Fecal Microbial Transplantation in Relapsing Multiple Sclerosis Patients.

Study Protocol

Protocol Number: MSFMT - 001

Test Product: Fecal Microbiota

Phase: II study

Sponsor: Dr. Michael Silverman

Principal Investigator: Michael Silverman, M.D., FRCP, FACP

Protocol Date: 09 OCT2018

Amendments - Version: 3.0

CONFIDENTIALITY STATEMENT

THE INFORMATION IN THE FOLLOWING DOCUMENT IS PRIVILEGED AND CONFIDENTIAL AND IS PROVIDED TO YOU AS AN INVESTIGATOR, POTENTIAL INVESTIGATOR, CONSULTANT OR CONTRACT ORGANIZATION FOR REVIEW BY YOU, YOUR DESIGNATE, YOUR STAFF AND APPROPRIATE ETHICAL REVIEW COMMITTEE. BY ACCEPTING THIS DOCUMENT, YOU AGREE THAT THE INFORMATION CONTAINED HEREIN WILL NOT BE DISCLOSED TO OTHERS WITHOUT WRITTEN AUTHORITY FROM THE SPONSOR. EXCEPT TO THE EXTENT NECESSARY TO OBTAIN APPROVAL OF THIS PROTOCOL BY AN ETHICAL REVIEW COMMITTEE OR GOVERNMENT AGENCY OR IN THE PROCESS OF OBTAINING PATIENT CONSENT TO PARTICIPATE IN THIS STUDY.

PROTOCOL/AMENDMENT APPROVAL PAGES

Protocol Version: 3.0

Date of Version: 10 SEPT 2018

Authored by:

_____

Signature

_____

Date (dd/mm/yy)

Approved by:

_____

Signature

_____

Date (dd/mm/yy)

ABREVIATIONS

MS multiple sclerosis

CNS central nervous system

RMS relapsing multiple sclerosis

MRI magnetic resonance imaging

FMT fecal microbiota transplantation

TLR toll-like receptor

CD cluster designation

IBD irritable bowels disease

EAE experimental acute encephalomyelitis

PSA polysaccharide

IL interleukin

SCFA short chain fatty acids

Th T helper

KVC kamamicina, colistina and vancomycin

ROR tyrosine kinase-like orphan receptor

CDI *Clostridium difficile* infection

UC ulcerative colitis

DNA deoxyribonucleic acid

RCT randomized clinical trial

SAE severe adverse event

EDSS expanded disability status scale

RN registered nurse

BMI body mass index

HIV human immunodeficiency virus

HTLV human T lymphotropic virus

Ig immunoglobulin

Ag antigen

Ab antibody

TTG transglutaminase

CMV cytomegalovirus

EBV Epstein-Bar virus

MRSA methicillin-resistant *Staphylococcus aureus*

VRE vancomycin-resistant *Enterococcus*

CRE carbapenemase producing *Enterobacteriaciae*

CMRTO College of Medical Radiation Technologists of Ontario

IUD intrauterine device

AST aspartate aminotransferase

ALT alanine transaminase

GM-CSF granulocyte-macrophage colony stimulating factor

TNF tumor necrosis factor

RBC red blood cell

WBC white blood cell

INF interferon

MIP macrophage inflammatory protein

EDTA Ethylene Diamine Triacetic Acid

PBS phosphate buffered saline

RNA ribonucleic acid

PCR polymerase chain reaction

TGF transforming growth factor

AE adverse events

CRF clinical research form

REB research ethics board

IEC independent ethics committee

GCP good clinical practice

TABLE OF CONTENTS

1. **Chris H Polman, Stephen C Reingold, Brenda Banwell, Michel Clanet, Jeffrey A Cohen, Massimo Filippi, Kazuo Fujihara, Eva Havrdova, Michael Hutchinson, Ludwig Kappos, Fred D Lublin, Xavier Montalban, Paul O'Connor, Magnhild Sandberg-Wollheim, Alan J Thompson, Emmanuelle Waubant, Brian Weinshenker, and Jerry S Wolinsky. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol. 2011 Feb; 69(2): 292–302. 4849**

CLINICAL STUDY SYNOPSIS

Funding: Chapman Chair in MS Clinical Research - LHSC and LHSF	Investigator: Marcelo Kremenchutzky
Name of test product: Fecal microbiota	
Title of the study: **Fecal Microbial Transplantation in Relapsing Multiple Sclerosis Patients**	

Principal Investigator and study center:

Michael Silverman, M.D., FRCP, FACP

St. Joseph's Health Care London

268 Grosvenor St.

London Ontario

N6A 4V2

Clinical phase: II

Primary objectives:

- To evaluate effects of fecal microbial transplant (FMT) from healthy donors on biological biomarkers as peripheral blood cytokines levels of relapsing multiple sclerosis [RMS] patients from baseline to up to 12 months

Secondary objective:

- To evaluate the safety of FMT in MS patients
- To evaluate tolerability of FMT in MS patients

Trial Design:

The proposed randomized, open label, controlled [treatment as usual control group], crossover study will be conducted in 40 patients (n=20 per group) diagnosed with the relapsing forms of multiple sclerosis according to the McDonald 2010 Criteria[1].

Based on the diagnostic criteria patients will be further screened to confirm eligibility and if eligible then consented.

Patients will be randomized 1:1 into two groups. One group will receive FMT, done by rectal enema administered by a RN, at baseline, month 1, month 2, month 3, month 4, and month 5 (early intervention group). The other group will receive treatment as per standard of care (treatment as usual control group) in the beginning of the study. For the last 6 months they will also receive FMT at month 6, month 7, month 8, month 9, month 10, and month 11 (late intervention group).

Clinical visits for both groups will be performed at baseline, and months 1 to 12 inclusive. During these visits a physical exam with vital signs, including a standardized neurological assessment Expanded Disability Status Scale – EDSS) will be done.

Patient's stools will be collected before each FMT procedure in both groups to evaluate gut microbial composition.

Urine will be collected to evaluate gut permeability at baseline, 6 and 12 months for both groups.

Peripheral blood samples also will be collected to evaluate circulating cytokine levels (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL- 15, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, GM-CSF, IFNγ, MIP-3α, TNFα and TNFβ) at baseline, months 1 to 12 inclusive for both groups.

Peripheral blood samples will also be collected to evaluate blood DNA bacteria at baseline, and months 1 to 12 inclusive for both groups.

Brain MRI with gadolinium [3T MRI machine at Robarts Research Institute] will be performed at baseline, 6 and 12 months for both groups as a safety biomarker [to rule out paradoxical effects; i.e. significant increase in MS activity as defined by Cutter's rule: 5 or more newly enhancing white matter lesions in the brain compared to the previous scan].

In the event of early termination, a visit will be performed whenever possible, including a final blood sample and all end of treatment assessments that should be completed as soon as possible. All patients with at least 1 week of treatment will be

eligible for analysis.

Number of Patients:

A maximum of 40 patients will be enrolled in intervention part of this study and randomized 1:1 into one of the two groups [20 patients per group] using an internet based randomizer software [https://www.randomizer.org/]. We may screen up to an extra 10 patients to compensate for possible drop outs, withdraw consent or those who discontinued for any other reason.

We will also recruit up to 10 healthy volunteers to provide a blood sample [1 tube, 5 ml] to serve as a reference arm for the cytokine analysis. This reference group will be seen only once and will not participate in any other study activities.

Consequently, the total number of participants in this study will be n=50.

Inclusion criteria for patients:

1. Have a confirmed diagnosis of relapsing MS defined by the 2010 Revised McDonald Criteria for the Diagnosis of Multiple Sclerosis[1]
2. Have a baseline Expanded Disability Status Scale (EDSS) ≤ 7.0
3. Age equal or older than 18 years of age
4. Be able to attend all clinic appointments without interruption
5. Be proficient in English
6. Be willing and able to give written informed consent
7. Negative blood pregnancy test at screening

Exclusion criteria for patients

1. Not meeting all of the above inclusion criteria
2. Pregnancy [current or planned for the near future] or breastfeeding
3. All qualified patients will not be currently or recently [90 days] treated with immunosuppressive therapy [i.e. chemotherapy] or high dose corticosteroids [i.e. 1g of prednisone or greater or equivalent]. Immune-Modulatory therapies are allowed as per standard of care.
4. Ongoing use of antibiotics
5. Presence of a chronic intestinal disease e.g. celiac, mal-absorption, colonic tumor
6. Concomitant inflammatory diseases
7. Standard of care exclusions for MRI scans as per LHSC standards

Healthy controls **Selection:**

1. Age equal or older than 18
2. Written informed consent
3. Self reportedly “healthy”, in particular not having a history of any disease or

disorder, or any treatment ever for such [ie lupus, cancer, etc], that in the opinion of the PI could be possibly related to changes in the immune system that would alter the results of a cytokine in blood analysis.

Test product, dose and mode of administration, batch number to be provided by Dr Silverman's Microbiology lab at St. Joseph's Health Care, London, ON. For this study, fecal samples from healthy donors will be screened as per Health Canada guidelines. About 50-70g of the fecal sample is blended in 220ml saline in a Stomacher and filtered and transferred to an enema bag for transplantation. From here on this is known as "FMT" or "study medication".

Lot no. XXXXXX

Duration of treatment/study:

FMT rectal enema will be administered as scheduled per group, for up to 6 months.

Criteria for evaluation:

- Circulating cytokines levels (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, GM-CSF, IFNγ, MIP-3α, TNFα and TNFβ).
- Gut microbiome through feces analysis
- Blood DNA bacteria
- Gut permeability through urine analysis
- All adverse events occurring during this study will be recorded. MS clinical functional status will be monitored using EDSS to assess any changes during the treatment period, as well as head MRI as a safety surrogate.

Statistical methods:

Adverse events will be coded using MedDRA dictionary. Incidence of adverse events by the severity, relationship to treatment, and outcome will be provided. Narratives of all serious adverse events and events causing death will be included. Laboratory parameters, vital signs and other safety parameters will be documented with the use of descriptive statistics.

1. GENERAL INFORMATION

Protocol Number and Title of the Study

Fecal Microbial Transplantation in Relapsing Multiple Sclerosis Patients.

Sponsor

Dr. Michael Silverman

Signature Authorization

Clinical Study Sites

London Health Sciences Centre

University Hospital

339 Windermere Road

London, Ontario, N6A 5A5

Robarts Research Institute

Western University

1151 Richmond St. N

London, Ontario, N6A 5B7

St. Joseph's Health Care London

268 Grosvenor St.

London, Ontario, N6A 4V2

Dean Cumming School of Medicine

University of Calgary

2500 University Dr NW,

Calgary, Alberta, T2N 1N4

Clinical Trial Leader

Michael Silverman, M.D., FRCP, FACP

Investigator

Michael Silverman, M.D., FRCP, FACPTel.: +1 519-646-6311

Email: Michael.silverman@sjhc.london.on.ca

Clinical Assessment

Sarah Morrow, M.D., FRCPC

Lynn McEwan, MS NP

Clinical Neurological Science Department
University of Western Ontario
London, ON, Canada

Cytokines Analytical Laboratory

Steven M Kerfoot, PhD
Assistant Professor

Kate Parham, PhD
Postdoctoral Associate

Department of Microbiology and Immunology
University of Western Ontario
London, Ontario, Canada

Microbiome Analysis

Jeremy P. Burton, BSc, MSc, PhD (Otago), dBA
Division of Urology, Department of Surgery/ Department of Microbiology & Immunology,
Lawson Health Research Institute and Canadian Centre for Human Microbiome and Probiotics

University of Western Ontario
London, Ontario, Canada

Urine Analytical Laboratory

Dr Jon Meddings, MD FRCPC
Dean Cumming School of Medicine
University of Calgary, Calgary, Alberta, Canada

Stool Analytical Laboratory

Michael S. Silverman, MD, FRCP

Seema Nair Parvathy, PhD

Department of Medicine, Division of Infectious Diseases
University of Western Ontario
London, Ontario, Canada

Biostatistics and Data Management

Prof Yves Bureau, PhD
Assistant Professor of Medical Biophysics Department
University of Western Ontario
Lawson Health Research Institute
London, Ontario, Canada

2. BACKGROUND INFORMATION

Multiple sclerosis (MS) is an autoimmune, inflammatory, demyelinating disease of the central nervous system (CNS) influenced by genetic susceptibility and some environmental factors not yet fully understood[3]. Relapsing forms of MS (RMS) are generally characterized by clearly defined periods of worsening neurological symptoms [i.e. relapse] followed by full or partial recovery [i.e. with sequelae and residual deficits upon recovery]. Relapsing forms of MS

include relapsing remitting or secondary progressive. In RMS, there is no clinically obvious disease progression during the periods between disease relapses[4].

Relapses are typically treated with a high dose of glucocorticoids over a short period of time [3-5 days]. Certain immunomodulatory agents, including interferon beta preparations, glatiramer acetate, natalizumab, fingolimod, dimethyl-fumarate, alemtuzumab and teriflunomide, have shown beneficial effects for patients with RMS (decreased relapse rate and a slower accumulation of brain lesions on MRI). However, these treatments may be associated with significant toxicity and limited efficacy over time[4].

2.1.Pathophysiology of Multiple Sclerosis and gut bacteria

The gut microbiome has recently emerged as a potential contributor to MS pathogenesis[5,6]. Previously reviewed data from murine model of autoimmune demyelinating disease[7,8] suggest that gut microbiota can powerfully modulate immune-mediated demyelination. Moreover, studies involving MS patients have shown that their microbial composition is different compared to healthy controls[9,10,11] and this could be related to disease development and activity[12]. Fecal microbial transplantation (FMT) from healthy donors is already used to restore normal fecal floral and treat colitis caused by *Clostridium difficile*. Therefore, we proposed that fecal microbial transplantation from a healthy donor could change the fecal flora composition of RMS patients, have a measurable effect on biomarkers of inflammation and possibly influence clinical or MRI disease activity.

We conducted a review about gut microbiome and MS accepted for publication (Mult Scler 2016 Dec 12. Breaking down the gut microbiome composition in multiple sclerosis).

2.2.Description of Fecal Microbial Transplantation

Fecal microbiota transplantation is the process of **fecal** bacteria transfer from a healthy donor into a recipient. The goal is to restore **colonic microflora** by transplanting healthy bacterial flora (e.g. by **enema**, **orogastric tube** or by mouth in the form of a capsule containing **freeze-dried** material)[12]. For this study we selected the enema that is a lower risk procedure compared to an upper tract endoscopy and more easily accepted for patients than an oral capsule of fecal bacteria. An enema will be done to infuse the study treatment into the **lower bowel** through the **rectum** by a trained nurse.

2.3. Summary of literature on MS and Gut Microbiome

There is a growing body of evidence that gut microbiota goes beyond classical physiological functions such as vitamin production, digestion of nutrients, or protection against pathogens. Under normal physiological conditions, the human gut microbiota is a homeostatic ecosystem.

Disruption of this equilibrium can result in what is known as "dysbiosis" and has been associated with several conditions, such as gastrointestinal diseases, obesity, diabetes, chronic periodontitis, vaginosis, atopic diseases, non-alcoholic steatohepatitis, and Alzheimer's disease[13].

Some studies have shown that MS patients have an alteration in their microbiota composition compared to healthy individuals. In a pilot study of the gut microbial in MS, Cantarel et al. found decreased *Faecalibacterium* among seven RMS patients compared to eight controls[14]. Miyake et al. compared 20 Japanese RMS patients to 40 controls and similarly found that that there was a depletion of species belonging to butyrate-producing *Clostridium* clusters XIVa and IV, including *Faecalibacterium prausnitzii* and *Eubacterium rectale*[14], among MS patients. Chen et al. found depletion of the butyrate-producing family *Erysipelotrichaceae*, which belongs to *Firmicutes*, among 31 patients with RMS compared to 36 controls[15]. Interestingly, however, the authors also noted an increased abundance of *Blautia* and *Dorea* genera, both belonging to *Clostridium* cluster XIVa, among their RMS patients[15]. Tremlett et al. also noted a depletion of butyrate-producing *Faecalibacterium*, *Lachnospiraceae, Ruminococcaceae* and *Butyricimonas* among 18 cases of early pediatric MS compared to 17 controls[16]. Two observational studies, the first by Gandhi et al. and the second by Jangi et al., reproducibly identified a reduction in the *Butryicimonas* genus among MS patients, which belongs to the *Bacteroidetes* phylum but is capable of producing butyrate[17,18]. In another pediatric study, Tremlett et al. followed 17 RRMS patients over a mean of 19.8 months and found that depletion of *Fusobacterium* genera, belonging to the *Fusobacteria* phylum, was associated with increased relapse risk[19]. Although presence of *Fusobacteria* has been associated with autoimmunity, including juvenile idiopathic arthritis and ulcerative colitis, *Fusobacterium* is also a butyrate producer[20-22]. The depletion of butyrate-producing bacteria in MS suggests that this pro-inflammatory alteration of the gut microbiota may be linked to disease pathogenesis.

Rumah et al. identified the first RMS patient with epsilon toxin (ETX)-producing *C. perfringens* Type B in her GI tract[23]. ETX is produced by *Clostridium perfringens* types B and D[35]. It disrupts the blood-brain barrier and binds myelin, leading to its consideration as a potential MS trigger[35]. The authors found reduced non-toxigenic *Clostridium perfringens* Type A in the gut microbial of MS patients, and suggest this may create an ecological niche for ETX-producing *Clostridium perfringens*[24]. Among their MS patients, 10% had antibodies specific to ETX in CSF and plasma compared to only 1% of controls, indicating increased immune system exposure to ETX among MS patients[24].

Furthermore, Miyake et al. found reductions of *Bacteroidetes,* including several *Bacteroides* species and *Prevotella copri*, among their RMS patients[16]. Similarly, Baum et al. compared 54 patients with MS to healthy controls and found reduced *Bacteroidaceae,* a family belonging to *Bacteroidetes*[26]. Tremlett et al. also found decreased *Bacteroidaceae* among their patients with early pediatric MS[27]. The relevance of these findings may relate to Lipid 654, a bacterially-derived lipodipeptide produced by a number of *Bacteroidetes* that was found to have lower serum levels among MS patients compared to healthy controls and Alzheimer's patients[15, 27].

Lipid 654 binds toll-like receptor 2 (TLR2)[28]; although TLRs are critical to innate immune system activation, low-level tonic TLR2 stimulation by Lipid 654 may be immunoregulatory via maintenance of immune system tolerization[28,29]. Decreased Lipid 654-producing *Bacteroidetes* may therefore be pro-inflammatory, again plausibly linking the gut microbial to MS immunopathogenesis.

Cekanaviciute et al. identified decreased *Parabacteroides distasonis*, which belongs to *Bacteroidetes,* among 51 MS patients compared to 55 controls[29]. Similarly, Chen et al. identified decreased *Parabacteroides* as well as *Prevotella* among their patients with RRMS compared to controls[30]. Oral administration of *Parabacteroides distasonis* antigens attenuates experimental murine colitis, with evidence of an immunomodulatory effect on cytokine production and increased mesenteric lymph node CD4+ T-regulatory cells[19]. Cekanaviciute et al. found *Parabacteroides distasonis* also augmented the CD4+ T-regulatory cell phenotype, suggesting that it may be linked to MS immunopathogenesis via its influence on CD4+ T-regulatory cell production[31].

Two studies, the first by Gandhi et al. comparing 53 patients with MS to 44 healthy controls, and the second by Jangi et al. comparing 60 patients with MS to 43 healthy controls, reproducibly found increased *Methanobrevibacter* in the gut microbial of MS patients[18,19]. Jangi et al. also identified higher breath methane among a cohort of MS patients compared to controls[19], which may be due to increased gut *Methanobrevibacter*. However, the association between increased methane production and constipation is a potential confounder that should be controlled for in future studies, given the high prevalence of constipation in MS[32, 33]. MS patients with antibody reactivity to *Methanobrevibacter*-derived lipids had increased T-cell expression of pro-inflammatory cytokines, and increased *Methanobrevibacter* was associated with reactivity to a myelin sheath component[19]. These findings suggest a link between *Methanobrevibacter* and T-cell auto reactivity causing inflammation and demyelination in MS.

Several other differences in the gut microbiota have been identified in MS. Cekanaviciute et al. found that MS patients had increased *Acinetobacter calcoaceticus*, which belongs to *Proteobacteria*[31]. Molecular database analysis identified similarity between *Acinetobacter calcoaceticus* and an encephalitogenic myelin peptide[34,35,]. Elevated anti-*Acinetobacter* antibodies have also been found in MS patients, implicating molecular mimicry as a potential immunopathogenic link between *Acinetobacter* and MS[35,36]. Cekanaviciute et al. identified a reduction in CD4+ T-regulatory cells after T-cell stimulation with *Acinetobacter calcoaceticus*, suggesting it may also promote autoimmunity by impairing T-regulatory cell function[31].

Chen et al. found increased abundance of a number of *Proteobacteria* among their patients with RMS, including *Pseudomonas* and *Mycoplana*[27]. *Proteobacteria* have been associated with IBD by driving inflammation and promoting gut dysbiosis, which may contribute to the development of other autoimmune diseases such as MS[37].

Jangi et al. found increased *Akkermansia* among MS patients, which belongs to the phylum *Verrucomicrobia*[19]. *Akkermansia muciniphila* has been found to facilitate infection and inflammation by *Salmonella Typhimurium* in gnotobiotic mice[38]. In contrast, however, reduced *Akkermansia muciniphila* has been observed in IBD and metabolic syndrome[39]. The implications of increased *Akkermansia* in MS are therefore unclear; there may be a direct immunopathogenic relationship, *Akkermansia* may indirectly facilitate the growth of other pro-inflammatory bacteria, or other mechanisms may contribute. Jangi et al. also found that increased *Akkermansia* and *Methanobrevibacter* positively correlated with expression of genes among T-cells and monocytes implicated in MS pathogenesis; in contrast, butyrate-producing *Butyricimonas* was inversely correlated with these genes, suggesting that gut microflora may contribute to MS pathogenesis by polarizing to an inflammatory rather than immunomodulatory T-cell and monocyte phenotype[19].

Baum et al. noted increased *Bifidobacterium* among MS patients, which belong to *Actinobacteria*[31]. Tremlett et al. similarly found an increase in *Bifidobacterium* among patients with early pediatric MS compared to controls[20]. *Bifidobacterium,* commonly administered as probiotic therapy, has been linked to IBD[40]. However, *Bifidobacterium animalis* and a probiotic mixture including *Bifidobacterium bifidum* significantly ameliorated EAE activity, with evidence of inhibited Th1/Th17 polarization and regulatory T-cell induction[41,42]. The reason for this conflicting data regarding *Bifidobacterium* in demyelinating disease is unclear; further study into the potential role of *Bifidobacterium* and other probiotic therapy in MS is needed.

Finally, fecal microbial transplantation is used to treat patients with recurrent *Clostridium difficile* infection (CDI), an antibiotic-associated bacterial colitis that may be life-threatening[43,44]. Antimicrobial therapy disrupts normal intestinal microflora and facilitates CDI; transplantation of healthy donor stool is believed to restore fecal microbial diversity in the recipient and prevent CDI recurrence[43,44]. As evidence of gut dysbiosis in MS mounts, FMT from donors free of autoimmune disease may be a consideration to restore microbial balance in those patients. Borody et al. reported neurologic benefit in three MS patients who underwent FMT for constipation[45]. The neurologic improvement was dramatic, including recovery of bowel and bladder function and regained ability to walk. Nevertheless, enthusiasm for these findings is tempered by the absence of controls, the diagnosis of 'atypical' MS in two patients, and the unclear biologic plausibility regarding how FMT would reverse established MS disease burden. On this ground, we propose that fecal microbial transplantation from healthy donors to MS patients could restore microbial gut balance and ultimately influence disease activity.

2.4 Pre-clinical studies

Previously reviewed animal research has examined the role of the gut microbiota in experimental autoimmune encephalomyelitis (EAE), a murine model of autoimmune

demyelinating disease[46]. It was shown that germ-free mice devoid of gut microbiota are resistant to EAE, but generally immunodeficient[47,8].

The zwitterionic capsular polysaccharide A (PSA) produced by *Bacteroides fragilis* was shown to be protective against EAE, with evidence of a PSA-dependent decrease in the pro-inflammatory cytokine IL-17 and enhanced conversion of IL-10-producing CD4+ T-regulatory cells[16]. PSA-mediated induction as well enhanced immunosuppressive function of human T-regulatory cells has also been shown *in vitro*[30,49]. PSA uniquely exemplifies a single gut microbial-derived product that has reproducibly demonstrated anti-inflammatory properties and seems protective against immune-mediated demyelination, suggesting therapeutic potential in MS[30,49]. Furthermore, *Firmicutes* producing the SCFA butyrate are increased in patients assigned to a plant-based rather than animal-based diet, and such SCFAs may have a beneficial anti-inflammatory effect in EAE and MS[50,51].

Some probiotic bacteria have been shown to improve EAE, with less pro-inflammatory and increased CD4+ T-regulatory cell responses[41,42,52-55]., *Bifidobacterium animalis* and a probiotic mixture including *Bifidobacterium bifidum* significantly ameliorated EAE activity, with evidence of inhibited Th1/Th17 polarization and regulatory T-cell induction[41,42].Whether there is any benefit to dietary probiotic supplementation in MS, however, is unknown, and caution may even be warranted given increased *Bifidobacterium* was identified in some MS gut microbiomestudies for example.[26,31]

The use of antibiotics to reduce the gut bacterial load is protective against EAE, with reduced pro-inflammatory cytokine production and enhanced CD4+ T-regulatory cell proliferation[30,48]. Yokote[56] et al. studied the possible effects of altering gut flora by antibiotic treatment on EAE. First, mice treated with kamamicina, colistina and vancomycin (KCV) had a change in their gut flora compared to the control group proved by DNA microarray analysis after KCV. The experimental mice group was treated starting one week before immunization. After the EAE induction, the experimental group had less clinical manifestations, less mononuclear cells infiltrations on lumbar spinal cord and reduced demyelination compared to the control group. Moreover, they also studied the cells response in vitro with antigen stimulation. Interestingly, the proliferation response of the mononuclear cells were not affected in the experimental group but they produced less inflammatory cytokines (IL-6 and 17) and showed a reduced expression of ROR, an important transcription factor for Th17, together with enhance production of IL-10.

In summary, animal models suggest that the gut microbiota can powerfully modulate immune-mediated demyelination. Moreover, that inflammatory cytokines can be influenced by microbiota changes in a measurable fashion.

2.5 Rationale for the use of FMT for the treatment of multiple sclerosis patients

Gastrointestinal disorders have more data published in the literature on this matter. A recent systematic review by Chapman et al. of FMT for CDI included 29 studies, 2 of them randomized control trials, 4 prospective and 23 retrospective. The authors concluded that FMT is a highly effective therapy for refractory or recurrent CDI with a success rate of 83-100% with an acceptable safety profile[57]. Another systematic review and meta-analysis by Sun et al. of FMT for ulcerative colitis (UC) identified 2 RCTs, 1 open-label case-control study, and 8 non-control cohort studies about the efficacy and safety of FMT in UC patients. They concluded that FMT may be an efficacious and safe alternative therapy for UC, at least when the standard therapy has failed or is unacceptable[58].

FMT has also been studied in metabolic disorders by Vrieze et al. This was a double-blind, randomized controlled study where half of the 18 male patients with metabolic syndrome were transplanted their own feces (control group), while the other half were transplanted feces from thin donors (experimental group). The experimental group showed significant increase in insulin sensitivity after six weeks from FMT[59].

Fecal matter transplantation in patients has been tested in a few medical conditions. Most reports consist in open label or non-controlled studies with a small number of subjects. Moreover, the fecal matter transplant may vary the way the transplant is prepared as the route of administration which makes comparisons even harder. Even though more robust evidence about the efficacy and application of this procedure is lacking, one can conclude that FMT is feasible and safe for a phase II clinical study.

The rationale for treatment of MS patients with FMT in a phase II study is to restore a gut microbiome composition to one similar to a healthy person, and measure its possible effects in biomarkers as pro and anti-inflammatory cytokines related to MS pathophysiology. We plan to further study gut bacteria interaction in MS by DNA blood bacteria and gut permeability analysis.

2.6 Patient population and study rationale

This is essentially an immunological study, and therefore patients with RMS, regardless of the disease duration, will be enrolled in this study. They will be in a standard of care treatment for their MS and may or may not be on treatment with disease modifying therapy as per their own individual case. On the other hand, patients must be off of systemic high dose corticosteroids for at least 90 days.

The primary aim of this study is to determinate if FMT can affect the immunological cytokine profile of MS patients. We also aim to evaluate the safety and tolerability of this treatment in this population. To our knowledge, a study like this has not yet been published anywhere else in the world.

2.7 Potential risks for FMT and study procedures

Two randomized control Trials (RCTs) reported mild adverse events attributed to FMT, including diarrhea, cramping, belching, nausea, abdominal pain, bloating, transient fever, and dizziness [41]. Serious adverse events were also described, including a case of Fournier gangrene[61], but none were attributed to the FMT.

Eight case-series studies (reporting on 70 patients) did not explicitly mention harms; all others specifically commented on harms (or lack thereof). Possible procedure-related harms, including micro perforation with colonoscopy[62] and gastrointestinal bleeding[63],peritonitis[64], and pneumonia[64] with use of the upper gastrointestinal tract route, were rarely reported. One case-series study reported on FMT use in 80 immuno compromised patients[65]. Although serious adverse events (2 deaths and 10 hospitalizations) occurred in 12 patients (15%); 4 were considered related to FMT, and 5 were possibly related.

Because rare adverse events may be first reported as a case report, we examined case reports for such events. One report[66] described a patient with abdominal pain and hypotension 3 days after FMT by means of a gastro jejunostomy tube placed through an indwelling gastric tube. The patient had pneumoperitoneum, toxic megacolon, and polymicrobial bacteremia and subsequently died. Long-term data on harms were not reported.

According to published articles, transient adverse responses after FMT have been reported, including mild fever, abdominal pain, diarrhea, exhaust, flatulence, and fatigue. However, these adverse effects are self-limiting. De Leon et al[66] reported a UC patient quiescent for more than 20 years who developed a flare of UC after FMT. This case gives us cautionary information concerning FMT being used to treat CDI with UC. Moreover, a recent paper reported a UC patient who had a cytomegalovirus infection after performing home FMT without donor screening[67]. As extracts of feces are mediators between the donor and recipient, FMT has the potential for transmitting occult infections even when strict donor screening is performed.

More recently Wang et al published a systematic review on safety of FMT. A total of 50 studies including original full-text articles, letters to the editor, abstracts of scientific conferences, case reports and case series which were published between 1913 and 2015 were reviewed. Totally 78 kinds of AEs were revealed with a total incidence rate of 28.5%. Among the 42 publications, 5 kinds were definitely and 38 kinds were probably related to FMT. The commonest FMT attributable AE was abdominal discomfort, which was reported in 19 publications. For upper gastrointestinal routes of FMT, 43.6% (89/204) patients were compromised by FMT-attributable AE, while the incidence dropped to 17.7% (76/430) for lower gastrointestinal routes. In contrast, the incidences of serious adverse events (SAEs) were 2.0% (4/196) and 6.1% (40/659) for upper and lower gastrointestinal routes, respectively. A total of 44 kinds of SAEs occurred in 9.2% patients, including death (3.5%, 38/1089), infection (2.5%, 27/1089), relapse of inflammatory bowel diseases (0.6%, 7/1089) and Clostridium difficile infection (0.9%, 10/1089).

The paucity of RCTs is notable. As a result, there is a dearth of high-quality evidence to guide clinicians and policymakers on how to apply this promising but largely untested therapy. The overall low quality of the available evidence evaluating FMT is important and indicates that additional research is needed. However, the information available suggests that FMT can be safely done.

3. STUDY OBJECTIVES AND PURPOSE

This is a prospective, cross-over, open label, randomized 1:1 early vs delayed groups, interventional [FMT] vs treatment as usual controlled trial to explore the effects of FMT from a healthy donor to RMS patients and investigate whether this can influence disease activity based on a panel of biological markers.

3.1 Primary outcome

To evaluate if FMT from healthy donors can significantly change cytokine profile in peripheral blood samples of RMS patients.

3.2 Secondary outcomes

We are also aiming to evaluate if FMT can possibly alter gut permeability through urinalysis and peripheral blood DNA bacteria. We also aim to evaluate treatment safety using the Expanded Disability Status Scale (EDSS) and MRI as surrogates for any paradoxical worsening in MS disease activity. We are monitoring adverse events to assess tolerability.

4. STUDY DESIGN

The proposed randomized, open label, with treat as usual control group, crossover phase II study will be conducted in 40 patients (n=20 per group) with the relapsing forms of multiple sclerosis according to the McDonald 2010 Criteria (Pollman 2011).

Patients will be randomized into 2 intervention groups. One will receive the FMT from baseline and for the first 6 months (early intervention group). On the other hand, the other group will be a control group during the first 6 months and will receive the FMT for the last 6 months of the study (fFigure 1). The schedule of study procedures and evaluations for both groups is given in Tables 1 and 2. Patients will be screened for eligibility based on MS diagnosis and EDSS and if eligible then consented. All qualified patients will not be currently or recently treated with high dose steroids.

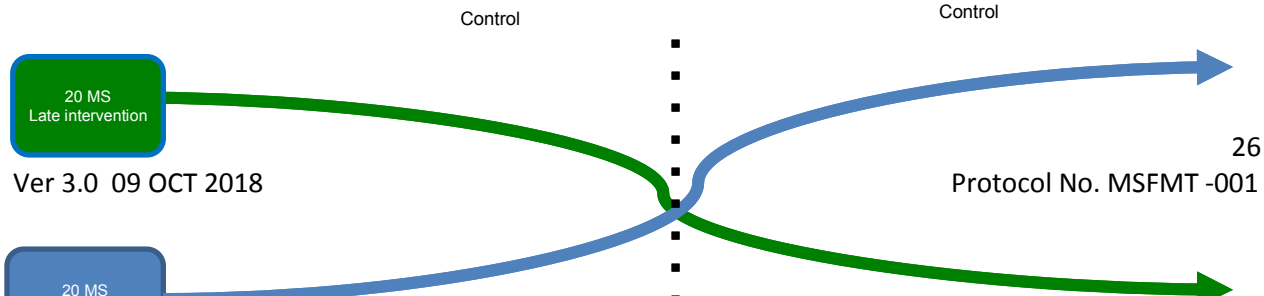


Figure 1. Study Design

At Visit 1, before FMT, patients will be evaluated for their vital signs, medical history and concomitant medications. Also before transplantation, patient's stool will be collected to study their microbial profile, blood analysis to evaluate cytokines levels as well as blood DNA bacteria and finally, urinalysis to assess gut permeability (baseline).

Stool samples for all microbiome analysis will be collected in the form of a used toilet paper packed in a black pouch and transferred in ambient conditions to the laboratory at the Lawson Health Research Institute at St. Joseph's Hospital and stored at -80 °C until the time of DNA extraction.

Peripheral blood will also be collected by standard technique venipuncture by a venipuncture certified and experienced RN. Cytokines levels will be assessed by Luminex in the Department of Microbiology and Immunology at the University of Western Ontario and blood DNA bacteria will be evaluated in St. Joseph Microbiology Laboratory.

For gut permeability patients will be instructed to drink lactulose solution and collect the urine throughout the night and first thing in the morning. A proper collecting bottle will be provided. Once the urine sample bottle reaches the laboratory at St. Joseph's Hosptial, London, Ontario the total volume will be measured and an aliquot of 30ml total, 10mL in each sterile urine container (no other additives) will be separated and stored at -20C and sent on dry ice to Dr. Meddings laboratory at Calgary, Alberta. All biological material will be transported according to biosafety regulations.

Patients will also undergo a contrast-enhanced brain MRI scan at Robarts Institute in London Ontario. Other baseline assessments (prior to the first dose of therapy) include an EDSS, pregnancy test (if applicable), and physical exam. Blood samples are also taken at this visit in order to establish a baseline for routine chemistry/hematology. After all this assessments FMT will be performed by a trained nurse via a rectal enema

Patients will return to the clinic for visit 4 weeks after the first FMT. Another stool sample to evaluate the microbial before the second FMT will be collected. Also peripheral blood samples for cytokines and blood bacterial DNA analysis. This same routine procedure will be

repeated at month 2, 3, 4 and 5 for the early intervention group and at month 6, 7, 8, 9, 10 and 11 for the late intervention group. Both groups will come to clinic at baseline andmonth 1 to 12 inclusive, even if they are not receiving FMT. During all visits, a stool sample, a cytokine sample and a blood DNA bacteria sample will be collected. It is worth mentioning that at 6 and 12 months another urine analysis will be done to evaluate gut permeability and MRI study at Robarts Institute, London, Ontario for safety assessment will be performed for both groups.

Another safety assessment in each visit after FMT is to review of any adverse events that may have occurred. All patients receiving at least 1 week of treatment will be eligible for analysis.

Table 1. Early Intervention Group Schedule

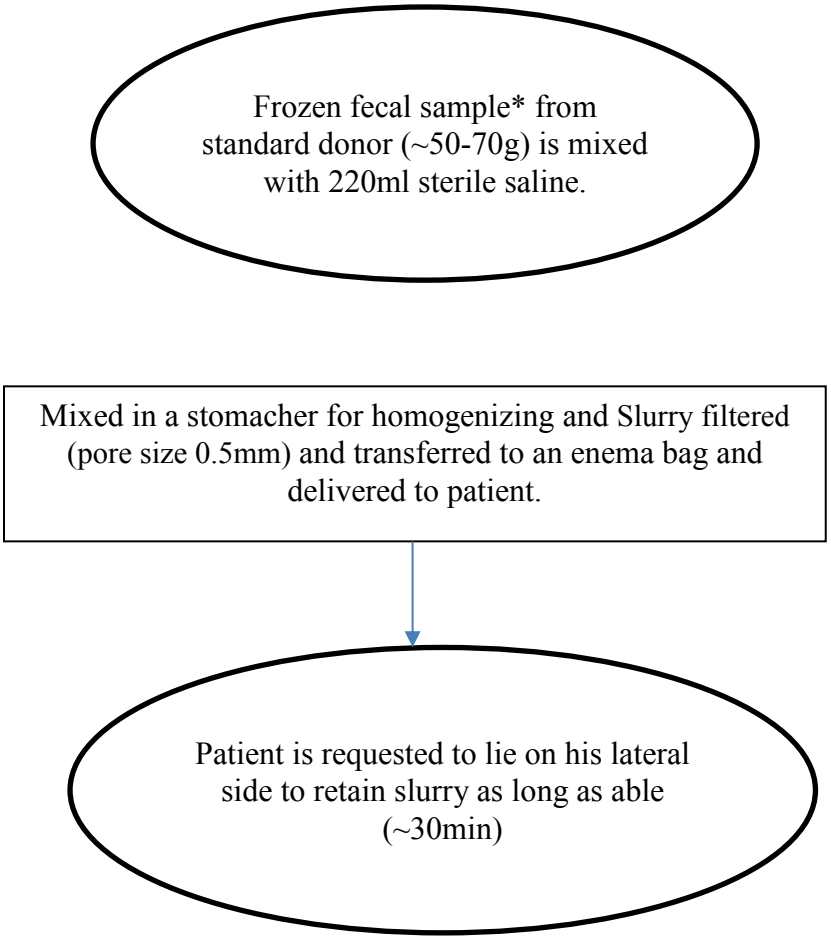
Study Months	Pre-Visit	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Visit Duration	1.5hours	1.5hours	1.5hours	1.5hours	1.5hours	1.5hours	1.5hours	45min	45min	45min	45min	45min	45min	45min
Informed Consent Form	X													
Fecal Transplantation		X	X	X	X	X	X							
Height		X												
Weight, Vital Signs		X	X	X	X	X	X	X	X	X	X	X	X	X
Stool Sample		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine: Gut Permeability		X						X						X
Circulating Cytokine Levels and Blood DNA		X	X	X	X	X	X	X	X	X	X	X	X	X
MRI		X						X						X
Routine Hematology and Urinalysis		X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam/ EDSS		X	X	X	X	X	X	X	X	X	X	X	X	X

Table 2. Late Intervention Group Schedule

Study Months	Pre-Visit	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Visit Duration	1.5 hours	45mins	45mins	45mins	45mins	45mins	45mins	1.5hours	1.5hours	1.5hours	1.5hours	1.5hours	1.5hours	1.5hours
Informed Consent Form	X													
Fecal Transplantation								X	X	X	X	X	X	
Height		X												
Weight, Vital Signs		X	X	X	X	X	X	X	X	X	X	X	X	X
Stool Sample		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine: Gut Permeability		X						X						X
Circulating Cytokine Levels and Blood DNA		X	X	X	X	X	X	X	X	X	X	X	X	X
MRI		X						X						X
Routine Hematology and Urinalysis		X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam / EDSS		X	X	X	X	X	X	X	X	X	X	X	X	X

4.1 Rationale for FMT preparation

The processing of stool samples for FMT via enema is shown in the chart below**.** Sample processing shall be done in sterile conditions in Biosafety cabinet level[67].



FMT will be administered via rectal enema. Microflora will be studied from stool samples collected prior to administration of the FMT and will be stored at -80C. Before and after procedure the patients will be tested as per the chart given above. Data than shall be recorded periodically and analyzed for statistical significance.

4.2 Duration of therapy and study

Patients will be screened prior to starting treatment with FMT and randomized between 2 groups. One, early intervention group, will receive FMT for the first 6 months and the other will be a control group treated as standard only. After 6 months, the early intervention will not receive the transplantation anymore and the control group will receive FMT for the remaining 6 months. Study drug will be administered for a period of 6 months for each group total.

Patients may withdraw consent at any time. Study drug may be discontinued at any time by the investigator if doing so is deemed to be in the best interest of the patient. However, the patient should be encouraged to continue in the study for safety follow-up.

4.3 Study Termination

The Investigator reserves the right to terminate this study at any time. In terminating the study, the Investigator will ensure that adequate consideration is given to the protection of the patients' safety and interests.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1 Donor Screening Criteria

Donors will be selected according to a standard protocol (appendix A). This is included in this protocol for information only. Donors will have been already selected and material will be already processed by Dr. Silverman's Microbiology lab at St Joseph's Hospital, London, Ontario, as per their standard of care.

5.2 Patient selection

5.2.1 Patient Inclusion criteria

- Have a confirmed diagnosis of relapsing (relapsing-remitting or secondary progressive) MS defined by the 2010 Revised McDonald Criteria for the Diagnosis of Multiple Sclerosis[1].Any disease duration will be accepted.
- Have a baseline EDSS of = or <7.0
- Older than 18 years of age.
- Be able to attend all clinic appointments without interruption
- Patients must be able to understand English sufficiently well to understand and comply with the clinic and medication schedules and procedures.
- Be willing and able to give written informed consent

- Negative blood pregnancy test at screening

5.2.2 *Patient Exclusion criteria*

- Not meeting all of the above inclusion criteria
- Pregnancy or breastfeeding
- Current or recent [in the last 90 days] exposure to high dose corticosteroids
- Ongoing use of antibiotics
- Presence of a chronic intestinal disease e.g. Celiac, malabsorption, Colonic tumor
- Inability to provide informed written consent.
- Immunosuppression from transplantation, HIV, Cancer chemotherapy or ongoing use of any immunosuppressive agents.
- Concomitant inflammatory diseases
- Any contra-indications for MRI. Participants are to be screened by a CMRTO certified MRI Technologist in order to determine the MRI compatibility or exclusion of implantable/external devices according to the manufacturer's safety guidelines. The devises include cerebral aneurysm clips, neuro-stimulator, mechanical heart valves, cardiac stents, IUDs, vena cava filters, shunts, embolization coils, cochlear implants, non-removable prosthesis/artificial limbs. Contraindications are pacemaker of defibrillator, shrapnel/metallic fragments, previous brain surgery, seizure, severe claustrophobia, weight or body index that will prevent a successful MRI study.

5.3 Recruitment of trial patients.

Successful recruitment will be 3-6 patients per month on average. The goal is a total recruitment of 20 patients/12 months given the high numbers of competing studies, missed patients, consent failure rates and the refusal of an FMT strategy. We expect to monitor success with recruitment throughout the study and make revisions to recruitment procedures and eligibility criteria as necessary.

5.3.1 Recruitment of Healthy Volunteers.

Advertising will be posted throughout campus and hospitals in London announcing this study and calling for self-reported healthy individuals 18 y/o and older to participate in this study as a "control group". We will recruit up to 10 [ten] volunteers. Participation will entail a single visit to the clinic for proper written informed consent prior to obtaining 1 tube, 5 ml of blood via standard of care venipuncture in one arm administered by a qualified registered nurse or lab technician. The tube with blood will be labeled with a study ID and sent to Dr Kerfoot's Immunology lab for analysis in a completely de-identified fashion. This "control group" will not have any further participation in this study.

5.4 Adherence to protocol.

Successful adherence will be defined as =90% of patients progressing to FMT and all follow-up. Preliminary estimates of non-administration of the trial intervention are needed, along with strategies that maximize exposure to the intervention.

5.5 Safety

We will monitor patients closely for potential adverse events. We plan to administer the FMT through a standard rectal enema done by a trained Registered Nurse. Full screening of the donor to rule out transmissible blood-borne, or fecal pathogens, as well as history and physical examination to rule out any underlying health conditions [as performed in previous studies[70,71] and as recommended in the recent Guidance document from Health Canada], will assure the safety of the procedure. It is important to note that FMT has been found to be extremely safe in multiple previous studies, with no documented complications ever reported when performed in medically stable patients.

6. TREATMENT OF PATIENTS

6.1 Study Drug

Fecal microbiota.

6.2 Study Evaluations

All summary of study procedures and evaluations at each visit were provided in Tables 1 and 2 (previous chapter).

6.2.1 Screening, Enrolment and Baseline

An informed consent form must be signed prior to initiating any procedures or evaluations for the purpose of the study (Appendix B). After the informed consent form has been signed patients will be randomized to receive FMT for the first 6 months of the study or to be a standard per treatment control group that will received treatment at the last 6 months of the study.

The following evaluations at baseline visit and prior to FMT will be performed in both groups:

- Medical history;
- Vital signs (including pulse, respiration rate, blood pressure and temperature);
- Physical Exam (including weight in kg);
- Concomitant medications;
- Dietary history;

- Expanded Disability Status Score (EDSS);
- Blood samples will be taken for haematology (including haemoglobin, red blood cell count, white blood cell count, differential and platelets);
- Blood samples will be taken for serum chemistry (sodium, potassium, chloride, uric acid, creatinine, blood urea nitrogen, total protein, calcium, albumin, total bilirubin, AST, ALT, alkaline phosphatase, glucose);
- Blood sample for multiplex cytokine profiling (includes pro-inflammatory (IL-6, IL-15, IL-17, GM-CSF, TNFα) and anti-inflammatory (IL-4, IL-10 and IL-14) cytokines).
- Clean catch, mid-stream urine for urinalysis and micro analysis (including pH, protein, glucose, ketones, nitrites, leukocyte esterase, RBCs, WBCs) and gut permeability;
- Blood sample for blood bacterial DNA
- Head MRI as baseline

After evaluation, the FMT via rectal enema will be performed by a RN in each visit.

6.2.2 Subsequent visits

On all subsequent visits, prior to FMT the following evaluations will be performed:

- Physical examination
- Rapid (urine) pregnancy test for women of childbearing potential;
- Vital signs (including pulse, respiration rate, blood pressure and temperature);
- Concomitant medications;
- EDSS;
- Blood samples will be taken for haematology (including haemoglobin, red blood cell count, white blood cell count, differential and platelets);
- Blood samples will be taken for serum chemistry (including: sodium, potassium, chloride, uric acid, creatinine, blood urea nitrogen, total protein, calcium, albumin, total bilirubin,, AST, ALT, alkaline phosphatase, glucose);
- Clean catch, mid-stream urine for urinalysis and micro analysis (including pH, protein, glucose, ketones, nitrites, leukocyte esterase, RBCs, WBCs,);
- Blood sample for blood bacterial DNA
- Blood sample for multiplex cytokine profiling (includes pro-inflammatory (IL-6, IL-15, IL-17, GM-CSF, TNFα) and anti-inflammatory (IL-4, IL-10 and IL-14) cytokines).

After evaluation, the FMT via rectal enema will be performed by a RN in each visit.

At baseline, 6 and 12 months a head MRI will be performed. At these time points urine will be also collected to perform gut permeability analysis.

At any time during the study, if a patient has experienced an adverse event then they may be asked to return to the clinic for an unscheduled visit. Patients with adverse events will be followed to resolution.

6.2.3 Peripheral blood cytokines levels

Cytokine levels will be measures as previously described[72]. Peripheral blood will be collected into EDTA blood collection tubes ensuring complete mixing with anticoagulant to avoid clotting. Within 60 minutes of blood collection samples will be spun for 10 minutes at 4500xg. The plasma will be collected and aliquoted into cryovials. All samples will be collected and stored at -80°C before the plasma is measured for 25 immunological cytokines using a luminex-based mulitiplexed immunoassay. The Human Th17 Magnetic bead panel (HTH17MAG-14K, Millipore) will be used in this study to detect changes in Interleukin 1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, granulocyte macrophage colony stimulating factor (GM-CSF), interferon γ (IFNγ), macrophage inflammatory protein 3α (MIP-3α), tumour necrosis factor α (TNFα) and TNFβ. Plasma samples will be run in duplicate and assays will be standardized as per product specifications. Plasma cytokine levels in healthy controls will be compared to levels in MS patients at all time points. Moreover, cytokine levels of MS patients at baseline (D0) will be compared to levels at 12 months to determine whether intervention (FMT) had an effect on pro- and anti-inflammatory cytokine levels.

6.2.4 Blood DNA bacteria

Up to 10mL blood will be collected in a 3mL K_2EDTA Whole Blood Collection Tube manufactured by BD (BD Product number: 367856). Isolation of bacterial DNA from blood is obtained by adding 1.5 ml of whole blood to 6 ml of 0.17 M ammonium chloride. The sample are incubated for 20 minutes at 37° C and centrifuged for 10 minutes at 10 000 rpm. The supernatant is removed and the precipitate is suspended in 100µl of solution of lysozyme (2 mg/ml) and lysostaphin (0.2 mg/ml) in PBS buffer. Sample are then transferred to Eppendorf tubes with glass beads (700-1100 µm) and subjected to mechanical disruption for 20 seconds at a speed of 4.0 m/s. Again, sample are incubated for 30 minutes at 37° C and centrifuged for 10 minutes at 12 000 rpm.

6.2.5 Stool collection

Fecal samples will be collected from patients at baseline, and months 1 to 12 inclusive prior to receiving their scheduled FMTs. To collect the stool samples the participants will collect a piece of visibly soiled toilet paper and place it into a DNA-free bag. The bag will be transported to the Lawson Health Research Institute at St. Joseph's Hospital and stored at -80 °C until the time of DNA extraction when all samples are collected. DNA will be extracted using the MO BIO Powersoil 96-well soil isolation kits, as adapted for the Human Microbiome Project. After DNA extraction, the V4 region of the 16S rRNA gene will be amplified using validated bar-coded PCR primer sets. After amplification, the samples will be sent for Illumina MiSeq sequencing (London Regional Genomics Centre). The reads generated from sequencing will be demultiplexed, taxonomically assigned by BLAST, and analyzed using the program R. Microbiome analysis will follow the protocol as described in Bisanz et al.[4] A principal component analysis will be conducted and ALDEx2 tests will be performed to determine what the main differences in bacterial composition are between samples. The microbial composition at baseline will be compared to the other time points to determine how long it takes for the recipient to revert to their original microbiota. The donor microbiota will also be compared to each time point to determine how similar the patient's bacterial composition is to the donors after transplant.

6.2.6 Urine analysis for gut permeability

Patients will be instructed not to consume alcohol on the day of the test or 5 days previously. Also, to eat supper before 6:00 pm, and then no food or drink is to be consumed after this time. At bedtime, patients will be advised to empty bladder and discard urine.

A packet containing sugar (lactulose) and mannitol will be provided. Patients will be instructed to empty the packet into a glass of water according to the table below.

Weight	Lactulose	Mannitol	Sucrose	Tap water
45kg-up	5 grams	2.0 grams	100 grams	450ml

Throughout the night and first thing in the morning, all urine must be collected and brought to St Joseph's Health Care.

6.3 Sample Size analysis

It was assumed that the acceptable Type I error will be 0.05 and power will be 0.8. Moderate effect size effect is expected. This is a study of a kind that has never been done before in this population. From similar studies in other populations or other interventions on a similar population, a 40% change in cytokine levels (ie TGFbeta1) analysis is expected, as reported on other cytokine study with no pharmacological intervention, such as vitamin D supplementation[73].

6.4 Study duration

One year.

7.0 ASSESSMENT OF EFFICACY

As this study is an immunological study, the following efficacy outcomes will be evaluated:

- Changes in peripheral blood cytokines within RMS patients between baseline and months 1 to 12 inclusive, for both groups. Luminex kit will be used.
- Changes on microbiome after FMT by analyzing the gut bacterial composition through stool analysis and blood bacteria DNA at baseline, and months 1 to 12 inclusive for both groups.
- Gut permeability through urine analysis at baseline, 6 and 12 months for both groups.

8.0 ASSESSMENT OF SAFETY

8.1 Head MRI

A contrast head MRI will be performed at baseline, 6 months and 12 months for both groups as a safety measure to evaluate any subclinical activity or any paradoxical effect potentially caused by the FMT.

8.2 Adverse events

8.2.1 Definition of an Adverse Event

An adverse event (AE) is defined as any undesirable physical, psychological, or behavioural event experienced by a subject in conjunction with their participation in the clinical trial, regardless of whether the event is procedure- or study product-related. This includes any condition (including a pre-existing condition) that was:

- Not present prior to study treatment initiation but appeared or reappeared following initiation of study treatment.
- Present prior to study treatment, but worsened during study treatment. (This could include any condition resulting from concurrent illness, reactions to concomitant medications, or progression of disease states).

The occurrence of an AE will be based on changes in the subject's physical examination, laboratory results, and/or signs and symptoms.

8.2.2 Reporting of an Adverse Event

All AEs regardless of causal relationship are to be recorded in the case report form (CRF) and other source documentation.

The Investigator must determine the intensity of any AEs according to a three-point scale: mild, moderate, severe, and reported on specific AE pages of the CRF. If the severity of an AE worsens during study drug administration, only the worst intensity should be reported on the AE page. If the AE lessens in intensity, no change in the severity is required.

Mild
Event may be noticeable to subject; does not influence daily activities; the AE resolves spontaneously or may require minimal therapeutic intervention;

Moderate
Event may make subject uncomfortable; performance of daily activities may be influenced; intervention may be needed; the AE produces no sequelae.

Severe
Event may cause noticeable discomfort; usually interferes with daily activities; subject may not be able to continue in the study; the AE produces sequelae, which require prolonged therapeutic intervention.

A mild, moderate or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event (as in mild, moderate, or severe myocardial infarction). However, a severe event may be of relatively minor medical significance (such as severe headache) and is not necessarily serious. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

The Investigator must also determine the causal relationship using the guidelines listed in Table 8.1 and Section 8.1.1. Life threatening AEs will be recorded as serious adverse events (SAEs) in the CRF and will be reported to the medical monitor.

All AEs will be followed until resolution. Resolution is defined as the disappearance of all signs or symptoms related to the adverse event or the stabilization of the adverse event with residual signs or symptoms that are not expected to improve.

Criteria for Determining Category of Relationship of Clinical Adverse Events to Treatment

Not related This category applies to those adverse events which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)

Unlikely (must have two) In general, this category can be considered applicable to those adverse events which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test drug. An adverse event may be considered unlikely if or when:

- It does not follow a reasonable temporal sequence from administration of the test drug.
- It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the test drug.
- It does not reappear or worsen when the drug is re-administered.

Possibly (must have two) This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when:

- It follows a reasonable temporal sequence from administration of the test drug.
- It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It follows a known pattern of response to the test drug.

Probably (must have three)

This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug. An adverse event may be considered probably related if or when:

- It follows a reasonable temporal sequence from administration of the test drug.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or any other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists (e.g. bone marrow depression, fixed drug eruptions, tardive dyskinesia).
- It follows a known pattern of response to the test drug.

Definitely (must have all)

This category applies to those adverse events which, the Investigator feels are incontrovertibly related to test drug. An adverse event may be assigned an attribution of definitely related if or when:

- It follows a reasonable temporal sequence from administration of the test drug.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dose with re exposure to drug. (Note: this is not to be construed as requiring re-exposure of the subject; however, a category of definitely related can only be used when a recurrence is observed.)
- It follows a known pattern of response to the test drug.

8.2.3 Definition of a Serious Adverse Event

Adverse events that are to be considered serious include the following:

- Death, regardless of cause, which occurs within 30 days of the last dose of study drug or if after 30 days is a result of delayed toxicity due to administration of the study drug.
- Immediately life-threatening AE
- Permanently disabling event.
- Hospitalization or prolonged hospitalization excluding those for protocol-specific procedures.
- Congenital anomaly.

"Life threatening" means that the study participant was, in the opinion of the Investigator, at immediate risk of death from the reaction as it occurred. Any other event thought by the Investigator to be serious should also be reported.

The term "severe" is used to grade intensity and is not synonymous with the term "serious".

8.4 Reporting of a serious adverse event

In the event of a SAE, regardless of any opinions on relationship to the study drug, the Medical Monitor is to be notified. In the event that the Medical Monitor cannot be reached, other personnel will be available to receive the report.

All SAEs that are unexpected (i.e., not identified in nature, severity, or frequency in the current Investigator's Brochure), whether considered to be drug related or not, require a SAE Report Form be completed. All SUSARS (suspected unexpected serious adverse events) must be reported in writing to the Concerned Research Ethics Board (REB).

A death occurring during the study or which comes to the attention of the Investigator within 30 days after discontinuation of study drug, whether considered treatment related or not, must be reported.

Preliminary reports of SAEs will be followed by detailed descriptions which will include copies of the hospital case reports, autopsy reports, and other documents as requested and applicable.

8.5 Reporting of a pregnancy during the study

In principle, pregnancy and the lactation period are exclusion criteria for clinical studies involving investigational drugs, which are not directly related to the respective conditions. In the event of a pregnancy occurring during the course of this particular study, the subject should be

withdrawn from study, but closely followed-up during the entire course of the pregnancy and postpartum period. All recommendations described in the investigational drug brochure during pregnancy and lactation have to be carefully considered.

Parental and neonatal outcomes must be recorded even if they are completely normal and without Adverse Events. Off-spring should be followed up for at least 8 weeks after delivery. Longer observation periods may be considered if an adverse outcome of the pregnancy was observed.

9. STATISTICAL ANALYSIS

9.1 Sample Size Selection

This is an exploratory proof of concept immunological study. As such the number of patients in each treatment group was arbitrarily chosen in lieu of a convenience sampling for this type of study.

9.2 Demographics

Demographic variables such as age, race, etc. and baseline status (medical history, physical exam, etc.) will be documented by dose level and/or overall, as applicable.

9.3 Biomarkers

We will analyze mean differences between two independent groups whilst subjecting participants to repeated measures overtime to assess variability between- and within-subjects variable. Therefore, we will use the mixed-design ANOVA model.

9.4 Safety Analysis

Adverse events will be coded using MedDRA dictionary. Incidence of adverse events by the severity, relationship to treatment, and outcome will be provided. Narratives of all serious adverse events and events causing death will be included. Laboratory parameters, vital signs and other safety parameters will be documented with the use of descriptive statistics.

9.5 Criteria for Study Termination

The study continues until all 40 patients have been treated.

9.6 Missing Values

No imputation for missing values will be utilized.

9.7 Evaluable Patients

All patients who received 1 week of study treatment will be included in all analyses and listings.

10. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURE ANALYSIS

10.1 Protocol Amendments

In the event that a protocol change is proposed for all patients, the following procedure for a protocol amendment will be followed. Each investigator(s) must sign and date the amendment prior to implementation. All major protocol amendments must be submitted to and approved by health authorities. In addition, the investigator(s) must report all protocol amendments to, and receive all required approvals from, Independent Ethics Committee (IEC) prior to the implementation of any protocol amendment at the study center. All protocol amendments will be listed in the amended protocol.

Administrative changes to the protocol which do not impact the design or conduct of the study will be submitted to health authorities and IEC as notifications.

10.2 Protocol Deviations

No deviations from this protocol will be permitted without the prior written approval of the investigators. Any such changes which may affect a patient's treatment or informed consent, especially those increasing potential risks, must receive prior approval by the IEC. Other administrative revisions which may impact the clinical portion of a study will be duly reported to the IEC by the Investigator

11. ETHICAL CONSIDERATIONS

11.1 Independent Ethics Committee (IEC)

Good Clinical Practice (GCP) requires that the Investigator's Brochure, clinical protocol, any protocol amendments, the informed consent and all other forms of patient information related to the study (e.g., advertisements used to recruit patients) and any other necessary documents for each participating site be reviewed by an IEC. Any amendments to the protocol will require IEC approval prior to implementation of any changes made to the study design. All expedite Serious Adverse Events should be reported to the IEC. During the conduct of the study, the investigator should promptly provide written reports to the IEC on any changes that affect the conduct of the study and/or increase the risk to patients.

11.2 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, the International Conference on Harmonization Harmonized Tripartite Guidelines E6 for Good Clinical Practice, ethical principles that have their origin in the Declaration of Helsinki, 2013 and all applicable local regulations.

11.3 Patient Information and Consent

Patients attending a clinic specializing in the care of patients with MS will be approached by their Physician or nurse regarding their interest in participation. If they express interest and consent they will then meet with the investigator and his representative. At this time, the patient will be questioned regarding inclusion and exclusion criteria, and if eligible will request informed consent for participation. The consent will contain all the essential elements of informed consent set forth in ICH E6. The investigator or his/her representative will explain the nature of the study to the patient, and answer all questions regarding this study. The informed consent must be reviewed, signed and dated by the patient and the person administering the consent prior to screening the patient for the study. A copy of the informed consent form will be given to the patient and the original will be placed in the patient's medical record if in accordance with the institution's standard operating procedures. An entry must also be made in the patient's chart to confirm that informed consent was obtained prior to any study-related procedures and that the patient received a signed copy. To protect the confidentiality of the patient, all reports and communications relating to the study will identify patients by assigned patient identifier only. Potential donors will be recruited from student groups or staff volunteers in Western University.

11.4 Benefits of the study

11.4.1 Participant's benefit

MS patients may or may not be benefitted by the outcome of the study. We hope to demonstrate that human gut microbiome may impact MS cytokines profile and that FMT is feasible. We aim to further increase the understanding about MS pathology.

11.4.2 Benefits to the society:

The goal of this project is to establish a comprehensive research team consisting of Neurologists, Neuroradiologists, Immunologists and Microbiome scientists to develop local expertise which would enable the present as well as future studies. Potential future interventions would be planned based on the study. In particular we will look at how long the transplanted microbiome persists and whether certain organisms are critical in the success of the intervention and thus could be selected for in a subsequent more focused microbial supplementation interventions (probiotics) or with selective antibiotics to remove harmful bacteria.

11.5 Potential risks/harms/inconveniences

We will monitor closely for potential adverse events. Full screening of the donor to rule out transmissible blood, or fecal pathogens, as well as history and physical examination to rule out any underlying health conditions, will assure the safety of the procedure. It is important to note that FMT has been found to be extremely safe in multiple previous studies. All details of the study shall be discussed with the patient's and donor and included in the informed consent forms. There is the potential of a previously unrecognized illness in the donor, to be identified through screening. Potential donors will be counseled regarding this and will receive pre and posttest counseling for HIV and other screened diseases. Any potential donors who are identified to have a significant health issue during screening will be referred for appropriate medical care.

12. DATA HANDLING AND RECORD KEEPING

12.1 Study Documentation and Record Retention

For this study all data will be collected in a case report form (CRF). CRFs will be maintained for each patient. The Investigator will be responsible for the recording of all data on the CRFs provided to them, as certified by the Investigator's signature and date. Should any value be significantly different from normal, the Investigator will comment in the appropriate sections provided in the CRFs.

All CRF corrections are to be made by the investigator or his/her study site designee and need to be documented by date.

Data recorded in each patient's CRF must have first been documented in the patient's medical record or other source documentation (e.g., laboratory reports, progress notes, etc.) CRFs will be reviewed and compared with corresponding source documents by the Clinical Monitor who will determine the CRFs acceptability. Copies of completed CRFs will be reviewed by the Clinical Monitor.

The Investigator Site File (ISF) must be maintained in an organized fashion with no co-mingling of any other non-study-related materials. The study file must contain all study-related correspondence (e.g., Ethics Committee correspondence, correspondence to/from the Sponsor and/or its designee, patients' signed informed consents, copy of the protocol and any/all amendments, copy of signed regulatory agency forms. etc.) In addition, the study file must contain contact information (i.e., name, address, telephone) for each patient who participates in the study. The patient contact information is for emergency or medical follow-up purposes related to this study. It is not submitted to the Sponsor, unless specifically required for the regulatory purposes.

All case report forms and all source documents (e.g., informed consent forms; adverse event reports; drug dispensing/disposition records; medical record information and reports as described above) must be retained in the ISF by the investigator for the maximum period of time defined as:

1. Twenty-five years or
2. For a minimum of two(2) years following last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or
3. At least 2 years after the discontinuation of clinical development of this product

12.2 Duties of the Investigational Staff

In addition to the specifications of this protocol, the investigator and all individuals responsible to the investigator who assist in the conduct in the study are obliged to conduct this study in accordance with currently applicable regulations.

The investigator will provide copies of the protocol and all pertinent information as necessary to the study staff, and will discuss the material with them to ensure they are fully informed regarding the drug and the conduct of the study.

12.3 Use of Information and Publication

Publication of the interim analysis and final study report are planned. The Sponsor-Investigator (Dr. Kremenchutzky) will determine authorship for each manuscript based on contributions to the study design, study execution, and manuscript completion. No author will be included without prior authorization but the intention is to be broadly inclusive of all study investigators who make active contributions as outlined by The ICJME criteria for authorship (Authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3).

13. REFERENCES

1. Chris H Polman, Stephen C Reingold, Brenda Banwell, Michel Clanet, Jeffrey A Cohen, Massimo Filippi, Kazuo Fujihara, Eva Havrdova, Michael Hutchinson, Ludwig Kappos, Fred D Lublin, Xavier Montalban, Paul O'Connor, Magnhild Sandberg-Wollheim, Alan J Thompson, Emmanuelle Waubant, Brian Weinshenker, and Jerry S Wolinsky. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol. 2011 Feb; 69(2): 292–302.
2. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale(EDSS). Neurology. 1983 Nov;33(11):1444-52.
3. Mkhikian H, Grigorian A, Li CF, Chen HL, Newton B, Zhou RW, Beeton C, Torossian S, Tatarian GG, Lee SU, Lau K, Walker E, Siminovitch KA, Chandy KG, Yu Z, Dennis JW, Demetriou M. Genetics and the environment converge to dysregulate N-glycosylation in multiple sclerosis. Nat Commun. 2011;2:334.
4. Olek MJ. Multiple sclerosis--Part I. Overview, pathophysiology, diagnostic evaluation, and clinical parameters. J Am Osteopath Assoc. 1999 Nov;99(11):574-88. Review.
5. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE.. Metagenomic Analysis of the Human Distal Gut Microbial. Science. 2006 Jun 2;312(5778):1355-9.
6. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635-1638. doi:10.1126/science.1110591.
7. Mielcarz DW, Kasper LH. The Gut Microbial in Multiple Sclerosis. Curr Treat Options Neurol. 2015;17(4). doi:10.1007/s11940-015-0344-7.
8. Cantarel BL, Waubant E, Chehoud C, et al. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. J Investig Med. 2015;63(5):729-734.
9. Miyake S, Kim S, Suda W, et al. Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. PLoS One. 2015;10(9):e0137429.
10. Chen J, Chia N, Kalari KR, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep. 2016;6:28484.
11. Tremlett H, Fadrosh DW, Faruqi AA, et al. Gut microbiota in early pediatric multiple sclerosis: a case−control study. Eur J Neurol. May 2016.

12. Kelly CR; De Leon L; Jasutkar N (2012). "Fecal Microbiota Transplantation for relapsing Clostridium difficile infection in 26 patients: methodology and results". J Clin Gastroenterol. 46 (2): 145–149.

13. Jessica D. Forbes, Gary Van Domselaar, and Charles N. Bernstein. The Gut Microbiota in Immune-Mediated Inflammatory Diseases. Front Microbiol. 2016; 7: 1081.

14. Miyake S, Kim S, Suda W, et al. Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. PLoS One. 2015;10(9):e0137429.

15. Chen J, Chia N, Kalari KR, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep. 2016;6:28484.

16. Tremlett H, Fadrosh DW, Faruqi AA, et al. Gut microbiota in early pediatric multiple sclerosis: a case−control study. Eur J Neurol. May 2016.

17. Gandhi R, Von Glehn F, Mazzola M, et al. Gut microbial is linked to immune cell phenotype in multiple sclerosis. Mult Scler J. 2014;20(1 Suppl):338-339.

18. Jangi S, Gandhi R, Cox LM, et al. Alterations of the human gut microbial in multiple sclerosis. Nat Commun. 2016;7:12015.

19. Tremlett H, Fadrosh DW, Faruqi AA, et al. Gut microbiota composition and relapse risk in pediatric MS: A pilot study. J Neurol Sci. 2016;363:153-157.

20. Tejesvi M V, Arvonen M, Kangas SM, et al. Faecal microbial in new-onset juvenile idiopathic arthritis. Eur J Clin Microbiol Infect Dis. 2016;35(3):363-370.

21. Sasaki M, Klapproth J-MA. The role of bacteria in the pathogenesis of ulcerative colitis. J Signal Transduct. 2012;2012:704953.

22. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon.FEMS Microbiol Lett. 2002 Dec 17;217(2):133-9. Review.

23. Canani RB, Costanzo M Di, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol. 2011;17(12):1519-1528.

24. Miyake S, Kim S, Suda W, et al. Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. PLoS One. 2015;10(9):e0137429.

25. Baum K, Rejmus R, Dorffel Y. Commensal gut flora in MS: Spatial organization and composition. Mult Scler J. 2015;21(11 Suppl):458-459.

26. Farrokhi V, Nemati R, Nichols FC, et al. Bacterial lipodipeptide, Lipid 654, is a microbial-associated biomarker for multiple sclerosis. Clin Transl Immunol. 2013;2(11):e8.

27. Perkins DJ, Polumuri SK, Pennini ME, Lai W, Xie P, Vogel SN. Reprogramming of murine macrophages through TLR2 confers viral resistance via TRAF3-mediated, enhanced interferon production. PLoS Pathog. 2013;9(7):e1003479.

28. Ochoa-Repáraz J, Mielcarz DW, Ditrio LE, et al. Central nervous system demyelinating disease protection by the human commensal Bacteroides fragilis depends on polysaccharide A expression. J Immunol. 2010;185(7):4101-4108.

29. Cekanaviciute E, Runia T, Debelius J, et al. The influence of microbiota on the adaptive immune response in MS. Mult Scler J. 2015;21(11 Suppl):454-454.

30. Aminov RI. Role of archaea in human disease. Front Cell Infect Microbiol. 2013;3:42.

31. Chatterjee S, Park S, Low K, Kong Y, Pimentel M. The degree of breath methane production in IBS correlates with the severity of constipation. Am J Gastroenterol. 2007;102(4):837-841.

32. Ebringer A, Hughes L, Rashid T, Wilson C. Acinetobacter immune responses in multiple sclerosis: etiopathogenetic role and its possible use as a diagnostic marker. Arch Neurol. 2005;62(1):33-36.

33. Ebringer A, Thorpe C, Pirt J, Wilson C, Cunningham P, Ettelaie C. Bovine spongiform encephalopathy: is it an autoimmune disease due to bacteria showing molecular mimicry with brain antigens? Environ Health Perspect. 1997;105(11):1172-1174.

34. Hughes LE, Smith PA, Bonell S, et al. Cross-reactivity between related sequences found in Acinetobacter sp., Pseudomonas aeruginosa, myelin basic protein and myelin oligodendrocyte glycoprotein in multiple sclerosis. J Neuroimmunol. 2003;144(1-2):105-115.

35. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD—what role do Proteobacteria play? Nat Rev Gastroenterol Hepatol. 2012;9(4):219-230.

36. Ganesh BP, Klopfleisch R, Loh G, Blaut M. Commensal Akkermansia muciniphila exacerbates gut inflammation in Salmonella Typhimurium-infected gnotobiotic mice. PLoS One. 2013;8(9):e74963.

37. Png CW, Lindén SK, Gilshenan KS, et al. Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. Am J Gastroenterol. 2010;105(11):2420-2428.

38. Wang W, Chen L, Zhou R, et al. Increased proportions of Bifidobacterium and the Lactobacillus group and loss of butyrate-producing bacteria in inflammatory bowel disease. J Clin Microbiol. 2014;52(2):398-406.

39. Kwon H-K, Kim G-C, Kim Y, et al. Amelioration of experimental autoimmune encephalomyelitis by probiotic mixture is mediated by a shift in T helper cell immune response. Clin Immunol. 2013;146(3):217-227.

40. Ezendam J, de Klerk A, Gremmer ER, van Loveren H. Effects of Bifidobacterium animalis administered during lactation on allergic and autoimmune responses in rodents. Clin Exp Immunol. 2008;154(3):424-431.

41. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368(5):407-415.

42. Shahinas D, Silverman M, Sittler T, et al. Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene deep sequencing. MBio. 2012;3(5).

43. Borody T, Leis S, Campbell J, Torres M, Nowak A. Fecal Microbiota Transplantation (FMT) in Multiple Sclerosis (MS). Am J Gastroenterol. 2011;106(Supplement 2):S352-S352.

44. Mielcarz DW, Kasper LH. The Gut Microbiome in Multiple Sclerosis. Curr Treat Options Neurol. 2015;17(4).

45. Berer K, Mues M, Koutrolos M, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature. 2011;479(7374):538-541.

46. Hooper L V, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012;336(6086):1268-1273.

47. Telesford KM, Yan W, Ochoa-Reparaz J, et al. A commensal symbiotic factor derived from *Bacteroides fragilis* promotes human CD39 $^{+}$ Foxp3 $^{+}$ T cells and T $_{reg}$ function. Gut Microbes. 2015;6(4):234-242.

48. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559-563.

49. Haghikia A, Jörg S, Duscha A, et al. Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. Immunity. 2015;43(4):817-829.

50. Lavasani S, Dzhambazov B, Nouri M, et al. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One*. 2010;5(2):e9009.

51. Takata K, Kinoshita M, Okuno T, et al. The lactic acid bacterium Pediococcus acidilactici suppresses autoimmune encephalomyelitis by inducing IL-10-producing regulatory T cells. PLoS One. 2011;6(11):e27644.

52. Maassen CBM, Claassen E. Strain-dependent effects of probiotic lactobacilli on EAE autoimmunity. Vaccine. 2008;26(17):2056-2057.

53. Rezende RM, Oliveira RP, Medeiros SR, et al. Hsp65-producing Lactococcus lactis prevents experimental autoimmune encephalomyelitis in mice by inducing CD4+LAP+ regulatory T cells. J Autoimmun. 2013;40:45-57.

54. Hiroaki Yokote,Sachiko Miyake, J. Ludovic Croxford, Shinji Oki, Hidehiro Mizusawa, Takashi Yamamura P. NKT Cell-Dependent Amelioration of a Mouse Model of Multiple Sclerosis by Altering Gut Flora. December 2008Volume 173, Issue 6, Pages 1714–1723.

55. Chapman BC, Moore HB, Overbey DM, Morton AP, Harnke B, Gerich ME, Vogel JD. Fecal microbiota transplant in patients with Clostridium difficile infection: A systematic review. J Trauma Acute Care Surg. 2016 Oct;81(4):756-64.

56. Sun D1, Li W, Li S, Cen Y, Xu Q, Li Y, Sun Y, Qi Y, Lin Y, Yang T, Xu P, Lu Q. Fecal Microbiota Transplantation as a Novel Therapy for Ulcerative Colitis: A Systematic Review and Meta-Analysis. Medicine (Baltimore). 2016 Jun;95(23):e3765.

57. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A,Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolicsyn drome. Gastroenterology. 2012 Oct;143(4):913-6.e7.

58. Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, et al. Fecal microbiota transplant for relapsing Clostridium difficile infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. Clin Infect Dis. 2014;58(11):1515-22.

59. Patel NC, Griesbach CL, DiBaise JK, Orenstein R. Fecal microbiota transplant for recurrent Clostridium difficile infection: Mayo Clinic in Arizona experience. Mayo Clin Proc. 2013;88(8):799-805.

60. MacConnachie AA, Fox R, Kennedy DR, Seaton RA. Faecal transplant for recurrent Clostridium difficile-associated diarrhoea: a UK case series. Qjm. 2009;102(11):781-4.

61. Aas J, Gessert CE, Bakken JS. Recurrent Clostridium difficile colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. Clin Infect Dis. 2003;36(5):580-5.

62. Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, Afzali A, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. Am J Gastroenterol. 109. United States2014. p. 1065-71.

63. Solari PR, Fairchild PG, Noa LJ, Wallace MR. Tempered enthusiasm for fecal transplant. Clin Infect Dis. 59. United States 2014. p. 319.

64. De Leon LM, Watson JB, Kelly CR. Transient flare of ulcerative colitis after fecal microbiota transplantation for recurrent Clostridium difficile infection. Clin Gastroenterol Hepatol. 2013;11(8):1036-8.

65. Hohmann EL, Ananthakrishnan AN, Deshpande V. Case Records of the Massachusetts General Hospital. Case 25-2014. A 37-year-old man with ulcerative colitis and bloody diarrhea. N Engl J Med. 2014;371(7):668-75.

66. Sinan Wang, Mengque Xu, Weiqiang Wang, Xiaocang Cao, Meiyu Piao, Samiullah Khan, Fang Yan, Hailong Cao, and Bangmao Wang. Systematic Review: Adverse Events of Fecal Microbiota Transplantation. PLoS One. 2016; 11(8): e0161174.

67. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium difficile* infection. Am J Gastroenterol. 2012; 107(5):761-7.

68. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008;57(6):1470-81.

69. Horton F, Wright J, Smith L, Hinton PJ, Robertson MD. Increased intestinal permeability to oral chromium (51 Cr) -EDTA in human Type 2 diabetes. Diabet Med. 2014;31(5):559-63.

70. Barra L, Summers K, Bell D, Cairns E. Serum cytokine profile of unaffected first-degree relatives of patients with rheumatoid arthritis. The Journal of rheumatology 2014, 41(2): 280-285.

71. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol. 2003 Jan;134(1-2):128-32.

APPENDIX A

Donor Screening Criteria

Donor inclusion criteria:

A healthy donor who has a normal body mass index (BMI of 18.5 – 24.9) and who satisfies the following criteria will be selected for the study and screened for all known potential transmissible agents.

Donor exclusion criteria:

- Any underlying metabolic disease including; hypertension, hyperlipidemia, diabetes, insulin insensitivity, atherosclerosis
- A history of any gastrointestinal or liver disorders or cancers. Including but not limited to; gastroesophageal reflux, peptic ulcer disease, celiac disease, inflammatory bowel disease (Crohn's disease or ulcerative colitis), microscopic colitis, motility disorders (including gastropariesis and irritable bowel syndrome) and diverticular disease.
- Previous surgery to the intestine, liver or gallbladder (except remote appendectomy).
- History of any malignancy
- Use within 3 months of any antibiotics or probiotics.
- Hospitalization within 3 months.
- Recent travel to a developing country (within 3 months)
- New Sexual Partner (within 3 months).
- Street drug use
- Family history of diabetes, early onset coronary disease or gastrointestinal or liver disease, colon cancer, familial malignancy
- Psychiatric history (major affective disorder, psychotic illness, ongoing use of any psychiatric medications)
- Any positive laboratory results for a transmissible pathogen
- Alcohol intake with a cut off value of <10g/d in women and <20g/d in men.

Donor laboratory screening: Summary of Tests and Procedures for Donor Screening is given below. For information purposes only.

Visit	**Tests and Procedures** *(as applicable based on the protocol)*
Screening/ Visit 1 30min-1hr	Assess body mass index (BMI of 18.5–24.9) Physical examination Use within 3months of any antibiotics or probiotics Hospitalization within 3 months Collect past medical history, family history of diabetes or coronary disease, psychiatric history Recent travel to a developing country (within 3 months) New Sexual Partner (within 3 months). Street drug use Alcohol intake with a cut off value of <10g/d in women &<20g/d in men. Stool (1g): Microbiology/Immunology tests: Bacterial culture, Ova and Parasites and *C. difficile.* If donor has travelled to areas where other infections occur we will screen for those as well. Stool for Transplantation - ~50 - 70g/transplantation Urine (5ml): Chlamydia and Gonorrhea Blood screening (5-10ml): Biochemical assays:, liver function tests Microbiology/Immunology tests: Screen for HIV 1/2, HTLV 1/2, Hepatitis A IgM, Hepatitis BsAg, Hepatitis BcAb, Hepatitis C, *H. pylori* antibody and Anti TTG antibody. Serology for Syphilis, Strongyloides, Schistosomiasis, Amebiasis, CMV and EBV. Throat and Rectal swab: for Chlamydia and Gonorrhea Nasal swab and rectal swab: for drug resistant organisms including MRSA, VRE

	and CRE (carbapenemase producing Enterobacteriaciae) To drop off Stool samples collected fresh or within 3days of the transplantation.
Visit 2 *10min*	