® Carba NP biochemical assay (bioMérieux S.A.) and/or the GeneXpert® quantitative real-time PCR method (Cepheid, Sunnyvale, CA, USA). The identified pathogens will be designated as ARB. Colonization will be defined as the detection of an ARB in at least two consecutive rectal swabs.

References:


Appendix E


<table>
<thead>
<tr>
<th>Stage</th>
<th>Skin (involves head only)</th>
<th>Liver (bilirubin)</th>
<th>Upper GI</th>
<th>Lower GI (oral output)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None or not present</td>
<td>&lt; 0.2 mg/dl</td>
<td>Not or mildly impaired</td>
<td>Not impaired</td>
</tr>
<tr>
<td>1</td>
<td>Patchy erythema or rash</td>
<td>0.2-1 mg/dl</td>
<td>Mild</td>
<td>Severe bloody diarrhoea</td>
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<tr>
<td>2</td>
<td>Erythema or rash</td>
<td>1-3 mg/dl</td>
<td>Moderate</td>
<td>Bile-stained diarrhoea</td>
</tr>
<tr>
<td>3</td>
<td>Erythema or rash</td>
<td>&gt;3 mg/dl</td>
<td>Severe</td>
<td>Severe bloody diarrhoea</td>
</tr>
<tr>
<td>4</td>
<td>Erythema or rash</td>
<td>&gt;5 mg/dl</td>
<td>Severe</td>
<td>Severe bloody diarrhoea</td>
</tr>
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<table>
<thead>
<tr>
<th>Confidence Levels</th>
<th>Pathology evidence</th>
<th>Clinician assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed</td>
<td>Unequivocal evidence of GVHD</td>
<td>GVHD is the likely diagnosis for symptoms</td>
</tr>
<tr>
<td>Probable</td>
<td>Not required</td>
<td>GVHD is a differential diagnosis but no treatment is being provided</td>
</tr>
<tr>
<td>Possible</td>
<td>Not required</td>
<td>GVHD is not considered as an explanation for the symptoms</td>
</tr>
<tr>
<td>Negative</td>
<td>Not required</td>
<td>GVHD is not present without GVHD symptoms</td>
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</table>

Table: GVHD Target Organ Involvement

<table>
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<tr>
<th>GVHD Target Organ Involvement</th>
<th>Skin (involves head only)</th>
<th>Liver (bilirubin)</th>
<th>Upper GI</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Grade 0: No skin involvement</td>
<td>None or not present</td>
<td>&lt; 0.2 mg/dl</td>
<td>Not or mildly impaired</td>
<td>Not impaired</td>
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<tr>
<td>Grade 1: Skin involvement</td>
<td>Patchy erythema or rash</td>
<td>0.2-1 mg/dl</td>
<td>Mild</td>
<td>Severe bloody diarrhoea</td>
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<td>Grade 2: Erythema or rash</td>
<td>Erythema or rash</td>
<td>1-3 mg/dl</td>
<td>Moderate</td>
<td>Bile-stained diarrhoea</td>
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<tr>
<td>Grade 3: Erythema or rash</td>
<td>Erythema or rash</td>
<td>&gt;3 mg/dl</td>
<td>Severe</td>
<td>Severe bloody diarrhoea</td>
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<tr>
<td>Grade 4: Erythema or rash</td>
<td>Erythema or rash</td>
<td>&gt;5 mg/dl</td>
<td>Severe</td>
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Table: Treatment for acute GVHD

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<th>Treatment for acute GVHD</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Prednisone</td>
<td>Yes</td>
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<tr>
<td>Cyclosporine</td>
<td>Yes</td>
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<td>Tacrolimus</td>
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<tr>
<td>Methotrexate</td>
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<td>Azathioprine</td>
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<tr>
<td>Mycophenolate</td>
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<td>Rituximab</td>
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<td>Anti-thymocyte antibodies</td>
<td>Yes</td>
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<tr>
<td>Anti-T-cell antibodies</td>
<td>Yes</td>
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<tr>
<td>Anti-lymphocyte antibodies</td>
<td>Yes</td>
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<tr>
<td>Other immunosuppressants</td>
<td>Yes</td>
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