MS-BIOME

Fecal Microbiota Transplantation (FMT) of FMP30 in Relapsing-Remitting Multiple Sclerosis: A Phase 1b Clinical Trial to Evaluate Feasibility, Safety, Tolerability and Effects on Immune Function

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BRIEF PROTOCOL SUMMARY

Study Protocol Title	Fecal Microbiota Transplantation (FMT) in Relapsing-Remitting Multiple Sclerosis: A Phase 1b Trial to Evaluate Feasibility, Safety, Tolerability, Dosing and Effects on Immune Function
Short title	***MS-BIOME***
Clinical Phase	Phase 1b
Study Center	UCSF MS Center
Investigational Product	Fecal Microbiota Transfer of donor stool (dosed as a single dose of 90 mL of FMP30) administered via colonoscopy. FMP30 donor stool will be obtained from OpenBiome, a Massachusetts-based nonprofit stool bank. Screening by OpenBiome excludes donors with known Multiple Sclerosis (MS) or other known autoimmune conditions and screened for transmissible pathogens. . Subjects will receive a 5-day oral antibiotic course of vancomycin, neomycin and metronidazole to precondition the gut as part of the FMT protocol, and FMT will then be delivered via a single colonoscopy.
Methodology/Design	Prospective, open label, single-group cohort study of a single fecal microbiota transplantation (FMT) of FMP30 donor stool administered via colonoscopy following preconditioning with oral antibiotics and standard bowel preparation for colonoscopy. The primary endpoints will be measured through 12 weeks; safety and biomarker follow-up will be collected through 48 weeks. A parallel observational control group of MS patients who otherwise satisfy study inclusion criteria based on their MS phenotype, demographics, disease duration and prior use of allowable MS therapies, will be recruited as a comparison/control arm to measure stability of the microbiome and immunological markers for 12 weeks or until time of initiation of MS disease modifying therapy clinically. Control subjects will not receive oral antibiotics, bowel preparation, colonoscopy or study MRI.
Objectives	**Primary Objective** To demonstrate that FMT in patients with relapsing-remitting MS is feasible, safe and tolerable To measure donor microbiome engraftment via changes in fecal microbiota community

	structure at multiple time points following a single cycle of FMP30 delivered via colonoscopy in people with relapsing-remitting MS **<u>Secondary objectives</u>** To evaluate whether FMT of FMP30 administered via colonoscopy can induce a shift from pro-inflammatory to immunomodulatory T cell profiles in patients with relapsing-remitting MS To evaluate whether FMT changes humoral function in patients with relapsing-remitting MS To begin to evaluate whether FMT favorably influences clinical and radiological endpoints in patients with relapsing-remitting MS
Number of patients	-10 patients treated with FMP30 -10-20 patients in the observational comparison group control arm
Diagnosis and inclusion criteria	1. Age 18-60 inclusive (at time of screening) 2. Diagnosis of relapsing-remitting multiple sclerosis (MS) by International Panel McDonald Criteria (2010)(1) incorporating 2017 revisions which reclassify select high-risk Clinically Isolated Syndromes under 2010 criteria as RRMS under 2017 criteria, and Lublin criteria (2014)(2) 3. Recent documented MS disease activity, defined as at least 1 clinical relapse within the past 1 year prior to baseline OR 2 clinical relapses in the past 2 years prior to baseline OR at least 1 new T2/FLAIR lesion on brain or spine MRI OR at least 1 gadolinium enhancing lesion on brain or spine MRI in the past 1 year prior to baseline 4. Expanded Disability Status Scale (EDSS) less than or equal to 6.0; EDSS 5.5 or less if MS disease duration is greater than 15 years (no other disease duration restriction) 5. Must have positive serology for Epstein-Barr Virus (EBV) (IgG anti-EBNA positive) at screening, indicating prior exposure 6. No prior MS disease modifying therapy or a 12 week washout period for subjects on glatiramer acetate or interferon-beta therapy 7. At least 4 weeks from baseline since last use of IV or oral glucocorticoids

	8. Agree to maintain a stable diet during the course of the study (over the counter probiotics are allowable) 9. Premenopausal women and women <12 months after the onset of menopause must have a negative serum pregnancy test unless they have undergone surgical sterilization 10. Female subjects of childbearing potential who are sexually active with a non-sterilized male partner must agree to use a highly effective method of contraception; non-sterilized male subjects who are sexually active with a female partner of childbearing potential must agree to use a highly effective method of contraception 11. Not actively participating in another *interventional* MS clinical trial (participation in other observational research studies is allowable)
Exclusion Criteria	1. Prior use of fingolimod, dimethyl fumarate, teriflunomide, natalizumab, alemtuzumab, mitoxantrone, cyclophosphamide, rituximab, ocrelizumab, daclizumab, methotrexate, azathioprine, mycophenolate mofetil, cyclosporine, leflunomide or induction chemotherapy 2. No use of diuretics like furosemide (Lasix) 1 week before the first dose oral antibiotics. The use of hydrochlorothiazide (HCTZ) for hypertension at a dose $\leq$ 50 mg/day is allowable. 3. Progressive MS by Lublin criteria (2014) 4. No oral or IV antibiotics within 8 weeks of screening and 12 weeks of scheduled of the planned FMT procedure if in the FMT arm or first stool collection if in control arm (note that topical, otic, ocular antibiotics are specifically allowable which is consistent with the IMSMS.org protocol for collaborative gut microbiome research in MS) 5. Hypersensitivity or allergy to study antibiotics, conscious sedation medications or bowel preparation 6. Contraindication to study procedures including MRI, anesthesia (ASA criteria IV and V), colonoscopy, phlebotomy 7. History of inflammatory bowel disease (Crohn's Disease, Ulcerative Colitis)

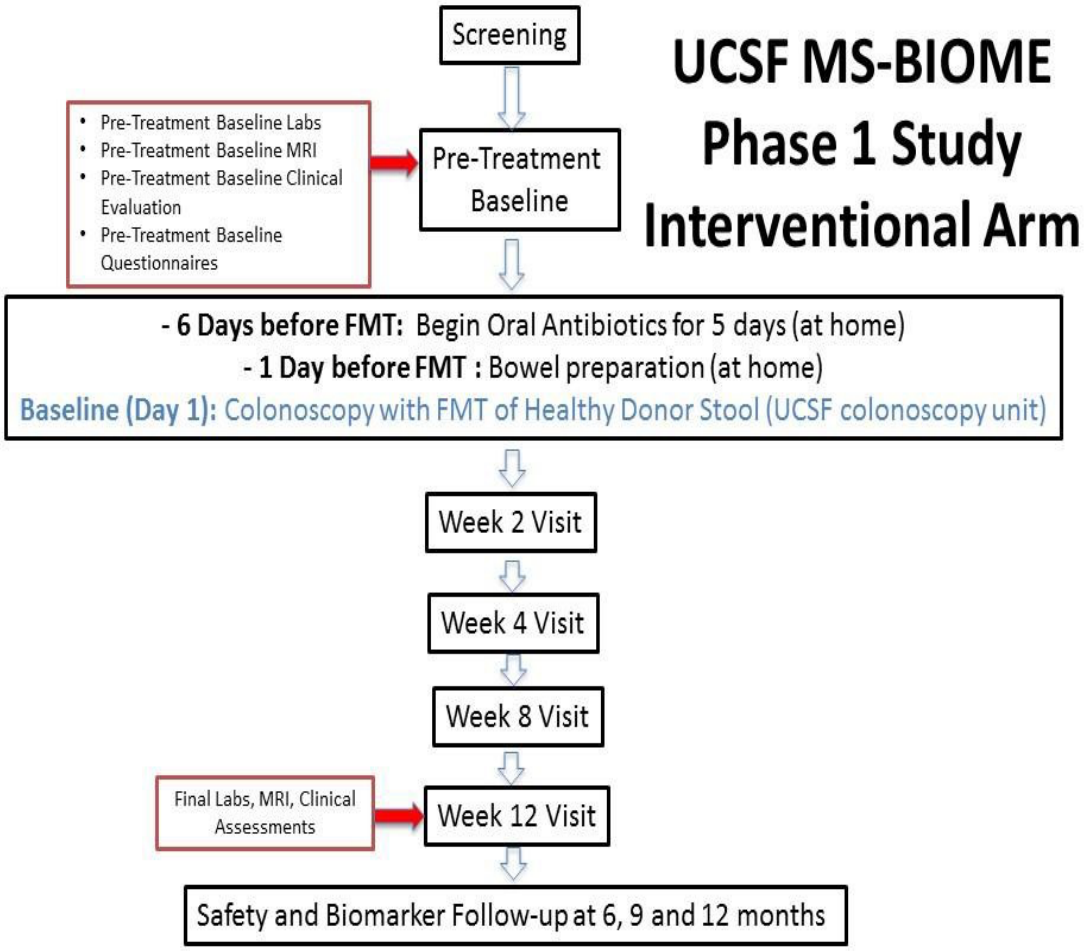
	8. Active symptomatic *C. Difficile* infection (colonization is not an exclusion) 9. Active gastrointestinal condition being investigated (i.e. GI bleeding, colon cancer, active GI workup); history of known or suspected toxic megacolon and/or known small bowel ileus, major gastrointestinal surgery (e.g. significant bowel resection) within 3 months before enrollment (note that this does not include appendectomy or cholecystectomy); or history of total colectomy or bariatric surgery 10. History of malignancy (except excised cutaneous basal cell carcinoma or squamous cell carcinoma which are allowable) including no concurrent induction chemotherapy, radiation therapy or biological treatment for active malignancy 11. Pregnant or lactating women or intention of getting pregnant during the trial period 12. Active infection including untreated latent or active tuberculosis, HIV, hepatitis, syphilis or other major active infection 13. Known immunodeficiency including CVID 14. INR>1.5, Platelets<100, Hemoglobin <8.5, WBC<2.0, Absolute lymphocyte count <0.8, Absolute Neutrophil Count <0.5, CD4<200, eGFR<45. 15. Any condition that in the opinion of the study PI could jeopardize the safety of the subject, would make it unlikely for the subject to complete the study or could confound the results of the study 16. Unable or unwilling to comply with study protocol requirements
Concomitant and Exclusionary Therapies	Standard *symptomatic* MS medications are allowable as concomitant therapy, including dalfampridine or 4-aminopyridine, botulinum toxin, oral spasticity agents/muscle relaxants, pain medication, antidepressant and anti-anxiety medication, modafinil, armodafinil and amphetamines for fatigue/alertness. Enrolled subjects will be asked to consent to delay initiation of an MS disease modifying therapy (DMT) until the end of the 12-week trial period. The length of this feasibility study was specifically designed to be short in order to minimize time not on a MS DMT and is consistent with other delayed start protocol

	designs in MS (for example, https://clinicaltrials.gov/show/NCT02688985). Subjects who experience breakthrough clinical worsening of MS after screening will be allowed to go on disease modifying therapy (DMT) at the discretion of their treating neurologist per routine medical management. If the decision to start a MS DMT occurs before the study intervention (antibiotics and/or FMT) this will be considered a screen failure and subjects will be excluded from the study and not included in further safety monitoring or in the pre-planned primary analysis. If the decision to start a MS disease modifying therapy occurs after any study intervention (antibiotics and/or FMT) has been administered, further study intervention will be discontinued but subjects will be invited and encouraged to complete the remaining study visits per protocol for microbiome, immunological and safety monitoring. For acute relapse management, PO or IV steroids will be allowable as part of routine medical management at the discretion of the treating neurologist.
Duration of administration	- 5 day oral antibiotic preconditioning initiated 6 days before colonoscopy - GoLytely bowel prep initiated at day 6 - Single dose of 90mls of FMP30 via colonoscopy at day 7
Evaluation Criteria	*Primary Feasibility Endpoint* - Proportion of subjects who complete the study protocol *Primary biomarker endpoint* - Engraftment → Change in fecal microbiota community structure at weeks 0, 2, 4, 8, and 12 - Engraftment -> Comparison of change in fecal microbiota community structure between FMT treated patients and the observational control arm at weeks 0, 4, 8, and 12 *Primary Safety endpoint* Treatment-emergent adverse events including treatment-emergent serious and non-serious adverse events through week 12 defined as proportion of subjects who develop an adverse event of severity grade 2 or more by *NIH CTCAE criteria*

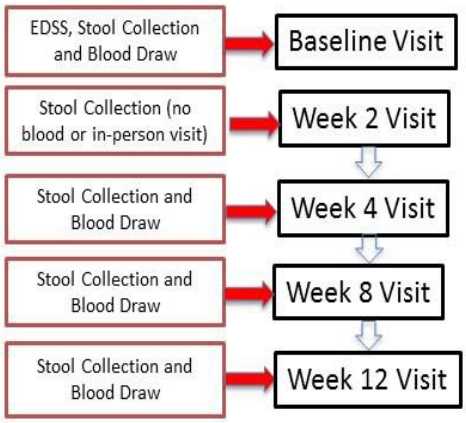
	Primary biomarker endpoint - Engraftment → Change in fecal microbiota community structure at weeks 0, 2, 4, 8, 12 - *Secondary biomarker endpoints* - Induction of T regulatory or Th2 cells and/or reduction of Th1 or Th17 cells at 0, 2, 4, 8 and 12 weeks - Plasma CD19+ and CD20+ B cell counts and serum immunoglobulin levels at weeks 0, 2, 4, and 12 weeks - New T2/FLAIR lesions, T2/FLAIR lesion load, T2/FLAIR lesion number at baseline and week 12 - New gadolinium enhancing lesions, total gadolinium enhancing lesions at baseline and week 12 *Secondary clinical endpoints* - Relapse rate through week 12 - EDSS and sub scores at the screening visit, pre-treatment baseline visit, week 2, week 4, week 8 and week 12 - Fatigue score (abbreviated MFIS) at baseline, week 4, week 8 and week 12 - Quality of life (MSQOL-54)] at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12 - Bowel symptoms (BWCS) at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12 - Bladder symptoms (BLCS) at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12 - Timed 25 foot walk at, pre-treatment baseline visit, week 2, week 4, week 8 and week 12 - Mood and Anxiety (MHI-5) and Columbia Suicide Rating Scale at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12
Statistical Methodology	Summary statistics will be calculated for the primary feasibility and safety analyses. For the primary biomarker analysis, Wilcoxon signed-rank (or paired T-test as appropriate) for paired pre-post analyses on immunological function in the FMT group will be used for the primary analysis. For the between group comparison of FMT treated vs the observational control arm, a t-test or Mann-Whitney/Wilcoxon rank sum non-parametric equivalent will be used as appropriate. For the paired analysis, we will have 80% power with an alpha of 0.05 to

	detect at least a 1 SD change in pre-post outcomes. For secondary outcomes that have repeated measures we will utilize multivariable linear regression mixed effects models with time as an interaction term for repeated-measures analyses and also compare outcomes in the FMT treatment arm with the non-interventional control comparison arm.
Duration of subject participation	FMT Treatment arm: 16 weeks in the active trial portion (up to 4 weeks for screening and baseline + 12 weeks post-FMT) with safety and biomarker follow-up for 1 year. Observational control comparison arm: 12 weeks (8 weeks of observation and up to 4 weeks for screening)

STUDY SCHEMA



UCSF MS-BIOME Phase 1 Study: Observational Control Arm for Stool and Blood Biomarker Measurement



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1. INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory/autoimmune disease of the central nervous system characterized by inflammatory-induced demyelination and neurodegeneration. In the United States, MS prevalence ranges from about 40 to 191 per 100,000 population[3]. MS is a leading cause of non-traumatic disability in working age adults. About 85-90% of people with MS present with a relapsing-remitting phenotype, whereas 10-15% have primary progressive disease[4].

There are now several licensed injectable, oral and infusible disease modifying treatments for relapsing MS that reduce relapse rates, MRI T2/FLAIR and T1 gadolinium enhancing lesions and disability progression, and all such therapies to date have a mechanism of action that works by modulating or suppressing systemic inflammation, particularly lymphocytes[5]. Currently available MS disease modifying therapies are designed to be taken chronically over the course of many years, if not decades, and all are only partially effective. Furthermore, safety, tolerability and perception of risk of currently available MS therapies varies widely[5] leading some MS patients to decline to take currently available therapies.

Genetics contributes to MS susceptibility, but a substantial proportion of MS disease risk remains unaccounted for by genetic factors alone and there has been increasing focus on environmental factors and gene-environmental interactions that contribute to MS risk and pathogenesis[6].

The human gastrointestinal system contains a massive population of microbes, and gut microbial organisms interact with the host immune system in a complex interplay that is commonly referred to as the gut microbiome. Changes in gut microbiota can affect immune function locally in the GI system, systemically, and in effector organs such as the central nervous system. The gut microbiome has been implicated in the pathogenesis of several autoimmune or inflammatory human conditions, including inflammatory bowel disease[7], Type 1 Diabetes[8,9], autoimmune hepatitis[10], systemic lupus erythematosus[11], juvenile idiopathic arthritis[12], and neuromyelitis optica[13].

Emerging evidence has now converged to support the hypothesis that the gut microbiome contributes to MS pathogenesis[14-16]. In murine models of experimental autoimmune encephalomyelitis (EAE), the gut microbiome is essential for regulation and activation of immunity. Commensal gut bacteria are essential for triggering experimental autoimmune demyelination in mice driven by myelin-specific CD4 T cells, and recruitment and activation of autoantibody producing B-cells appears to depend in part on commensal microbiota[17]. Mice maintained under germ-free conditions exhibit significantly attenuated EAE compared to conventionally colonized mice, with germ-free animals producing less proinflammatory cytokines in the intestine and spinal cord (and reciprocally

greater T regulatory cells), whereas intestinal colonization of segmented filamentous bacteria in germ free mice restored ability to get EAE[18] Alteration of gut microbiota with antibiotics reduces susceptibility to EAE[19] and ameliorates EAE[20]. Oral treatment with polysaccharide A (PSA) a bacterial product expressed in the commensal bacteria Bacteroides fragilis attenuates EAE by affecting T regulatory function and migration[21]. Interferon-I produced by astrocytes in mice induced with EAE can combine with metabolites made by commensal gut flora derived from dietary tryptophan to suppress CNS inflammation[22]. Several studies in adult[23-26] and pediatric-age[27, 28] humans with MS also demonstrate dysbiosis of gut microbiota compared to unaffected controls that are associated with alterations in peripheral immune mechanisms implicated in MS pathogenesis. This has led to a "Chicken and Egg Dilemma" in the field about whether "dysbiosis precedes disease or, if in the contrary, an autoimmune disease such as MS can lead to gut dysbiosis[29]."

Work at UCSF (in the laboratory of co-investigator Dr. Sergio Baranzini) showed that in the microbiomes of 71 treatment-naïve MS patients and 71 healthy controls, specific bacterial taxa were significantly enriched in untreated relapsing MS patients (Fig. 1, manuscript submitted). In addition, *Akkermansia muciniphila* and *Acinetobacter calcoaceticus*, both increased in MS patients, induce proinflammatory responses in human PBMCs and in mono-colonized mice (Fig. 2)[39]. These observations suggest that the gut microbiome may be a viable target for therapy in multiple sclerosis, and measuring gut microbiota composition and host immunologic responses may provide insights into the patient's response to therapy.

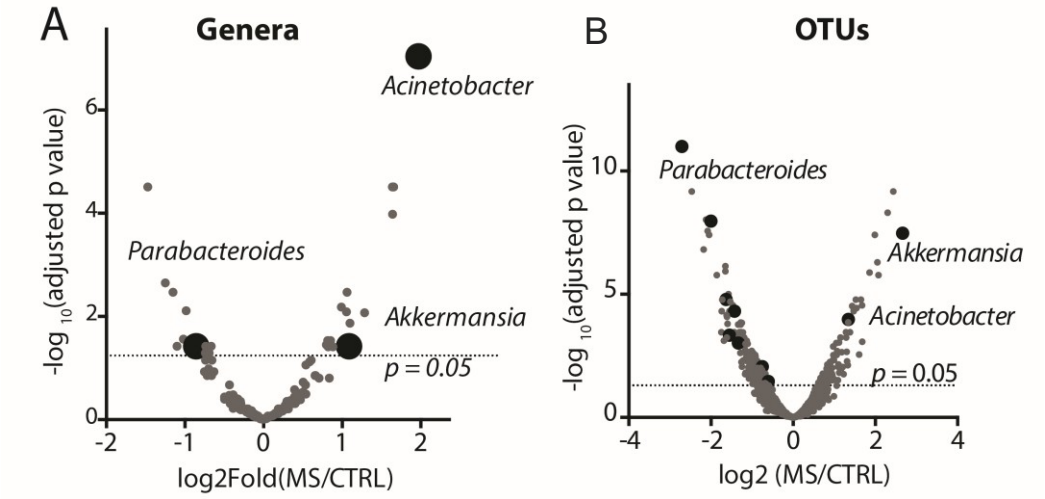

Fig. 1: Changes in gut microbiota in relapsing MS. Systematic examination of relative abundances of individual microbial taxa between 71 untreated relapsing MS patients and 71 controls at the genus (A) and operational taxonomic unit (OTU) levels (B) by Wald test with Benjamini-Hochberg correction for multiple comparisons showed that 247 OTUs (16.89% of total) and 25 bacterial genera

(19.38% of total) were significantly increased or reduced in MS. Bacteria found to be significantly more prevalent in MS include *Akkermansia* and *Acinetobacter*.

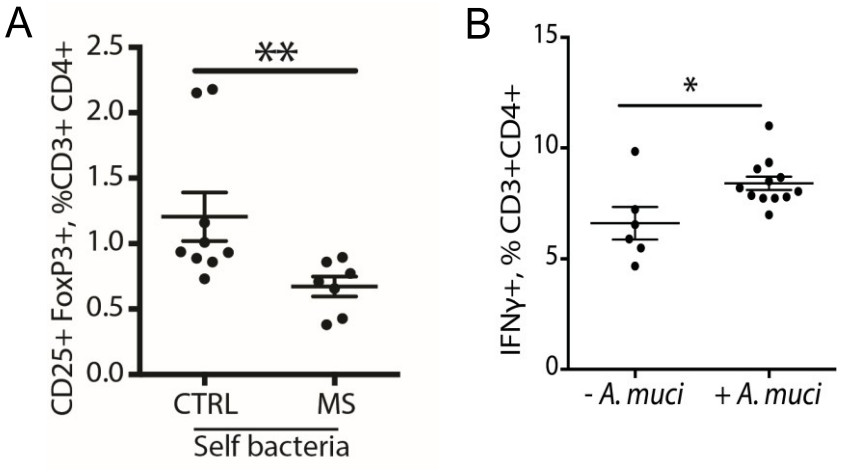

Fig. 2: Immunologic impact of MS-specific gut microbiota. (A) Peripheral blood mononuclear cells (PBMCs) from MS patients showed an impaired ability to differentiate or expand anti-inflammatory CD25+ FoxP3+ Treg populations. (B) *Akkermansia muciniphila*, which is increased in MS populations, significantly increased healthy donor PBMC differentiation into pro-inflammatory Th1 lymphocytes.

Fecal microbiota transplantation (FMT) is a medical procedure in which donor stool is transplanted into a host in order to alter the composition of gut microbiota. FMT is approved in the U.S. as a treatment for refractory *C. Difficile* infection, and several trials have been published documenting its safety and efficacy[30, 31.] It is now also established that frozen donor stool (which is much easier to store and maintain) is non-inferior to fresh donor stool for efficacy treating *C. Difficile* infection[32]. FMT has also been studied for treatment of several other indications, including autoimmune diseases like Ulcerative Colitis in which FMT appears to be a promising rescue therapy[33], whereas other studies (including those without antibiotic preconditioning before the FMT procedure) showed lower level engraftment after a single FMT[34]. FMT in HIV-treated individuals was well-tolerated but led to limited donor engraftment in a regimen without antibiotic preconditioning[35]. FMT from a single “rational” universal donor was beneficial and well-tolerated in a randomized study for treatment of hepatic encephalopathy[36].

In June 2019, the United States Food and Drug Administration (FDA) notified study investigators about two cases of serious multidrug-resistant organism (MRDO) infection in recipients of FMT. MDRO infection in those cases was reportedly transplanted from donor stool to the recipient through DMT and the infection then traveled from the gut into the bloodstream of the recipient causing serious infection. One patient died. The FDA instituted a mandate about

screening FMT donors for MDRO infection risk and to test FMT donor stool for MDRO infections. Our FMT supplier (OpenBiome) adheres to the FDA mandates but with any FMT procedure there may still be risk of transmitted MDRO infection despite such donor screening and testing, and these bacteria which are resistant to some antibiotics could be transmitted through FMT and cause serious infection or death. Informed consent forms were updated to reflect this FDA guidance and above warnings and responses to the advisory letter were provided to the FDA.

In March 2020 the FDA issued a safety alert regarding the possible transmission of Shiga Toxin-Producing *E. coli* (STEC) and Enteropathogenic E. Coli (EPEC). STEC is a type of *E. coli* that can produce a toxin called Shiga toxin. Shiga toxin can cause symptoms like abdominal pain, diarrhea (often bloody), vomiting, and mild fever. Most people develop symptoms within 3-4 days of acquiring the bacteria, and most get better within 5-7 days. EPEC is a type of *E. coli* that is generally carried asymptomatically but can sometimes cause transient watery diarrhea, similar to traveler's diarrhea. Symptoms typically resolve in a matter of days.

Several adverse event reports from OpenBiome have been received related to patients who were treated with investigational FMT for *Clostridiodes difficile* infection (rCDI). Four patients tested positive for STEC after being treated with FMT with material from a single donor. Two were hospitalized and have since been discharged; two were treated as outpatients and their symptoms have resolved. In addition, two patients who also received FMT from this donor have passed away. These individuals had multiple pre-existing conditions. Their deaths are likely unrelated to their FMT. Two additional adverse event reports for patients who received material from this donor were received that are likely unrelated to their FMT. Both had multiple pre-existing conditions. Both have passed away from unrelated causes.

In addition, two immunocompromised patients who were treated with FMT for rCDI later tested positive for EPEC. The patients were treated with material from two different donors. One patient was hospitalized for related symptoms; one was hospitalized for unrelated causes. Both have been discharged.

In response to these events and FDA guidance, OpenBiome notified sites with product that has tested positive and recalled unused units of product that tested positive. The study team has notified the one patient who received product that tested positive. OpenBiome has also made several changes to their routine donor screening, including a change in detection methods to PCR-based methods and the inclusion of additional stool pathogens and new organisms to their donor screening panel (see Appendix N: OpenBiome Amendments to BMF17195).

On March 23, 2020 the FDA issued a safety alert regarding the potential for of

COVID-19 via stool. OpenBiome reports that their protocols and screening measures are compliant with all FDA guidance related to COVID-19.

A potential risk of fecal microbiota transplantation (FMT) is the transmission of SARS-CoV-2, a novel coronavirus that causes the disease COVID-19. Infection with SARS-CoV-2 could be transmitted through stool and could cause serious infection or death. It is possible for healthy, asymptomatic stool donors to potentially be infected with SARS-CoV-2.

OpenBiome's donors who provide stool for FMT routinely undergo regular stool, blood, and nasal screenings for many different infectious agents, including a nasal swab test for SARS-CoV-2 at a minimum of every 14 days. Each FMT is only made available when these screens do not detect these infectious agents in the donor at each of these time points. Donors also undergo regular clinical assessments for any risk factors associated with carrying SARS-CoV-2, such as review of recent travel to areas considered high risk for the virus, including visiting certain healthcare facilities, or other behaviors which may increase the risk of exposure. OpenBiome will continue to update its screening guidelines and procedures as additional data, assays, and information becomes available.

For study subjects who received FMT from stool donations provided prior to December 1st, 2019, it is believed that the risk of SARS-CoV-2 virus being present in the FMT is very low and therefore no testing for the virus has been performed. All FMT products that were shipped to our site were manufactured before December 1, 2019, and we have not nor intend to use any FMT products donated after that date.

To our knowledge, there are no peer-reviewed published studies of FMT in MS. A search of clinicaltrials.gov for "Fecal Microbiota Transplantation" revealed 365 trials studying FMT, of which to date only two other studies are listed for relapsing-remitting MS. One study in the United Kingdom was listed as a Phase II trial with a crossover design treating with FMT (delivered via rectal enema by a trained nurse) in an early vs. delayed start at 6 months design. The study completion date is posted as January 25, 2019 and recruitment status has since been terminated (https://clinicaltrials.gov/ct2/show/NCT03183869, accessed March 12, 2021). Another study from Rush University Medical Center was an observational study of a single subject who underwent FMT outside the United States for the treatment of their MS. This study was completed May 1, 2020 (https://clinicaltrials.gov/ct2/show/NCT03975413, accessed March 12, 2021).

In 2016 and 2017, our UCSF MS Center team held detailed discussions with key experts and stakeholders, including UCSF gastroenterology and infectious disease collaborators (including Dr. Sarah Doernberg, director of antibiotic stewardship at UCSF, and Dr. Richard Jacobs in infectious disease), which

greatly contributed to development of this study protocol.

A 2017 systematic review published in *Annals of Internal Medicine*[37] of 85 FMT trials found that many trials suffer from gaps in reporting details, including "eligibility criteria for donors (47%), materials used for collecting stools and the period of collection (96%), methods used for conservation of stools (76%), the amount and type of stools used (for example, fresh or frozen), and duration of stool conservation (67%). Many (58%) did not report an analysis of microbiota composition." We agree with the authors that FMT is a complex intervention and that detailed reporting is essential for advancing research in this field, particularly when applied to novel indications such as MS. Our trial is designed to try to collect such details with the intention of comprehensive reporting.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVES

i. To demonstrate that FMT of FMP30 in patients with relapsing-remitting MS is feasible, safe and tolerable
ii. To measure donor microbiome engraftment via changes in fecal microbiota community structure at multiple time points following FMT in people with relapsing-remitting MS. Stability of the microbiome in FMT treated patients will also be compared with an observational control arm of untreated MS patients not treated with FMT.

2.2 SECONDARY OBJECTIVES

iii. To evaluate whether FMT of FMP30 donor stool administered via colonoscopy can induce a shift from pro-inflammatory to immunomodulatory T cell profiles in patients with relapsing-remitting MS
iv. To evaluate whether FMT changes humoral function in patients with relapsing-remitting MS
v. To evaluate whether FMT favorably influences clinical and radiological endpoints in patients with relapsing-remitting MS

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

In this Phase 1b open-label prospective clinical trial, patients with relapsing-remitting MS will undergo FMT of FMP30 (donor stool) via colonoscopy and immunological efficacy endpoints will be assessed at various time points. The active phase of the study will continue for 12 weeks post-FMT with safety and biomarker (engraftment) follow-up for 48 weeks. A parallel observational control arm of MS patients who otherwise satisfy study inclusion criteria based on their MS phenotype, demographics, disease duration and prior use of allowable MS

therapies, will be recruited as a comparison observational group to measure stability of stool and serum immunological measures.

The primary hypotheses are that:

1) FMT will be safe and tolerable in patients with MS.

2) FMT preceded by antibiotic preconditioning will lead to a change in fecal microbiota community structure

Secondary hypotheses are that:

1) FMT preceded by antibiotic preconditioning will induce a favorable shift from pro-inflammatory to immunomodulatory T cell profiles in patients with relapsing-remitting MS

2) That engraftment will not appreciably decay over time

3) That FMT will favorably change humoral function

4) That FMT will favorably influence short-term clinical and radiological endpoints.

FMP30 donor stool will be obtained from OpenBiome (Somerville, MA; OpenBiome.org), an established nonprofit stool bank with stringent safety protocols and quality control. Donor stool will be obtained from donors without MS and without other known autoimmune diseases. OpenBiome will screen for transmissible pathogens according to their protocol (see *Appendix L: OpenBiome Donor Screening Testing*). As of January 25, 2021, OpenBiome has made amendments to their donor screening to include new organisms and change detection methods (see Appendix N: OpenBiome Amendments to BMF17195). UCSF has obtained an IND from the FDA for FMT of FMP30 donor stool in MS.

After providing written informed consent and reviewing inclusion and exclusion criteria, subjects will participate in either the FMT Treatment Arm or the Observational Control Arm.

Subjects in the FMT Treatment Arm will first undergo screening assessments according to the study schedule of activities and provide blood samples for eligibility and research, and stool samples for research. Subjects who pass screening will have their pre-treatment baseline visit with 21 days of their screening visit where they will have an MRI, safety and biomarker research blood sample collection, stool sample collection for research, complete study activities according to the study visit schedule, be given antibiotics, bowel preparation, a medication compliance diary and directions on when and how to start the antibiotics and bowel preparation before their scheduled FMT colonoscopy procedure.

The week before their Baseline FMT visit, subjects will be contacted by study staff to review directions of when and how to initiate the 5 day oral antibiotic regimen, and to take the GoLytely bowel preparation to precondition the gut for

FMT and optimize engraftment of the donor microbiome. Study staff will ensure that the subjects understand how to complete their oral antibiotic regimen, compliance diary, and bowel preparation correctly.

At the study Baseline Visit, following standard bowel preparation for colonoscopy, subjects will then undergo colonoscopy with FMT of FMP30 by an experienced gastroenterologist. Subjects will return 2 weeks, 4 weeks, 8 weeks, and 12 weeks after baseline for scheduled assessments of stool and blood sampling, questionnaires, physical examination, EDSS and MS rating scales. A follow-up MRI will be conducted at the Week 12 visit. The active study time of 12 weeks was designed to be short to minimize time off MS disease modifying therapies (should the subject wish to go on a MS disease modifying therapy). After the end of the 12 week active study time, subjects will be able to transition to other therapies such as disease modifying therapies (DMTs). Safety follow-up visits at weeks 24, 36, and 48 will include additional questionnaires, assessments, and safety and biomarker blood sample collection, and stool collection. The total study time will be 52 weeks (4 weeks screening + 12 weeks Active Treatment + 36 weeks safety follow-up).

Subjects participating in the Observational Control Arm will not undergo the interventional FMT treatment. Participants in this arm will have a total of 5 visits over the course of 12 weeks. At the Screening/Baseline visit, subjects will be asked about their Demographics and medical history, concomitant medications, provide blood samples to determine eligibility, undergo an EDSS neurological exam and MS Relapse assessment, and provide blood and stool samples for research along with other study activities according to the study visit schedule. Subjects will be provided a stool kit and pre-paid mailing label to collect their screening stool sample within 3 days after their screening visits and send it to Dr.

Baranzini's lab. At 2 weeks (± 2 days) post baseline/screening, subjects will collect and mail in another stool sample with a provided collection kit and prepaid mailing label. At weeks 4, 8, and 12, subjects will undergo an MS Relapse Assessment, review concomitant medications, and provide blood and stool samples.

3.2 RATIONALE FOR STUDY DESIGN

We hypothesize that the gut microbiome influences MS disease activity and that modifying the gut microbiome via colonoscopically-delivered FMT sourced from donors without MS or other autoimmune disease will be favorable in MS. Data gained from this Phase 1b safety, tolerability and feasibility study will be instrumental for determining whether FMT can be developed into a promising new treatment strategy for MS and informing the design of a Phase 2 randomized-controlled placebo-controlled trial of FMT in MS.

3.3 OUTCOME MEASURES

3.3.1 Primary Outcome Measures

Primary Feasibility Endpoint

- Proportion of subjects who complete the study protocol

Primary biomarker endpoint

- Engraftment → Change in fecal microbiota community structure at weeks 0, 2, 4, 8, and 12
- Engraftment -> Comparison of change in fecal microbiota community structure between FMT treated patients and the observational control arm at weeks 0, 4, 8, and 12

Primary Safety endpoint

-Treatment-emergent adverse events including treatment-emergent serious and non-serious adverse events through week 12 defined as proportion of subjects who develop an adverse event of severity grade 2 or more by NIH CTCAE criteria

3.3.2 Secondary Outcome Measures

Secondary biomarker endpoints

- Induction of T regulatory or Th2 cells and/or reduction of Th1 or Th17 cells at 0, 2, 4, 8 and 12 weeks
- Plasma CD19+ and CD20+ B cell counts and serum immunoglobulin levels at weeks 0, 2, 4, and 12 weeks
- New T2/FLAIR lesions, T2/FLAIR lesion load, T2/FLAIR lesion number at baseline and week 12
- New gadolinium enhancing lesions, total gadolinium enhancing lesions at baseline and week 12

Secondary clinical endpoints

- Relapse rate through week 12
- EDSS and sub scores at the screening visit, pre-treatment baseline visit, week 4, week 8 and week 12
- Fatigue score (abbreviated MFIS) at baseline, week 4, week 8 and week 12
- Quality of life (MSQOL-54)] at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12
- Bowel symptoms (BWCS) at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12
- Bladder symptoms (BLCS) at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12
- Timed 25 foot walk at, pre-treatment baseline visit, week 2, week 4, week 8 and week 12
- Mood and Anxiety (MHI-5) and Columbia Suicide Rating Scale at the screening, pre-treatment baseline visit, week 2, week 4, week 8 and week 12

3.4 SAFETY PLAN

Subjects will be evaluated at each study visit for the duration of their participation in the study (see Section 4.5 Study Assessments and Appendix A, Schedule of Assessments).

Specific potential safety issues for this trial include antibiotic adverse reactions, complications and tolerability of colonoscopy, adverse reactions and worsening of the existing underlying disease, specifically MS relapse or MS MRI disease activity.

See *Section 5: Assessment of Safety*, for complete details of the safety evaluation for this study.

3.5 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in accordance with U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), and local ethical and legal requirements, including approval by the UCSF Institutional Review Board.

4. MATERIALS AND METHODS

4.1 SUBJECTS

4.1.1 Subject Selection

Subjects will be recruited through the UCSF MS Center clinic and clinical research program.

4.1.2 Inclusion Criteria

1) Age 18-60 inclusive (at time of screening)
2) Diagnosis of relapsing-remitting multiple sclerosis (MS) by International Panel McDonald Criteria (2010)[1] incorporating 2017 revisions which reclassify select high-risk Clinically Isolated Syndromes under 2010 criteria as RRMS under 2017 criteria, and Lublin criteria (2014)[2]
3) Recent documented MS disease activity, defined as at least 1 clinical relapse within the past 1 year prior to baseline OR 2 clinical relapses in the past 2 years prior to baseline OR at least 1 new T2/FLAIR lesion on brain or spine MRI OR at least 1 gadolinium enhancing lesion on brain or spine MRI in the past 1 year prior to baseline
4) Expanded Disability Status Scale (EDSS) less than or equal to 6.0; EDSS

5.5 or less if MS disease duration is greater than 15 years (no other disease duration restriction)
5) Must have positive serology (IgG anti-EBNA positive) for Epstein-Barr Virus (EBV) at screening (indicating prior exposure)
6) No prior MS disease modifying therapy or a 12 week washout for subjects on glatiramer acetate or interferon-beta
7) At least 4 weeks from baseline since last use of IV or oral glucocorticoids
8) Agree to maintain a stable diet during the course of the study (over the counter probiotics are allowable)
9) Premenopausal women and women <12 months after the onset of menopause must have a negative serum pregnancy test unless they have undergone surgical sterilization
10) Female subjects of childbearing potential who are sexually active with a nonsterilized male partner must agree to use a highly effective method of contraception; nonsterilized male subjects who are sexually active with a female partner of childbearing potential must agree to use a highly effective method of contraception
11) Not actively participating in another *interventional* MS clinical trial (participation in other observational research studies is allowable)

4.1.3 Exclusion Criteria

1) Prior use of fingolimod, dimethyl fumarate, teriflunomide, natalizumab, alemtuzumab, mitoxantrone, cyclophosphamide, rituximab, ocrelizumab, daclizumab, methotrexate, azathioprine, mycophenolate mofetil, cyclosporine, leflunomide or induction chemotherapy
2) No use of diuretics like furosemide (Lasix) 1 week before the first dose of oral antibiotics. The use of hydrochlorothiazide (HCTZ) for hypertension at a dose ≤ 50 mg/day is allowable.
3) Progressive MS by Lublin criteria (2014)
4) No oral or IV antibiotics within 8 weeks of screening and 12 weeks of the planned FMT procedure (note that topical, otic, ocular antibiotics are specifically allowable) (which is consistent with the IMSMS.org protocol for collaborative gut microbiome research in MS)
5) Hypersensitivity or allergy to study antibiotics, conscious sedation medications or bowel preparation
6) Contraindication to study procedures including MRI, anesthesia (See *Appendix K:* ASA *Criteria IV and V*), colonoscopy, phlebotomy
7) History of inflammatory bowel disease (Crohn's Disease, Ulcerative Colitis)
8) Active symptomatic *C. difficile* infection (colonization is not an exclusion)
9) Active gastrointestinal condition being investigated (i.e. GI bleeding, colon cancer, active GI workup); history of known or suspected toxic megacolon and/or known small bowel ileus, major gastrointestinal surgery (e.g. significant bowel resection) within 3 months before enrollment (note that

this does not include appendectomy or cholecystectomy); or history of total colectomy or bariatric surgery
10) History of malignancy (except excised cutaneous basal cell carcinoma or squamous cell carcinoma which are allowable) including no concurrent induction chemotherapy, radiation therapy or biological treatment for active malignancy
11) Pregnant or lactating women or intention of getting pregnant during the trial period
12) Active infection including untreated latent or active tuberculosis, HIV, hepatitis, syphilis, or other major active infection
13) Known immunodeficiency including CVID
14) INR>1.5, Platelets<100, Hemoglobin <8.5, WBC<2.0, Absolute lymphocyte count <0.8, Absolute Neutrophil Count <0.5, CD4<200, eGFR<45.
15) Any condition that in the opinion of the study PI could jeopardize the safety of the subject, would make it unlikely for the subject to complete the study or could confound the results of the study
16) Unable or unwilling to comply with study protocol requirements

4.2 METHOD OF TREATMENT ASSIGNMENT

Eligible participants who wish to participate in the study and pass screening will receive the study intervention. Eligible participants who do not wish to receive FMT will be invited to enroll in the observational control arm to measure stool and blood biomarkers.

4.3 STUDY TREATMENT

4.3.1 Supply, Preparation, Administration and Storage

4.3.1.1 Donor Stool Formulation

FMP30 donor stool will be obtained from OpenBiome (Somerville, MA; OpenBiome.org), a nonprofit stool bank. (See the Drug Master File BBMF-17195 from OpenBiome,).

Donors are screened by OpenBiome for the following risk factors per OpenBiome protocol and are excluded if they have any of the following (see also *Appendix L: OpenBiome Donor Screening Testing*):

A. Infectious risk factors:
1. Known human immunodeficiency virus (HIV), hepatitis A, B, or C infections or exposure.
2. High-risk sexual behaviors.
3. Use of illicit drugs.

4. Tattoo or body piercing within 6 months.
5. Incarceration or history of incarceration.
6. Known current communicable disease (e.g., upper respiratory tract infection).
7. Risk factors for variant Creutzfeldt Jakob disease.
8. Travel within the last 3 months to areas of the world where diarrheal illnesses are endemic or risk of traveler's diarrhea is high.

B. Gastrointestinal and systemic comorbidities:

1. History of inflammatory bowel disease, irritable bowel syndrome, idiopathic chronic constipation, or chronic diarrhea.
2. History of GI malignancy or known polyposis
3. Major immunosuppressive medications or systemic antineoplastic agents.
4. Antibiotic use in the last 12 weeks.
5. History of major gastrointestinal surgery (e.g., gastric bypass).
6. Past history of *Clostridium difficile* infection.
7. Metabolic syndrome with BMI > 30.
8. Allergies and systemic autoimmunity (e.g., multiple sclerosis, connective tissue disease) and atopic diseases including asthma, eczema, and eosinophilic disorders.
9. Past history of cancer including leukemia.

As of January 25, 2021, OpenBiome has made amendments to their donor screening to include new organisms and change detection methods. (see Appendix N: OpenBiome Amendments to BMF17195).

C. Laboratory Testing:

OpenBiome has an established protocol for screening stool donors. This does not include all known viruses including cytomegalovirus (CMV) or Epstein-Barr virus (EBV) or unknown viruses or unknown pathogens (see *Appendix L: Donor Screening List and Appendix N: OpenBiome Amendments to BMF17195)*. Our Phase 1 protocol excludes for pregnancy (acute *cytomegalovirus* (CMV) can be of major concern in pregnant women) and we exclude individuals who are EBV IgG anti-EBNA seronegative indicating prior exposure (rates of EBV positivity in adults with MS are very high as they are in the general population). Please also note that to our knowledge and per OpenBiome there have been no reported

cases of CMV or EBV infection following allogenic FMT, including in immunocompromised patients[40].

4.3.1.2 Donor Stool Administration

The decision to deliver FMT via colonoscopy (as opposed to by enema or with oral capsules) was based on guidance from gastroenterology collaborators based on hypothesized better engraftment with colonoscopic delivery as well as tolerability (including with potential oral delivery given number of capsules required). The dosing selection of 3 x FMP30 was based on trying to maximize

potential engraftment while minimizing volume of stool needed to transplant and based on guidance from the OpenBiome Scientific Advisory board in designing this study protocol.

According to FMP30 preparation instructions provided by OpenBiome, donor stool may be stored locally for up to 6 months in a standard -20°C laboratory freezer or up to 24 months in a -80°C laboratory freezer. FMP30 will be prepared and thawed according to these instructions (see *Appendix B: Storage Controls and Material Specifications*).

Once thawed, the material is ready for immediate administration. After thawing, material may remain at room temperature for up to 4 hours (or refrigerated/on ice for up to 8 hours). Samples should never be re-frozen. If thawed and not used within 8 hours, the material should be disposed of, as freeze thaw cycles may compromise viability.

Recipients will undergo standard bowel preparation the day preceding FMT. The stool suspension will be introduced via colonoscopy and delivered by a study gastroenterologist into the cecum and ascending colon.

4.3.1.3 Antibiotics

Emerging data from FMT trials for non-MS, non-Clostridium difficile indications demonstrates that engraftment of donor stool can be low when antibiotic preconditioning is not utilized[34, 35]. This protocol will therefore utilize antibiotic preconditioning prior to FMT and careful measurement of degree of engraftment as a primary biomarker endpoint. Antibiotic preconditioning using various regimens has been used in other FMT studies and is also widely used in the context of preparation for elective colorectal surgery (in which oral antibiotics have been shown to reduce surgical site infection rates and readmission rates)[38]. The selection of Neomycin and Metronidazole was based on standard usage in the context of bowel preparation for colorectal surgery and familiarity of use for other GI indications. (Ciprofloxacin, a fluoroquinolone, was considered as an alternative to neomycin and is sometimes used in the FMT context, but there are emerging safety concerns with fluoroquinolones as a class). Furthermore, both neomycin and vancomycin when administered orally tend to have poor systemic absorption and will therefore be expected to act mostly locally on colonic microbiota and potentially reduce risk of systemic side effects. Oral vancomycin was included with the goal of covering additional gram positive bacteria that are postulated to be important as part of the mechanism of action of FMT and are widely used in combination temporally with FMT in the Clostridium Difficile setting). Note that this same antibiotic protocol was also selected by UCSF GI investigators studying FMT in the context of ulcerative colitis, see https://clinicaltrials.gov/ct2/show/NCT03006809, accessed June 25, 2017).

Summary Antibiotic Preconditioning Regimen:

Vancomycin	**500 mg PO BID**	**X 5 days**
Metronidazole	**500 mg PO BID**	**X 5 days**
Neomycin	**500 mg PO BID**	**X 5 days**

All antibiotics to be started 6 days prior to FMT

Oral antibiotics will be obtained through the UCSF Research pharmacy and delivered in secured packaging with labeling per pharmacy protocols. Subjects will be asked to complete a medication diary to document taking of home antibiotics. Subjects will be required to complete the antibiotic regimen and the medication diary to receive the FMT study treatment. Failure of the subject to do so will result in exclusion from the study.

4.3.2 Dosage Modification

Antibiotic dosages are protocolized; dosage modifications will not be permitted.

FMT dosage via colonoscopy may include a lower amount of transplanted stool at the discretion of the study gastroenterologist if there are any peri-procedural safety or technical considerations. Total FMT dose (in mL) will be documented.

4.1.3 Over Dosage

There is no data about over-dosage of FMT in humans.

4.4 CONCOMITANT AND EXCLUDED THERAPY

Routine *symptomatic* MS therapies will be allowable, including dalfampridine or 4-aminopyridine, botulinum toxin, oral spasticity agents/muscle relaxants, pain medications, antidepressants and anti-anxiety medication, modafinil and armodafinil or amphetamines for fatigue/alertness.

Subjects will be asked to consent to not being on a MS disease modifying therapy during the 12 week study period. The length of this feasibility study was specifically designed to be short in order to minimize time not on a MS DMT. Subjects with a new clinical MS relapse or breakthrough clinical worsening of MS will be allowed to go on DMT at the discretion of their treating neurologist per routine medical management. If the decision to start a MS DMT occurs before the study intervention (antibiotics and/or FMT) this will be considered a screening failure and subjects will be excluded from the study and not included in further safety monitoring or in the pre-planned primary analysis. If the decision to start a MS disease modifying therapy occurs after any study intervention (antibiotics and/or FMT) has been administered, further study intervention will be discontinued but subjects will be invited and encouraged to complete the remaining observational period of the protocol for engraftment, immunological and safety monitoring. For acute relapse management, PO or IV steroids will be

allowable as part of routine medical management at the discretion of the treating neurologist.

4.5 STUDY ASSESSMENTS

After providing written informed consent and reviewing inclusion and exclusion criteria, subjects will participate in either the FMT Treatment Arm or the Observational Control Arm. Study visits will take place at the Neuroscience Clinical Research Unit (NCRU) at the Sandler Neuroscience Center (675 Nelson Rising Lane, San Francisco, CA) on the UCSF Mission Bay Campus. Colonoscopy will take place at the UCSF Parnassus Endoscopy Unit (505 Parnassus Avenue, San Francisco, CA).

FMT Treatment Arm:

After providing written informed consent and reviewing inclusion and exclusion criteria, subjects will undergo screening visit assessments. Screening can be extended to a total of 5 weeks if deemed necessary by the PI to repeat require screening diagnostic testing. These assessments include:

Screening:

- Medical history including documentation of MS history, past medical problems and diagnoses, past surgical history, family history, medication review, medication allergy review, social history, smoking history, and contraception data collection for subjects that are heterosexually active
- Measurements of vital signs, weight and height
- Electrocardiogram (ECG)
- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Concomitant medication review
- Physical examination including a general physical examination and neurological examination
- MS relapse assessment
- Expanded Disability Status Scale (EDSS) (a structured neurological exam)
- MS symptom and disability questionnaires including the MFIS (Fatigue), MSQOL-54 (Quality of Life), BWCS (bowel symptoms), BCLS (bladder symptoms), MHI-5 (Mood and Anxiety) and the Columbia Suicide Rating Scale (suicidality)
- Food Frequency Questionnaire
- Stool collection (2 stool collection kits will be provided at screening visit. Stool sample expected to be collected within 3 days of visit and sent in by the patient in pre-labeled packaging. The subject has the option of providing a stool sample at the pre-treatment baseline visit if one is not provided at screening.)
- Urine collection for urinalysis and culture to assess kidney function
- Blood draw via venipuncture: Up to a maximum of 150mL of venous blood

samples will be obtained at each visit for the assessments described below and as listed in the schedule of assessments for subsequent visits.

About 15 ml of blood will be taken at the screening visit and subsequent visits according to the schedule of study activities for safety laboratory tests. Samples for the following screening safety and eligibility laboratory tests will be sent to UCSF's Medical Center laboratory for analysis at the UCSF Mission Bay campus at 1975 4th Street, San Francisco, CA.

- Serum pregnancy test (if the subject can become pregnant)
- Hematology (hemoglobin, hematocrit, RBCs, WBC absolute and differential, ANC, and quantitative platelet count)
- Prothrombin Time and International Normalized Ratio (PT/INR)
- Chem-10 (sodium, potassium, CO2, chloride, BUN, Creatinine, Glucose, Magnesium, Calcium, phosphorus)
- Lymphocyte Subsets
- Liver function tests(Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Total Bilirubin, Alkaline phosphatase, Creatinine)
- Immunoglobulins (IgG, IgM, IgA) to evaluate for immunodeficiency
- Tuberculosis screening (will be primarily analyzed by UCSF Mission Bay labs. Will be analyzed by Quest Labs if needed, as back up)
    - Must be negative a screening
- Viral serology
    - HIV (Must be negative at screening)
    - Hepatitis
        - Must be negative for HBsAg and HepBAb, and HepCAb at screening visit
    - Treponemal screen (must be negative for syphilis at screening visit)
    - EBV IgG anti-EBNA must be positive at screening (indicating prior exposure)

About 100 mL of blood will be drawn for biomarker and immunology research at screening and subsequent visits according to the study schedule of activities.

Blood and stool research samples will be analyzed at the UCSF MS Immunology Research Lab located in the Sandler Neurosciences Center at 675 Nelson Rising Lane.

<u>Pre-treatment Baseline Visit:</u>

Within 21 days after screening, subjects will partake in pre-treatment baseline visit assessments. These assessments include:

- Review of inclusion/exclusion criteria
- Vital signs
- Weight

- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Subjects will receive an Adverse Event Diary to record any adverse events that they subject experience throughout the study. Study staff will review this with the subject at all subsequent visits.
- Concomitant medication review
- Physical examination including a general physical examination and neurological examination
- Brain, Cervical and Thoracic spine MRI, with and without contrast.
- MS Relapse Assessment
- Expanded Disability Status Scale (a structured neurological exam)
- Timed 25 foot walk
- MS symptom and disability questionnaires including the MFIS (Fatigue), MSQOL-54 (Quality of Life), BWCS (bowel symptoms), BCLS (bladder symptoms), MHI-5 (Mood and Anxiety) and the Columbia Suicide Rating Scale (suicidality)
- Stool collection (A stool sample is not required at this visit if the subject has already provided one at screening)
- Urine pregnancy test (if the subject can become pregnant)
- Blood draw via venipuncture for immunological research
- Subjects will be given an oral antibiotic regimen and written instructions to take at home 6 days before their FMT colonoscopy procedure date. They will also be given an antibiotic compliance diary, and are required to record the dates and times they take their antibiotics and GoLytely Bowel Preparation. This will be reviewed by study staff on the FMT colonoscopy procedure date.
- Subjects will receive GoLytely bowel preparation, a bowel preparation compliance diary and standardized instructions for bowel preparation per usual UCSF colonoscopy unit protocol.

Subjects will be treated with oral antibiotics for gut preconditioning for 5 days before their FMT colonoscopy. The antibiotics will be provided by the UCSF research pharmacy and given to subjects at their pre-treatment baseline study visit. Subjects will be given written instructions to start taking them exactly 6 days prior to their scheduled FMT colonoscopy. Subjects will also receive a phone call by the study coordinator 1-3 days before they should start taking their antibiotics to confirm that the subject understands the instructions and are not experiencing any new health problems.

GoLytely bowel preparation for colonoscopy will be self-administered at home the night before and morning of their FMT. Subjects will receive GoLytely bowel prep solution and standardized instructions for bowel preparation per usual UCSF colonoscopy unit protocol at their pre-treatment baseline visit. These instructions will also be reviewed via the reminder phone call before antibiotics have been initiated.

To ensure bowel preparation compliance, subjects will be given a compliance diary to record the dates and times that they took their prescribed antibiotics and GoLytely bowel preparation. This will be collected and reviewed on the day of

their FMT colonoscopy procedure. If the compliance diary is incomplete the FMT procedure is subject to cancellation under the discretion of the Principle Investigator and study gastroenterologist.

FMT Treatment Colonoscopy Baseline Visit:

Depending on the availability, subjects may be asked to complete the following study assessments up to a week prior to their scheduled colonoscopy:

- Stool Collection
- Urine pregnancy test (if the patient can become pregnant)
- Blood draw via venipuncture for safety labs
- Urinalysis and culture

The following assessments will be completed on the day of the FMT colonoscopy:

- Vital signs
- Weight
- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Concomitant medication review
- Antibiotic compliance diary collected and confirmed completed
- Confirmation that bowel preparation was completed
- Blood draw via venipuncture for immunology research samples

After these assessments have been completed subjects will undergo the research-based FMT colonoscopy by a study gastroenterologist, per standard UCSF GI colonoscopy unit protocols to include administration of conscious sedation. At the discretion of the study gastroenterologist, up to 90 ml of FMP30 donor stool will be administered for FMT via colonoscopy. A colonoscopy report generated by the study gastroenterologist will be included in the medical record with note of any abnormal findings. If there is a concerning finding during the colonoscopy that in the opinion of the gastroenterologist warrants timely biopsy, a biopsy will be performed. The sample will be sent to UCSF pathology for review and for a written report. The patient will be informed in the event of an abnormal finding. If a biopsy is conducted FMT will not be performed and the subject will be excluded from further active FMT treatment but offered to complete study visits for safety and biomarker follow-up.

The study gastroenterologist and study P.I. will be notified at any point during recovery if the subject experiences:

- Heart rate less than 60 or greater than 120 beats/min
- Respiratory rate less than 12 or greater than 25 breaths/min
- Systolic blood pressure is less than 90 mmHg or greater than 140 mmHg
- If Glucose greater than 200
- Diastolic blood pressure is less than 60 mmHg or greater than 90 mmHg
- Temperature greater than 38.5 degrees Celsius

When endoscopy discharge criteria are met, the subject will be discharged from the endoscopy center.

FMT Treatment Arm Post FMT Visits:

Subjects in the FMT Treatment arm will come in for follow-up study visit assessments at weeks 2, 4, 8, and 12 following colonoscopy. These visit will include:

- Vital signs
- Weight
- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Concomitant Medication Review
- Physical examination including a general physical examination and neurological examination
- Brain, Cervical and Thoracic spine MRI, with and without contrast at week 12
- MS Relapse Assessment
- Expanded Disability Status Scale (a structured neurological exam)
- Timed 25 foot walk
- MS symptom and disability questionnaires including the MFIS (Fatigue), MSQOL-54 (Quality of Life), BWCS (bowel symptoms), BCLS (bladder symptoms), MHI-5 (Mood and Anxiety) and the Columbia Suicide Rating Scale (suicidality)
- Food Frequency Questionnaire at week 12
- Stool collections
- Pregnancy Test at weeks 2, 8, and 12 (if patient can become pregnant)
- Blood draw via venipuncture for lab safety values and immunological research

Safety Follow-Up Visits:

After subjects complete the active portion of the study, they will enter the Safety Follow-Up period at weeks 24, 36, and 48. Assessments at these visits include:

- Vital signs
- Weight
- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Concomitant medication review
- Physical examination including a general physical examination and neurological examination (at week 48)
- EDSS
- Timed 25 foot walk
- MS symptom and disability questionnaires including the MFIS (Fatigue), MSQOL-54 (Quality of Life), BWCS (bowel symptoms), BCLS (bladder symptoms), MHI-5 (Mood and Anxiety) and the Columbia Suicide Rating Scale (suicidality)
- Food Frequency Questionnaire
- Stool collection
- Urine pregnancy test (if the subject can become pregnant)

- Blood draw via venipuncture for clinical laboratory safety values and immunological studies for research

After their week 48 safety follow-up visit, subjects in the FMT Treatment arm will have completed the study.

Unscheduled Visits:

In the case of a MS relapse or any other AE that the PI deems as a safety concern during the study, subjects will be asked to come in for an unscheduled visit. Assessments at this visit will include:

- Vital signs
- Weight
- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Concomitant Medication Review
- Physical examination including a general physical examination and neurological examination
- Optional brain, cervical and thoracic spine MRI, with and without contrast
- MS relapse assessment
- EDSS
- MS symptom and disability questionnaires including the MFIS (Fatigue), MSQOL-54 (Quality of Life), BWCS (bowel symptoms), BCLS (bladder symptoms), MHI-5 (Mood and Anxiety) and the Columbia Suicide Rating Scale (suicidality)
- Stool collection
- Optional urine pregnancy test (if the subject can become pregnant)
- Blood draw via venipuncture for lab safety values and optional immunology study samples

Early Termination Visit:

In the case of early termination, subjects will be asked to have an early termination visit. Assessments during this visit include:

- Vital signs
- Weight
- Assessment of Adverse Events (AEs) and Serious Adverse Events (SAEs)
- Concomitant Medication Review
- Physical examination including a general physical examination and neurological examination
- Brain, Cervical and Thoracic spine MRIs, with and without contrast
- EDSS
- Timed 25 foot walk
- MS symptom and disability questionnaires including the MFIS (Fatigue), MSQOL-54 (Quality of Life), BWCS (bowel symptoms), BCLS (bladder symptoms), MHI-5 (Mood and Anxiety) and the Columbia Suicide Rating Scale (suicidality)
- Stool collection
- Urine collection for pregnancy test and urinalysis lab safety values
- Blood draw via venipuncture clinical laboratory safety values, and immunological studies for research

Observational Control Arm:

Screening/Baseline Week 0 Visit:
After providing written informed consent and reviewing inclusion and exclusion criteria, subjects will undergo the Screening/Week 0 visit assessments. These assessments include:

- Demographics and medical history
- Concomitant medication review
- MS Relapse Assessment
- EDSS
- Food Frequency Questionnaire
- Stool collection (Stool collection kits for screening, week 2 and week 4 will be provided at screening visit. The stool sample for screening will be expected to be collected within 3 days of visit and sent in by the patient in pre-labeled packaging.)
- Blood draw via venipuncture: Up to a maximum of 150mL of venous blood will be obtained at each visit for the assessments described below and as listed in the schedule of assessments for subsequent visits. About 15 ml of blood will be taken at the screening visit and subsequent visits according to the schedule of study activities for safety laboratory tests. Samples for the following safety and eligibility laboratory tests will be sent to UCSF's Medical Center laboratory for analysis at the UCSF Mission Bay campus at 1975 4th Street, San Francisco, CA.
    - Hematology (hemoglobin, hematocrit, RBCs, WBC absolute and differential, ANC, and quantitative platelet count)
    - Lymphocyte Subsets
    - Immunoglobulins (IgG, IgM, IgA) to evaluate for immunodeficiency
    - Tuberculosis screening (will be analyzed by Quest Labs)
        - Must be negative a screening
    - Viral serology
        - HIV
            - Must be negative at screening
        - Hepatitis
            - Must be negative for HBsAg and HepCAb prior to enrollment;
        - Treponemal screen (must be negative for syphilis)
        - EBV IgG anti-EBNA positive at screening, indicating prior exposure

- About 100 mL of blood will be drawn for biomarker and immunology research at screening and subsequent visits according to the study schedule of activities. Blood and stool research samples will be analyzed at the UCSF MS Immunology Research Lab located in the Sandler Neurosciences Center at 675 Nelson Rising Lane.

Week 2:

Participants will collect and mail in a stool sample in the packaging with a pre-paid air-bill provided by the study team at the screening visit.

Weeks 4, 8, and 12 Visits:
Subjects will return for follow up assessments at weeks 4, 8 and 12. These assessments include:

- Concomitant medication review
- MS Relapse Assessment
- Food Frequency Questionnaire at week 12
- Stool collection
- Blood draw via venipuncture for safety labs and Immunological samples for research

After their Week 12 visit, subjects in the observational control arm will have completed the study.

4.3.1 Blood and Stool Samples for Research

4.3.1.1 Blood Samples

While the primary endpoint of this study (as in all phase 1 studies) is safety and tolerability, we also thought it important to include a scientific and mechanistic biomarker endpoint, in this case the T cell in vitro assay and a measurement of microbiome structure change. They are generally accepted as a method to determine the overall inflammatory properties of microbiota and in immune dysregulation in people with MS.

Immune function will be measured in T cell in-vitro assays. Microbiota extracts will be co-cultured with blood peripheral mononuclear cells under different immune polarizing conditions to determine the pro-inflammatory properties of a given gut microbiota sample. The potential clinical applicability of the T cell in-vitro assay has been observed in preclinical studies of microbiome differences between people with and without MS including as published by Dr. Baranzini's lab at UCSF.

4.3.1.2 Stool Samples

The clinical relevance of the fecal microbiota community structure is that it provides a mechanism of how microbiome alterations affect immune function in Multiple Sclerosis. It will be used as a measure of whether the subject's microbiome changes with FMT intervention. Microbiota structure will be determined at each available time point by 16S ribosomal RNA sequencing on an Illumina Miseq instrument. Raw data files from the sequencer will be analyzed for

quality, and processed with the QIIME2 pipeline to obtain measures of alpha diversity (the richness of bacterial communities within a given sample), beta-diversity (how different two samples are when compared) (PcoA), individual organism testing, taxa representation, and differential abundance. With this method, the precise relative proportion of each identified organism can be assessed by creating a unique bacterial signature for each subject. If changes in community structure are detected, we will also explore how long these changes persist after the intervention.

4.6 DISCONTINUATION OF PROTOCOL-SPECIFIED THERAPY

Protocol-specified therapy may be discontinued for any of the following reasons:

- Unacceptable toxicity
- Patient election to discontinue therapy (for any reason)
- Patient election to start another MS disease modifying therapy
- Physician's judgment

4.7 SUBJECT DISCONTINUATION

Subjects will be removed from the study if there are any emergent safety concerns that in the opinion of the study investigators could negatively affect the welfare of the participant.

Subjects with clinical MS relapses or breakthrough clinical worsening of MS will be allowed to go on disease modifying therapy (DMT) at the discretion of their treating neurologist per routine medical management. If the decision to start a MS DMT occurs before the study intervention (antibiotics and/or FMT) subjects will be considered a screening failure and excluded from the study and not included in further safety monitoring or in the pre-planned primary analysis. If the decision to start a MS disease modifying therapy occurs after any study intervention (antibiotics and/or FMT), any further study intervention will be discontinued and subjects will be invited to complete remaining study visits per protocol for safety and immunological monitoring. PO or IV steroids will be allowable as part of routine medical management for relapse activity.

4.8 STUDY DISCONTINUATION

The Principal Investigator may elect to terminate the study at any time, including but not limited to unexpected adverse events that indicate the potential for a health hazard to subjects, failure to enroll or loss of financial support.

As of February 18, 2021, the study team decided to stop further recruitment early due to the challenges presented by the COVID-19 pandemic and in consideration that this is a Phase 1 study.

4.9 STATISTICAL METHODS

4.9.1 Analysis of the Conduct of the Study

For study integrity, the following variables will be analyzed using descriptive/summary statistics: Enrollment, Dropouts, and Protocol Violations.

Descriptive statistics will be used to describe baseline characteristics and demographics of the cohort. The t-test (or Wilcoxon Rank sum) or chi-squared (or Fisher exact) tests as appropriate will be used to analyze differences between the interventional and control groups.

4.9.2 Efficacy Analysis

a. Primary Endpoint

Summary statistics will be used to report primary safety and feasibility outcomes. For the primary biomarker immunology outcome, the Wilcoxon signed-rank (or paired T-test) will be used as a paired pre-post analysis of change in immunological function in the FMT treated arm. The t-test or Mann-Whitney- U/Wilcoxon rank sum or chi-squared to Fisher Exact test will be used as appropriate to compare differences between the FMT treated arm and observational control arm.

b. Secondary Endpoints

Mixed effects linear regression modeling with time as the interaction term to leverage repeated measures will be used to analyze the following:

- Fecal microbiota community structure at weeks 0, 2, 4, 8, 12 and for safety follow-up through an open label extension through 1 year
- Induction of T regulatory or Th2 cells and/or reduction of Th1 or Th17 cells at 0, 2, 4 8 and 12 weeks and for safety follow-up through 1 year
- Plasma CD19+ and CD20+ B cell counts and serum immunoglobulin levels at weeks 0, 2, 4, 8 and 12 weeks and for safety follow-up through 1 year
- Fatigue score (abbreviated MFIS) at baseline, week 4, week 8 and week 12
- Quality of life (MSQOL-54)] at baseline, week 4, week 8 and week 12
- Bowel symptoms (BWCS) at baseline, week 4, week 8 and week 12
- Bladder symptoms (BLCS) at baseline, week 4, week 8 and week 12

- Timed 25 foot walk at baseline, week 4, week 8 and week 12

The Wilcoxon signed rank (or paired T test) will be used for the following pre-post comparisons in the interventional, FMT treated arm:

- New T2/FLAIR lesions, T2/FLAIR lesion load, T2/FLAIR lesion number at baseline and week 12
- New gadolinium enhancing lesions, total gadolinium enhancing lesions at baseline and week 12
- EDSS and sub scores at baseline and week 12
- Timed 25 foot walk at baseline, week 4, week 8 and week 12
- Mood and Anxiety (MHI-5) and Columbia Suicide Rating Scale and baseline, week 8 and week 12

We will also analyze using comparison of means for pre-post treatment (student's T-test or Wilcoxon rank sum or chi-squared or Fisher Exact as appropriate)

4.9.3 Safety Analysis

Descriptive/summary statistics will be reported for safety endpoints and will include:

- Summary of Adverse Events and Serious Adverse Events
- Timing of AEs and Serious AEs

4.9.4 Missing Data

Available data will be reported along with an accounting of missing data. There are no plans to use imputation or related strategies.

4.9.5 Sample Size

We intend to recruit 10 subjects into the FMT treatment arm of this feasibility study. Using a Wilcoxon signed-rank (or paired T-test) for paired pre-post analyses on immunological function with a sample size of 10 subjects with complete data, we will have 80% power with an alpha of 0.05 to detect at least a 1 SD change in pre-post outcomes of change in T cell function.

An observational control group of up to 20 subjects will be recruited to measure stool and serum biomarkers.

As of February 18, 2021, the study team decided to stop further recruitment due to the challenges presented by the COVID-19 pandemic and in consideration that this is a Phase 1 study. Investigators are currently analyzing data obtained. Given this, data analyses will primarily be descriptive based on the number of subjects enrolled.

4.10 DATA QUALITY

Data quality will be ensured through the use of standard practices and procedures through the UCSF MS Clinical research program.

5. ASSESSMENT OF SAFETY

5.1 SAFETY VARIABLES

Patients will be evaluated clinically and with standard laboratory tests before the study and at regular intervals during the study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, and standard laboratory measurements.
Adverse events (AEs) and serious adverse events (SAEs) that are considered related to the FMT intervention will be monitored and reported.

5.2 DEFINITION OF AN ADVERSE EVENT (AE)

Adverse events (AEs) will be recorded at each regular scheduled study visit in the study patient record (source document) as well as on a specific AE case report form (CRF). An AE is any untoward medical occurrence in a study patient or clinical investigation participant administered a pharmaceutical product. An AE does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product, e.g.:

- any new clinical diagnosis
- any symptom that requires medical clarification or leads to in-patient admission (surgery or accident)
- any suspected adverse drug reaction (ADR)
- any symptom that appears on the study patient's medical records
- any event related in time with the application of the study medication and affecting the health of the study patient (including laboratory value changes)

If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

AEs must be graded for severity and relationship to study product. Adverse events of special interest (AESI) will be defined as newly acquired transmissible infectious diseases, newly acquired autoimmune disease, and worsening of the underlying disease state.

AEs will be assessed by the clinician using the NIH Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 defined grading system (Briefly, the criteria for estimating adverse event severity grade:

- Grade 1, Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2, Moderate: Minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL).
- Grade 3, Severe: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4, Life threatening: Places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- Grade 5, Death

Based on the most recent version of NCI NIH CTCAE (v4.0), which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

All adverse events, regardless of relatedness should be reported. All adverse events should be evaluated for relatedness when reporting and documenting on the CRF. The following guidelines of relatedness will be utilized:

- Definitely Related: The adverse event is clearly related to the FMT material - i.e. an event that follows a reasonable temporal sequence from administration of the FMT material or follows a known or expected response pattern to the FMT material, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure, and that could not be reasonably explained by the known characteristics of the patient's clinical state.
- Possibly Related: An adverse event that follows a reasonable temporal sequence from administration of the FMT material or follows a known or expected response pattern to the suspected intervention, but that could have been produced by other factors.

In addition to open-ended questions on adverse events meeting the above definitions, specific potential adverse events will be inquired about during the safety follow up period (following the 12 week active portion of the study through to 48 weeks.

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the site PI or lead PI, it results in any of the following outcomes:

- Death
- Life-threatening adverse event*
- Inpatient hospitalization or prolongation of existing hospitalization
- A congenital anomaly/birth defect
- Persistent or significant disability or incapacity or substantial disruption of

the ability to conduct normal life function

- Important medical events that, may not result in death, be life-threatening, or require hospitalization may be considered when, based upon appropriate medical judgement, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

**Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the site PI or lead PI, its occurrence places the patient or participant at immediate risk of death. It does not include an adverse event which, had it occurred in a more severe form, might have caused death.*

Any adverse event or suspected adverse reaction that meets the criteria for serious adverse event will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

5.3 UNSOLICITED ADVERSE EVENTS

On enrollment in the study, the study participants will be instructed to contact the site PI if an AE occurs. These include fever, chills, abdominal pain, abdominal distention, excessive flatulence, constipation, nausea, and vomiting. These unsolicited, unrelated non-serious adverse events occurring from the time of FMT until 12 weeks (for the active period of the study) and 52 weeks (for the safety follow-up). Patients will be given a patient diary with date, time, details and action taken to help with data collection. This diary be taken to the site PI for evaluation at each follow-up visit and patients instructed to seek immediate medical attention if indicated.

5.4 REPORTING OF ADVERSE EVENTS

Study participants will be instructed to contact the study team if any serious or unexpected adverse event occurs. Study staff will enquire about AEs at each study visit. Reported AE's will be recorded in detail in an AE CRF.

AE information to be collected in the AE CRF:

- Nature of the event
- Time of onset: date, time
- Concomitant treatment: product (generic name), indication, dosage, dosage interval, presentation, mode of administration, administration regimen

- Duration of the AE
- Severity
- Seriousness
- Causality
- Outcome

The course and outcome of the adverse event will be commented on as follows:

- Recovered without sequelae
- Not yet recovered
- Recovered with sequelae
- Fatal

Any SAE (including death, irrespective of the cause) occurring during the study will be immediately reviewed by the study PI.

If determined to be a SUSAR (Suspected Unexpected Serious Adverse Reaction) by the study PI, the event will be reviewed by the Data Safety Committee. All unexpected fatal or life-threatening events associated with the use of the trial drugs will be reported to the regulatory agencies and competent authorities via telephone or fax within 7 calendar days after being notified of the event. Also, all related but unexpected SAEs including non-death/non-life-threatening related but unexpected SAEs associated with the use of the trial medication will be reported to the appropriate authorities and investigators by a written safety report within 15 calendar days of notification. The UCSF IRB will be notified of SUSARs in accordance to the Post-Approval Reporting Requirements policy of the UCSF Office of Ethics and Compliance (see *Appendix M: UCSF Post-Approval Reporting Requirements*).

In case of a SAE (non-SUSAR), the study PI will record the event for review and compilation. The Data Safety Committee will review all SAE every 6 months or *ad hoc* depending on the clinical case at the discretion of the study PI. A specific SAE CRF will be provided. The report must contain a detailed description of the symptoms observed and the concomitant treatment administered. Furthermore, the report must comment on a possible causative relationship between the AE and the trial medication. Each SAE must be followed until it is resolved or can be explained satisfactorily.

For non-serious adverse reactions (definitely related, possibly related, not related) the study PI will record the event for review and compilation. All non-serious adverse reactions will be reviewed by the Data Safety Committee every 6 months or *ad hoc* depending on the clinical case at the discretion of the study PI.

In accordance with safety requirements, the study PI will inform the local IRB. The following instructions must be heeded:

- In the case of an intolerable SAE, the study patient must, at the decision of the study PI, be withdrawn from further treatment, and symptomatic treatment must be administered. The participant may opt

to voluntarily provide additional samples for duration of study.
- The measures taken must be recorded on the CRF.

In accordance with local legislation, the study PI will submit copies of the final SAE-report to the Regulatory Authorities concerned, if necessary.

5.5 RECORDING SAFETY VARIABLES

The principal investigator will be responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the UCSF IRB and the FDA in accordance with CFR 312.32 (IND Safety Reports).

5.5.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and study intervention initiation, defined in this study as the first dose of antibiotic pre-treatment. AE monitoring ends 16 weeks following the last administration of study treatment or study discontinuation/termination, whichever is earlier.

5.5.2 Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to FMT procedure and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes
There is a plausible temporal relationship between the onset of the AE and administration of FMT procedure (including antibiotic preconditioning and bowel preparation), and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the FMT procedure.

No
Evidence exists that the AE has an etiology other than the FMT procedure (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to the FMT procedure.

5.6 REPORTING ADVERSE EVENTS

A. Deaths

All deaths that occur during the protocol-specified AE reporting period will be reported.

B. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions will be reported as part of the medical and surgical history. Preexisting conditions will be reassessed throughout the trial and reported as an AE or SAE if the frequency, severity, or character of the condition worsens during the study.

C. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization will be documented and reported as an SAE. Hospitalization for diagnostic or elective surgical procedures for preexisting conditions will not be reported.

D. Pregnancy

If a female subject becomes pregnant during the course of the study, this will be reported as an AE, and appropriate reporting will also be made to the UCSF IRB.

5.7 DATA SAFETY MONITORING PLAN

This study will adhere to a pre-specified IRB-approved data safety monitoring plan with the goal of ensuring participant and monitoring data quality. As this study is a single-center open label Phase 1 clinical trial for which the local investigator will have access to all data, an independent data safety monitoring board is not required per UCSF IRB guidance (https://irb.ucsf.edu/data-and-safety-monitoring-plans-and-boards). The study PI will review patient safety and data on a near real-time basis as part of routine study conduct.

The study will be halted pending review and discussion by the Data Safety Committee if at any point during the study a serious adverse event (SAE) at least possibly related to study treatment or a suspected or proven infection at least possibly related to the study treatment is identified.

In addition, to ensure participant safety, the data safety monitoring plan will include additional review by a Data Safety Committee consisting of the study PI (Dr. Jeffrey Gelfand), Dr. Sergio Baranzini and GI co-investigators (Drs. James Ostroff and Priya Kathpalia) at the following intervals for interim analyses:

■ Within 2 weeks of when the first 3 FMT treated patients have reached 4 week post-FMT

- Within 3 weeks after each 3 sequential FMT-treated subjects reach 12 weeks post-FMT
- Semi-annually to review all study progress up to that date
- At study completion

The Data Safety Committee will review clinical data, adverse events, and any anticipated or unanticipated problems involving risk to participants and will have access to all primary study data upon request. PI can request additional ad hoc meetings as needed. Immunological efficacy outcomes as available will be shared with the committee as available but may not be available at every timeline, especially earlier time points, given the need for batched analyses. The study PI provides assurance that the research team will adhere to all required UCSF IRB reporting requirements as well as those that may be required by the FDA.

6. REGULATORY CONSIDERATIONS

6.1 IND

An IND will be obtained from the FDA for FMT of donor stool in MS. Reporting of AEs will be in accordance with IND and UCSF IRB guidelines.

6.2 INFORMED CONSENT

Written informed consent will be obtained from all subjects. A copy of the ICF will be provided to each subject along with a UCSF IRB approved Human Subjects Bill of Rights. Subjects will also be required to sign appropriate HIPAA release documentation for medical record review.

6.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE APPROVAL

This study protocol, informed consent forms and supporting documentation will be submitted to the UCSF IRB for review before the study is initiated. The study will be conducted in accordance with U.S. FDA, applicable national and local health authorities, and UCSF IRB requirements, including for applicable reporting.

6.4 DATA COLLECTION

Data will be collected using a combination of hard copy case report forms and electronic case report forms with database programs using REDCap, a web-based platform approved and encouraged by UCSF for use in human subject research.

6.5 DISCLOSURE AND PUBLICATION OF DATA

Disclosure will be in accordance with UCSF IRB guidelines and approved protocols. Some data created by the study will be added to the medical record, including clinical laboratory results and MRI reports (which are read out by UCSF neuro-radiologists for safety). Medical information obtained from the study may also be given to the subject's treating physician or health-care provider with the subject's permission.

Data generated by this study will be made available to relevant authorities, including the U.S. FDA, local or national health authorities or the UCSF IRB.

It is the intention of the investigators that the results of this study will be presented at scientific meetings and published in peer-reviewed medical journals. No names or identifying information will be used in presenting trial results.

The study will be registered with clinicaltrials.gov.

6.6 RETENTION OF RECORDS

Records will be obtained per current UCSF IRB and U.S. FDA guidelines, with the current standard to retain records for at least 2 years after completion of the study.

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8. APPENDICES

Appendix A-1: Table of Assessments for Interventional FMT Treatment Group

	Screening	***Treatment Period***									***Early Termin-ation Visit***	***Safety Follow-Up***		
Week	***- 4***	***Pre-Treatment Baseline Visit***	***Pre-FMT Phone Call***	***Baseline FMT Week 0***	***Post FMT Phone Call***	***2***	***4***	***8***	***12 (End of Active Portion)***	***Unsched-uled visit***		***24 (OLE SAFETY FOLLOW-UP)***	***Week 36 (OLE SAFETY FOLLOW-UP)***	***Week 48 (OLE SAFETY FOLLOW-UP)***
Day	***-28***	***Within 21 Days After Screening***	***-7(±1)***	***0***	***2 (±1)***	***14 (±2)***	***28 (±7)***	***56 (±7)***	***84 (±7)***			***168 (±7)***	***252 (±7)***	***336 (±7)***
Informed Consent	X													
Review Inclusion/Exclusion Criteria	X	X												
Demographics, Medical History, Smoking History, Reproductive Status, & Contraception	X													
Vital Signs	X	X		X		X	X	X	X	X	X	X	X	X
Weight, Height (height only collected at screening)	X	X		X		X	X	X	X	X	X	X	X	X
ECG (for safety) with cardiology read	X													
Adverse Event Reporting	X	X (Provide Subject with AE Diary)	X (telephone visit)	X	X (telephone visit)	X	X	X	X	X	X	X	X	X
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X	X				X	X	X	X	X	X			X
IMAGING														
MRI Brain, Cervical and Thoracic Spine with contrast (includes 3 formal neuroradiology reads) – also includes SF SIGMA spine sequences		X							X	X (optional)	X			

	Screening	Treatment Period									Early Termination Visit	Safety Follow-Up		
Week	**- 4**	**Pre-Treatment Baseline Visit**	**Pre-FMT Phone Call**	**Baseline FMT Week 0**	**Post FMT Phone Call**	**2**	**4**	**8**	**12 (End of Active Portion)**	**Unscheduled visit**		**24 (OLE SAFETY FOLLOW-UP)**	**Week 36 (OLE SAFETY FOLLOW-UP)**	**Week 48 (OLE SAFETY FOLLOW-UP)**
Day	**-28**	**Within 21 Days After Screening**	**-7(±1)**	**0**	**2 (±1)**	**14 (±2)**	**28 (±7)**	**56 (±7)**	**84 (±7)**			**168 (±7)**	**252 (±7)**	**336 (±7)**
CLINICAL ASSESSMENTS														
MS Relapse Assessment	X	X				X	X	X	X	X	X	X	X	X
EDSS	X	X				X	X	X	X	X	X	X	X	X
Timed 25 foot walk		X				X	X	X	X		X	X	X	X
Questionnaires (MSQOL-54, MFIS, MHI-5, C-SSRS, BWCS, BLCS)	X (Use Baseline/Screening Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)				X (Use Since Last Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)		X (Use Since Last Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)	X (Use Since Last Visit version of C-SSRS)
Food Frequency Questionnaire	X								X			X	X	X
LABORATORY ASSESSMENTS														
Stool Collection (microbiome)	X (can be collected at pre-treatment visit)	X (optional if not collected at screening)		X		X	X	X	X	X	X	X	X	X
Pregnancy test (Serum test at screening. Urine test at all other visits)	X	X		X		X		X	X	X (optional)	X	X	X	X
CBC/diff	X			X		X		X	X	X	X			X
Urinalysis + culture	X			X					X	X (optional)	X			
HIV	X								X		X			
Treponemal Screen	X								X		X			
EBV IgG anti-EBNA Screen	X													
Immunoglobulins (IgG, IgM, IgA)	X			X		X	X	X	X		X			
Hepatitis B and C screening	X								X		X			

	Screening	Treatment Period									Early Termination Visit	Safety Follow-Up		
Week	**- 4**	**Pre-Treatment Baseline Visit**	**Pre-FMT Phone Call**	**Baseline FMT Week 0**	**Post FMT Phone Call**	**2**	**4**	**8**	**12 (End of Active Portion)**	**Unsched-uled visit**		**24 (OLE SAFETY FOLLOW-UP)**	**Week 36 (OLE SAFETY FOLLOW-UP)**	**Week 48 (OLE SAFETY FOLLOW-UP)**
Day	**-28**	**Within 21 Days After Screening**	**-7(±1)**	**0**	**2 (±1)**	**14 (±2)**	**28 (±7)**	**56 (±7)**	**84 (±7)**			**168 (±7)**	**252 (±7)**	**336 (±7)**
Lymphocyte Subsets (CD 3, 4, 8, 19)	X			X		X	X	X	X	X	X			
INR/PT(Blood Clotting)	X													
LFTs (AST, ALT, AlkP, T bili)	X			X		X	X	X	X	X (optional)	X			
Quantiferon Gold	X													
Chem-10	X			X		X	X	X	X		X	X	X	X
Immunology studies (Baranzini Lab)	X	X		X		X	X	X	X	X (optional)	X	X	X	X
Intervention														
Oral Antibiotic Regimen (taken at home)		X (give meds, directions, and diary to subject)	X (telephone call; review antibiotic directions)	X (antibiotic diary collection)										
Bowel Preparation (taken at home)		X (give to subject with directions)	X (telephone call; review bowel prep directions)	X (bowel regimen taken at home)										
Colonoscopy				X										
FMT with donor stool				X										
Conscious sedation for FMT				X										

Appendix A-2: Schedule of Assessments for Observational Control Arm (no FMT)

	Screening/Baseline	Observation Period			
Week	**Week 0**	**Week 2**	**Week 4**	**Week 8**	**Week 12** END OF OBSERVATIONAL PORTION
Day	**0**	**14 (±2)**	**28 *(±7)***	**56 *(±7)***	**84*(±7)***
Informed Consent	X				
Review Inclusion/Exclusion Criteria	X				
Demographics and Medical History	X				
EDSS	X				
Medication Review	X		X	X	X
MS Relapse Assessment	X		X	X	X
Frequency Questionnaire	X				X
LAB ASSESSMENTS					
Stool Collection (microbiome)	X	X (subject will mail in sample)	X	X	X
Immunology studies (Baranzini Lab)	X		X	X	X
HIV	X				
Treponemal Screen	X				
EBV IgG anti-EBNA Screen	X				
Hepatitis B and C screening	X				
CBC/diff	X		X	X	X
Immunoglobulins (IgG, IgM, IgA)	X		X	X	X
Lymphocyte Subsets (CD 3, 4, 8, 19)	X		X	X	X

Appendix B: Storage Controls and Material Specifications

OPENBIOME

Storing and Administering FMT Microbiota Preparations

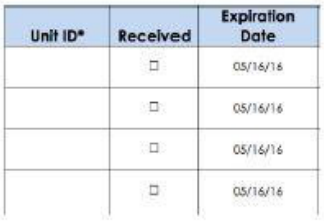
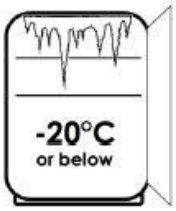
Instructions for FMT Upper Delivery (30 mL) or FMT Lower Delivery (250 mL)

Temperature Requirement: Treatment units must be stored frozen at **-20°C (-4°F) or below**; transient fluctuations of +5°C (+9°F) are acceptable.

Expiration Dates: Store treatment units at -20°C for up to 6 months, or at -80°C for up to 12 months. Both expiration dates can be found on the bottle (FMT Lower Delivery) or box (FMT Upper Delivery).

Handling: Wear medical-grade disposable gloves when handling bottles directly.

When you receive treatment unit(s):

Step 1	Step 2	Step 3
 Confirm that the treatments arrived frozen. Check the temperature tag on the inside lid of the shipping cooler or visually inspect treatment for any sign of thawing.	 Note date treatments were received and whether treatments were frozen on delivery on your Unit List.	 Transfer units to your freezer OR begin to thaw unit(s) if needed immediately. **If treatments did not arrive frozen**, dispose of them following your facility's protocols for handling human waste and contact OpenBiome.

Unit ID*	Received	Expiration Date
	☐	05/16/16
	☐	05/16/16
	☐	05/16/16
	☐	05/16/16

A note on sharing freezer space with other material: The exterior of each bottle is cleaned with a BruTab 6S® broad-spectrum disinfecting and sanitizing solution after the cap is sealed and before the bottle is frozen. Contact OpenBiome for specifics.

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www.openbiome.org

2067 Massachusetts Avenue
Cambridge, MA 01240

Phone/Fax: 617-575-2201
info@openbiome.org

OPENBIOME

When you are ready to thaw FMT Upper Delivery or FMT Lower Delivery:

Step 1	Step 2	Step 3
		 4h room temperature 8h refrigerated
Remove bottle from box, check that tamper-evident screw cap is intact. If the seal has been damaged, contact OpenBiome.	Thaw using ONE of the methods outlined in the chart below.	Once thawed completely, treatments can be left at room temperature for up to 4 hours, or kept refrigerated for up to 8 hours.

Method	Approx. Thaw Time by Item	Max. Time Until Use After Completely Thawed
Water Bath (30°C/86°F)	FMT Lower (FMP250): 1 hour FMT Upper (FMP30): 15 minutes	Room Temp.: 4 hours Refrigerated: 8 hours
Room Temp. (21°C/70°F)	FMT Lower (FMP250): 4 hours FMT Upper (FMP30): 45 minutes	
Refrigerator (2°C/36°F)	FMT Lower (FMP250): 16 hours FMP Upper (FMP30): 90 minutes	
Thaw times do not differ significantly for samples stored at -20°C or -80°C.		

WARNING: Freeze-thaw cycles compromise the viability of the bacterial communities in the preparation. Do not refreeze thawed treatments. Dispose of unused or expired material according to your internal protocols for handling human waste.

Please contact info@openbiome.org or 617-575-2201, option 3, with any questions.

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www.openbiome.org

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Cambridge, MA 01240

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Appendix C: Subject Adverse Event Diary

Subject Adverse Event Diary

Subject Initials _____ Page____of _____

Instructions: Please record any symptoms or changes in your health you may be feeling throughout this study. Please bring this diary to all study appointments. Contact study staff for any questions or concerns that you may have.

AE #	AE Description	Onset Date/Time	Resolution Date/Time	Action Taken to Resolve	Outcome

Appendix D-1: Colombia-Suicide Severity Rating Scale (C-SSRS): Baseline/Screening

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline/Screening Version

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103-130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

Appendix D1: Colombia-Suicide Severity Rating Scale (C-SSRS): Baseline/Screening (cont.)

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past 6 Months**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g. thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
INTENSITY OF IDEATION		
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. Lifetime - ***Most Severe Ideation:*** _____ ***Type # (1-5)*** _____ ***Description of Ideation*** Past X Months - ***Most Severe Ideation:*** _____ ***Type # (1-5)*** _____ ***Description of Ideation***	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____	____
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____	____

Appendix D1: Colombia-Suicide Severity Rating Scale (C-SSRS): Baseline/Screening (cont.)

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**	**Past 1 Year**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe:	Yes ☐ No ☐ Total # of Attempts _____	Yes ☐ No ☐ Total # of Attempts _____
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes ☐ No ☐	Yes ☐ No ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes ☐ No ☐ Total # of interrupted _____	Yes ☐ No ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes ☐ No ☐ Total # of aborted _____	Yes ☐ No ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes ☐ No ☐	Yes ☐ No ☐

Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____

Appendix D-2: Colombia-Suicide Severity Rating Scale (C-SSRS): Since Last Visit

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

Appendix D2: Colombia-Suicide Severity Rating Scale (C-SSRS): Since Last Visit (cont.)

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Since Last Visit**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** ☐ **No** ☐
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** ☐ **No** ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** ☐ **No** ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** ☐ **No** ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** ☐ **No** ☐
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). ***Most Severe Ideation:*** ____ _____ ***Type # (1-5)*** ***Description of Ideation***	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration ***When you have the thoughts, how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____

Appendix D2: Colombia-Suicide Severity Rating Scale (C-SSRS): Since Last Visit (cont.)

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	Since Last Visit
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	Yes No ☐ ☐ Total # of Attempts ____ Yes No ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of interrupted ____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of aborted ____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes No ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes No ☐ ☐
Suicide:	Yes No ☐ ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____

Appendix E: Mental Health Inventory (MHI-5)

MHI-5

Patient's Initals: _____ **Date:** ____/____/____
dd mmm yyyy

ID#: **Visit:**

MENTAL HEALTH INVENTORY (MHI-5)

The next set of questions are about how you feel, and how things have been for you during the past 4 weeks. If you are marking your own answers, please circle the appropriate response (0, 1, 2,...). If you need help in marking your responses, tell the interviewer the number of the best response. Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

During the past 4 weeks, how much of the time...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little bit of the time	None of the time
1. have you been a happy perosn?	1	2	3	4	5	6
2. have you felt calm and peaceful?	1	2	3	4	5	6
3. have you been a very nervous person?	1	2	3	4	5	6
4. have you felt downhearted and blue?	1	2	3	4	5	6
5. have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6

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Appendix F: Modified Fatigue Impact Scale (MFIS)

Patient's Initials: _____ **Date:** _____/____/____
dd mmm yyyy

ID#: _____ **Visit:**

MODIFIED FATIGUE IMPACT SCALE (MFIS)

Following is a list of statements that describe how fatigue may affect a person. Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. In medical conditions like MS, feelings of fatigue can occur more often and have a greater impact than usual. Please read each statement carefully, and then circle the one number that best indicates how often fatigue has affected you in this way during the past 4 weeks. (If you need help in marking your responses, tell the interviewer the number of the best response.) Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

Because of my fatigue during the past 4 weeks....

		Never	Rarely	Sometimes	Often	Almost always
1.	I have been less alert.	0	1	2	3	4
2.	I have had difficulty paying attention for long periods of time.	0	1	2	3	4
3.	I have been unable to think clearly.	0	1	2	3	4
4.	I have been clumsy and uncoordinated.	0	1	2	3	4
5.	I have been forgetful.	0	1	2	3	4
6.	I have had to pace myself in my physical activities.	0	1	2	3	4
7.	I have been less motivated to do anything that requires physical effort.	0	1	2	3	4

Appendix F: Modified Fatigue Impact Scale (MFIS) (cont.)

Because of my fatigue during the past 4 weeks....

		Never	Rarely	Sometimes	Often	Almost always
8.	I have been less motivated to participate in social activities.	0	1	2	3	4
9.	I have been limited in my ability to do things away from home.	0	1	2	3	4
10.	I have had trouble maintaining physical effort for long periods.	0	1	2	3	4
11.	I have had difficulty making decisions.	0	1	2	3	4
12.	I have been less motivated to do anything that requires thinking.	0	1	2	3	4
13.	my muscles have felt weak.	0	1	2	3	4
14.	I have been physically uncomfortable.	0	1	2	3	4
15.	I have had trouble finishing tasks that require thinking.	0	1	2	3	4
16.	I have had difficulty organizing my thoughts when doing things at home or at work.	0	1	2	3	4
17.	I have been less able to complete tasks that require physical effort.	0	1	2	3	4
18.	my thinking has been slowed down.	0	1	2	3	4
19.	I have had trouble concentrating.	0	1	2	3	4

Appendix F: Modified Fatigue Impact Scale (MFIS) (cont.)

Because of my fatigue during the past 4 weeks....

		Never	Rarely	Sometimes	Often	Almost always
20.	I have limited my physical activities.	0	1	2	3	4
21.	I have needed to rest more often or for longer periods.	0	1	2	3	4

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54)

Multiple Sclerosis Quality of Life

(MSQOL)-54 Instrument

For Further Information, Contact:

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Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

INSTRUCTIONS:

This survey asks about your health and daily activities. Answer every question by circling the appropriate number (1, 2, 3, ...).

If you are unsure about how to answer a question, please give the best answer you can and write a comment or explanation in the margin.

Please feel free to ask someone to assist you if you need help reading or marking the form.

1. In general, would you say your health is:
(circle one number)

Excellent……1

Very good……2

Good……3

Fair……4

Poor……5

2. **Compared to one year ago**, how would you rate your health in general **now**?

(circle one number)

Much better now than one year ago..... 1

Somewhat better now than one year ago……2

About the same 3

Somewhat worse now than one year ago..... 4

Much worse now than one year ago 5

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

3-12. The following questions are about activities you might do during a typical day. Does **your health** limit you in these activities? If so, how much? (Circle 1, 2, or 3 on each line)

	Yes, Limited a Lot	Yes, Limited a Little	No, Not Limited at All
3. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
4. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
5. Lifting or carrying groceries	1	2	3
6. Climbing several flights of stairs	1	2	3
7. Climbing one flight of stairs	1	2	3
8. Bending, kneeling, or stooping	1	2	3
9. Walking more than a mile	1	2	3
10. Walking several blocks	1	2	3
11. Walking one block	1	2	3
12. Bathing and dressing yourself	1	2	3

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

13-16. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

(Circle one number on each line)

	YES	NO
13. Cut down on the amount of time you could spend on work or other activities	1	2
14. Accomplished less than you would like	1	2
15. Were limited in the kind of work or other activities	1	2
16. Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

17-19. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious).

(Circle one number on each line)

	YES	NO
17. Cut down on the amount of time you could spend on work or other activities	1	2
18. Accomplished less than you would like	1	2
19. Didn't do work or other activities as carefully as usual	1	2

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

(circle one number)

Not at all..... 1

Slightly 2

Moderately 3

Quite a bit 4

Extremely..... 5

Pain

21. How much **bodily** pain have you had during the **past 4 weeks**?

(circle one number)

None 1

Very mild..... 2

Mild 3

Moderate..... 4

Severe 5

Very severe..... 6

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

(circle one number)

Not at all..... 1

A little bit 2

Moderately 3

Quite a bit 4

Extremely..... 5

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

23-32. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**... (Circle one number on each line)

	All of the Time	Most Of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6
32. Did you feel rested on waking in the morning?	1	2	3	4	5	6

Subject ID:_____ Vist:_____ 6 of 16

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Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

33. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

(circle one number)

All of the time.....1

Most of the time.....2

Some of the time 3

A little of the time.....4

None of the time.....5

Health in General

34-37. How TRUE or FALSE is each of the following statements for you.

(Circle one number on each line)

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
34. I seem to get sick a little easier than other people	1	2	3	4	5
35. I am as healthy as anybody I know	1	2	3	4	5
36. I expect my health to get worse	1	2	3	4	5
37. My health is excellent	1	2	3	4	5

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Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Health Distress

How much of the time during the **past 4 weeks**...

(Circle one number on each line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
38. Were you discouraged by your health problems?	1	2	3	4	5	6
39. Were you frustrated about your health?	1	2	3	4	5	6
40. Was your health a worry in your life?	1	2	3	4	5	6
41. Did you feel weighed down by your health problems?	1	2	3	4	5	6

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Cognitive Function

How much of the time during the **past 4 weeks...**

(Circle one number on each line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
42. Have you had difficulty concentrating and thinking?	1	2	3	4	5	6
43. Did you have trouble keeping your attention on an activity for long?	1	2	3	4	5	6
44. Have you had trouble with your memory?	1	2	3	4	5	6
45. Have others, such as family members or friends, noticed that you have trouble with your memory or problems with your concentration?	1	2	3	4	5	6

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Sexual Function

46-50. The next set of questions are about your sexual function and your satisfaction with your sexual function. Please answer as accurately as possible about your function **during the last 4 weeks only.**

How much of a problem was each of the following for you **during the past 4 weeks?**

(Circle one number on each line)

MEN	Not a problem	A Little of a Problem	Somewhat of a Problem	Very Much a Problem
46. Lack of sexual interest	1	2	3	4
47. Difficulty getting or keeping an erection	1	2	3	4
48. Difficulty having orgasm	1	2	3	4
49. Ability to satisfy sexual partner	1	2	3	4

(Circle one number on each line)

WOMEN	Not a problem	A Little of a Problem	Somewhat of a Problem	Very Much a Problem
46. Lack of sexual interest	1	2	3	4
47. Inadequate lubrication	1	2	3	4
48. Difficulty having orgasm	1	2	3	4
49. Ability to satisfy sexual partner	1	2	3	4

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

50. Overall, how satisfied were you with your sexual function **during the past 4 weeks**?

(circle one number)

Very satisfied..... 1

Somewhat satisfied..... 2

Neither satisfied nor dissatisfied 3

Somewhat dissatisfied 4

Very dissatisfied..... 5

51. During the **past 4 weeks**, to what extent have problems with your bowel or bladder function interfered with your normal social activities with family, friends, neighbors, or groups?

(circle one number)

Not at all 1

Slightly..... 2

Moderately 3

Quite a bit..... 4

Extremely 5

52. During the **past 4 weeks**, how much did *pain* interfere with your enjoyment of life?

(circle one number)

Not at all 1

Slightly..... 2

Moderately 3

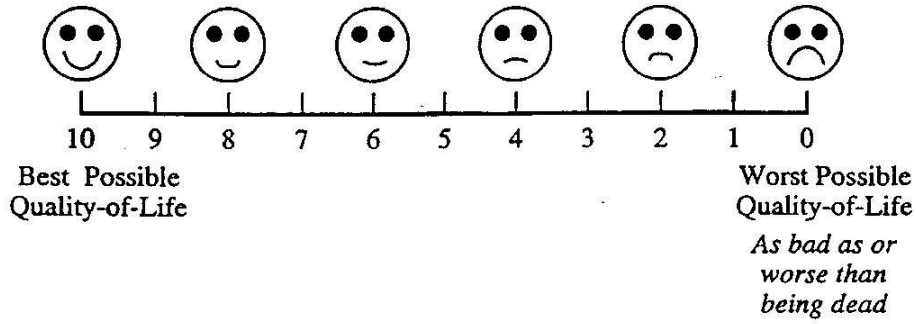
Quite a bit..... 4

Extremely 5

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

53. Overall, how would you rate your own quality-of-life?

Circle one number on the scale below:



54. Which best describes how you feel about your life as a whole?

(circle one number)

Terrible	1
Unhappy	2
Mostly dissatisfied	3
Mixed - about equally satisfied and dissatisfied	4
Mostly satisfied	5
Pleased	6
Delighted	7

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Scoring Forms for Multiple Sclerosis Quality of Life (MSQOL) -54

Table 1
MSQOL-54 Scoring Form

Table 2
MSQOL-54 Physical Health Composite Score

Table 3
MSQOL-54 Mental Health Composite Score

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Table 1

MSQOL-54 Scoring Form

Scale/Item Number	Response 1	2	3	4	5	6		Subtotal	Final Score 0-100 point scale
Physical Health									
3.	0	50	100					_____	
4.	0	50	100					_____	
5.	0	50	100					_____	
6.	0	50	100					_____	
7.	0	50	100					_____	
8.	0	50	100					_____	
9.	0	50	100					_____	
10.	0	50	100					_____	
11.	0	50	100					_____	
12.	0	50	100					_____	
							Total:	_____ ÷ 10 =	_____
Role limitations due to physical problems									
13.	0	100						_____	
14.	0	100						_____	
15.	0	100						_____	
16.	0	100						_____	
							Total:	_____ ÷ 4 =	_____
Role limitations due to emotional problems									
17.	0	100						_____	
18.	0	100						_____	
19.	0	100						_____	
							Total:	_____ ÷ 3 =	_____
Pain									
21.	100	80	60	40	20	0		_____	
22.	100	75	50	25	0			_____	
52.	100	75	50	25	0			_____	
							Total:	_____ ÷ 3 =	_____
Emotional well-being									
24.	0	20	40	60	80	100		_____	
25.	0	20	40	60	80	100		_____	
26.	100	80	60	40	20	0		_____	
28.	0	20	40	60	80	100		_____	
30.	100	80	60	40	20	0		_____	
							Total:	_____ ÷ 5 =	_____
Energy									
23.	100	80	60	40	20	0		_____	
27.	100	80	60	40	20	0		_____	
29.	0	20	40	60	80	100		_____	
31.	0	20	40	60	80	100		_____	
32.	100	80	60	40	20	0		_____	
							Total:	_____ ÷ 5 =	_____

Table 1 (cont.) Scale/Item Number	Response 1	2	3	4	5	6	Subtotal	Final Score 0-100 point

Subject ID:_____ Vist:_____

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Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Health Perceptions									
	1.	100	75	50	25	0			_____
	34.	0	25	50	75	100			_____
	35.	100	75	50	25	0			_____
	36.	0	25	50	75	100			_____
	37.	100	75	50	25	0			_____
								Total:	_____ ÷ 5 = _____
Social function									
	20.	100	75	50	25	0			_____
	33.	0	25	50	75	100			_____
	51.	100	75	50	25	0			_____
								Total:	_____ ÷ 3 = _____
Cognitive function									
	42.	0	20	40	60	80	100		_____
	43.	0	20	40	60	80	100		_____
	44.	0	20	40	60	80	100		_____
	45.	0	20	40	60	80	100		_____
								Total:	_____ ÷ 4 = _____
Health distress									
	38.	0	20	40	60	80	100		_____
	39.	0	20	40	60	80	100		_____
	40.	0	20	40	60	80	100		_____
	41.	0	20	40	60	80	100		_____
								Total:	_____ ÷ 4 = _____
Sexual function*									
	46.	100	66.7	33.3	0				_____
	47.	100	66.7	33.3	0				_____
	48.	100	66.7	33.3	0				_____
	49.	100	66.7	33.3	0				_____
								Total:	_____ ÷ 4 = _____
Change in health									
	2.	100	75	50	25	0			_____
Satisfaction with sexual function									
	50.	100	75	50	25	0			_____

	Response						
Overall quality of life	**1**	**2**	**3**	**4**	**5**	**6**	**7**
53.	(multiply response by 10)						
54.	0	16.7	33.3	50	66.7	83.3	100

Total: _____ ÷ 2 = _____

Note: The total number of items in each scale is listed as the divisor for each subtotal. However, due to missing data, the divisor might actually be less than that if not every item within a given scale has been answered. For example, if item 38 in the Health Distress scale was left blank and the other 3 items in the scale were answered, then the "Total" score for Health Distress would be divided by '3' (instead of '4') to obtain the "Final Score."
* Males and females can be combined in the analysis even though question 47 is different for the two groups. The scale scores can also be reported separately for males and females.

Appendix G: Multiple Sclerosis Quality of Life Questionnaire (MSQOL-54) (cont.)

Table 2
Formula for calculating MSQOL-54 Physical Health Composite Score

MSQOL-54 Scale	Final Scale Score	x	Weight	=	Subtotal
Physical function	_____	x	.17	=	_____(a)
Health perceptions	_____	x	.17	=	_____(b)
Energy/fatigue	_____	x	.12	=	_____(c)
Role limitations - physical	_____	x	.12	=	_____(d)
Pain	_____	x	.11	=	_____(e)
Sexual function	_____	x	.08	=	_____(f)
Social function	_____	x	.12	=	_____(g)
Health distress	_____	x	.11	=	_____(h)

PHYSICAL HEALTH COMPOSITE: Sum subtotals (a) through (h) = _____

Table 3
Formula for calculating MSQOL-54 Mental Health Composite Score

MSQOL-54 Scale	Final Scale Score	x	Weight	=	Subtotal
Health distress	_____	x	.14	=	_____(a)
Overall quality of life	_____	x	.18	=	_____(b)
Emotional well-being	_____	x	.29	=	_____(c)
Role limitations - emotional	_____	x	.24	=	_____(d)
Cognitive function	_____	x	.15	=	_____(e)

MENTAL HEALTH COMPOSITE: Sum subtotals (a) through (e) = _____

Appendix H: Bowel Control Scale (BWCS)

Patient's Name: _____ **Date:** _____/____/____
month day year

ID#: _____ **Test#:** 1 2 3 4

BOWEL CONTROL SCALE (BWCS)

INSTRUCTIONS

The next set of questions concerns bowel problems that can occur in MS. Many of these questions are very personal, but this is an important topic to cover. If you are marking your own answers, please circle the appropriate response (0, 1, 2,...) based on your bowel function during the past 4 weeks. If you need help in marking your responses, tell the interviewer the number of the best response. Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

During the past 4 weeks,
how often have you...

		Not at all	Once	Two to four times	More than weekly but not daily	Daily
1.	been constipated?	0	1	2	3	4
2.	lost control of your bowels or had an accident?	0	1	2	3	4
3.	almost lost control of your bowels or almost had an accident ?	0	1	2	3	4
4.	altered your activities because of bowel control problems?	0	1	2	3	4

5. **During the past 4 weeks, how much have bowel problems restricted your overall lifestyle? (Please circle one number.)**

Not at all										Severely
0	1	2	3	4	5	6	7	8	9	10

Appendix I: Bladder Control Scale (BLCS)

Patient's Name: _____ **Date:** _____/____/____
month day year

ID#: _____ **Visit (circle one):** **Screening** **Pre-treament**
wk 2 wk 4 wk 8 wk 12 wk 24 wk 36 wk 48

BLADDER CONTROL SCALE (BLCS)

INSTRUCTIONS
The next set of questions concerns bladder problems that can occur in MS. Many of these questions are very personal, but this is an important topic to cover. If you are marking your own answers, please circle the appropriate response (0, 1, 2,...) based on your bladder function during the past 4 weeks. If you need help in marking your responses, tell the interviewer the number of the best response. Please answer every question. If you are not sure which answer to select, please choose the one answer that comes closest to describing you. The interviewer can explain any words or phrases that you do not understand.

During the past 4 weeks,
how often have you...

		Not at all	Once	Two to four times	More than weekly but not daily	Daily
1.	lost control of your bladder or had an accident?	0	1	2	3	4
2.	almost lost control of your bladder or had an accident?	0	1	2	3	4
3.	altered your activities because of bladder problems?	0	1	2	3	4

4. **During the past 4 weeks, how much have bladder problems restricted your overall lifestyle? (Please circle one number.)**

Not at all										Severely
0	1	2	3	4	5	6	7	8	9	10

Appendix J: Food Frequency Questionnaire

Appendix J: Food Frequency Questionnaire

FOOD QUESTIONNAIRE

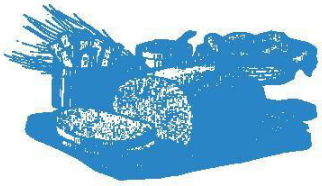
RESPONDENT ID #

TODAY'S DATE

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

DAY

YEAR 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024



ABOUT THIS SURVEY

This form is about the foods you usually ate in the past 3 months. It will take about 30 - 40 minutes to complete. Please answer each question as best you can. Estimate if you aren't sure.

- **USE ONLY A NO. 2 PENCIL.**
- **Fill in the circles completely, and erase completely if you make any changes.**

ABOUT YOU

SEX
- Male
- Female

If female, are you pregnant or breast feeding?
- No
- Yes
- Not female

AGE

WEIGHT pounds

HEIGHT ft. in.

INSTRUCTIONS

There are usually two kinds of questions to answer for each food:

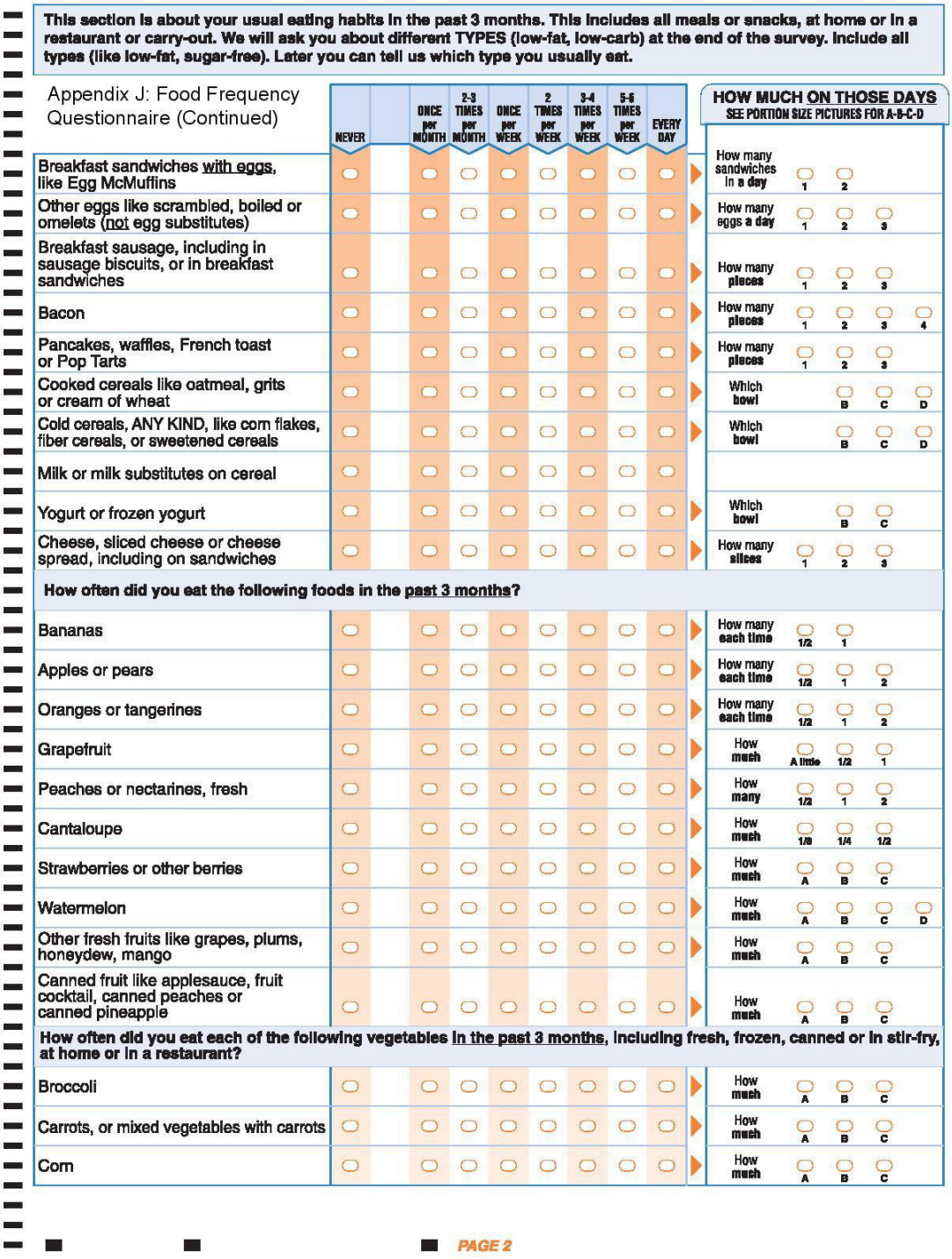
1. **HOW OFTEN,** on average, did you eat the food during the past 3 months?
   *Please DO NOT SKIP any foods. Mark "Never" if you didn't eat any of the food in the question.
2. **HOW MUCH** did you usually eat of the food?
   *Sometimes we ask how many you eat, such as 1 egg, 2 eggs, etc., ON THE DAYS YOU EAT IT.
   *Sometimes we ask "how much" as A, B, C or D. **LOOK AT THE ENCLOSED PICTURES.**
   For each food, pick the picture (bowls or plates) that looks the most like the serving size you usually eat. (If you don't have pictures: A=1/4 cup, B=1/2 cup, C=1 cup, D= 2 cups.)
3. **EXAMPLE:** This person drank apple juice twice a week, and had one glass each time. Once a week he ate a "C"-sized serving of rice (about 1 cup).

	HOW OFTEN IN THE PAST 3 MONTHS								HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D)			
	NEVER		ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY			
Apple juice	○		○	○	○	●	○	○	○	How many glasses each time	1 ●	2 ○	3 ○	4 ○
Rice	○		○	○	●	○	○	○	○	How much each time	A ○	B ○	C ●	D ○

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

Appendix J: Food Frequency Questionnaire (cont.)



This section is about your usual eating habits in the past 3 months. This includes all meals or snacks, at home or in a restaurant or carry-out. We will ask you about different TYPES (low-fat, low-carb) at the end of the survey. Include all types (like low-fat, sugar-free). Later you can tell us which type you usually eat.

Appendix J: Food Frequency Questionnaire (Continued)

	NEVER		ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D)
Breakfast sandwiches with eggs, like Egg McMuffins	○		○	○	○	○	○	○	○	How many sandwiches in a day: 1 2
Other eggs like scrambled, boiled or omelets (not egg substitutes)	○		○	○	○	○	○	○	○	How many eggs a day: 1 2 3
Breakfast sausage, including in sausage biscuits, or in breakfast sandwiches	○		○	○	○	○	○	○	○	How many pieces: 1 2 3
Bacon	○		○	○	○	○	○	○	○	How many pieces: 1 2 3 4
Pancakes, waffles, French toast or Pop Tarts	○		○	○	○	○	○	○	○	How many pieces: 1 2 3
Cooked cereals like oatmeal, grits or cream of wheat	○		○	○	○	○	○	○	○	Which bowl: B C D
Cold cereals, ANY KIND, like corn flakes, fiber cereals, or sweetened cereals	○		○	○	○	○	○	○	○	Which bowl: B C D
Milk or milk substitutes on cereal	○		○	○	○	○	○	○	○	
Yogurt or frozen yogurt	○		○	○	○	○	○	○	○	Which bowl: B C
Cheese, sliced cheese or cheese spread, including on sandwiches	○		○	○	○	○	○	○	○	How many slices: 1 2 3
How often did you eat the following foods in the past 3 months?										
Bananas	○		○	○	○	○	○	○	○	How many each time: 1/2 1
Apples or pears	○		○	○	○	○	○	○	○	How many each time: 1/2 1 2
Oranges or tangerines	○		○	○	○	○	○	○	○	How many each time: 1/2 1 2
Grapefruit	○		○	○	○	○	○	○	○	How much: A little 1/2 1
Peaches or nectarines, fresh	○		○	○	○	○	○	○	○	How many: 1/2 1 2
Cantaloupe	○		○	○	○	○	○	○	○	How much: 1/8 1/4 1/2
Strawberries or other berries	○		○	○	○	○	○	○	○	How much: A B C
Watermelon	○		○	○	○	○	○	○	○	How much: A B C D
Other fresh fruits like grapes, plums, honeydew, mango	○		○	○	○	○	○	○	○	How much: A B C
Canned fruit like applesauce, fruit cocktail, canned peaches or canned pineapple	○		○	○	○	○	○	○	○	How much: A B C
How often did you eat each of the following vegetables In the past 3 months, including fresh, frozen, canned or in stir-fry, at home or in a restaurant?										
Broccoli	○		○	○	○	○	○	○	○	How much: A B C
Carrots, or mixed vegetables with carrots	○		○	○	○	○	○	○	○	How much: A B C
Corn	○		○	○	○	○	○	○	○	How much: A B C

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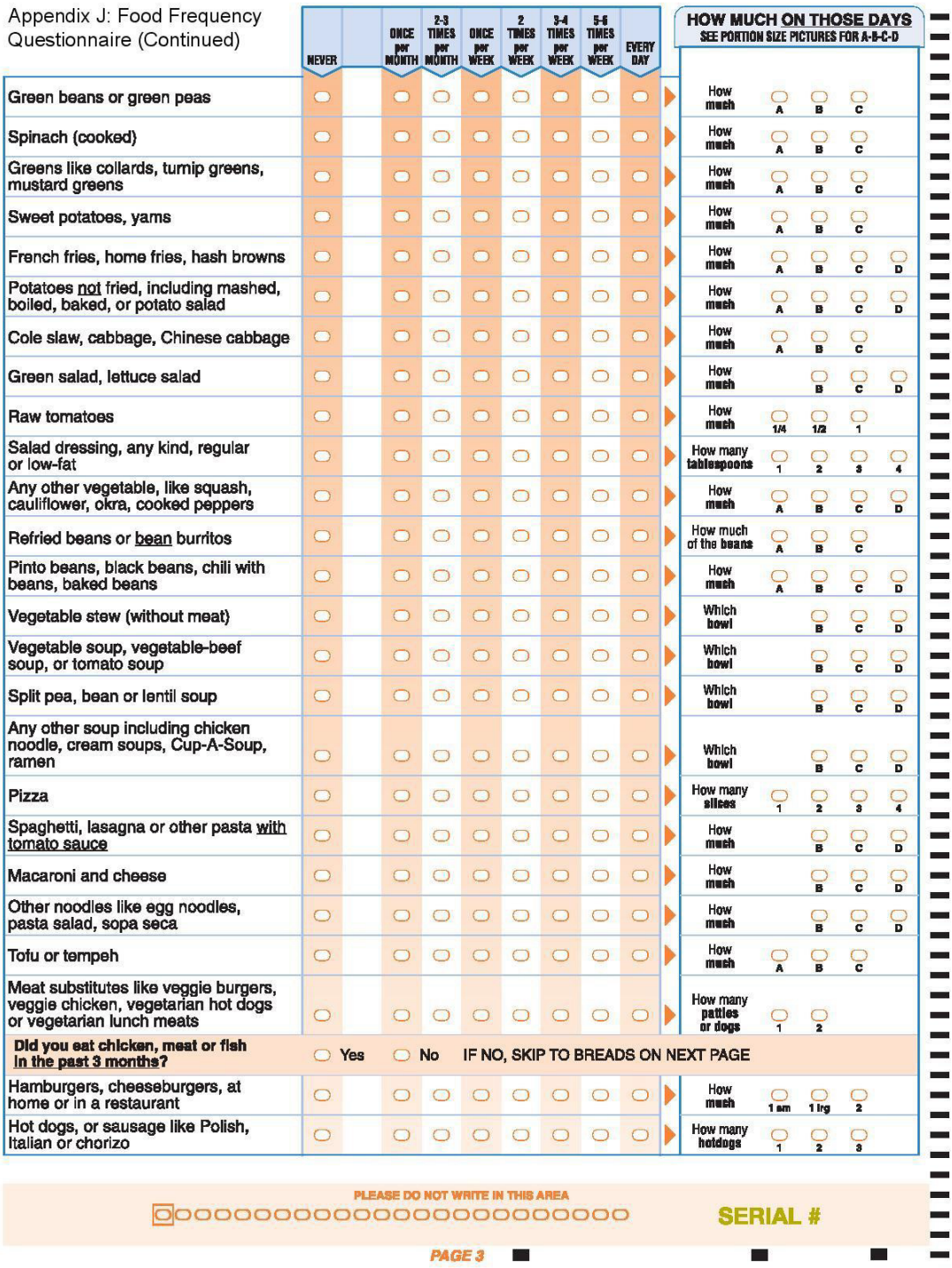

Appendix J: Food Frequency Questionnaire (Continued)

	NEVER		ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D)
Green beans or green peas	○		○	○	○	○	○	○	○	How much ○A ○B ○C
Spinach (cooked)	○		○	○	○	○	○	○	○	How much ○A ○B ○C
Greens like collards, turnip greens, mustard greens	○		○	○	○	○	○	○	○	How much ○A ○B ○C
Sweet potatoes, yams	○		○	○	○	○	○	○	○	How much ○A ○B ○C
French fries, home fries, hash browns	○		○	○	○	○	○	○	○	How much ○A ○B ○C ○D
Potatoes not fried, including mashed, boiled, baked, or potato salad	○		○	○	○	○	○	○	○	How much ○A ○B ○C ○D
Cole slaw, cabbage, Chinese cabbage	○		○	○	○	○	○	○	○	How much ○A ○B ○C
Green salad, lettuce salad	○		○	○	○	○	○	○	○	How much ○B ○C ○D
Raw tomatoes	○		○	○	○	○	○	○	○	How much ○1/4 ○1/2 ○1
Salad dressing, any kind, regular or low-fat	○		○	○	○	○	○	○	○	How many tablespoons ○1 ○2 ○3 ○4
Any other vegetable, like squash, cauliflower, okra, cooked peppers	○		○	○	○	○	○	○	○	How much ○A ○B ○C ○D
Refried beans or bean burritos	○		○	○	○	○	○	○	○	How much of the beans ○A ○B ○C
Pinto beans, black beans, chili with beans, baked beans	○		○	○	○	○	○	○	○	How much ○A ○B ○C ○D
Vegetable stew (without meat)	○		○	○	○	○	○	○	○	Which bowl ○B ○C ○D
Vegetable soup, vegetable-beef soup, or tomato soup	○		○	○	○	○	○	○	○	Which bowl ○B ○C ○D
Split pea, bean or lentil soup	○		○	○	○	○	○	○	○	Which bowl ○B ○C ○D
Any other soup including chicken noodle, cream soups, Cup-A-Soup, ramen	○		○	○	○	○	○	○	○	Which bowl ○B ○C ○D
Pizza	○		○	○	○	○	○	○	○	How many slices ○1 ○2 ○3 ○4
Spaghetti, lasagna or other pasta with tomato sauce	○		○	○	○	○	○	○	○	How much ○B ○C ○D
Macaroni and cheese	○		○	○	○	○	○	○	○	How much ○B ○C ○D
Other noodles like egg noodles, pasta salad, sopa seca	○		○	○	○	○	○	○	○	How much ○B ○C ○D
Tofu or tempeh	○		○	○	○	○	○	○	○	How much ○A ○B ○C
Meat substitutes like veggie burgers, veggie chicken, vegetarian hot dogs or vegetarian lunch meats	○		○	○	○	○	○	○	○	How many patties or dogs ○1 ○2
Did you eat chicken, meat or fish in the past 3 months?	○ Yes	○ No	IF NO, SKIP TO BREADS ON NEXT PAGE							
Hamburgers, cheeseburgers, at home or in a restaurant	○		○	○	○	○	○	○	○	How much ○1 sm ○1 lrg ○2
Hot dogs, or sausage like Polish, Italian or chorizo	○		○	○	○	○	○	○	○	How many hotdogs ○1 ○2 ○3

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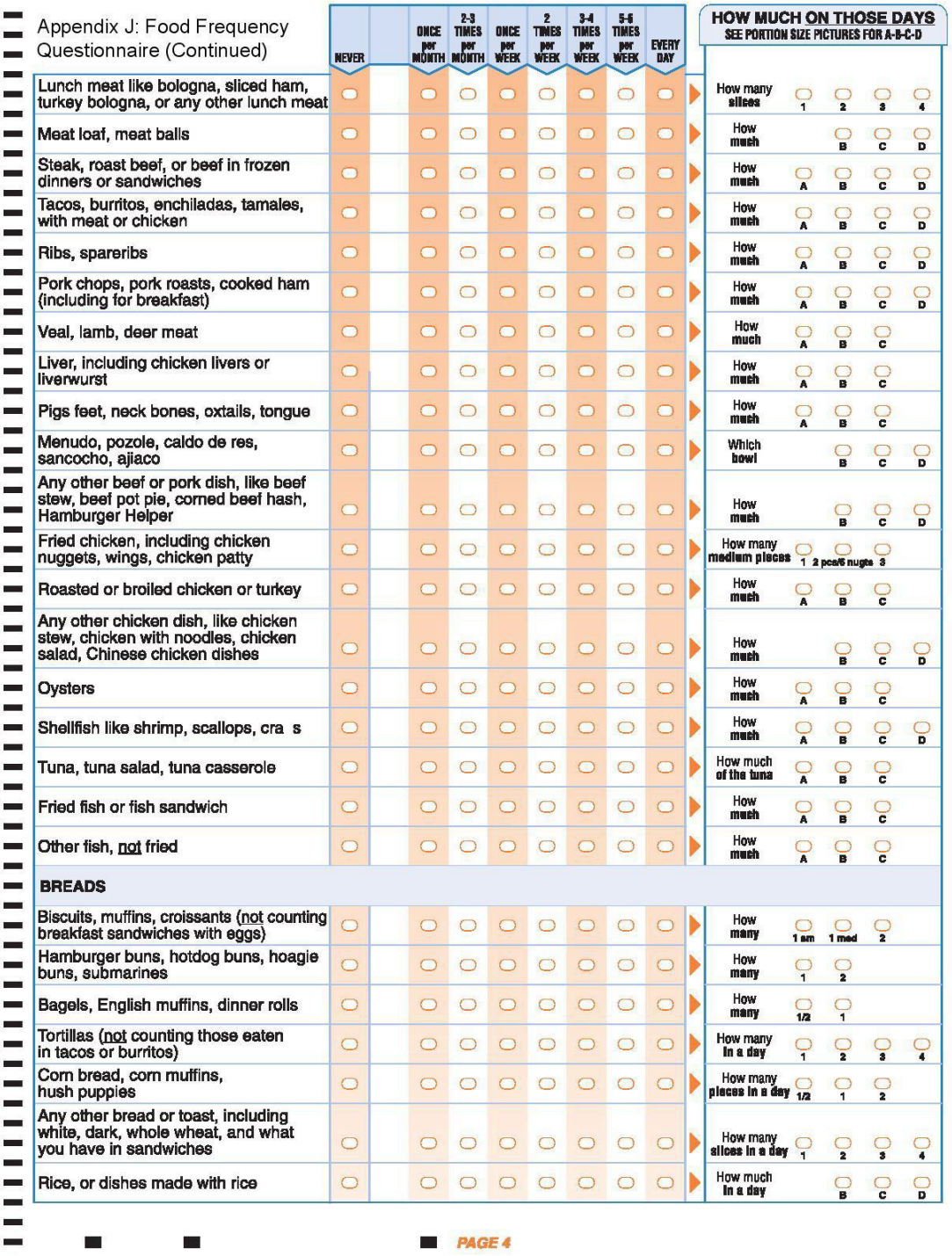
Appendix J: Food Frequency Questionnaire (cont.)



Appendix J: Food Frequency Questionnaire (Continued)

	NEVER		ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D)
Lunch meat like bologna, sliced ham, turkey bologna, or any other lunch meat										How many slices: 1 2 3 4
Meat loaf, meat balls										How much: B C D
Steak, roast beef, or beef in frozen dinners or sandwiches										How much: A B C D
Tacos, burritos, enchiladas, tamales, with meat or chicken										How much: A B C D
Ribs, spareribs										How much: A B C D
Pork chops, pork roasts, cooked ham (including for breakfast)										How much: A B C D
Veal, lamb, deer meat										How much: A B C
Liver, including chicken livers or liverwurst										How much: A B C
Pigs feet, neck bones, oxtails, tongue										How much: A B C
Menudo, pozole, caldo de res, sancocho, ajiaco										Which bowl: B C D
Any other beef or pork dish, like beef stew, beef pot pie, corned beef hash, Hamburger Helper										How much: B C D
Fried chicken, including chicken nuggets, wings, chicken patty										How many medium pieces: 1 2 pcs/6 nuggs 3
Roasted or broiled chicken or turkey										How much: A B C
Any other chicken dish, like chicken stew, chicken with noodles, chicken salad, Chinese chicken dishes										How much: B C D
Oysters										How much: A B C
Shellfish like shrimp, scallops, cra s										How much: A B C D
Tuna, tuna salad, tuna casserole										How much of the tuna: A B C
Fried fish or fish sandwich										How much: A B C
Other fish, not fried										How much: A B C
BREADS										
Biscuits, muffins, croissants (not counting breakfast sandwiches with eggs)										How many: 1 sm 1 med 2
Hamburger buns, hotdog buns, hoagie buns, submarines										How many: 1 2
Bagels, English muffins, dinner rolls										How many: 1/2 1
Tortillas (not counting those eaten in tacos or burritos)										How many in a day: 1 2 3 4
Corn bread, corn muffins, hush puppies										How many pieces in a day: 1/2 1 2
Any other bread or toast, including white, dark, whole wheat, and what you have in sandwiches										How many slices in a day: 1 2 3 4
Rice, or dishes made with rice										How much in a day: B C D

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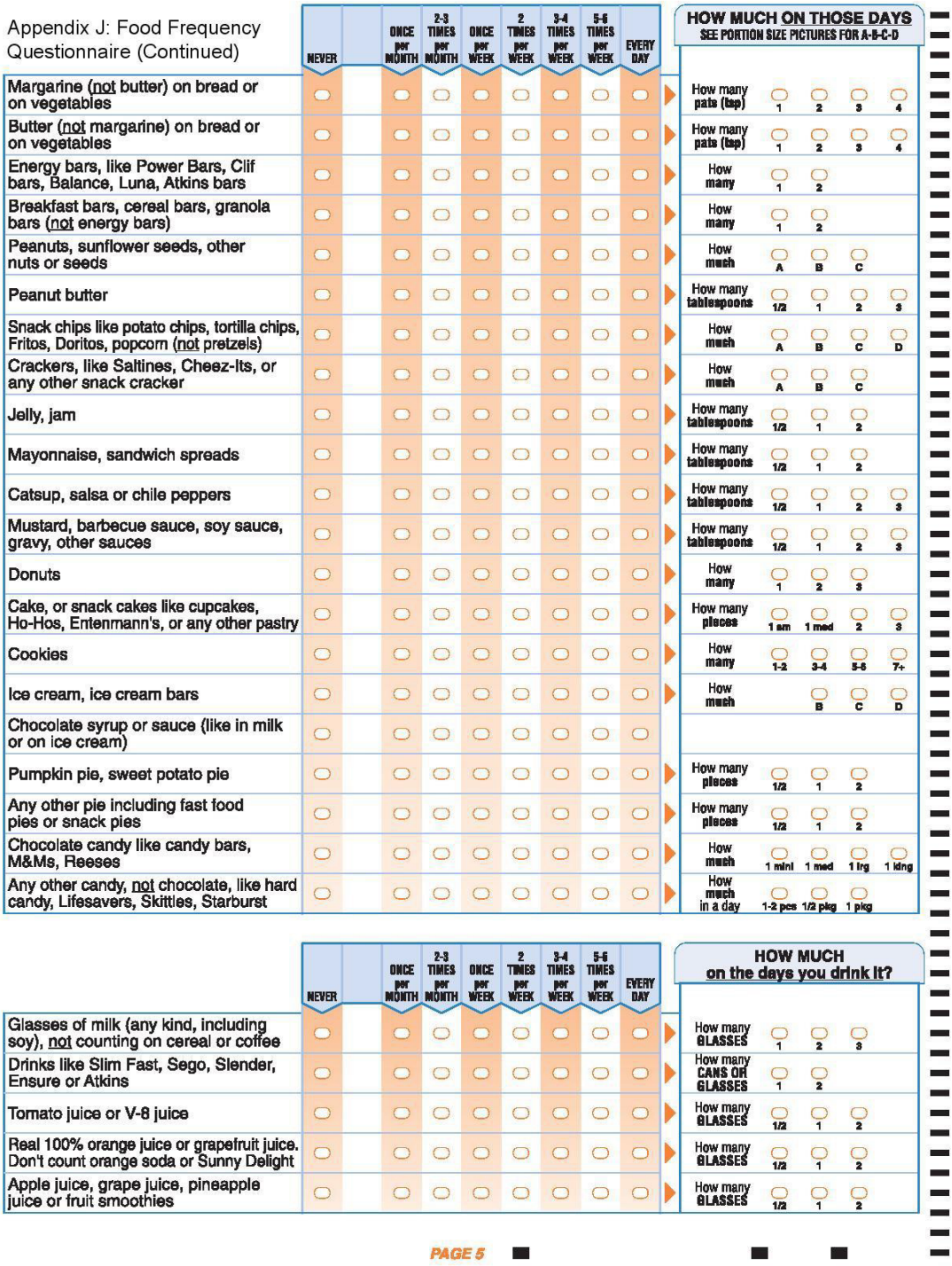
Appendix J: Food Frequency Questionnaire (cont.)



Appendix J: Food Frequency Questionnaire (Continued)

	NEVER		ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH ON THOSE DAYS (SEE PORTION SIZE PICTURES FOR A-B-C-D)
Margarine (not butter) on bread or on vegetables	○		○	○	○	○	○	○	○	How many pats (tsp) ○ 1 ○ 2 ○ 3 ○ 4
Butter (not margarine) on bread or on vegetables	○		○	○	○	○	○	○	○	How many pats (tsp) ○ 1 ○ 2 ○ 3 ○ 4
Energy bars, like Power Bars, Clif bars, Balance, Luna, Atkins bars	○		○	○	○	○	○	○	○	How many ○ 1 ○ 2
Breakfast bars, cereal bars, granola bars (not energy bars)	○		○	○	○	○	○	○	○	How many ○ 1 ○ 2
Peanuts, sunflower seeds, other nuts or seeds	○		○	○	○	○	○	○	○	How much ○ A ○ B ○ C
Peanut butter	○		○	○	○	○	○	○	○	How many tablespoons ○ 1/2 ○ 1 ○ 2 ○ 3
Snack chips like potato chips, tortilla chips, Fritos, Doritos, popcorn (not pretzels)	○		○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Crackers, like Saltines, Cheez-Its, or any other snack cracker	○		○	○	○	○	○	○	○	How much ○ A ○ B ○ C
Jelly, jam	○		○	○	○	○	○	○	○	How many tablespoons ○ 1/2 ○ 1 ○ 2
Mayonnaise, sandwich spreads	○		○	○	○	○	○	○	○	How many tablespoons ○ 1/2 ○ 1 ○ 2
Catsup, salsa or chile peppers	○		○	○	○	○	○	○	○	How many tablespoons ○ 1/2 ○ 1 ○ 2 ○ 3
Mustard, barbecue sauce, soy sauce, gravy, other sauces	○		○	○	○	○	○	○	○	How many tablespoons ○ 1/2 ○ 1 ○ 2 ○ 3
Donuts	○		○	○	○	○	○	○	○	How many ○ 1 ○ 2 ○ 3
Cake, or snack cakes like cupcakes, Ho-Hos, Entenmann's, or any other pastry	○		○	○	○	○	○	○	○	How many pieces ○ 1 sm ○ 1 med ○ 2 ○ 3
Cookies	○		○	○	○	○	○	○	○	How many ○ 1-2 ○ 3-4 ○ 5-6 ○ 7+
Ice cream, ice cream bars	○		○	○	○	○	○	○	○	How much ○ B ○ C ○ D
Chocolate syrup or sauce (like in milk or on ice cream)	○		○	○	○	○	○	○		
Pumpkin pie, sweet potato pie	○		○	○	○	○	○	○	○	How many pieces ○ 1/2 ○ 1 ○ 2
Any other pie including fast food pies or snack pies	○		○	○	○	○	○	○	○	How many pieces ○ 1/2 ○ 1 ○ 2
Chocolate candy like candy bars, M&Ms, Reeses	○		○	○	○	○	○	○	○	How much ○ 1 mini ○ 1 med ○ 1 lrg ○ 1 king
Any other candy, not chocolate, like hard candy, Lifesavers, Skittles, Starburst	○		○	○	○	○	○	○	○	How much in a day ○ 1-2 pcs ○ 1/2 pkg ○ 1 pkg

	NEVER		ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on the days you drink it?
Glasses of milk (any kind, including soy), not counting on cereal or coffee	○		○	○	○	○	○	○	○	How many GLASSES ○ 1 ○ 2 ○ 3
Drinks like Slim Fast, Sego, Slender, Ensure or Atkins	○		○	○	○	○	○	○	○	How many CANS OR GLASSES ○ 1 ○ 2
Tomato juice or V-8 juice	○		○	○	○	○	○	○	○	How many GLASSES ○ 1/2 ○ 1 ○ 2
Real 100% orange juice or grapefruit juice. Don't count orange soda or Sunny Delight	○		○	○	○	○	○	○	○	How many GLASSES ○ 1/2 ○ 1 ○ 2
Apple juice, grape juice, pineapple juice or fruit smoothies	○		○	○	○	○	○	○	○	How many GLASSES ○ 1/2 ○ 1 ○ 2

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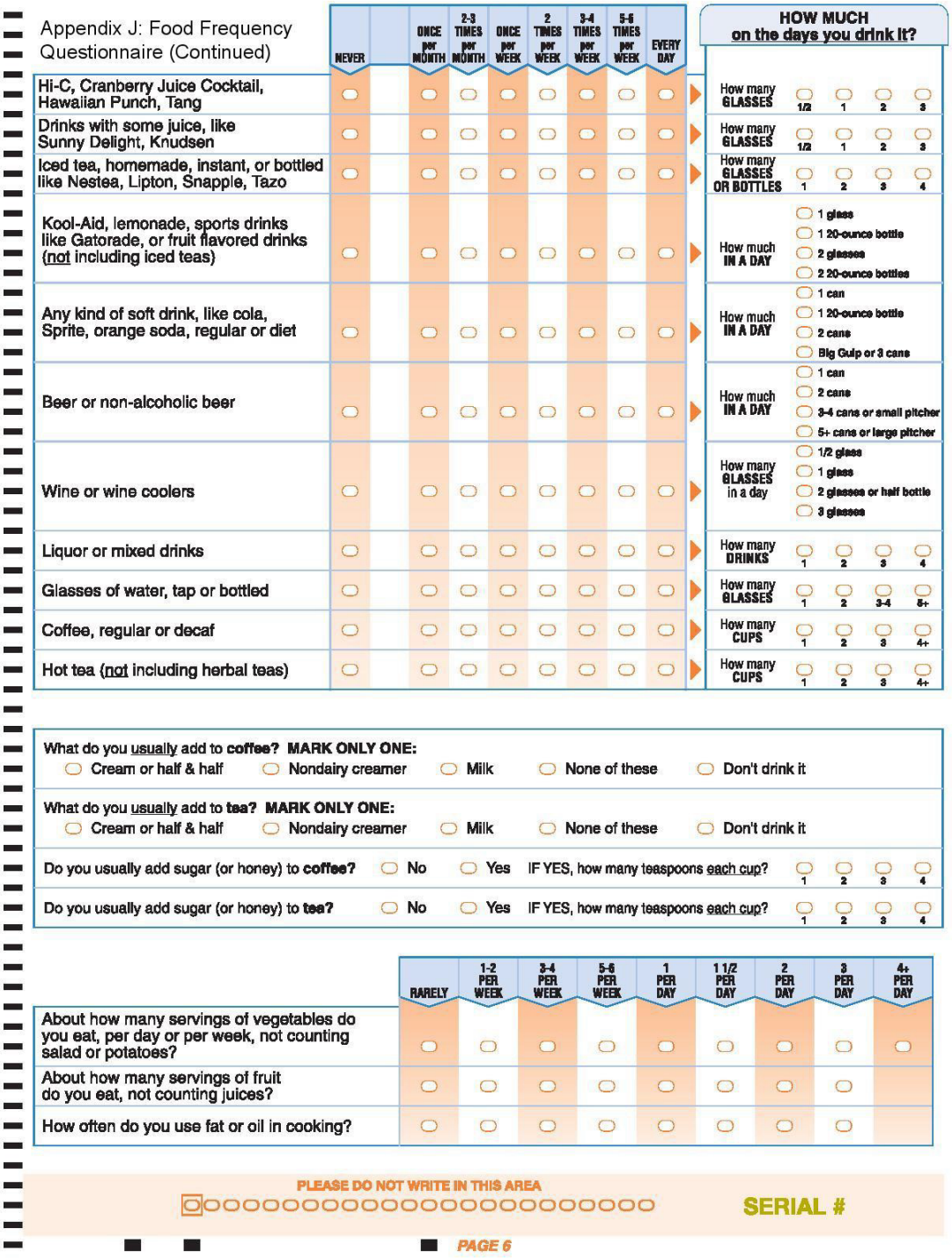
Appendix J: Food Frequency Questionnaire (cont.)



Appendix J: Food Frequency Questionnaire (Continued)

	NEVER	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on the days you drink it?
Hi-C, Cranberry Juice Cocktail, Hawaiian Punch, Tang	○	○	○	○	○	○	○	○	How many GLASSES: ○ 1/2 ○ 1 ○ 2 ○ 3
Drinks with some juice, like Sunny Delight, Knudsen	○	○	○	○	○	○	○	○	How many GLASSES: ○ 1/2 ○ 1 ○ 2 ○ 3
Iced tea, homemade, instant, or bottled like Nestea, Lipton, Snapple, Tazo	○	○	○	○	○	○	○	○	How many GLASSES OR BOTTLES: ○ 1 ○ 2 ○ 3 ○ 4
Kool-Aid, lemonade, sports drinks like Gatorade, or fruit flavored drinks (not including iced teas)	○	○	○	○	○	○	○	○	How much IN A DAY: ○ 1 glass ○ 1 20-ounce bottle ○ 2 glasses ○ 2 20-ounce bottles
Any kind of soft drink, like cola, Sprite, orange soda, regular or diet	○	○	○	○	○	○	○	○	How much IN A DAY: ○ 1 can ○ 1 20-ounce bottle ○ 2 cans ○ Big Gulp or 3 cans
Beer or non-alcoholic beer	○	○	○	○	○	○	○	○	How much IN A DAY: ○ 1 can ○ 2 cans ○ 3-4 cans or small pitcher ○ 5+ cans or large pitcher
Wine or wine coolers	○	○	○	○	○	○	○	○	How many GLASSES in a day: ○ 1/2 glass ○ 1 glass ○ 2 glasses or half bottle ○ 3 glasses
Liquor or mixed drinks	○	○	○	○	○	○	○	○	How many DRINKS: ○ 1 ○ 2 ○ 3 ○ 4
Glasses of water, tap or bottled	○	○	○	○	○	○	○	○	How many GLASSES: ○ 1 ○ 2 ○ 3-4 ○ 5+
Coffee, regular or decaf	○	○	○	○	○	○	○	○	How many CUPS: ○ 1 ○ 2 ○ 3 ○ 4+
Hot tea (not including herbal teas)	○	○	○	○	○	○	○	○	How many CUPS: ○ 1 ○ 2 ○ 3 ○ 4+

What do you usually add to **coffee?** **MARK ONLY ONE:**
○ Cream or half & half ○ Nondairy creamer ○ Milk ○ None of these ○ Don't drink it

What do you usually add to **tea?** **MARK ONLY ONE:**
○ Cream or half & half ○ Nondairy creamer ○ Milk ○ None of these ○ Don't drink it

Do you usually add sugar (or honey) to **coffee?** ○ No ○ Yes IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

Do you usually add sugar (or honey) to **tea?** ○ No ○ Yes IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

	RARELY	1-2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	1 1/2 PER DAY	2 PER DAY	3 PER DAY	4+ PER DAY
About how many servings of vegetables do you eat, per day or per week, not counting salad or potatoes?	○	○	○	○	○	○	○	○	○
About how many servings of fruit do you eat, not counting juices?	○	○	○	○	○	○	○	○	
How often do you use fat or oil in cooking?	○	○	○	○	○	○	○	○	

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Appendix J: Food Frequency Questionnaire (cont.)

Appendix J: Food Frequency Questionnaire (Continued)

If you ate the following foods in the past 3 months, what type did you usually eat? MARK ONLY ONE ANSWER FOR EACH QUESTION

Milk	○ Whole milk ○ Reduced-fat 2% milk	○ Low-fat 1% milk ○ Non-fat milk	○ Soy milk ○ Rice milk	○ Don't drink
Slim Fast, Sego, Slender or Ensure		○ Low-Carb like Atkins	○ Regular	○ Don't drink
Orange juice	○ Calcium-fortified	○ Not calcium-fortified	○ I don't know	○ Don't drink
Soda or pop	○ Diet soda, low-calorie	○ Regular	○ Don't drink	
Iced tea	○ Homemade, no sugar	○ Homemade, w/sugar	○ Bottled, no sugar	○ Bottled, regular ○ Don't drink
Beer	○ Regular beer	○ Light beer	○ Low-Carb beer	○ Non-alcoholic beer ○ Don't drink
Hamburgers or cheeseburgers		○ Hamburgers	○ Cheeseburgers	○ Don't eat
Hot dogs	○ Low fat or turkey dogs	○ Regular hot dogs	○ Don't eat	
Lunch meats	○ Low-fat or turkey lunch meats	○ Regular lunch meats	○ Don't eat	
Spaghetti or lasagna	○ Meatless	○ With meat sauce or meatballs		○ Don't eat
Cheese	○ Low Fat	○ Not Low Fat	○ Don't eat	
Salad dressing	○ Low-Carb	○ Low-fat	○ Regular	○ Don't use
Energy bars like Power Bar, Clif, Atkins	○ Low-Carb, low sugar	○ Low-fat	○ Regular	○ Don't eat
Breakfast bars, cereal bars, or granola bars	○ Low-Carb, low sugar	○ Low-fat	○ Regular	○ Don't eat
Bread	○ 100% whole wheat	○ Low-Carb	○ Regular	○ Don't eat
Tortillas	○ Corn	○ Flour	○ Don't know or don't eat	
Chocolate candy or chocolate candy bars	○ Low-Carb, low sugar	○ Low-fat	○ Regular	○ Don't eat
Cookies	○ Low-Carb, low sugar	○ Low-fat	○ Regular	○ Don't eat
Cake, snack cakes, and other pastries	○ Low-Carb, low sugar	○ Low-fat	○ Regular	○ Don't eat
Ice cream	○ Low-Carb, low sugar	○ Low-fat or ice milk	○ Regular	○ Don't eat
Jelly or jam	○ Low-Carb, low sugar	○ Regular	○ Don't use	
Beef or pork	○ Avoid eating the fat	○ Sometimes eat the fat	○ Often eat the fat	○ Don't eat
Chicken or Turkey	○ Avoid eating the skin	○ Sometimes eat the skin	○ Often eat the skin	○ Don't eat

What kinds of **fat or oil** do you usually use in cooking? **MARK ONLY ONE OR TWO**

○ Don't know, or Pam	○ Stick margarine	○ Corn oil, vegetable oil	○ Lard, fatback, bacon fat
○ Butter	○ Soft tub margarine	○ Olive oil or canola oil	○ Crisco
○ Butter/margarine blend	○ Low-fat margarine		

If you eat **cold cereals**, what do you eat? Choose one or two that you eat most often. (If you usually just eat one kind, just choose one.)

○ Low-carb cereals like Atkins, Low-Carb Special K	○ Total	○ Other fiber cereals like Raisin Bran, Fruit-n-Fiber
○ Cheerios, Grape Nuts, Shredded Wheat, Wheaties, Wheat Chex	○ Fiber One	○ Sweetened cereals like Frosted Flakes, Froot Loops
	○ Product 19, Complete	○ Other cold cereals, like Corn Flakes, Rice Krispies, Special K
	○ All Bran, Bran Buds	

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What vitamin supplements did you take fairly regularly in the past 3 months?

Multiple Vitamins. Did you take...	HOW OFTEN: DIDN'T TAKE	A FEW DAYS per MONTH	1-3 DAYS per WEEK	4-6 DAYS per WEEK	EVERY DAY	FOR HOW MANY YEARS?: LESS THAN 1 YEAR	1 YEAR	2 YEARS	3-4 YEARS	5-9 YEARS	10+ YEARS
Prenatal vitamins	○	○	○	○	○	○	○	○	○	○	○
Regular Once-A-Day, Centrum, Theragran, "senior" vitamins or house brands of multiple vitamins	○	○	○	○	○	○	○	○	○	○	○
Stress-tabs or B-Complex type	○	○	○	○	○	○	○	○	○	○	○
Single Vitamins, not part of multiple vitamins											
Vitamin A (not beta-carotene)	○	○	○	○	○	○	○	○	○	○	○
Beta-carotene	○	○	○	○	○	○	○	○	○	○	○
Vitamin C	○	○	○	○	○	○	○	○	○	○	○
Vitamin E	○	○	○	○	○	○	○	○	○	○	○
Folic Acid, Folate	○	○	○	○	○	○	○	○	○	○	○
Calcium or Tums	○	○	○	○	○	○	○	○	○	○	○
Vitamin D, alone or combined with calcium	○	○	○	○	○	○	○	○	○	○	○
Zinc	○	○	○	○	○	○	○	○	○	○	○
Iron	○	○	○	○	○	○	○	○	○	○	○
Selenium	○	○	○	○	○	○	○	○	○	○	○
Omega-3, fish oil, flax seed oil	○	○	○	○	○	○	○	○	○	○	○

If you took Once-a-day, Centrum or Thera-type multiple vitamins, did you usually take types that
○ contain minerals, iron, zinc, etc. ○ do not contain minerals ○ Don't know

If you took vitamin C, how many milligrams of **vitamin C** did you usually take, on the days you took it?
○ 100 ○ 250 ○ 500 ○ 750 ○ 1000 ○ 1500 ○ 2000 ○ 3000+ ○ Don't know

If you took vitamin E, how many IUs of **vitamin E** did you usually take, on the days you took it?
○ 100 ○ 200 ○ 300 ○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000+ ○ Don't know

Did you take any of these supplements at least once a week?
○ Ginkgo ○ St. John's Wort ○ Echinacea ○ DHEA ○ Didn't take these
○ Ginseng ○ Kava Kava ○ Melatonin ○ Glucosamine/Chondroitin

SOME LAST QUESTIONS ABOUT YOU

Would you say your health is ○ Excellent ○ Very good ○ Good ○ Fair ○ Poor

Are you currently trying to lose weight? ○ Yes ○ No

Was there ever a time in your life when you often drank more beer, wine or liquor than you do now? ○ Yes ○ No

Do you smoke cigarettes now? ○ Yes ○ No
IF YES, On average about how many cigarettes a day do you smoke now? ○ 1-5 ○ 6-14 ○ 15-24 ○ 25-34 ○ 35+

Are you ○ Hispanic or Latino ○ Not Hispanic or Latino

What race do you consider yourself to be? (MARK ALL THAT APPLY)
○ White ○ Asian ○ Native Hawaiian or Other Pacific Islander
○ Black or African American ○ American Indian or Alaska Native ○ Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

Appendix K: ASA Physical Status Classification System

ASA PHYSICAL STATUS CLASSIFICATION SYSTEM

Last approved by the ASA House of Delegates on October 15, 2014

Current definitions (NO CHANGE) and Examples (NEW)

ASA PS Classification	Definition	Examples, including, but not limited to:
ASA I	A normal healthy patient	Healthy, non-smoking, no or minimal alcohol use
ASA II	A patient with mild systemic disease	Mild diseases only without substantive functional limitations. Examples include (but not limited to): current smoker, social alcohol drinker, pregnancy, obesity (30 < BMI < 40), well-controlled DM/HTN, mild lung disease
ASA III	A patient with severe systemic disease	Substantive functional limitations; One or more moderate to severe diseases. Examples include (but not limited to): poorly controlled DM or HTN, COPD, morbid obesity (BMI ≥40), active hepatitis, alcohol dependence or abuse, implanted pacemaker, moderate reduction of ejection fraction, ESRD undergoing regularly scheduled dialysis, premature infant PCA < 60 weeks, history (>3 months) of MI, CVA, TIA, or CAD/stents.
ASA IV	A patient with severe systemic disease that is a constant threat to life	Examples include (but not limited to): recent (< 3 months) MI, CVA, TIA, or CAD/stents, ongoing cardiac ischemia or severe valve dysfunction, severe reduction of ejection fraction, sepsis, DIC, ARD or ESRD not undergoing regularly scheduled dialysis
ASA V	A moribund patient who is not expected to survive without the operation	Examples include (but not limited to): ruptured abdominal/thoracic aneurysm, massive trauma, intracranial bleed with mass effect, ischemic bowel in the face of significant cardiac pathology or multiple organ/system dysfunction
ASA VI	A declared brain-dead patient whose organs are being removed for donor purposes	

*The addition of "E" denotes Emergency surgery: (An emergency is defined as existing when delay in treatment of the patient would lead to a significant increase in the threat to life or body part)

These definitions appear in each annual edition of the ASA Relative Value Guide®. There is no additional information that will help you further define these categories.

Appendix L: OpenBiome Donor Screening Testing

Table 1: Donor Screening Testing

	Pathogen		Method
Serological Testing	HIV-1/2		IA
	Hepatitis A, IgM		IA
	Hepatitis B, anti-HBc, IgM antiHBc, HBsAg		IA
	Hepatitis C		IA
	Treponema pallidum		Antibody cascading reflex
	Strongyloides		EIA
Stool Testing	Multi-Drug Resistant Organisms	VRE	Culture
		CRE	Culture
		ESBL	Culture
	Salmonella spp		Culture
	Shigella spp		Culture
	Campylobacter spp		Culture
	Vibrio spp		Culture
	Rotavirus A		EIA
	Cryptosporidium spp		EIA
	Shiga Toxin		PCR
	Enteropathogenic Escherichia coli (EPEC)		PCR
	Giardia lamblia		EIA
	Adenovirus		EIA
	Norovirus		PCR
	Clostridium difficile Toxin B		RT-PCR
	Cryptosporidium spp		EIA
	Helicobacter pylori		EIA
	Ova and parasites		Microscopic exam
	Cyclospora and Isospora		Microscopic exam
	Microsporidia		Microscopic exam
	Bristol Stool Type assessment		Visual
Nasal Swab	Multi-Drug Resistant Organisms	MRSA	Culture
Additional Screening	Liver Function Panel		Serological testing
	ALT		
	AST		
	ALP		
	Albumin		
	Bilirubin (Total, direct, indirect)		
	CBC and Differential		
	Mini Health Questionnaire		Documentation
	Random Clinical Health Checks		Clinical assessment

IA = Immunoassay, EIA = Enzyme immunoassay; VRE = Vancomycin-resistant *enterococci*; CRE = carbapenem-resistant *Enterobacteriaceae*; ESBL = Extended-spectrum beta-lactamases; RT-PCR = reverse-transcription polymerase chain reaction; qPCR = qualitative polymerase chain reaction; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; ALP = Alkaline Phosphatase; CBC = Complete Blood Count

Appendix M: UCSF Post Approval Reporting Requirements

UCSF Office of Ethics and Compliance
Human Research Protection Program

Post-Approval Reporting Requirements Summary Sheet

Federal regulations and the UCSF IRB/HRPP require investigator reporting of any post-approval research-related event or information that may meet the HRPP's institutional definitions of "*unanticipated problem involving risk to participants or others*" or "*serious or continuing noncompliance*." The IRB determines whether these definitions apply when evaluating investigator event reports. The following table summarizes which types of events or information should be reported to the IRB/HRPP, the reporting window and appropriate reporting form to use.

See the Adverse Event and/or Protocol Violation or Incident sections of the UCSF HRPP website for definitions and details. Questions? Contact the IRB at 415-476-1814 or IRB@ucsf.edu and ask to speak with the QIU (Quality Improvement Unit) Analyst of the day.

What, When, and How to Report to the HRPP

Type of Event	When to Report*	Reporting Form
* The SFVAMC has a shorter timeline and different definitions than UCSF for reporting certain categories of post- approval events. (The website pages noted above include links to the relevant SFVAMC information.)		
ADVERSE EVENTS		
Internal (on-site) adverse event that PI determines to be 1. Definitely, probably or possibly related *AND* 2. Serious or unexpected	Within **5 working days** of UCSF PI awareness **Internal, related deaths and life-threatening events:** Report **immediately**	iRIS Adverse Event Reporting Form
External (off-site) adverse even that UCSF PI determines • changes the study risks or benefits, *OR* • necessitates modification to the IRB-approved consent document(s), and/or the IRB-approved application/protocol	Within **10 working days** of UCSF PI awareness	iRIS Adverse Event Reporting Form
OTHER TYPES of EVENTS or SAFETY INFORMATION		
Audit or Monitoring Report with significant findings	Within **10 working days** of awareness	iRIS Reporting Form
DSMB/DMC Report		
Hold on Study Activities due to unexpected risk or required by any oversight entity e.g. UCSF, FDA, OHRP		
Updated Investigator Brochure		
Other Safety Information or Publication	**Change** to risk language: Within **10 working days** of awareness **No change** to risk language: **Reporting not required**	iRIS Reporting Form
Pharmacy Packet Inserts		
PROTOCOL VIOLATIONS and RESEARCH-RELATED INCIDENTS		
Major Violation including, but not limited to incorrect intervention given, enrollment of ineligible participant, key safety procedure/lab not done or done outside window.	Within **10 working days** of awareness	iRIS Protocol Violation/Incident Report Form
Immediate Protocol Change to Protect Participant Safety	Within **10 working days** of occurrence	
Major Incident including, but not limited to problem with consent or recruitment process, significant complaint or concern, lapse in study approval, loss of adequate resources, potential breach of confidentiality or privacy.	**Potential breaches of privacy or confidentiality:** Within **48 hours** of awareness **Other Major Incidents:** Within **10 working days** of awareness	

Last updated Oct 2017

Appendix N: OpenBiome Amendments to BMF17195

Amendments to Biologics Master File 17195, "Liquid Stool Suspension for Infusion"

1. **Changes to routine donor stool screening:** Following a review of donor screening, several changes have been made to routine donor screening including the addition of new organisms and change of detection method to PCR-based methods. These changes are described further below.

   Addition of stool pathogens to routine donor screening:

   Diarrheagenic *E. coli* (EAEC, EIEC, and ETEC) by PCR: Following the reports of adverse events of diarrheagenic *E. coli* transmission, we undertook a review of our stool pathogen panel to assess our current methods of detection. As previously communicated to the Agency, and as covered in the FDA communication, "Information Pertaining to Additional Safety Protections Regarding Use of Fecal Microbiota for Transplantation -- Testing of Stool Donors for Enteropathogenic *Escherichia coli* and Shiga toxin-Producing *Escherichia coli*" issued on 06APR2020, Shiga-toxin producing *E. coli* (STEC) detection by enzyme immunoassay (EIA) with reflex to culture was insufficient to detect the presence of STEC compared to PCR-based methods. Further, reports of the detection of enteropathogenic *E. coli* (EPEC) by PCR following FMT led to the inclusion of EPEC for routine donor screening. Given these changes, we undertook a review of our donor screening program for inclusion of other diarrheagenic *E. coli* by PCR in stool, and have included the following organisms:

   - Enteroaggregative *E. coli* (EAEC)
   - *Shigella*/Enteroinvasive *E. coli* (EIEC)
   - Enterotoxigenic *E. coli* (ETEC)

Amendments to Biologics Master File 17195, "Liquid Stool Suspension for Infusion"

- Updated material release criteria for Drug Product produced on or after December 1, 2019 to replace 14-day donor nasopharyngeal swab testing with stool-based SARS-CoV-2 RT-PCR testing on every raw material lot (every stool sample)
- Added a new vendor to perform stool-based testing for SARS-CoV-2
- Updated the Stool Donation Agreement to incorporate updates to the SARS-CoV-2 testing activities
- Added the validation report for the SARS-CoV-2 RT-PCR stool assay
- Submitted Drug Product Lot Certificates of Analysis, as directed by the Agency

Addition of new organisms to our donor screening panel: In addition to including diarrheagenic *E. coli*, we have also included the following pathogens to our routine donor screening program for detection by PCR in stool:

- Plesiomonas shigelloides
- Astrovirus
- Sapovirus

Change in method of detection to PCR: In addition to reviewing the inclusion of new organisms to our donor screening panel, we also assessed the methods of detection for existing pathogens on our routine donor screening panel and have implemented the changes described below.

Methods have been changed from stool culture-based methods to PCR for:

- Campylobacter (C. jejuni/C. coli/C. upsaliensis)
- Salmonella spp.
- Shigella spp.
- Vibrio spp.

Methods have been changed from stool EIA to PCR for:

- Cryptosporidium
- Giardia lamblia
- Adenovirus
- Rotavirus

Methods have been changed from stool microscopic examination to PCR for:

- Cyclospora
- Entamoeba histolytica
- Microsporidia

2. ***Screening for presence of SARS-CoV-2 –*** OpenBiome has implemented changes to its Quality and Safety Program to mitigate the potential risk of transmission of SARS-CoV-2 to patients receiving FMT. These measures include:

    - Donor nasopharyngeal swab testing every 14 days using Quest Diagnostics SARS-CoV-2 RNA (COVID-19), Qualitative nucleic acid amplification assay (NAAT). This test has been authorized by the FDA under an Emergency Use Authorization (EUA).
    - With each stool donation, donors undergo:
        - Screening for symptoms of fever, cough, shortness of breath, sore throat, headache, myalgia, severe fatigue, new loss of smell or taste, nausea, vomiting or diarrhea.
        - Screening for exposure to known or suspected cases of COVID-19
        - Screening for travel
        - Screening for any COVID-19 tests in the last 30 days

    - Temperature checks
        - A body temperature greater than 100.4°F will result in clinical evaluation to determine if symptoms are compatible with possible COVID-19 or other process.
- Any prospective or active donor who has had a laboratory- or clinically-confirmed COVID-19 (SARS-CoV-2) infection or who has had any possible exposure to known or possible cases of COVID-19 regardless of whether they themselves are symptomatic or asymptomatic is deferred for 8 weeks.
- Stool remains in quarantine until a 4-week clinical follow-up occurs after the end of each bookend screen. This follow-up is used to confirm that, in the previous 4 weeks, the donor has not had:
    - Symptoms or diagnosis of COVID-19
    - Exposure to known or suspected cases of COVID-19

3. **In addition to the above broad changes, the following updates have also been made to MF 17195 since 07APR2020:**

    - Updated relevant sections to describe maximum routine donor screening intervals (including maximum 60-day screening intervals for pathogens listed above; maximum 14-day screening intervals for SARS-CoV-2)
    - Revised the Donor Health Questionnaire and Mini Health Questionnaire to incorporate further COVID-19 mitigation measures, summarized above.
    - Revised donor-facing Stool Donation Agreement to include information about added pathogen testing.
    - Updated donor-facing informational material to incorporate information about additional pathogen testing and updates to screening intervals.
    - Revised Standard Operating Procedures resulting from updates to routine donor screening.
    - Updated the Adverse Event Decision Making Algorithm, Adverse Event Reporting Protocol, and de-identified Patient Follow-Up Form to accurately reflect OpenBiome's safety reporting requirements and processes.
        - Reportability has been expanded to include Adverse Events of Special Interest (AESIs), defined as:
            - Suspected Transmission of an Infectious Agent: Any adverse event where transmission of an infectious organism via the drug product may have occurred, regardless of severity.
            - Suspected Transmission of a Multi-Drug Resistant Organism: Any adverse event where transmission of a multi-drug resistant organism via the drug product may have occurred, regardless of severity.
            - New onset Gastrointestinal Disease or Disorder diagnoses: Any new onset gastrointestinal disease or disorder, adverse event post-drug delivery which may have been caused by the drug product, including, but not limited to inflammatory bowel disease (including microscopic colitis and lymphocytic colitis), regardless of severity.
    - Updated Reference Standards and added Drug Product stability data to reflect the latest stability testing activities.
    - Updated Drug Product and placebo Drug Product production figures to provide continued insight into the annual scale of manufacturing.
    - Added new Regulatory Contact and corrected telephone contact.

- Submitted new and corrected Letters of Authorization (LOAs) for cross-referenced IND holders
- Submitted Certificates of Analysis for newly produced Drug Product lots