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Protocol Title: Oral supplementation of 2'-fucosyllactose in allogeneic bone marrow transplant recipients to maintain intestinal homeostasis

NCT Number: NCT04263597

Document Version Date: 04/16/2024

Oral supplementation of 2'-fucosyllactose in allogeneic bone marrow transplant recipients to maintain intestinal homeostasis

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Background:

High dose chemotherapy and radiation used as preparative regimens in patients undergoing an allogeneic hematopoietic stem cell transplant (HSCT) disrupts intestinal homeostasis by damaging the intestinal epithelium and altering the intestinal microbiome[1]. Altered microbiota further drives intestinal inflammation, resulting in life-threatening post-transplant complications such as blood stream infections (BSI)[2] and acute graft versus host disease (GVHD)[3, 4]. These complications of BSI and acute GVHD further disrupt the intestinal microbiota, establishing a vicious cycle of dysbiosis[5, 6]. Indeed, restoration of intestinal homeostasis is essential for resolution of tissue injury, establishment of immunological tolerance and overall survival of HSCT patients.
A critical microbial function which contributes to intestinal homeostasis is the production of the short chain fatty acid butyrate, which promotes intestinal epithelial cell and regulatory T-cell function[7]. We have previously shown that a progressive decline in intestinal butyrate levels correlates with loss of microbial diversity and higher incidence of acute GVHD in children undergoing allogeneic HSCT[8]. Additionally, butyrate producing microbiota are reduced in children with low intestinal butyrate levels, linking butyrate production with the intestinal microbiome[7]. Therefore, modulation of the intestinal microbiome and increasing intestinal butyrate levels may be a crucial strategy for maintaining intestinal homeostasis in HSCT recipients.

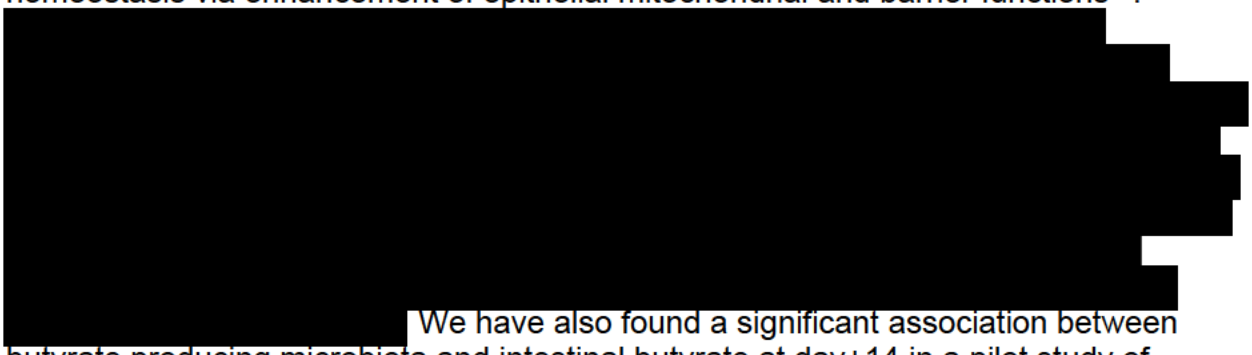
Prebiotics are dietary supplements with beneficial effects on the intestinal microbiome and are promising agents for restoring healthy intestinal microbiota[9]. Breast milk, the first diet for an infant, offers all the essential nutrients for growth and development and provides bioactive factors such as immune globulin, antimicrobial proteins and cytokines[10]. In addition, human milk also shapes the intestinal microbiome of the infant gut[11]. The major component of human milk that shapes the intestinal microbiome is known as human milk oligosaccharides (HMO)[12]. These HMOs form the third most abundant solid component of human milk with typical concentrations of 10-15 g/L in mature term milk[13]. HMOs are not digested in the upper gastrointestinal tract but reach the large intestine where they provide selective substrates for intestinal bacteria, modulate the immune system and prevent the epithelial adhesion of intestinal pathogens[14].

HMOs exist as either fucosylated or sialylated units, with the fucosylated component being the most prominent component (~77%)[15, 16]. In human milk, 2-fucosyllactose (2FL), a prebiotic trisaccharide, is the most abundant HMO with a typical concentration of ~ 2-3g/L[17]. Studies investigating the effects of 2FL supplementation in healthy adults have shown that 2FL supplementation at a range of doses was safe and well tolerated[18]. Additionally, healthy adult subjects receiving 2FL for 2 weeks showed an increase in the relative abundance of Bifidobacteria and Actinobacteria and a reduction in the relative abundance of Firmicutes and Proteobacteria[18]. Bifidobacteria are beneficial bacteria for human health because of their ability to digest complex carbohydrates and dietary fibers[19]. Low Bifidobacteria abundance has been linked to several gastrointestinal and metabolic diseases[20]. In patients with inflammatory bowel

disease, 2FL supplementation also did not support the growth of Enterobacter sp or Escherichia, which are increased in IBD patients with severe symptoms. Preclinical safety studies of 2FL in rats have established a no observed adverse effect level of 5 g/kgbw/day. This data has supported the first studies of 2FL supplemented infant formula. Infants fed with a 2FL supplemented formula exhibited improved growth velocity and lower plasma cytokines profiles compared to infants who were fed a control formula[21].

2FL is a product of the fucosyltransferase 2(FUT2) gene enzyme[22]. In the United States, approximately 23% of the population are FUT2 non-secretors[23], who are deficient in the innate gut carbohydrate containing fucose, which increases susceptibility to microbial dysregulation and chronic inflammation[24]. FUT2 non-secretors are also at increased risk of developing Crohn's disease[25]. We have also shown that pediatric HSCT recipients with an inactivating polymorphism in the FUT2 gene have a higher incidence of BSI[26].

Several factors make 2FL a promising prebiotic in HSCT patients. The safety and tolerability of 2FL has already been established in healthy infants and adults[18]. 2FL enhances the growth of Bifidobacterium[27], which via cross feeding of F. prausnitzi and other microbes promotes butyrate production[28]. Butyrate in turn promotes mucosal homeostasis via enhancement of epithelial mitochondrial and barrier functions[29].



We have also found a significant association between butyrate producing microbiota and intestinal butyrate at day+14 in a pilot study of pediatric HSCT patients[8]. Patients in this study with lower levels of intestinal butyrate also had a higher incidence of acute GVHD. This also provides evidence for the role of 2FL supplementation to boost the butyrate producing microbiota as a mechanism for reducing complications such as acute GVHD after HSCT.

Looking more specifically at the composition of the microbiota of patients who died after HSCT in a separate adult study, greater abundance of Proteobacteria correlated with increased mortality. Multiple studies also show that the expansion of Firmicutes is associated with increased severity of acute gastrointestinal GVHD in humans[30]. Studies of 2FL have shown reduction of relative abundances of Firmicutes and Proteobacteria suggesting anti-inflammatory benefits beyond modifying butyrate production, which could be relevant to the HSCT population (Figure 1).

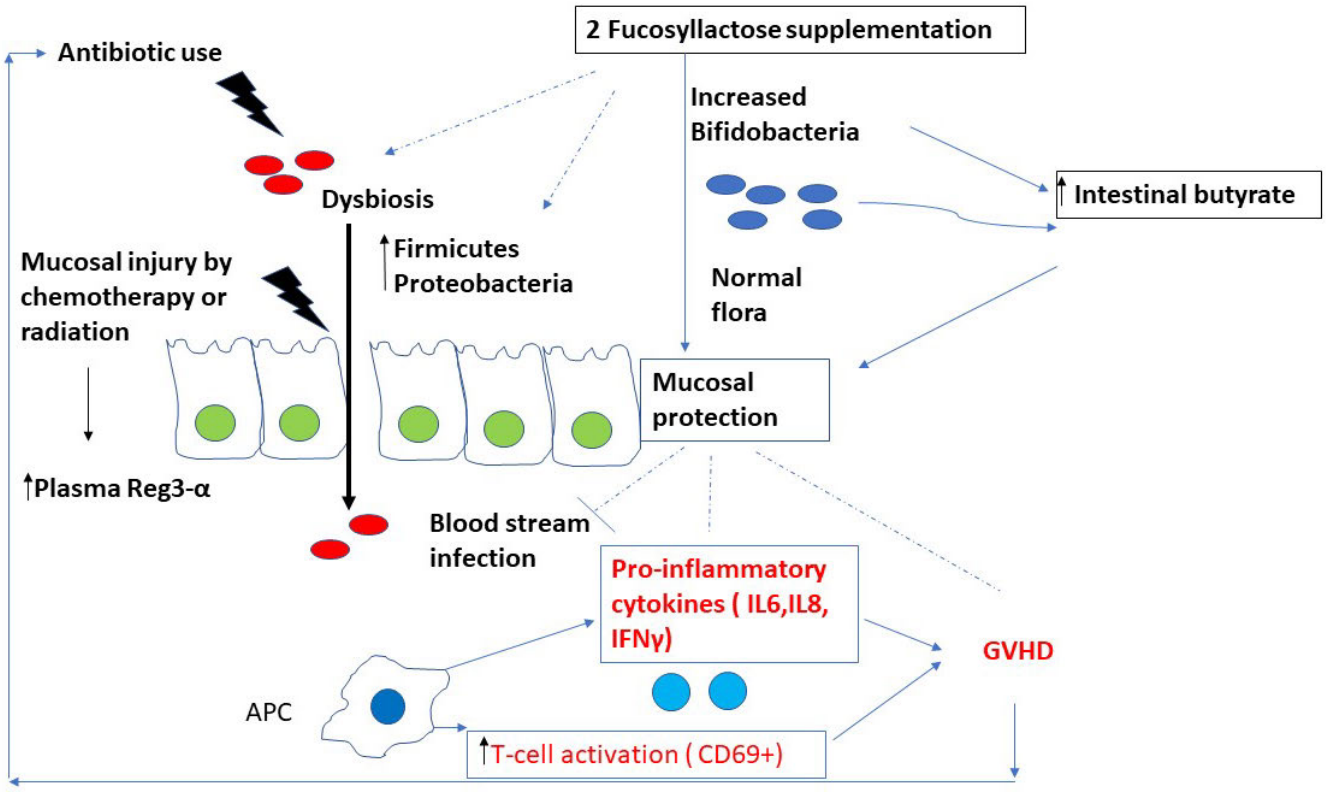


Figure 1. Graphical representation of background and hypothesis.

Hypothesis:

We hypothesize that 2FL supplementation will be safe and tolerable and result in an increase in the relative abundance of fecal short chain fatty acids such as butyrate, acetate and propionate at day+7 compared to baseline values.

Phase 1 Study objectives

a) To establish the dose dependent safety and tolerability of 2'-FL in allogeneic transplant patients
b) To demonstrate the dose dependent increase in fecal *Bifidobacteria* at day+30 in patients receiving 2'-FL
c) To demonstrate the dose dependent increase in fecal butyrate at day+30 in patients receiving 2'-FL

Phase 2 Study Objectives

d) To test efficacy of 2FL supplementation in children and young adult allogeneic HSCT patients with a goal to reduce intestinal inflammation and improve post HSCT outcomes such as acute GVHD and bloodstream infections.

Previous Studies:

There have been no prior studies of 2FL supplementation in HSCT patients. 2FL has been administered successfully to 100 healthy adult volunteers in a randomized controlled trial[18]. In this study, 2FL was safe and well tolerated with some increased gastrointestinal upset observed at the highest (20g) dosing. Patients who received 2FL at 10g and 5g dosing showed an increase in the relative abundance of *Bifidobacteria* and *Actinobacteria* while reducing *Firmicutes* and *Proteobacteria* in a dose dependent fashion. There was no statistically significant association indicating that the initial abundance of Bifidobacterium determined whether an individual was considered a responder or non-responder (response defined as an increase in sequence abundance of Bifidobacterium by ≥10%). In addition, no correlation was observed between initial abundance and the change in *Bifidobacteria*.

Safety and dosing of 2FL supplementation has also been established by the European Commission, the Panel on Dietetic Products, Nutrition and Allergies. This panel concluded that 2FL supplementation was safe for infants and children up to the age of 18 years.

We were interested in the role of HMOs early but did not have access to an oral supplement previously. We therefore conducted a randomized clinical trial of human milk in children undergoing transplant between 0-5 years of age with the hypothesis that human milk, by virtue of HMOs, would reduce intestinal inflammation after HSCT compared to children of the same age group who received standard nutrition. We enrolled 42 patients on this trial and showed that human milk administration was safe and feasible in young children and reduced intestinal inflammation by modifying the intestinal microbiome and reducing plasma cytokines and T-cell activation compared to patients who received standard nutrition[33].



Study Design:

This phase I/IIa study is a single center prospective study at Cincinnati Children's Hospital Medical Center (CCHMC).

Duration of study:

Six years to allow for patient enrollment and manuscript preparation.

Number of patients to be enrolled:

We will enroll approximately 90 patients on study. Initial 15 patients to establish safety as per the FDA have been enrolled. We have further enrolled 10 patients and performed interim analyses which have demonstrated safety, lack of any dose limiting toxicities and a positive signal of increase in fecal acetate and propionate at day+7 compared to baseline values. We now propose to enroll 65 additional patients on the phase II portion of this study to at least have 50 evaluable patients for analyses factoring in drop out, death, proceeding to second HSCT.

Duration of enrollment for patients:

Patients will remain on study until approximately day+100 after HSCT.

Inclusion Criteria:

Eligible patients will

- Be scheduled for allogeneic stem cell transplant
- All ages and underlying diagnoses, preparative regimens, stem cell sources and acute GVHD prophylaxes are eligible

Exclusion Criteria:

- Patients will be ineligible if they are unable to take anything orally or enterally (i.e. intestinal failure)
- Actively breastfeeding infants will be excluded from enrollment.
- Recent (within the week prior to enrollment) GI infection
- Patients receiving anti-diarrheal medications such as loperamide
- Patients who have received probiotics or prebiotics during the previous month
- Patients who have had any type of gut damage within the past three months; such as, previous bowel perforations, previous episode of Grade 4 neutropenic colitis or typhlitis
- Patients with inflammatory bowel disease, short bowel syndrome and patients with a history of bowel resections.

Study design:

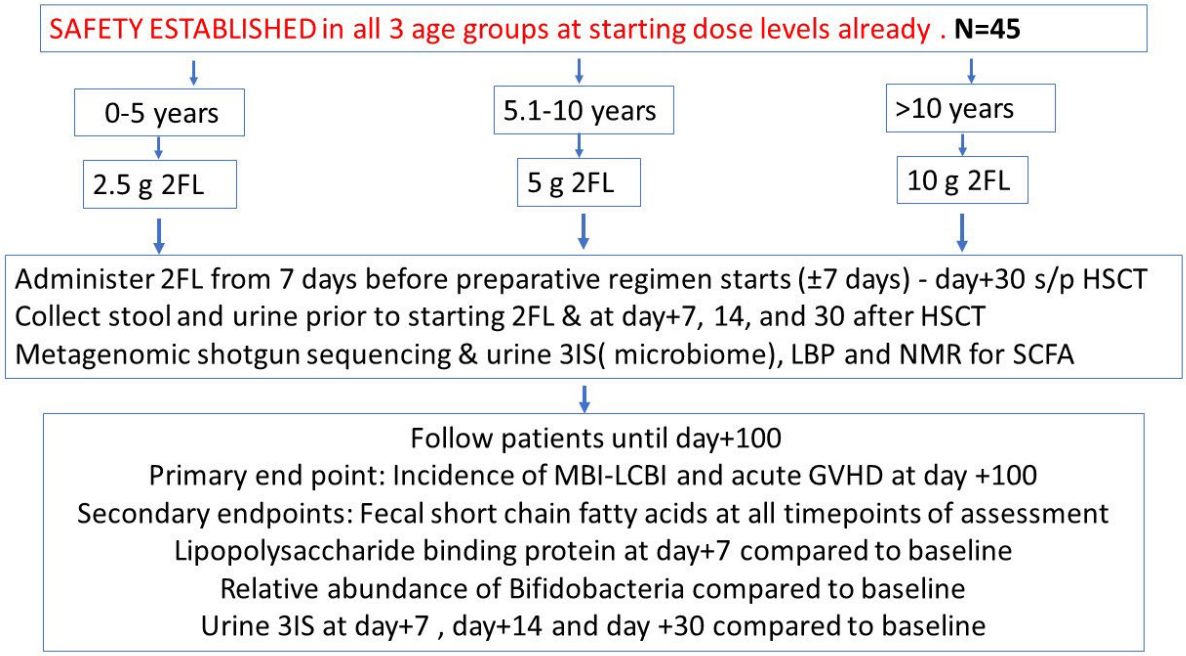


Figure 2. Study design of phase II study of patients

We will enroll eligible patients and allocate them into the following arms as determined by age at enrollment.

Arm 1: 0-5 years
Arm 2: 5.1-10 years
Arm 3: >10 years

Arm 1: 2FL will be initiated one week prior to starting preparative regimen (±7 days), doses may be given once or divided twice daily to assist in tolerability. Patients will continue 2FL until day+30 after HSCT (±7 days).

1) **Dose of 2FL:**

2FL:

	Dosing
Arm 1 (0-5 years)	2.5 g
Arm 2 (5.1-10 years)	5g
Arm 3 (>10 years)	10g

Table 1. Doses of 2'FL

Phase 1 Primary study end point: Tolerability of 2FL as determined by compliance to the medication.

Method of dose escalation and de-escalation: We have performed and concluded the initial safety phase of the trial prior to opening it for enrollment and have already explored the starting dose level and established safety. We will therefore enroll 3 patients at the starting dose level as described in Figure 2 and proceed with dose escalation within each age group after enrolling the first 3 patients without waiting to establish additional safety. Enrollment and dose escalation within each arm will happen independent of each other. Assessment for safety and DLTs will occur continuously on study.

Phase 2 Primary study end point: Fecal Butyrate/acetate/propionate levels increase from baseline at day+7

2) **Route of administration**: oral or enteral tube (NG, OG, G-tube, NJ tube, G-J tube).

3) **Frequency of administration**: Once daily or may be divided twice daily especially during mucositis.

4) **Duration of administration**: Approximately 1 week prior to start of preparative regimen (± 7 days) until day+30 after HSCT (± 7 days). If 2FL supplementation begins one week prior to start of preparative regimen but transplant is delayed for clinical reasons, we will halt 2FL supplementation once the decision to delay HSCT is made, and resume 2FL with start of preparative regimen.

Rationale for duration of administration:
The pathophysiological events leading to clinical manifestations of acute graft versus host disease and bloodstream infections occur early after allogeneic bone marrow transplant, estimated to occur between day 0 and day+25 after transplant[3]. These

events include microbial shifts, intestinal damage due to high dose chemotherapy and mucosal breakdown during the period of low bone marrow reserve, all of which contribute to the breakdown of intestinal homeostasis. Routine GVHD prophylactic medications such as calcineurin inhibitors or mycophenolate mofetil commence around day-3 to day+1 after BMT. In our pilot human milk trial [33], we began human milk administration on day-3 and continued until day+14 after HSCT, and on evaluation of our correlative data we observed maximal suppression of plasma cytokines and reduction in various markers of intestinal injury (plasma reg-3 -alpha levels and fecal human DNA levels) and changes in the intestinal microbiome at day+14 in patients receiving human milk but not at day+28, day+45 or day+60. We also did not see a statistically significant difference in the incidence of acute GVHD or bloodstream infections between the human milk recipients compared to controls, perhaps due to the shortened duration of our intervention at day+14, which ended prior to the accepted period when GVHD pathophysiological events occur. This provided rationale to extend our intervention of 2'-FL to day+30 to adequately allow for reduction in intestinal inflammation beyond the critical period of GVHD pathophysiology. 2'-FL is a prebiotic and is used by bacteria to support growth of existing beneficial bacteria either directly or via cross feeding interactions. We were concerned about starting 2'-FL too late if we chose day-3 as our starting point (as we did in our pilot human milk trial), because it is possible that most *Bifidobacteria* would be destroyed by the high dose chemotherapy and would not be present to utilize 2'-FL for their growth. We therefore wanted to start our intervention one week prior to initiation of chemotherapy to allow for adequate growth of beneficial bacteria.

5) **Method of administration**: The 2FL powder will be provided to participants in packets and they will be instructed to drink this daily/twice daily by adding the required amount to food or drink. This may also be mixed in standard feeds or mixed with 5-7 cc of water and administered by enteral tube, whenever applicable. Feeding tubes are only to be used for 2FL administration if already placed for other medical reasons. A feeding tube should not be placed or replaced in patients for the sole reason of 2FL administration. 2FL administration should not be prioritized over the receipt of other medications or feeds. Additional methods of administration including mixing 2FL in yogurt/pudding/apple sauce or making popsicles of 2FL and administering during mucositis may be pursued.

6) **Source of 2FL**: We will use 2FL manufactured by FrieslandCampina in partnership with Glycosan, LLC. The 2FL produced by this company utilizes a bioengineered strain of E. coli that was developed and patented by Glycosan LLC. The technology and manufacturing processes used to produce 2FL is considered the most efficient and stable at scale. The 2FL product consists of a white powder that is slightly sweet and contains no flavor, the composition is 93% 2FL 3% other sugars and 4% moisture as documented in the specification sheet. It has no identified microbial contaminants. We will administer 2FL under an IND. 2FL will be dispensed by investigational pharmacy to participants.

7) **Measurement of compliance**: Nursing documentation of 2FL administration will be used as documentation of compliance for patients who are inpatient.

Patients who are outpatient will be asked to record their 2FL supplementation doses in a study diary to confirm compliance. Directions on how to complete the diary will be provided. Study staff will review the diary for compliance at the end of the study dosing period (Day+30 ± 7 days).

An acceptable compliance will be defined as consumption of the dose on at least 60% of planned treatment days. Patients who meet this will be considered evaluable.

8) **Samples to be collected:** We will collect stool, blood, and urine samples according to the following table:

	Baseline (prior to 2FL supplement)	**Day+7 ±5 days**	**Day+14 ± 5 days**	**Day+30 ± 5 days**
5 mL whole blood in EDTA	**X**	**X**	**X**	**X**
Stool sample	**X**	**X**	**X**	**X**
Urine sample	**X**	**X**	**X**	**X**

Table 2. Sample collection while on study.

If patients have also consented to the institutional HSCT repository [redacted] we may obtain the samples from the repository. If patients are not enrolled in the HSCT repository or if a sample is not available from the repository for any reason, we will collect the samples according to the schedule noted above. If a participant chooses not to provide a sample or is unable to provide a sample for any reason, this will be documented in the study chart but will not be considered a protocol deviation for this study.

9) **Sample processing and storage**: Plasma and PBMCs will be stored from whole blood and cryopreserved while stool and urine samples will be stored at -80C.

10) **Diet while on 2FL supplementation**: Infant formula containing 2FL will not be allowed while on study. Routine nutritional supplementation after HSCT including enteral feeds and/or TPN will be allowed as part of standard of care. No dietary modifications will be necessary during the period of 2FL supplementation. There is a known influence of dietary fiber with 2FL responses, but we feel these will be less likely to influence our results since most patients have significant loss of appetite during and 2-3 weeks following chemotherapy.

11) **Assessment of safety**: In the event of patients requiring systemic antibiotics targeting anaerobes during 2FL supplementation, 2FL will be paused for the duration of the antibiotic and restarted when the antibiotic is discontinued if the discontinuation

occurs prior to day+30 after HSCT. This is to ensure additional safety for our immune compromised patients in whom 2FL might also facilitate the growth of pathogenic bacteria in the absence of beneficial bacteria.

The list of antibiotics used in our practice which target anaerobes which would trigger holding 2-FL include:

1. Meropenem/Imipenem
2. Piperacillin-tazobactam/ Ampicillin- sulbactam
3. Metronidazole
4. Clindamycin

13) **Generation of data**: Using the single nucleotide polymorphism rs601338 (428G > A), FUT2 secretor status will be genotyped. Metagenomic shotgun sequencing will be performed on stool samples at all time points collected. Fecal microbiome will be assessed for relative abundance of *Bifidobacteria, Actinobacter, Fimicutes* and *Proteobacteria* in addition to microbial diversity and human DNA as a measure of the degree of intestinal damage. Nuclear magnetic resonance will be performed on stool to assess levels of butyrate, acetate and propionate at all timepoints.

Clinical Data:

Patients' medical information will be systematically and prospectively collected as is usual for all HSCT patients. The data collected will include, but not be limited to-

1. Demographics including age, gender, weight, and height.
2. Underlying diagnosis
3. Specifics of HSCT (preparative regimen, type of graft, cell dose, GVH prophylaxis, HLA type and donor relation etc.).
4. Details of engraftment (timing, failure to engraft, loss of graft
5. Adverse effects directly related to 2FL
6. Onset of GVHD, grade, organ involved, and treatments used by day+100
7. Whole blood donor chimerism
8. Infectious complications which include asymptomatic viremias for EBV, Adenovirus, CMV, and viral disease, bacterial and fungal infections as documented by blood cultures or bronchoalveolar lavage cultures/PCRs.
9. Overall survival at day+100
10. Incidence of relapse of primary disease by day+100

Monitoring:

Patients will be monitored for safety of 2FL while they receive the 2FL supplementation as described above. Incidences of BSI will be documented. Compliance will be assessed.

Phase 1 Study End Points:

Primary study end point:

- Safety and tolerability of 2FL across dose ranges

Secondary Study End points:

- Increase in relative abundance of fecal Bifidobacteria at day+30 compared to baseline by >/=10%
- Decrease in relative abundance of fecal Firmicutes and Proteobacteria at day+30 compared to baseline for patients on 2FL
- Incidence of day+100 acute GVHD and BSI rates

Phase 2 Study End Points:

Primary study end point:

- Fecal Butyrate/acetate/propionate levels increase from baseline at day+7

Secondary study end point:

- Incidence of MBI-LCBI/GVHD
- Urine 3IS levels at day+7/day+14/day+30 compared to baseline

Stopping Rules:

1. Unexpected significant risks in 2'FL arm
2. An unexpected increase in grade 3-4 acute GVHD by 20%
3. Engraftment failure increase in 20% of patients on 2'-FL
4. Increase in GI infections by 20% in 2'-FL arm
5. Unexpected increase in nausea, vomiting, diarrhea that does not respond to supportive care after 48 hours in 30% of patients who receive 2'-FL

Performance Sites:

All study activities will take place on the main campus of Cincinnati Children's Hospital Medical Center.

Adverse effects of 2FL:

GI distress at higher doses of 2FL was observed in healthy volunteers. These include nausea, abdominal pain, indigestion, reflux, diarrhea, constipation, flatulence, rumbling and bloating. However, these were not severe enough to warrant discontinuation of 2FL.

Risk assessment recommendation:

This study will be of more than minimal risk but with potential for direct benefit to participants.

Potential Benefits:

Participating subjects may benefit from this study as 2FL could modulate their intestinal microbiome and lead to lesser intestinal inflammation after HSCT. This could in turn lead to lower incidence of acute GVHD and BSI.

Statistical analysis:

Descriptive statistics will be used to present difference in microbiota taxonomic and functional profiles, fecal short chain fatty acids, plasma cytokines, plasma reg-3-α, fecal human DNA, T-cell activation markers and incidences of acute GVHD and BSI at day+100. Our primary efficacy outcome is the difference across subjects in fecal short chain fatty acids before and after supplementation. We will model time as a categorical variable and test for any differences over follow-up. We will also compare the relative abundance of Bifidobacterium across subjects from baseline to after supplementation. We will assess differences in within-patient variation in Bifidobacterium using Flinger-Kileen test of homogeneity of variances. Differences in overall community composition will be explored using the Principal coordinate analysis with Bray-Curtis distance. This will be followed by identification of specific taxonomic and functional microbial features that are associated with 2'-FL supplementation. We will use a linear modeling system adapted for microbial community data and control for the major factors impacting the microbiome such as age, antibiotic exposure and account for multiple samples from the same patient.

Potential Risks, Discomforts, Inconveniences and Precautions:

There are risks to the patients which include allergic reaction to 2FL, and gastrointestinal discomfort (nausea, abdominal pain, indigestion, reflux, diarrhea, constipation, flatulence, rumbling and bloating). 2FL has been safely administered to infants and adults without complications but its safety is not determined in HSCT patients.

Concomitant medication and supportive care guidelines:

Should there be emesis within 30 minutes of taking 2FL, it should be re-administered. However, any emesis after 30 minutes of taking 2FL will not require the supplement to be re-administered.

All standard transplant related concomitant medications and supportive care are permitted with the exception of systemic antibiotics targeting anaerobes during 2FL

supplementation. 2FL will be paused for the duration of the antibiotic and restarted when the antibiotic is discontinued if the discontinuation occurs prior to day+30 after HSCT. This is to ensure additional safety for our immune compromised patients in whom 2FL might also facilitate the growth of pathogenic bacteria in the absence of beneficial bacteria.

Data and Safety Monitoring:

a. Adverse Events: An adverse event is any undesirable sign, symptom, medical condition, or experience that develops or worsens in severity in a subject who has received an intervention (drug, biologic, or other intervention).

b. Adverse Event Monitoring: Safety and tolerability for the study drug will be assessed according to the Common Terminology Criteria for Adverse Events v5.0 The principal investigator or designee will review adverse events closely and assess the event's relationship to study procedures to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study procedures, especially in relation to the underlying disease and baseline lab values for the patient. All adverse events determined to be at least possibly attributable to 2FL administration, regardless of grade, will be recorded on case report forms and reported in compliance with CCHMC IRB and federal guidelines. All expected and unexpected Grade 3 and higher adverse events (except as specified below) that occur starting at the first dose of 2FL up through 30 days following the last dose of 2FL will be recorded on the case report forms, unless directly attributable to the underlying disease. The principal investigator will review each event and assess its relationship to study events to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study therapy.

c. Additionally, for this study, events as previously identified as resulting from administration of a stem cell transplant will be considered as expected. The transplant regimen is well known to commonly affect systems such as the hematologic, immunologic and gastrointestinal systems. The following are known complications of transplant- disseminated intravascular coagulation, febrile neutropenia, hemolysis, thrombotic thrombocytopenic purpura, adrenal insufficiency, colitis, constipation, diarrhea, enterocolitis, gastrointestinal pain, malabsorption, mucositis, nausea, pancreatitis, vomiting, dyspepsia, fever, pain, cholecystitis, allergic reaction, serum sickness, upper respiratory infection, urinary tract infection, weight gain/loss, acidosis, alkalosis, anorexia, dehydration, glucose intolerance, iron overload, generalized muscle weakness, reversible posterior leukoencephalopathy syndrome, bladder spasms, cystitis, hematuria, urinary frequency, urinary urgency, irregular menstruation, pruritis, hypertension, purpura, and petechiae. For the purposes of the study, the listed events that are ≤ grade 3 which are expected toxicities of HSCT will not be considered adverse events.

d. Hematologic toxicities which are attributable to the underlying hematological disease will not be considered adverse events regardless of the grade.

e. In the event that any of these known complications of transplant or hematologic toxicities are thought to be at least possibly attributable to the study drug, the event will be recorded on case report forms and subsequently reported to the FDA and IRB according to current reporting requirements.

f. Adverse reactions must be reported to the principal investigator who is responsible for the reporting to the Cincinnati Children's Hospital Medical Center IRB according to current guidelines. All Adverse Event reporting is to comply with the current CCHMC IRB policy and applicable federal regulations. If any serious adverse events occur, current guidelines will be followed for expedited reporting to the IRB and FDA.

g. All serious and medically significant adverse events considered related to 2FL by the investigator will be followed until resolved or considered stable.

h. Expected events are those that have been previously identified as resulting from administration of the protocol therapy, those that can be contributed to the underlying condition, and/or those associated with transplant. An adverse event is considered unexpected, for reporting purposes only, when either the type of event or the severity of the event is not previously described for 2FL in the consent and/or protocol.

i. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy as determined by the PI.

Risk to unborn fetus:

There are no known adverse effects of 2FL on the unborn fetus. However, appropriate contraception methods are advised for unforeseen events from 2FL.

Risk associated with blood draws:

This study will require research blood to be drawn at specified time points as shown in Table 1. This blood will be drawn from a central line whenever possible and will be combined with routine clinical blood draws whenever possible and will not exceed maximum allowed blood volume for that patient per day per standard of care.

Regulatory Issues:

The protocol and informed consent document for this study will be approved in writing by the Institutional Review Board (IRB) prior to any patient being registered on the study.

Changes to the protocol must also be approved by the IRB. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to FDA inspection at any time during the study.

Informed consent will be obtained prior to initiating study procedures.
This protocol will be performed under an investigator-initiated IND.

Regulatory and Compliance Monitoring:

Monitoring of clinical trials is a continuous, ongoing review of the conduct of the trial to ensure that it is conducted, documented, and reported in accordance with the IRB-approved protocol, Title 21 CFR, the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements of the sponsor, CCHMC.

Specifically, source document verification of eligibility for all enrolled study participants including verification of appropriate documentation of consent will be performed. 100% Source Document Verification (SDV) of case records will be conducted for the first 2 participants enrolled on the study, as well as, approximately 10% randomly selected participants thereafter, by comparing data in the source documents to data entered in the Case Report Forms (CRF). In addition, 100% SDV may be performed for pivotal clinical parameters related to primary study endpoints.

Data Safety Monitoring Plan:

Data Safety Monitoring Board: Three physicians not associated with this protocol will be chosen as the DSMB for this study. The DSMB will meet with the PI or designee and review study data/progress a minimum of every 3 months; more often at the discretion of the PI and/or medical monitor. The DSMB is required to review all unanticipated problems involving risk to subjects or others, unanticipated serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the review. At a minimum, the DSMB should comment on the outcomes of the event or problem, and in the case of unexpected serious adverse event, comment on the relationship to participation in the study. Based on the review of these events, the DSMB should make a recommendation regarding study continuation. All decisions regarding study continuation, modification, or termination will be reported immediately or annually, as appropriate, to the IRB, FDA, and other appropriate agencies. Reports for events determined by either the investigator or medical monitor to be possible or definitely related to study participation and reports of events resulting in death should be forwarded in compliance with current IRB policy and applicable federal regulations.

Privacy:

There is a small risk that protected health information may be released to an unauthorized person. Study personnel will employ standard measures to protect patient privacy and the confidentiality of all patient information. Study subjects will be assigned a unique identification number not related to PHI and the link will be securely maintained by the PI or designee. All data collected will be stored on a secure, password-protected computer, and any paper copies or reports will be stored in a secure location. Access to the files will be limited to the Principal Investigator and designees. No research findings will be reported to individual patients or treating physicians and no identifiable data will be reported in publications.

Process of Obtaining Consent:

One of the study team members will consult with the patient and/or the parent/legal guardian to explain the procedures, risks and benefits of the study at the patient/parent's level of understanding. Opportunity will be given to consider the study and have questions answered. Information will be given in a written format in the form of a description of the study, which will include a signature space for consent to be given. Prior to the initiation of the study, acknowledgement of the receipt of this information and the subject's freely tendered offer to participate will be obtained in writing from each subject in the study. Participation is voluntary, and all subjects/parents/guardians will give informed consent to participate. Assent will be obtained from patients 11-17 years of age. Information will also be given verbally, and all patients and/or parents will have an opportunity to ask questions.

While we do not plan on targeting non-English speaking patients for enrollment on this study, we do periodically have patients who speak Spanish or Arabic. We will not exclude these patients from this study. We will use a Spanish or Arabic short form according to the process outlined in the CCHMC Standard Operating Procedures (SOP) or we will use a fully translated consent form.

The informed consent process may be conducted via telephone, video conference, web conference, etc. according to CCHMC SOP. Due to unforeseen circumstances (i.e. email or fax not readily available), the participant/LAR may not be able to provide a copy of the signed and dated consent to the study team at the time of consent. In this event, the participant/LAR may electronically transfer a copy of the signed and dated consent when he/she is able to, send the signed and dated consent to study staff via mail, or the participant/LAR may bring it with them to a scheduled visit. Once a copy of the signed and dated consent is received, the person obtaining consent will sign and date the document on the day the document is actually received. The reason for the discrepancy in dates will be documented in the informed consent process note and this will not be considered a protocol deviation. No research procedures will occur until the consent document has been received by the study team and applicable signatures and dates obtained.

Funding:

This study will be funded by the St. Baldricks Foundation.

Participant Compensation:

Financial compensation will be provided to patients to facilitate adherence to daily 2FL dosing. Participants will receive $50 on day +7 and an additional $50 on day +30 if they take 60% of their planned doses of 2FL prior to these defined timepoints. Nursing documentation will be utilized when patients are inpatient, and a study diary will be used for checking compliance when patients are outpatient. ClinCard will be utilized for payment. International participants who do not have a social security number or tax identification number will be offered gift cards in the amounts specified above. Subjects may decline to receive compensation. This will be documented in the subject's research chart but will not be considered a protocol deviation.

Future Use:

Data and samples will be stored indefinitely after subject study participation is complete. Data and samples will be retained unless the participant chooses to withdraw. Data and samples could be used for future research endeavors. Data and samples that have already been distributed to researchers prior to withdrawal of consent as well as any data obtained from the distributed data or samples will not be destroyed.

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