SPONSOR: Hassane Zarour

TITLE: Phase II Feasibility Study of Fecal Microbiota Transplant (FMT) in Advanced Melanoma Patients Not Responding to PD-1 blockade

IND NUMBER: 17603

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PI: Diwakar Davar

Co-PI: Hassane M. Zarour

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Version: 6 dated 05/12/2021

SUMMARY OF CHANGES

SUMMARY OF CHANGES TO PROTOCOL

Page 1, changed from version 5 dated 06-04-2020 **to** version 6 dated 5-12-2021

Page 21, #4.2, changed from Determination of "SARS-CoV-2 negative" status will be made on nasopharyngeal, and stool specimens obtained at two timepoints: 1st sample collected at least 14 days before stool sample donation, and a 2nd sample collected at least 14 days after stool sample donation; **changed to** Determination of "SARS-CoV-2 negative" status will be made on nasopharyngeal, blood and stool specimens obtained at two timepoints (every 2 weeks for up to 12 weeks): 1st sample collected at least 14 days before stool sample donation, and a 2nd sample collected at least 14 days after stool sample donation.

SUMMARY OF CHANGES TO DONOR CONSENT

Page 1, changed from version 06/04/2020 **to version** 05/06/2021

Page 3, changed from You will be asked to provide a blood sample of 90ml for testing; **changed to** You will be asked to provide a blood sample of 90ml for testing during each visit.

Page 5, Section- How long will I be in this study?, 1st paragraph, changed from You will be in this study for the duration of your follow-up for your cancer diagnosis. However, the time taken for blood and stool tests and stool donations is only 8 weeks. It is possible that you may be asked to donate more stool if a recipient who responds initially progresses and is considered a candidate for a second transplant or if there are more than a few individuals who are identified as possible recipients for samples originating from you; **changed to** You will be in this study for the duration of your follow-up for your cancer diagnosis. However, the time taken for blood, nasal swabs and stool studies will be every 2 weeks for up to 12 weeks. It is possible that you may be asked to donate more stool if a recipient who responds initially progresses and is considered a candidate for a second transplant or if there are more than a few individuals who are identified as possible recipients for samples originating from you.

Page 6, Section- Will I be paid if I take part in this research study, 2nd paragraph, added You will be reimbursed monthly in the amount of $25.00 for travel expenses.

1.0 TRIAL SUMMARY

Abbreviated Title	Phase II feasibility study of fecal microbiota transplant (FMT) in advanced melanoma patients not responding to PD-1 blockade
Trial Phase	II
Clinical Indication	PD-1 refractory advanced melanoma
Trial Type	Phase II Simon 2-stage
Type of control	N/A
Route of administration	pembrolizumab – IV; FMT – endoscopically administered
Trial Blinding	N/A
Treatment Groups	N/A
Number of trial subjects	13 (stage 1), 7 (stage 2); total 20
Estimated enrollment period	15-20 months
Average length of treatment per patient	12 weeks (minimum; FMT with 4 cycles of pembrolizumab) up to 24 weeks (8 cycles of pembrolizumab)
Duration of therapy	Up to 2 years (24 months; 35 cycles of pembrolizumab)
Duration of Participation	Up to 2 years (24 months)

2.0 TRIAL DESIGN

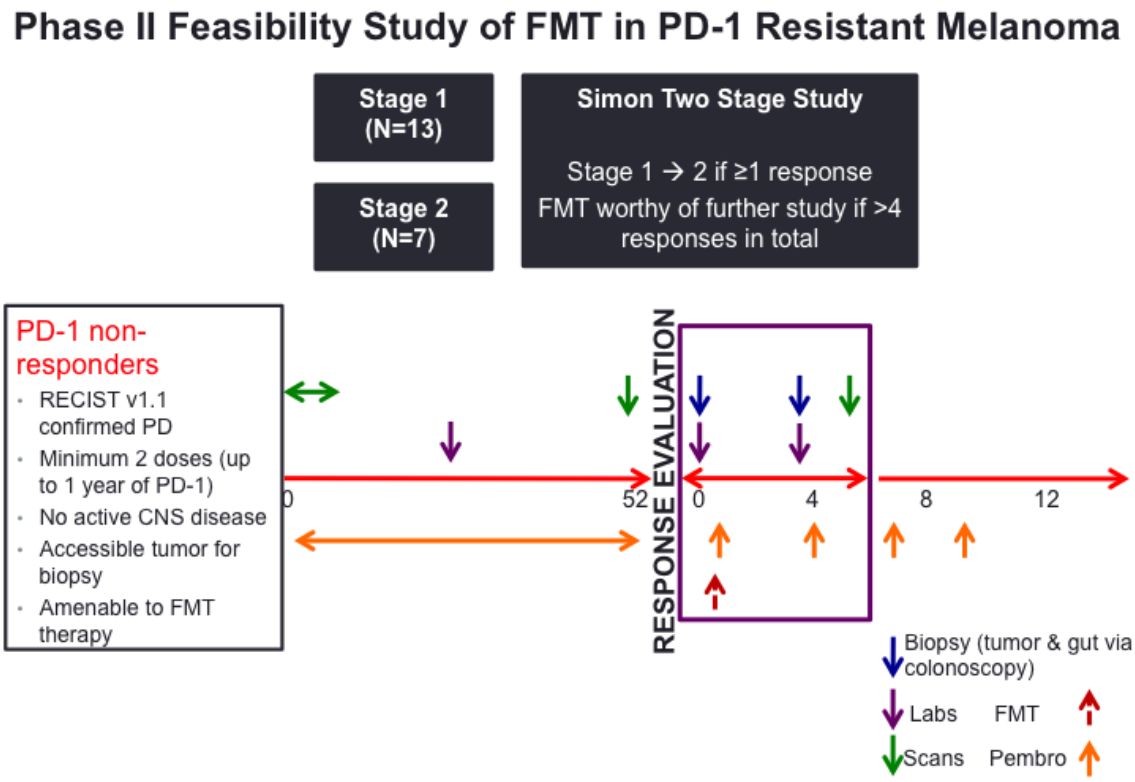
2.1 Trial Design

This is a phase II Simon two-stage single-center study of concurrent FMT with pembrolizumab in patients with PD-1 resistant/refractory melanoma The study will be conducted over a 12-week period (and up to 24-weeks in responding patients).

Recipient eligibility is based upon prior exposure to PD-1 inhibitor therapy and response at 1st (or subsequent) restaging scan(s) from week 12 up to week 52. Patients must have received a minimum of 2 cycles to be considered eligible. Patients who have received either nivolumab or pembrolizumab are eligible. Patients who have received pembrolizumab/nivolumab in combination with other investigational agent(s) **may be** eligible at the discretion of the treating investigator. PD-1 refractory disease is defined as progressive disease (PD) at the first (or subsequent) radiographic evaluation while receiving PD-1 inhibitor treatment as assessed by RECIST v1.1 on a restaging scan. Patients with stable disease as their best response are eligible; but patients with complete (or partial) response as their best response are ineligible. Other eligibility criteria include absence of active CNS disease, presence of disease amenable to biopsy and lack of contra-indications to FMT administration. Patients will be stratified on the presence or absence of liver metastases.

Suitable patients will be identified following first (or subsequent) restaging study that documents progressive disease (RECIST v1.1) at any time after a minimum 2 cycles of pembrolizumab or nivolumab up to week 52 (17 cycles of pembrolizumab; 26 cycles of nivolumab). Patients will undergo a screening evaluation consisting of imaging (including CNS if clinically suspected), tumor biopsy, and serological/stool studies to confirm suitability for FMT administration. Eligible patients will receive FMT endoscopically (along with intestinal biopsy) with cycle 1 pembrolizumab (+/- 3 days) followed by 3 further cycles of pembrolizumab (cycles 2-4) following which restaging will be performed. Patients with stable and/or responding disease will continue to receive pembrolizumab on study for 4 cycles. Patients with stable and/or responding disease after 8 cycles of pembrolizumab will continue to receive therapy off study until disease progression or up 2 years from FMT administration (35 cycles total).

2.2 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

- **Objective:** To investigate whether combination of single PD-1 responder-derived FMT with Pembrolizumab converts PD-1 non-responders to responders.

  Hypothesis: Failure to respond to PD-1 blockade in patients with advanced melanoma is associated with intestinal dysbiosis and PD-1 responder-derived FMT can convert PD-1 non-responders to responders.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: To investigate how FMT administration affects composition and function of T-cells and innate/adaptive immune system subsets.

Hypothesis: PD-1 responder-derived FMT augments tumor antigen-specific CD8+ and CD4+ T-cell responses, DC maturation/function and innate immune responses to melanoma.

3.3 Exploratory Objective

(1) **Objective:** To determine whether PD-1 response in responding patients is associated with common gut microbiota profile.

Hypothesis: PD-1 responders and non-responders have distinct gut commensal microbiota profiles; and that administration of single responder-derived FMT can reconstitute responder-associated microbiota profile in PD-1 non-responders.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab.

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T cells and the ratio of CD8+ effector T cells/FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by

which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 regulate tumor-specific T-cell expansion and function in vitro (Fourcade et al. 2009). In subjects with melanoma, PD-1 response appears to be associated with preexisting CD8+ T cell responses to melanoma (Tumeh et al. 2014). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab was first approved in the United States by the Food & Drug Administration (FDA) on September 4, 2014, for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab (IPI) and, if BRAF V600 mutation positive, a BRAF inhibitor. Pembrolizumab was approved for a broader indication as monotherapy for the treatment of unresectable or metastatic melanoma in adults by the FDA on December 18, 2015.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Intestinal microbiome comprises entire compilation of microorganisms dwelling in the gastrointestinal tract. Intestinal microbiome exists in a symbiotic relationship with the host, actively contributing to intestinal health and disease. Intestinal dysbiosis has been implicated in a wide range disease states including inflammatory bowel disease (IBD), obesity, allergic disorders, Type 1 diabetes mellitus, autism, obesity, and cancer in both human and animal models. *Clostridium difficile* is an infectious Gram-positive spore-forming bacillus microorganism that is capable of causing toxin-mediated gastrointestinal illness with a wide spectrum of severity. *C. difficile* is an intestinal commensal, growth of which is normally

suppressed by more dominant anaerobes. Antibiotic use can result in loss of commensal microbiota barrier effect and release of previously unavailable niches; and this dysbiosis sets up an inflammatory cascade. Fecal microbiota transplants (FMT) are highly effective in treating relapsed/refractory *Clostridium difficile* infection - an improvement linked to the increased fecal bacterial diversity in recipients following donation inoculation from healthy volunteers (van Nood et al. 2013).

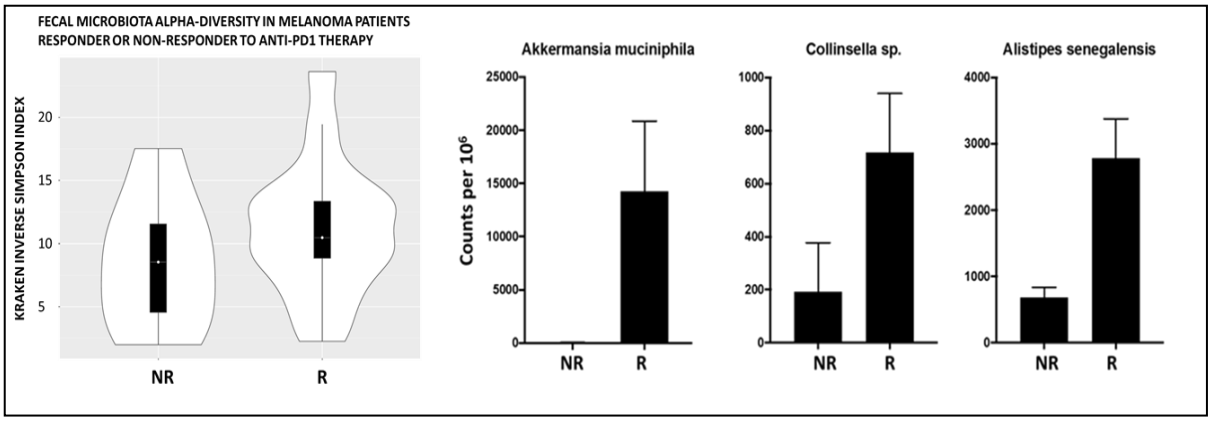
In models of intestinal infections, microbiota-derived signals are required to sustain mucosal immune homeostasis; loss of which results in chronic inflammation. Specifically in cancer, intestinal dysbiosis has been linked to both colorectal carcinogenesis (Schneider et al. 2017) and response to anti-cancer therapies (Tsilimigras et al. 2017; Roy and Trinchieri 2017); including chemotherapy (Iida et al. 2013), radiotherapy and immunotherapy (Roy and Trinchieri 2017). In the setting of anti-cancer immunotherapies, intestinal dysbiosis has been linked to differential responses in models of adoptive T-cell therapy, intra-tumoral CpG-oligodeoxynucleotide therapy, and CTLA-4 and PD-1/PD-L1 immune checkpoint inhibition. The exact mechanisms underlying the role of intestinal microbiota in modulating anti-cancer immune responses are complex and likely involve many interlinked pathways; published data suggests that commensal microbes promote crosstalk between innate myeloid/lymphoid cells and dendritic cells that collectively improves immune homeostasis in intestine and sensitizes tumor cells to anti-cancer immune therapies (Mortha et al. 2014).

Two recent studies have shown the role of the gut microbiome in regulating clinical responses to CTLA-4 and PD-1/PD-L1 blockade in murine melanoma models (Vétizou et al. 2015; Sivan et al. 2015). Vétizou *et al* showed that efficacy of anti–CTLA-4 therapy was dependent on where mice were housed and use of broad-spectrum antibiotics; although efficacy was restored when antibiotic-treated or germ-free–housed mice were fed *Bacteroides* isolates. Stool from melanoma patients with *Bacteroidales* species had a similar effect. Sivan *et al* demonstrated that mice from difference facilities (Jackson Laboratory and Taconic Farms) known to have distinct microbiota profiles exhibited differential tumor growth - a difference that disappeared upon cohousing. This difference was transmittable, mediated efficacy of anti-PD-1 therapy and was identified as being related to presence of *Bifidobacterium* species in the gut flora of the Jackson Laboratory mice. Although both groups used similar murine models of melanoma, their findings implicated different microbiota (CTLA-4 *Bacteroidales*; PD-1 *Bifidobacterium*).

Two recent studies of intestinal microbiome samples from patients with NSCLC, renal cancers (RCC), and melanoma patients (MPs), have shown that the presence of certain commensals correlated with better clinical outcome upon PD1 blockade (Gopalakrishnan et al. 2018; Routy et al. 2018). Metagenomics studies of stools from 100 cancer patients (60 NSCLCs and 40 RCCs) revealed correlations between better clinical outcome upon PD-1 blockade and the relative abundance of *Akkermensia muciniphila* (Routy et al. 2018). Oral administration of *Akkermensia* to non-responding tumor-bearing germ-free mice restored the efficacy of PD-1 blockade. In addition, 16S RNA sequencing of stools obtained from a limited number of MPs (n=43, 30R, 13NR) showed increased alpha diversity and relative abundance of *Ruminicoccaceae/Faecalobacterium* bacteria in PD-1 responders and lower Bacteroidales abundance in PD-1 non-responders (Gopalakrishnan et al. 2018). Fecal

microbiota transplant (FMT) from human PD-1 responders to melanoma-bearing germ-free mice reduced significantly tumor growth as compared to FMT from PD-1 non-responders.

To the best of our knowledge, the evaluation of the role of the gut microbiome in modulating immunological and clinical responses to anti-cancer therapy in vivo has been conducted only in syngeneic mice. These studies have significant limitations including the inability of syngeneic murine systems to completely recapitulate the features of spontaneous human tumors and significant differences in intestinal microbiota between mice with identical genetic backgrounds. Given these factors along with the significant greater complexity and inter-individual variability in human microbiota relative to mice, translating these findings to improving outcomes of patients failing PD1/PDL1 blockade is likely to involve more than inoculating non-responders with *Bacteroidales* or *Bifidobacterium*. Under the aegis of a tissue/blood banking protocol (UPCI 96-099) supported by the Skin Cancer SPORE, we have prospectively banked pre-treatment biopsy, pre- and on-treatment blood [serum, processed peripheral blood mononuclear cells (PBMC)] and stool specimens on **PD1Rs** – defined as patients who are alive and demonstrate an ongoing response (complete or partial) at 2 years or greater to pembrolizumab or nivolumab given as standard of care (SOC) therapy for advanced melanoma. As controls, we used patients with non-response to PD-1 monotherapy (**PD1 NR**). This cohort is representative of a "real-world" advanced melanoma population with an overall response rate of 33%. Stool specimens from these **PD1 R** and **PD1 NR** were banked within 4-8 hours at -80^{0}C. In order to minimize variability, stool and oral cavity samples were obtained from age-matched (by decade) and sex-matched PD1Rs and PD1NRs. Prior to sample collection, investigators ensured that patients had not had any recent hospitalizations (within 1 month) and/or receipt of recent antibiotic therapy (within 1 month).



We observed increased relative abundance of *Akkermensia, Collinsella and Alistipes* species in PD1Rs as compared to PD1NRs (Figure 2), but no differences were observed in the

relative abundance of *Bifidobacterium* species and *Bacteroides fragilis* (data not shown). Because of the limited number of pre-treatment and NR samples, the significance of these findings still needs to be determined with sequential microbiome analysis of larger numbers of PD1Rs and PD1NRs.

It is important to understand that the vast majority of the studies evaluating the role of intestinal dysbiosis in response to anti-cancer therapy have been conducted in mice; and the translation of these experimental findings to the clinic is an ongoing challenge. Mouse tumor models provide an excellent means by which to dissect the molecular mechanisms of carcinogenesis and anti-tumor treatments; but often fail to completely recapitulate the features of spontaneous human tumors. Further, intestinal microbiota varies even between mice with identical genetic backgrounds and housed in similar conditions; with profound physiologic effects. Separately, the physiological and immune responses of mice are similar but not identical to those of humans. Given these factors along with the significant greater complexity and inter-individual variability in human microbiota relative to mice, translating these findings to improving outcomes of patients failing PD-1/PD-L1 blockade is likely to involve more than inoculating non-responders with *Bacteroidales* or *Bifidobacterium*.

With immune checkpoint blockade, unique response patterns have been observed in which both objective responses and prolonged disease stabilization can be seen after an initial increase in tumor size. Immune-related response criteria (irRC) have been developed to better characterize these atypical responses (pseudo-progression) (Wolchok et al. 2009); but is important to note that atypical responses only account for 3-7% of responses seen in Pembrolizumab treated patients (Wolchok et al. 2009; Hodi et al. 2016). Thus, 93-97% of all eventual responders are identified within 12 months of commencing PD-1 monotherapy. There is no accepted standard therapy for melanoma patients who fail PD-1 therapy.

The above data suggest that **PD-1 R** and **PD-1 NR** have distinct intestinal microbiota; and that these differences are present pre-treatment and may account for the differential response to PD-1 therapy in **PD-1 R** compared to **PD-1 NR**. Based on this data, we propose to investigate whether FMT from **PD-1 R** patients may convert **PD-1 NR** patients into **PD-1 R** with evidence of anti-melanoma T cell responses. We will also investigate whether response to PD-1 is associated with a specific gut microbiota profile.

4.2.2 Rationale for Dose Selection/Regimen/Modification (Pembrolizumab)

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of

pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

4.2.3 Rationale for Dose Selection/Regimen/Modification (FMT)

FMT as defined in this study is derived from PD-1 responders (PD-1 R), hereafter termed "**PD-1 R FMT**" and administered under IND 17063. PD-1 R donor selection is detailed in Section 4.2.3.1.

PD-1 R FMT samples are collected and stored in a -80°C freezer. Each FMT unit is marked with an expiration date of 6 months after collection, assuming storage in a -80°C freezer. Normal temperature fluctuations in freezer temperature of up to 5°C are acceptable. Upon opening, standard protocols for handling biohazardous material will be followed at all times. Sterile microbiological technique will be followed when handling material to avoid contamination. If treatments need to be destroyed, internal protocols for disposal of human stool should be followed. All FMT storage units are cleaned with a sporicidal disinfectant prior to freezing.

FMT samples from suitable donors (**PD-1 R FMT**) will be obtained and screened. FMT will undergo processing to produce **PD-1 R FMT infusate** (see **Appendix: FMT infusate – thawing and infusate preparation**). Generally 30g of **PD-1 R** stool will be used to generate 1 **PD-1 R FMT infusate**. Upon initial collection and processing, PD-1 R FMT infusate will be passed through a 330-micron filter to exclude particulate matter. Given the complexities of bacterial composition, the exact quantities of various bacteria may differ from batch to batch and an exact "FMT dose" cannot be calculated.

PD-1 R FMT will be administered colonoscopically. PD-1 R FMT infusate will be thawed prior to administration according to prepared guidelines (see **Appendix: FMT infusate – thawing and infusate preparation**). Using universal precautions, PD-1 R FMT infusate will be pre-loaded into standard 50cc syringes that have a tip compatible with the endoscope port for direct delivery of material through channel.

4.2.3.1 Donor Selection

PD-1 R are defined as patients with advanced unresectable stage IIIB/IIIC or metastatic melanoma treated with PD-1 checkpoint inhibitors (either nivolumab or pembrolizumab) who have experienced a durable partial or complete response. Median duration of follow up should be ≥24 months (for partial responders) or ≥12 months (for complete responders). Published data indicates that the likelihood of relapse in melanoma patients following this duration of follow up is negligible (Ribas et al. 2016; Robert et al. 2015).

PD-1 R identified as being suitable donors – designated as "PD-1R - donors" - will be separately consented under a donor-specific consent form (see **Appendix: Donor ICF**). PD-1 R donor inclusion and exclusion criteria are enumerated below.

Patients with classical Hodgkin lymphoma (Ansell et al. 2015), gastric cancer (Muro et al. 2016), hepatocellular carcinoma (Feng et al. 2017), head and neck squamous cell carcinoma (Ferris et al. 2016), Merkel cell carcinoma (Kaufman et al. 2016; Nghiem et al. 2016), microsatellite instable (MSI-H) tumors (Le et al. 2015), non-small cell lung cancer (NSCLC) (Gettinger et al. 2015; Garon et al. 2015), renal cell carcinoma (RCC) (Motzer et al. 2015), and urothelial carcinoma (UC) (Balar et al. 2017; Plimack et al. 2017) have all experienced

durable responses to PD-1/PD-L1 therapy. However, the durability of responses in this patient population is unknown. Study investigators will also collect samples from PD-1 R patients with these histologies – designated as "PD-1R– at interest population".

Inclusion criteria "PD-1R - donors:

1. Documented history of metastatic melanoma (excluding uveal melanoma) treated with pembrolizumab or nivolumab administered singly or in combination.

2. Currently in remission from melanoma with median duration of remission lasting ≥12 months (for complete responders) or ≥24 months (for partial responders) measured since initiation of therapy.

3. Willingness to complete donor-specific questionnaire (Appendix 1) to assess exposure to infectious agents including but not limited to SARS-CoV-2.

    3.1. Questionnaire (Appendix 1) will include an assessment of whether donor was diagnosed with laboratory-confirmed SARS-CoV-2 infection, experienced symptoms of COVID-19 (e.g., fever, cough, shortness of breath) not explained adequately by another diagnosis, or was exposed to a suspected or confirmed case of COVID-19 or SARS-CoV-2 infection.

4. Willingness to complete donor-specific serologic testing to evaluate infectious agents including SARS-CoV-2.

    4.1. To evaluate for the possibility of SARS-CoV-2 exposure and/or infection, all candidate donors will undergo **nasopharyngeal** (NP) and **stool testing** for SARS-CoV-2 using a RT-PCR intended for qualitative detection of SARS-CoV-2 nucleic acids. RT-PCR assay is composed of two principal steps: (1) extraction of nucleic from patient NP and stool specimens, (2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and real-time detection with 2019-nCoV specific probes. Test will be performed by Diagnostic Solutions Laboratory, located at 5895 Shiloh Rd Ste 101, Alpharetta, GA.

        4.1.1. NP test: DSL COVID-19 NP assay evaluates for the presence of two coronavirus genes, the nucleocapsid and the spike gene. A total of three primer/probe sets were designed to detect nucleocapsid and spike RNA. A total of two primer/probe sets (CDC's N1 and N3 primers) were designed to detect the viral nucleocapsid gene; 1) N1 is SARS-CoV-19 specific, 2) N3 is a coronavirus generic primer and probe set that can also detect other coronaviruses. The third primer/probe set was designed by Diagnostic Solutions Laboratory using the published SARS-CoV-2 reference genome to detect SARS-CoV-2 specific sequence of the spike gene. Blasting the spike primer and probe set indicated 100% homology with SARS-CoV-2 but no homology with any other nucleotide sequence present in the database, including both SARS-CoV (taxonomy ID 694009) and MERS-CoV (taxonomy ID 1335626). Each primer and probe sets are tested in individual reactions.In addition, the test also detects the human RNase P gene as a sample internal extraction and

amplification control using primers and probe sequences published by CDC. In addition, the test utilizes external controls as described below (low titer positive control and a negative control).

4.1.1.1. A Negative Template Control (NTC) serves as a negative process control to monitor for any reagent contamination and sample carryover that could occur during the extraction and amplification process. The NC consists of elution buffer and is run once for every batch of extracted specimens.

4.1.1.2. A Positive Control (PC) is used to verify that the assay run is performing as intended. The PC is based on genomic RNA from SARS-Related Coronavirus 2and will be positive for N1, N3, and S. The positive control is used once for each batch of extracted specimens.

4.1.1.3. An Internal Control will be performed on every clinical sample as an endogenous control. The internal control is the human Ribonuclease P (RNase P) gene isolated with the sample RNA of each specimen. It will be used to confirm isolation efficiency as well as amplification.

Interpretation of DSL COVID-19 Assay

Interpretation	**RNaseP**	**SARS-CoV-2 N1**	**SARS-CoV-2 N3**	**SARS-CoV-2 spike**	**Action**
COVID Negative	+	-	-	-	Report result
COVID Positive	+	+	+	+	Report result
	+	+	-	+	
	+	+	-	-	
	+	-	-	+	
	+	-	+	+	
	+	+	+	-	
COVID Inconclusive	+	-	+	-	
Repeat testing	-	+/-	+/-	+/-	
*For samples with a repeat inconclusive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecoviruses currently unknown to infect humans, for epidemiological purposes or clinical management.					

4.1.2. Stool test: DSL COVID-19 stool assay evaluates for the presence of three coronavirus genes: SARS-CoV-2 membrane (M), SARS-CoV-2 spike (S) and SARS-CoV-2 nucleocapsid (N). Following adequate detection of RNaseP in a test sample, detection of 1 of the viral genes is sufficient for a positive test result. Due to the discontinuous transcription of all coronaviruses that results in the presence of the capsid gene in a vast majority of the mRNA populations as well as in the full-length viral genome, detection of the capsid gene in the absence of additional genes is expected. The lower limit of detection (LLoD) for each viral gene target was determined by testing serial dilutions of the SARS-CoV-2 genomic isolate in triplicate and is summarized in the DSL COVID-19 validation summary. These data indicate that the LLoD for viral spike (S) and membrane (M) detection is 104 genomic equivalents/mL while the LLoD for viral capsid (N) detection is 103 genomic equivalents/mL. In addition, the observation that the mean CT value for each of the determined LLoDs are very low (close to 40 cycles), it is likely that any signal obtained below a CT of 38 is below the LLoD for the viral target genes and should be disregarded for analytic purposes.

4.2. Determination of "SARS-CoV-2 negative" status will be made on nasopharyngeal, blood and stool specimens obtained at two timepoints (every 2 weeks for up to 12 weeks): 1st sample collected at least 14 days before stool sample donation, and a 2nd sample collected at least 14 days after stool sample donation.

4.3. As the turnaround of the test is 48-72 hours, the stool sample will be released for FMT use no sooner than 48-72 hours after 2nd test results and donor is deemed "SARS-CoV-2 negative".

4.4. Candidate donors with discordant stool and NP SARS-CoV-2 results will be excluded.

4.5. Candidate donors excluded based on based on screening and/or testing as described in 3.1 and 4.1.1-4.1.2 above will not be allowed to donate stool specimens for use in this study at this time. This is subject to subsequent revision should stool-specific SARS-CoV-2 testing become available.

Inclusion criteria “PD-1R– at interest population”:

1. Documented history of metastatic melanoma (or other malignancies) treated with pembrolizumab or nivolumab or other PD-1/PD-L1 inhibitors.

2. Currently in complete/partial remission with duration of complete/partial remission ≥6 months since initiation of therapy.

Exclusion criteria:

1. History of suspected/confirmed SARS-CoV-2/COVID infection/exposure.

1.1. Any instances of suspected/confirmed SARS-CoV-2/COVID infection/exposure will result in exclusion of candidate donor from further donations and exclusion from clinical use of any FMT manufactured from stool donated by candidate donor beginning 4 weeks prior to the suspected/confirmed SARS-CoV-2/COVID infection/exposure

2. History of antibiotic treatment during the 1 month preceding donation.

3. History of intrinsic gastrointestinal illnesses, including inflammatory bowel disease, irritable bowel syndrome, chronic diarrheal disorder (celiac disease), active primary gastrointestinal malignancies or major gastrointestinal surgical procedures.

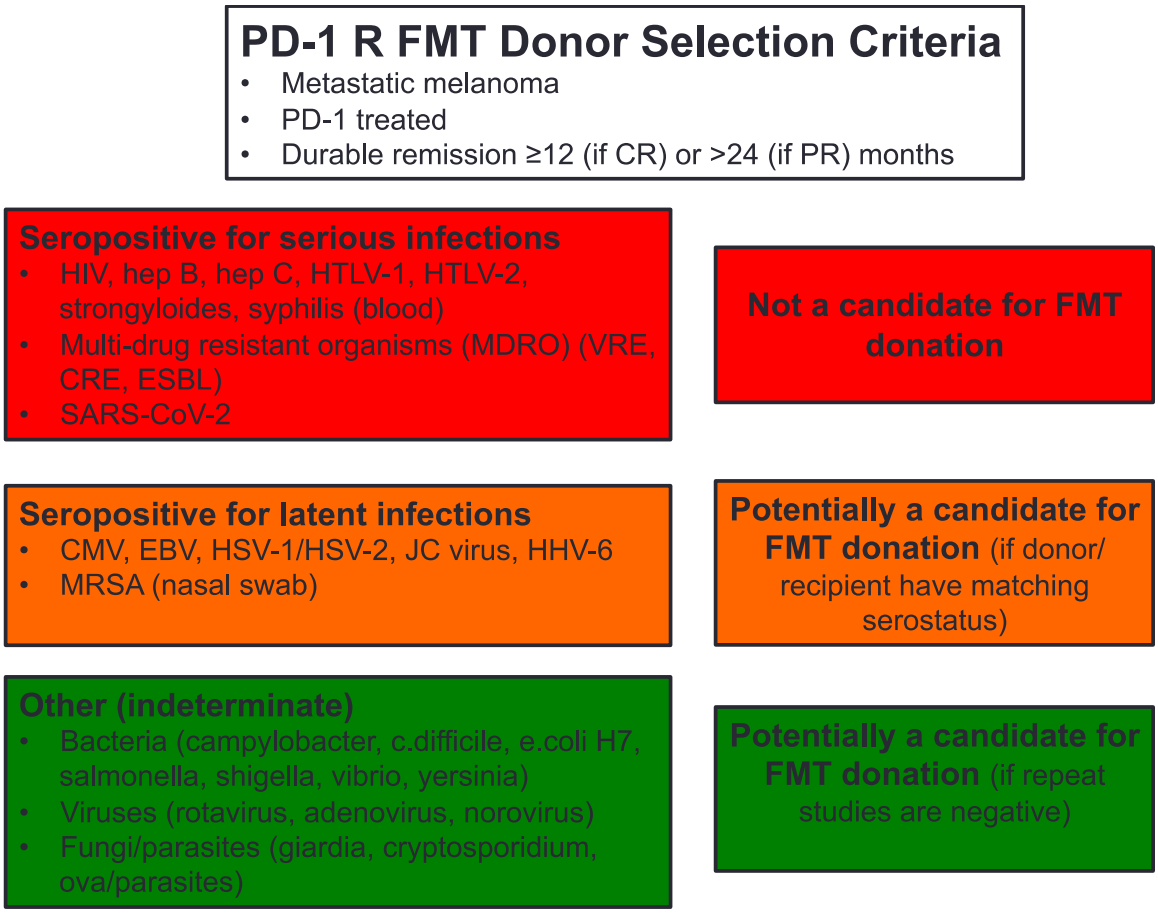
4. History of symptomatic autoimmune illness.

5. History of documented chronic pain syndromes (fibromyalgia, chronic fatigue) or neurologic, neurodevelopmental disorders.

6. History of metabolic syndrome, obesity (BMI of >35), or moderate-to-severe malnutrition (as assessed clinically).

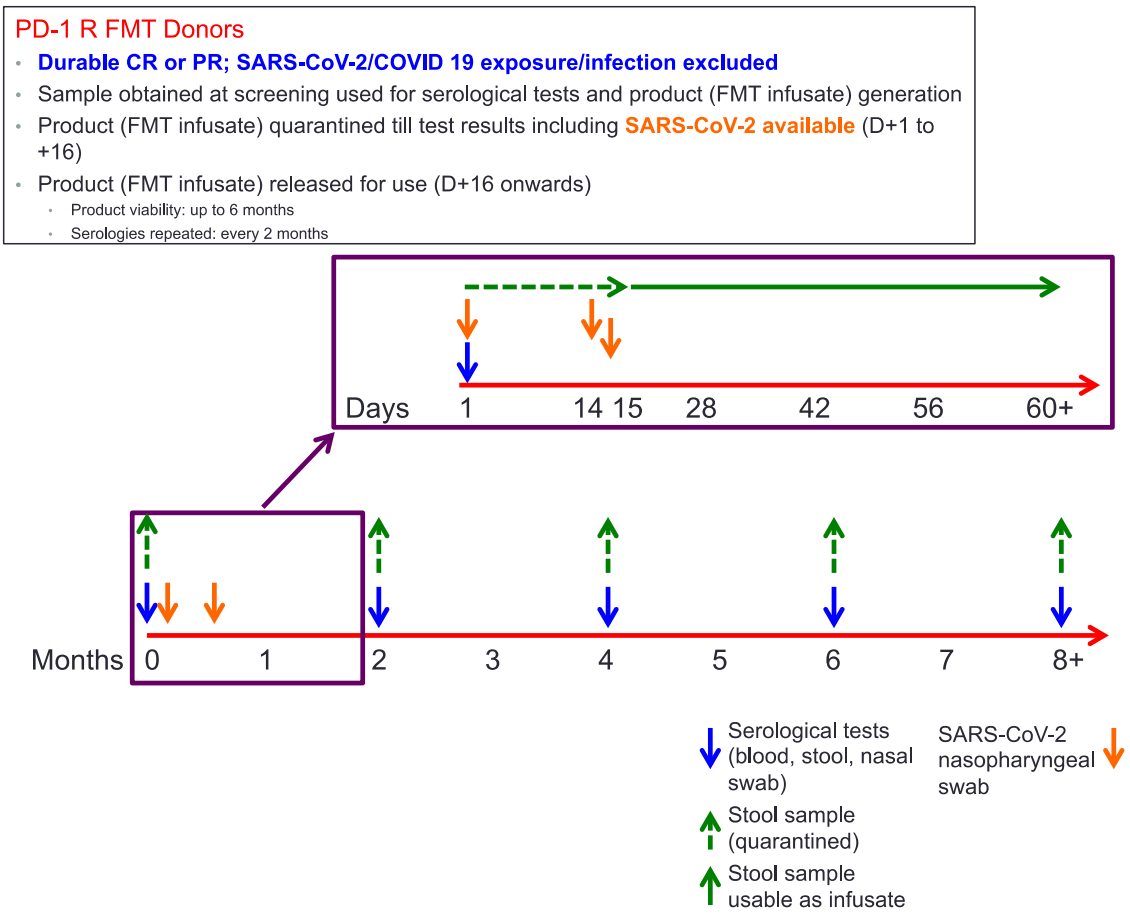
7. Documented pregnancy (serum/urine pregnancy test to be performed on women of childbearing potential within 1 week of donation for transplant).

PD-1 R FMT donors will undergo extensive screening including a detailed questionnaire and serological/stool/nasal swab tests (see **Appendices: FMT Donor Screening Questionnaire** and **FMT Donor Lab Testing**) to eliminate possibility of transmitting infectious agents prior to FMT sampling. Only patients deemed suitable by screening questionnaire and serological/stool/nasal swab tests are deemed suitable for FMT donation (see Figure below).



PD-1 R donors will be divided into patients with and patients without liver metastases based on initial staging imaging obtained immediately prior to initiation of PD-1 therapy.

PD-1 R who agree to be donors will donate stool samples which will undergo sample processing and administration as detailed in Section 4.2.3. Serial serological testing will be performed and repeated (see figure below).



4.2.4 Rationale for Endpoints

4.2.4.1 Efficacy Endpoints

Primary efficacy endpoint: safety/toxicity and objective response rate (ORR) by RECIST 1.1.

Secondary efficacy endpoints: include incidence of grade III/IV toxicities, safety of FMT administration endoscopically, progression-free survival (PFS), and overall survival (OS).

4.2.4.2 Biomarker Research

Biomarker endpoints include:

Analysis of bacterial taxa occurrence (within-sample and between-sample differences) in PD-1 responders, FMT responders and FMT non-responders.

Evaluation of anti-tumor T-cell responses, DC function/maturity by multi-parameter flow cytometry, multiplex immunohistochemistry, analysis of T-cell receptor (TCR) clonality/diversity and genetic mutation/expression studies (RNAseq, exome sequencing).

5.0 METHODOLOGY

5.1 FMT Recipient Entry Criteria

PD-1 resistant/refractory melanoma (excluding uveal melanoma) as defined as progressive disease (PD) at first (or subsequent) radiographic evaluation that demonstrates disease progression while receiving PD-1 inhibitor treatment. Other eligibility criteria include presence of disease amenable to biopsy and lack of contra-indications to FMT administration.

5.1.1 Subject Inclusion Criteria

A patient must meet all of the following criteria to be eligible to participate in this study:

1. Have a histologically or cytologically confirmed diagnosis of unresectable stage III or IV melanoma. Patient may not have a diagnosis of uveal melanoma.

2. Have received any number of prior systemic therapies for metastatic disease. Prior radiation therapy (any number) and interferon use (any formulation and/or duration) in the adjuvant or metastatic disease settings is permitted. Vaccine therapy will be counted as systemic therapy.

3. **Patient must currently be receiving systemic PD-1 immunotherapy with pembrolizumab or nivolumab to be eligible**. **Patients who have received PD-1 immunotherapy in the adjuvant setting and then relapsed are eligible.**

4. Must be PD-1 inhibitor refractory/resistant – defined as having received at least 2 doses of pembrolizumab with documented systemic disease progression on staging imaging.

    a. PD is defined as increase in tumor burden > 20% relative to nadir (minimum recorded tumor burden) by RECIST v1.1.

    b. Once PD is confirmed, initial date of PD documentation will be considered as the date of disease progression.

    c. Patients can be enrolled at any time following initiation of PD-1 therapy assuming patients have not had (at any time) a record of response to PD-1 therapy (prior best response of stable disease – acceptable; prior best response of complete/partial response – unacceptable).

    d. For patients receiving adjuvant PD-1 inhibitor therapy, initial date of PD documentation will be considered as the date of disease progression.

5. Patients with CNS disease (parenchymal but not leptomeningeal) are eligible if CNS metastases are treated and deemed stable (with a repeat CT/MRI imaging study) prior to the enrollment date. If radiation is used to treat CNS parenchymal disease, a 2 week washout period will apply (counted from D1 treatment).

a. Stability must be confirmed with a repeat CT/MRI imaging study performed as part of the screening evaluation (at least 2 weeks after radiation).

b. Patients with new CNS metastases identified during screening are ineligible.

6. Willingness to receive FMT administered endoscopically (colonoscopically) and undergo necessary bowel preparation pre-procedure.

    a. Understand infectious risks associated with FMT administration. Although FMT infusate has been screened for bacteria, viruses, fungi and parasites there is a risk of transmission of known and unknown infectious organisms contained in the donor stool. Post-FMT bacteremia (e.g. E. coli), sepsis and fatal events may rarely occur.

    b. Understand non-infectious risks associated with FMT administration.

        i. Possible allergy and/or anaphylaxis to antigens in donor stool.

        ii. Theoretical risk of developing disease possibly related to donor gut microbiota including but not limited to: obesity, metabolic syndrome, cardiovascular disease, autoimmune conditions, allergic/atopic disorders, neurologic disorders, psychiatric conditions and malignancy.

    c. Understand risks associated with colonoscopy including risk of infection transmission, colonic perforation, aspiration pneumonia, and death.

    d. Understand that data regarding the long-term safety risk of FMT are lacking.

7. Amenable to participate in the correlative studies and should have available tumor tissue for tumor biopsies. Acceptable biopsies include surgical biopsy, core biopsy or punch/surgical tumor biopsies (of accessible lesions).

8. Have measurable disease as per RECIST version 1.1. At least 1 of the tumor sites must be amenable to biopsy and this may not be the site of disease used to measure antitumor response.

9. Be willing and able to provide written informed consent for the trial.

10. Be ≥ 18 years of age on day of signing informed consent.

11. Have a performance status of 0 or 1 on the ECOG Performance Scale.

12. Demonstrate adequate organ function as defined in **Table 5.1.1**, all screening labs should be performed within 14 days of treatment initiation.

Table 5.1.1-1 Adequate Organ Function Laboratory Values

System	Laboratory Value

Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

13. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

14. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

15. Male subjects of childbearing potential (Section 5.7.1) must agree to use an adequate method of contraception as outlined in Section 5.7.1- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

5.1.2 Subject Exclusion Criteria

A patient meeting any of the following criteria is not eligible to participate in this study:

1. Presence of absolute contra-indications to FMT administration

   1.1. Toxic megacolon

   1.2. Severe dietary allergies (e.g. shellfish, nuts, seafood)

   1.3. Inflammatory bowel disease

1.4. Anatomic contra-indications to colonoscopy

2. Patients receiving PD-1 therapy whose disease is responding (as defined by RECIST v1.1).

3. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

4. Highly symptomatic patients (e.g., declining ECOG performance status; rapidly worsening symptoms; rapid progression of disease; progression of tumor at critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention) are not eligible.

5. Expected to require any other form of systemic or localized antineoplastic therapy while on study.

6. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy (> 10 mg prednisone daily or equivalent) or any other form of immunosuppressive therapy prior to trial treatment. Patients receiving systemic steroids at physiologic doses are permitted to enroll assuming steroid dose is not above the acceptable threshold (> 10 mg prednisone daily or equivalent).

7. Has a known history of a hematologic malignancy, primary brain tumor or sarcoma, or of another primary solid tumor, unless the patient has undergone potentially curative therapy with no evidence of that disease for five years.

    7.1. **Note:** The time requirement also does not apply to patients who underwent successful definitive resection of basal or squamous cell carcinoma of the skin, superficial bladder cancer, in situ cancers including cervical cancer, breast cancer, melanoma, or other in situ cancers.

8. Active central nervous system (CNS) metastases and/or leptomeningeal involvement.
    8.1. **Note:** Patients with treated brain metastases will need repeat CNS imaging (MRI brain or CT head with IV contrast) to document stability. Patients with previously treated brain metastases may participate provided they are stable (without evidence of progression by MRI/CT brain imaging performed during screening period), have no evidence of new or enlarging brain metastases and are off systemic steroids (≤ 10 mg/day prednisone or equivalent) for at least one week prior to enrollment.
    8.2. **Note:** Patients with leptomeningeal disease (leptomeningeal enhancement on MRI/CT imaging and/or positive CSF cytology) are not eligible to enroll.
    8.3. **Note:** Patients with no history of CNS disease will not require staging MRI brain unless they have symptoms to suggest new brain metastases.

9. Had a severe hypersensitivity reaction to treatment with pembrolizumab or any of its excipients.

10. Has an active autoimmune disease or a documented history of autoimmune disease or syndrome that requires systemic steroids or immunosuppressive agents. Patients with vitiligo, type I diabetes, resolved childhood asthma/atopy are exceptions to this rule. Patients who require intermittent use of bronchodilators or local steroid injections are not excluded from the study. Patients with hypothyroidism stable on hormone replacement are not excluded from the study.

11. Has a history of (non-infectious) pneumonitis that was life-threatening and/or required invasive support (CTCAE grade 4 or greater) or current pneumonitis.

12. Has serious concomitant illnesses, such as: cardiovascular disease (uncontrolled congestive heart failure, uncontrolled hypertension, recent myocardial infarction within prior month, and severe cardiac arrhythmia), bleeding disorders, symptomatic autoimmune diseases, severe obstructive or restrictive pulmonary diseases, active systemic infections, and inflammatory bowel disorders. This includes HIV or AIDS-related illness, or active HBV and HCV.

13. Has an active infection requiring systemic therapy.

14. Has active COVID-19 infection and/or exposure to SARS-CoV-2 as defined below:

    14.1. Positive SARS-CoV-2 result on nasopharyngeal and/or stool specimens (by RT-PCR test)
    14.2. Active COVID-19 infection (per CDC guidelines)
    14.3. Exposure to active COVID-19 infected patient (as confirmed using SARS-CoV-2 RT-PCR test or other approved test) as defined per CDC guidelines.

15. Has active human immunodeficiency virus (HIV) infection (as manifested by presence of HIV 1/2 antibodies and/or positive HIV ELISA/Western Blot assays).

16. Has active Hepatitis B or Hepatitis C infection. Patients with a history of Hepatitis B/C infection who have received anti-viral therapy and are disease free (Hep B - negative HBsAg and HBV DNA; Hep C – negative HCV RNA) may be considered for enrollment after discussion with Principal Investigator.

17. Has a known history of active TB (Bacillus Tuberculosis).

18. Patient has received a live vaccine within 4 weeks prior to the first dose of treatment.
    18.1. *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.*

19. Has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier. Patients with prior adverse events on replacement therapy who are asymptomatic or minimally symptomatic are not excluded from the study (i.e. hypothyroidism, hypopituitarism, adrenal insufficiency).

20. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

21. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

22. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in **Table 5.2**.

Table 5.2-1 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 (+/- 3 days) of each 3-week cycle for 8 cycles (Cycles 1 to 8)	Standard
FMT	Single administration	Once	Endoscopically administered	Cycle 1 day 1 (+/- 3 days)	Experimental

5.2.1 Dose Selection/Modification (Pembrolizumab)

5.2.1.1 Dose Selection

Pembrolizumab will be dosed at a fixed dose of 200mg every 3 weeks. Rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

5.2.1.2 Dose Modification (Escalation/Titration/Other)

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 7-1 (see **Appendix 5: Dose Modifications for Pembrolizumab**).

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued after consultation with the Principle Investigator. With investigator and Sponsor agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. For information on the management of adverse events, see Section 5.6.1.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab should be discontinued from trial treatment.

5.2.2 Timing of Dose Administration (Pembrolizumab)

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (**Section 6.0**). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons. Pembrolizumab will be given on study for 4 cycles (minimum) and up to 8 cycles in patients who are responding (or receiving clinical benefit as assessed by Principle Investigator). In patients who continue to respond (or receive clinical benefit), pembrolizumab will be administered by treating investigator(s) until disease progression or up to 2 years from FMT administration (35 cycles total).

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

5.2.3 Dose Selection/Modification (FMT)

Rationale for FMT as defined and used in this trial is provided in Section 4.2.3. – Rationale for Dose Selection/Regimen/Modification (FMT).

5.2.4 Timing of Dose Administration (FMT)

FMT will be administered on cycle 1, day 1 (+/-3 days).

5.2.5 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

Not applicable.

5.4 Stratification

N/A

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or

vaccination may be required. The investigator should discuss any questions regarding this with the Principle Investigator. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and Events of Clinical Interest (ECI) as defined in Section 7.2.

5.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy
- Investigational agents other than PD-1 R FMT
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6 Rescue Medications & Supportive Care

5.6.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of immune related adverse events (irAE) are outlined (see **Appendix 5, Table 5-1**). Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined in **Appendix 5, Table 5-1**). Refer to Section 5.2.1 for dose modification.

Note: Although FMT administration has not previously been associated with irAEs, given the novel combination, treating investigator(s) should attempt to delineate whether AEs that occur within a reasonable duration of FMT administration could have an underlying immune-related etiology. If so determined, criteria for managing irAE (see **Appendix 5, Table 5-1**) should be extended to managing these irAEs. Occurrence of these novel irAEs should be reported to the Principal Investigator.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

5.6.2 Management of Infusion Reactions

Pembrolizumab may cause severe or life-threatening infusion reactions, including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines to treat pembrolizumab-associated infusion reactions are provided in **Appendix 6** (see **Appendix 6**).

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

Subjects should maintain a high fiber diet (daily dietary fiber intake $\geq$30 grams) unless modifications are required to manage an AE such as diarrhea, nausea or vomiting. To assess this, patients will formally be assessed by a dietician at study entry. To accurately assess effect of FMT, enrolled patients are required to maintain a dietary log (see **Appendix 3: Dietary Intake Questionnaire**) to track dietary intake including supplements, probiotics. Treating physicians are required to minimize prescribing oral antibiotic(s) unless absolutely

required while patients are required to report antibiotic treatment anytime for the duration of the study.

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection.

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed

immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.7.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.8 Duration of Therapy

Patients who are responding or deriving clinical benefit (at the determination of the Principal Investigator) at the first restaging interval (after Cycle 4 pembrolizumab) will stay on study. Patients who continue to respond or derive clinical benefit (at the determination of the Principal Investigator) at the second restaging interval (after Cycle 8 pembrolizumab) will continue to receive pembrolizumab on study for 2 years (or 35 cycles total), counted from the time of FMT initiation. Patients who development a confirmed CR will be allowed to discontinue therapy after a minimum of 6 months since confirmation of CR in discussion with Principal Investigator.

- **Discontinuation of Study Therapy after CR**

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR and have treated for at least 24 weeks with pembrolizumab (from time of FMT administration) and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared.

5.8.1 2nd FMT Administration

Patients who initially respond to but subsequently progress will be offered a second FMT administration at the time of confirmed progression (RECIST v1.1) at the discretion of the Principal Investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Patients will be required to resign informed consent.

5.9 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 – Other Procedures.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative withdraws consent.
- Confirmed radiographic disease progression

- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Repeated or serious noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.5.5

- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.1). Subjects who discontinue for reasons other than PD will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.10 Subject Replacement Strategy

Patients are deemed evaluable for safety if they were screened and received FMT and at least one dose of pembrolizumab. Patients are deemed evaluable for efficacy if they completed screening and received FMT/pembrolizumab and 1st re-staging scan. Patients who drop out of the study for reasons related to disease progression **prior to 1st re-staging scan** may be replaced.

5.11 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the FMT infusate

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Screening Phase**	**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**	
Treatment Cycle/Title:	Study Screening (Visit 1)	1*	2*	3*	4*	To be repeated beyond 4 cycles 5*	6*	7*	8+*	Discontinuation	Safety Follow-up[A]	Follow Up Visits[B]	Survival Follow-Up[C]
Scheduling Window (Days):	**-28 to -1**	**± 3**	**± 3**	**± 3**	**± 3**	± 3	± 3	± 3	± 3	At time of discontinuation	30 days post discontinuation	Every 12 weeks	Every 12 weeks
Administrative Procedures													
Informed Consent	+												
Inclusion/Exclusion Criteria	+												
Demographics and Medical History	+												
Prior and Concomitant Medication Review	+												
Pembrolizumab administration		+	+	+	+	+	+	+	+				
Post-study anticancer therapy status											+	+	+
Survival Status										+	+	+	+
Administration of Investigational Agents													
FMT administration (colonoscopic)		+											
Clinical Procedures/Assessments													
Review Adverse Events	+	+	+	+	+	+	+	+	+				
Full Physical Examination	+	+	+	+	+	+	+	+	+				
Vital Signs and Weight	+	+	+	+	+	+	+	+	+				
ECOG Performance Status	+	+	+	+	+	+	+	+	+				
Patient self-monitoring[D]		+	+	+	+	+	+	+	+				
Screening questionnaire prior to FMT administration (see Appendix 1)	+												

Product: Pembrolizumab
Protocol/Amendment No.:

Trial Period:	**Screening Phase**	**Treatment Cycles[a]**									**End of Treatment**	**Post-Treatment**	
Treatment Cycle/Title:	Study Screening (Visit 1)	1*	2*	3*	4*	To be repeated beyond 4 cycles 5*	6*	7*	8+*	Discontinuation	Safety Follow-up[A]	Follow Up Visits[B]	Survival Follow-Up[C]
Scheduling Window (Days):	**-28 to -1**	**± 3**	**± 3**	**± 3**	**± 3**	± 3	± 3	± 3	± 3	At time of discontinuation	30 days post discontinuation	Every 12 weeks	Every 12 weeks
Screening labs prior to FMT administration **(see Appendix 2)**	+												
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													
Hematology and serum chemistry[E]	+	+	+	+	+	+	+	+	+				
Coagulation studies and urinalysis[F]	+												
Thyroid studies[G]	+	+		+		+		+					
Efficacy Measurements													
Tumor Imaging [H]	+				+				+				
CNS Imaging (if not previously performed) *CNS imaging will be incorporated into restaging assessments if prior CNS disease is known/stable[H]	+*				+*				+*				
Tumor Biopsies; Archival Tissue Collection; Correlative Studies Blood/Stool													
Tissue Collection (pre-treatment and C4; biopsy at progression is optional)	+				+								
Correlative studies (blood)[I]	+	+	+	+	+	+	+	+	**+ (q12)**			+	
Correlative studies (stool)[J]	+	+	+	+	+	+	+	+	**+ (q6)**			+	
Dietary questionnaire (Appendix 3)[K]	+	+	+	+	+	+	+	+	**+ (q12)**				

Product: Pembrolizumab
Protocol/Amendment No.:

Study Calendar Notes:
[A] Safety follow up: For all patients (responders and non-responders) at 30 days (+/- 10 days) following cycle 8 pembrolizumab on study.
[B] Follow up visits: For all responder patients only every 12 weeks (+/- 14 days) following cycle 4 pembrolizumab on study. Will include correlative stool sampling.
[C] Survival follow up visits: For all patients (responders and non-responders) every 12 weeks (+/- 14 days) following cycle 8 pembrolizumab on study
[D] Patients self-monitoring: while on study, patients will be provided a memory card and digital thermometer to facilitate the standardized collection of AEs particularly AEs commonly associated with FMT including fever, diarrhea, vomiting, abdominal discomfort, constipation, and flatulence.
[E] CBC w/diff, Comprehensive Serum Chemistry Panel (allowed within 28 days)
[F] PT/INR and aPTT; urinalysis; Pregnancy Test; Stool and Blood Screening (allowed within 28 days)
[G] FT4 and TSH (allowed within 28 days) To be repeated at every odd cycle.
[H]Restaging imaging will be obtained after 4 cycles of therapy (12 weeks) comprising of CT or PET/CT imaging. Scans will be repeated every 12 weeks. If CNS lesions were present at staging, restaging imaging should include MRI brain or CT head with contrast. If CNS lesions were not present at staging scans, this need not be repeated.
[I]Correlative laboratory studies will be performed at Screening, during each cycle (Cycles 1-8) and q12 weekly afterwards till EOT.
[J]Correlative stool studies will be performed at Screening, weekly (Cycles 1-8), and q6 weekly afterwards till EOT.
[K]Following FMT, dietary history questionnaire (Appendix 3) will be monitored q12 weekly afterwards till EOT.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Principal Investigator and/or Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB approval in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB requirements, applicable laws and regulations and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. This will include the intake of any oral antibiotic(s) for any indication. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before

the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.6 Assignment of Screening Number

Not applicable.

7.1.1.7 Assignment of Randomization Number

Not applicable.

7.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

Antibiotic use and/or intake of probiotics is discouraged. Use will be recorded.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a full physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

Vital signs will be taken at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

On this study, response will be first assessed after FMT administration and 4 cycles of pembrolizumab (cycle 4; week 21-22) and after cycle 8 (week 33-34). Assessment of antitumor response will be based on RECIST 1.1. Because tumor pseudo-progression is a well-recognized consequence of PD-1 based immunotherapies **and use of FMT as a therapeutic agent in cancer is highly novel**, confirmation of PD requires continued presence of PD on 2 consecutive assessments of response done at least 4 weeks apart during which pembrolizumab will be continued.

Computed tomography (CT) of chest, abdomen, pelvis and CT/MRI brain scans (if clinically indicated) will be performed at screening and after cycle 4 (Day 1 ± 7 days). Following cycle 4, reimaging will occur every 4 cycles (12 weeks) starting with cycle 8 (cycles 12, 16, 20 etc.).

PD documented at any evaluation time point **will be followed** with a repeated radiographic evaluation at the Principal Investigator's discretion to rule out tumor pseudo-progression.

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood/Stool Sampling

Tumor biopsies will be performed at screening (Day -21 to -1) and at cycle 4 (Day 1 ± 7 days). Surgical, core, or punch biopsies are permitted. Post-progression tumor biopsy is recommended but not required.

Correlative blood samples will be obtained as directed in Table 6.1 (Screening, cycles 1-8 and every 12 weeks starting after cycle 9 (Day 1 ± 3 days).

Correlative stool samples along with dietary history questionnaire will be obtained as directed in Table 6.1 (Screening, weekly cycles 1-8 and every 6 weeks starting after cycle 9 (Day 1 ± 3 days).

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 7.1.3.

Prior to receiving FMT, specific laboratory tests to screen for infectious etiologies will be performed as listed in **Appendix 2**. Results of this must be available prior to FMT administration.

Table 7.1.3-1 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonado
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Free tyroxine (T4)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Thyroid stimulating hormone (TSH
Absolute Lymphocyte Count	Creatinine		†Serum pregnancy test
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies (at sp
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only.

‡ If considered standard of care in your region.

7.1.3.1 Pharmacokinetic/Pharmacodynamic Evaluations

7.1.3.1.1 Blood Collection for Serum Pembrolizumab

Not applicable. No pembrolizumab pharmacokinetic sampling will be performed.

7.1.3.1.2 Blood Collection for Anti-Pembrolizumab Antibodies

Not applicable. No pembrolizumab pharmacokinetic sampling will be performed.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in **Section 7.2 - Assessing and** Re**cording Adverse Events**.

Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in **Section 7.1.5.5**. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in **Section**

7.1.5.4.1) and then proceed to the Follow-Up Period of the study (described in **Section 7.1.5.6**).

7.1.4.2 Blinding/Unblinding

Not applicable. This is an open-label study, neither the patients nor the treating physicians are blinded.

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening: Donor

Donor screening will commence upon signing ICF. Donor screening will consist of stool sampling and **FMT Recipient (or Donor) Laboratory Testing** (as delineated in **Appendix 2**). Once collected, donor sample will be treated as outlined in **Section 4.2.3.1**.

7.1.5.2 Screening: Recipient

Screening will commence once screen-eligible patients are found to have PD on first restaging scan following 2 cycles of pembrolizumab (or nivolumab). Screening procedures are outlined in Section 6.1: Study Flow Chart. Screening procedures include: administrative procedures (particularly informed consent wherein patients are only considered enrolled **after** protocol-specific informed consent is signed), clinical assessments and laboratory assessments (particularly **Appendix 1: FMT Recipient (or Donor) Screening Questionnaire** and **Appendix 2: FMT Recipient (or Donor) Laboratory Testing**). There is a 4-week (day -28 to day -1) window during which screening will take place.

7.1.5.3 Treatment Period

Treatment period will commence once eligible patients have signed informed consent and have completed all administrative procedures including clinical assessments and laboratory assessments as outlined above and have been deemed eligible by Principal Investigator.

Treatment period will commence with colonoscopic administration of FMT by gastroenterologists. Following FMT, pembrolizumab (cycle 1) will be administered. There is a 3-day window (+/- 3 days) for administration of pembrolizumab. Prior to restaging, 3 further cycles will be administered. Schedule of activities during each treatment visit will be as outlined in **Section 6.1: Study Flow Chart**.

Patients will be restaged after 4 cycles of pembrolizumab. Patients with stable or responding disease will continue to receive pembrolizumab for an additional 4 cycles. Patients with stable or responding disease will continue to be followed by study team until completion of cycle 8 pembrolizumab and 2nd restaging study. Pembrolizumab will be administered for up to 24 months, progression or response (as outlined in Section 5.8.). Patients who have an initial response but develop subsequent PD, a 2nd administration of FMT (same or different

donor) will be considered at the discretion of the investigator and sponsor if the study remains open (as outlined in section 7.1.5.4.).

7.1.5.4 Post-Treatment Visits

End of treatment (EOT) is defined as the visit where the decision is made to discontinue the participant from treatment. Evaluations will be performed prior to study discharge, or for participants who are prematurely discontinued. or participants who complete all scheduled cycles of therapy, the EOT visit will be the same as the last scheduled and completed on-treatment visit.

For participants who complete all scheduled cycles of therapy, the EOT visit will be the same as the last scheduled and completed on-treatment visit (eg, Cycle 9 Day 1) and the start of the safety follow-up period. For participants that do not complete all scheduled cycles of therapy, the EOT visit will be the most recent on-treatment visit (with all available safety and response data), does not need to be repeated and will be considered the start of the safety follow-up period.

7.1.5.4.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days (+/- 10 days) after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 (or baseline) or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.1.5.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.5.5 Re-Treatment Following Initial Response

Following initial FMT with cycle 1 pembrolizumab, all patients will continue to receive further cycles of pembrolizumab as outlined in Section 5.8 and undergo periodic tumor assessment(s) as outlined in Section 7.1.2.6.

Re-treatment with FMT in patients who initially respond (RECIST v1.1-defined response) may be allowed in selected participants with disease progression during the safety, response or survival follow-up period. Eligibility will be dependent upon participants having confirmed disease progression and will be considered on a case by case basis following discussion with Principal Investigator.

Participants who meeting criteria for retreatment will be required to reconsent to study. Patients will be treated with a second course of FMT along with pembrolizumab. No more than 2 (two) FMT treatments will be permitted. Participants entering retreatment will follow

the same treatment schedule as outlined in Section 6.1. Biopsy and correlative analyses requirements will follow same schedule as well.

7.1.5.6 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and be treated per standard-of-care. Patients should be assessed every 12 weeks by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 12 weeks (± 7 days). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.5.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

7.1.5.6.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be followed every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.5.7 Second Course Phase

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

1. Either

a. Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and

i. Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy

ii. Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

b. Had SD, PR or CR and stopped pembrolizumab treatment after 24 months for reasons other than disease progression or intolerability.

AND

2. Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab

3. Did not receive any anti-cancer treatment since the last dose of pembrolizumab

4. Has a performance status of 0 or 1 on the ECOG Performance Scale

5. Demonstrates adequate organ function as detailed in Section 5.1.2

6. Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.

7. Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.

8. Male subject should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

9. Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in **Section 6.0 – Trial Flow Chart**.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use pembrolizumab and/or FMT is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Adverse events may occur any time during the course of the clinical trial. Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment initiation must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment initiation through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the screening period as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this study, reporting of adverse events will commence after administration of study agents (day +1 and onwards). Adverse events occurring prior to this including those during pre-screening period, screening period and during prior pembrolizumab (or nivolumab) administration will be captured as part of the medical record but will not be submitted.

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose); while an overdose of FMT will be defined as any dose exceeding 3 administrations at a given time (dose ≥3 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. No specific information is available on the treatment of overdose of FMT. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") pembrolizumab and/or FMT, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of pembrolizumab and/or FMT meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-993-1220).

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-993-1220)

7.2.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.2.3.1 Serious Adverse Events

An SAE is any AE that fulfills one of the criteria outlined in **Table 6**.

Examples of such "important medical events" include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as with important medical events described above.

Events that meet SAE criteria must be recorded and reported regardless of expectedness or assessed association with study drug.

Note: Planned hospital admissions or surgical procedures for elective procedures or for an illness or disease that existed before the signing of the ICF or before the subject was enrolled in the study will not be captured as SAEs. If planned admissions or procedures occur at a

time other than what was planned (i.e., due to an exacerbation in the preexisting illness or disease), they should be reported as SAEs.

For the time period beginning when study drugs (FMT and pembrolizumab combination on day +1) are administered, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.3 for additional details) that occurs to any subject must be reported within 24 hours to the Principal Investigator and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning study drugs (FMT and pembrolizumab combination on day +1) are administered through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.3 for additional details), whether or not related to the pembrolizumab and/or FMT, must be reported within 24 hours to the Principal Investigator and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator to be related to pembrolizumab and/or FMT that is brought to the attention of the investigator at any time following C1D1 through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Principal Investigator and to Merck Global Safety.

All subjects with serious adverse events must be followed up for outcome.

The causal relationship to study drugs is determined by a physician and should be used to assess all adverse events (AE). The causal relationship can be one of the following:

- Unrelated:
    - Exposure to investigational agents (FMT and/or pembrolizumab) has not occurred; *or*
    - Administration of investigational agents (FMT and/or pembrolizumab) and occurrence of AE are not reasonably related in time; *or*
    - AE is considered likely to be related to an etiology other than the use of the investigational agents (FMT and/or pembrolizumab); and there are no facts or arguments to suggest a causal relationship to the investigational agents (FMT and/or pembrolizumab).
- Possibly related:
    - Administration of the investigational agents (FMT and/or pembrolizumab) are reasonably related in time; *and*

- AE could not be explained equally well by factors or causes other than exposure to investigational agents (FMT and/or pembrolizumab).

- Probably related:

  - Administration of investigational agents (FMT and/or pembrolizumab) and occurrence of AE are reasonably related in time; *and*

  - AE is more likely explained by exposure to the investigational agents (FMT and/or pembrolizumab) than by other factors or causes.

Every effort should be made to discern whether any reported AE/SAE are related to either pembrolizumab or FMT. If it is deemed equally likely for AE/SAE to be related to both, that should be reflected as well.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-993-1220) at the time of submission to FDA.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-993-1220).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to pembrolizumab, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. Any use of oral and/or intravenous antibiotics for any duration/indication must be reported within 24 hours to the Principal Investigator. Reporting should include the name, dose and duration of antibiotic prescribed and the indication(s) it was prescribed for.

2. Any use of oral and/or intravenous steroids for any duration/indication must be reported within 24 hours to the Principal Investigator. Reporting should include the name, dose and duration of steroid prescribed and the indication(s) it was prescribed for.

3. An overdose of pembrolizumab and/or FMT, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Principlal Investigator, that is not associated with clinical symptoms or abnormal laboratory results.

4. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.2.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Suspected/actual events as outlined in this section will not be reported to Merck as described in Section 7.2.3.- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Principal Investigator within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Principal Investigator will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g.transportation issues etc.) will not be considered a SAE.

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness and attribution to pembrolizumab and/or FMT.

Table 7.2.4-1 Evaluating Adverse Events

An investigator who is a qualified physician or qualified designee, will evaluate all adverse events as to:

V5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of pembrolizumab and/or FMT that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a pembrolizumab and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days..	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes	

	listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause pembrolizumab and/or FMT to be discontinued?	
Relationship to pembrolizumab	Did pembrolizumab and/or FMT cause the adverse event? The determination of the likelihood that pembrolizumab and/or FMT caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between pembrolizumab and/or FMT and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely pembrolizumab and/or FMT caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to pembrolizumab and/or FMT such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of pembrolizumab and/or FMT? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to pembrolizumab and/or FMT (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was pembrolizumab discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to pembrolizumab in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY pembrolizumab , OR IF REEXPOSURE TO pembrolizumab POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding pembrolizumab or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of pembrolizumab and/or FMT relationship).**
Yes, there is a reasonable possibility of pembrolizumab and/or FMT relationship.		There is evidence of exposure to pembrolizumab and/or FMT. The temporal sequence of the AE onset relative to the administration of pembrolizumab is reasonable. The AE is more likely explained by pembrolizumab than by another cause.
No, there is not a reasonable possibility of pembrolizumab and/or FMT relationship		Subject did not receive the pembrolizumab and/or FMT OR temporal sequence of the AE onset relative to administration of pembrolizumab is not reasonable OR the AE is more likely explained by another cause than the pembrolizumab . (Also entered for a subject with overdose without an

	associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1. Overview of the Study

This study is designed to assess the objective response rate (ORR) and toxicity of the addition of FMT to PD-1/PD-L1 treated patients. The patients will be evaluated for response and toxicity using the Simon's two-stage phase II design.

8.2. Sample Size

13 patients will be accrued in the first stage. If the study continues to the second stage, an additional 7 patients will be accrued. The detailed decision rule and sample size justification are in the following Section 8.5.1.

8.3. Dose Limiting Toxicity (DLT) Definition and Monitoring

Dose limiting toxicity (DLT) will be defined based on incidence of AEs considered **possibly related** to pembrolizumab and/or FMT that occur any time from the initial dose of study treatment of FMT in combination with pembrolizumab, with severity graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0. Any occurrence of CTCAE grade 3 or greater adverse event in four weeks following the administration of the FMT will be considered a DLT.

DLT will be monitored during DLT monitoring period, latter defined as 4-week period following administration of FMT/pembrolizumab. During DLT monitoring period, no further patient enrollment will occur. DLTs will be monitored for the first 3 patients in each cohort. During the DLT monitoring period, only 1 patient will be enrolled per month.

Given that this is a novel combination study, in the event of AE, treating investigator(s) will make every effort to attribute AE to either pembrolizumab or FMT. In the event that AE attribution is "possibly" related to both pembrolizumab or FMT, this will be reflected in the AE attribution form.

8.4. Stopping Criteria

Occurrence of any death occurring within 30 days of receiving the investigational agent that is considered at least possibly related to the agent will be treated as a study stopping criterion.

During this period of time, the study investigators and independent DSMB will evaluate death to determine causality (relationship to either pembrolizumab or FMT or both). Autopsy will be encouraged.

Study will not recur till after 30 day period has passed. Study will only resume if study investigators and independent DSMB collectively determine that death was unlikely related to investigational agents (FMT and/or pembrolizumab).

8.5. Analysis Plan

8.5.1. Decision Rule and Sample Size Justification

8.5.1.1. Patient Evaluability and Definition of Objective Response

All patients meeting the eligibility criteria who have signed a consent form and begun pembrolizumab and/or FMT will be considered evaluable for estimation of the response probability. For evaluable patients, objective response will be assessed by RECIST 1.1.

8.5.1.2. Decision Rule:

Based on prior data on response rates in this patient population, the largest ORR where the proposed treatment would be considered unpromising in this population is 5%. The smallest success proportion that would warrant further subsequent studies with the proposed treatment in this patient population is 25%. The following design is based on Simon's minimax two-stage study design and uses to test the null hypothesis that the true success rate in each patient population is at most 5%.

In Stage 1: Enter 13 evaluable patients and follow for at least 12 weeks. If 0 patients have a confirmed response in this timeframe, we will consider this early evidence that this treatment regimen is not sufficiently active in this patient population. If 1 or more patients have a response, then accrual will continue in the second stage of this trial.

In Stage 2: Enter an additional 7 evaluable patients to the trial. If 2 or fewer successes are observed in the 20 evaluable patients, we will consider this sufficient evidence that this treatment is not sufficiently effective in this patient population. If 3 or more successes are observed in the first 20 evaluable patients, then this treatment will be considered promising in this patient population and will be evaluated further in future studies.

Power and Significance Level: Assuming that the number of successes is binomially distributed, this decision rule has a significance level of 0.07; i.e., there is a 7% chance of finding the drug to be effective when it truly is not. This decision rule has 90% power to detect an effective treatment given that the true response rate is at least 25% using this treatment.

8.5.2. Analysis of the Primary Endpoint

The ORR in each cohort will be estimated by the number of objective responses divided by the total number of evaluable patients. Exact 95% confidence intervals for the true ORR will be calculated.

Toxicity: As per NCI CTCAE Version 5.0, the term toxicity is defined as adverse events that are classified as either unrelated, possibly or probably related to study treatment. The maximum grade for each type of toxicity will be recorded for each patient, and frequency tables will be reviewed to determine toxicity patterns. Although the addition of FMT to PD-1/PD-L1 treated patients is novel, as FMT is being administered only once, no true "dose escalation" design is required. However, to accurately capture toxicities for this novel combination, we will be monitoring toxicities closely. The detailed data and safety monitoring plan is in Section 10.5.

8.5.3. Analysis of Secondary and Exploratory Endpoints

The secondary objective is to investigate how FMT administration affects composition and function of T-cells and innate/adaptive immune system subsets. The pre-post difference of these endpoints will be compared between responders and non-responders with the two sided t-test or Wilcoxon rank sum test where appropriate. The exploratory objective is to determine whether PD-1 response in responding patients is associated with common gut microbiota profile. The same statistical analysis methods will be used as for the secondary endpoints.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Pembrolizumab is supplied by the HCC pharmacy. FMT is manufactured as described in Section 4.2.3. No clinical supplies are provided by Merck.

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the FMT infusates generated during the context of this study, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules applicable to regulatory requirements, the subjects' signed ICF and, in the US, the subjects' signed HIPAA Authorization. The consent form must also include a statement that Merck and regulatory authorities have direct access to participant records. The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

10.2 Compliance with Financial Disclosure Requirements

Study investigators will ensure compliance with US Food and Drug Administration (FDA) financial disclosure regulations as set forth in 21 CFR Part 54.

10.3 Compliance with Law, Audit and Debarment

The study may be evaluated by Sponsor or designee internal auditors and government inspectors who must be allowed access to CRFs, source documents, other study files, and study facilities. All audit reports will be kept confidential.

10.4 Compliance with Trial Registration and Results Posting Requirement

The data collected during this study are confidential and proprietary to Sponsor or designee. Any publications or abstracts arising from this study must adhere to the publication requirements set forth in the clinical trial agreement (CTA) governing Investigator participation in the study. These requirements include, but are not limited to, submitting proposed publications to Sponsor or designee at the earliest practicable time prior to submission or presentation and otherwise within the time period set forth in the CTA.

10.5 Data Safety Monitoring Plan

Sponsor-Investigator, sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in Melanoma Center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

a. Serious adverse events

b. Subject safety issues

c. Recruitment issues

d. Accrual

e. Protocol deviations

f. Unanticipated problems

g. Breaches of confidentiality

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA) (21 CFR 312.50, 21 CFR 312.56), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

Minutes from the DSMB meetings are available to anyone unable to attend the center DSMB.

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 5. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB. Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPCI Data Safety and Monitoring Committee (DSMC), which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse

events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed twice a year rather than monthly by the disease center DSMB.

Both the UPCI DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

10.6 Quality Management System

Independent monitoring of the clinical study for protocol and Guidelines on Good Clinical Practice compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

The Investigator (i.e., the study site principal investigator) and the University of Pittsburgh and University of Pittsburgh Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

10.7 Data Management

The Investigator (i.e., the study site principal investigator) will maintain records in accordance with Good Clinical Practice.

The investigator will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

10.8 Institutional Review Board (IRB) Approval

The investigator (i.e., the study site principal investigator) will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment, if applicable.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the investigator will promptly notify the University of Pittsburgh IRB of the deviation.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice.

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of an sponsor's decision to modify the previously accepted clinical protocol, the sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of protocol changes requiring the submission of a Protocol Amendment include:

- Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.
- Any significant change in the design of the protocol (such as the addition or deletion of a control group).
- The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or AE; or the dropping of a test intended to monitor the safety of the investigational drug.

10.9 Ethical and Scientific Conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on Guidelines on Good Clinical Practice; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and University of Pittsburgh Medical Center, Commonwealth of Pennsylvania, and applicable federal agencies.

10.10 Informed Consent

The investigator (i.e., the study site principal investigator) will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The investigator, or a sub-investigator(s) designated by the sponsor, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The investigator or sub-investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical

study or the risk-to-benefit ratio of study participation, the investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

11.0 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

11.1 Tumor Biopsies

Tumor biopsies from at least one accessible tumor lesion will be obtained from all patients during screening and after 4 doses of pembrolizumab (+/- 1 week). Where possible, tumor biopsies will be obtained at tumor progression.

Punch biopsies, surgical excisional biopsies or core biopsies are acceptable. Patients with internal visceral disease may require image guided needle biopsies.

11.2 Correlative Studies

Correlative studies will be performed using PBMCs and tumor tissues collected from the patients included in the study as detailed in the laboratory manual.

These studies will include:

- Multiparemeter flow cytometry to evaluate T cell phenotype and function
- Nanosotring studes of tumor biopsies
- RNAseq and exome sequencing
- Studies of T cell repertoire
- Stool samples will be used for genomic evaluation of microbiota
    - 16S ribosomal RNA (rRNA) and metagenomic sequencing will be used to characterize the complexity of the human microbiome in the stool and at multiple body sites. 16S rRNA gene sequencing is a well-established method for studying phylogeny and taxonomy of samples from complex microbiomes or environments that are difficult or impossible to study.

Prior biopsy samples including primary tumor shave/excision biopsies, sentinel lymph node biopsies, completion lymph node dissection samples will be requested from pathology. Prior tissue obtained to establish metastatic disease, as part of definitive surgical management or as part of standard of care testing prior to current therapy will also be requested. These samples will be used for immunohistochemical (IHC) and RNA sequencing studies to evaluate immune responses and gene signatures associated with improved clinical outcome.

APPENDICES

Appendix 1: FMT Recipient (or Donor) Screening Questionnaire

Dear *** :

You have been identified as a potential donor for a medical procedure called fecal microbiota transplantation (FMT, or stool transplantation). FMT is a relatively new medical procedure that has been used to treat patients with C. difficile infections (CDI) – a diarrheal illness caused by antibiotics that kill the normal stool bacteria of the human gastrointestinal tract. The composition of gut bacteria has been shown to mediate outcomes to PD-1 inhibitor therapy in animal models of melanoma. You have been identified by Dr. Diwakar Davar and Dr. Hassane Zarour as a potential stool donor.

Your doctors are conducting a study in which patients with advanced melanoma whose cancer **is not responding** to PD-1 therapy receive FMT. In this study, selected patients will receive an extract from your frozen stool sample. Are you interested in learning more about how you can participate as a donor for FMT? **YES, PLEASE / NO THANKS**

If no, you do not need to answer any more questions. Please return this form to the person who gave it to you or to the return address at the end of this form. If yes, please continue to answer the following questions:

Name *(please print):*
Date of birth:
Gender: **MALE / FEMALE**
Telephone number(s):

Date Completed:
Time Completed:

The answers to the following questions will be used by our stool transplant physician to determine if your feces can be used for a stool transplant. The answers to some but not all questions may disqualify you as a donor. Please answer the questions truthfully. The answers that you give will NOT be shared with the person you are donating to or appear in his/her chart.

1. Are you at least 18 years old? **YES/NO**
2. Except for birth control, do you take any prescription medications? **YES/NO**
3. What is your height in feet and inches?

4. What is your weight in pounds?

5. Have you ever donated blood before?
    a. If yes, have you even been notified by the blood bank of any positive testing for infectious conditions such as hepatitis C or HIV?
    b. If no, have you attempted to donate blood previously but asked not to donate for medical reasons ?

Please answer the following **YES / NO** questions to the best of your ability:

In the past 6 weeks

6. Female donors: Have you been pregnant or are you pregnant now? **YES / NO / I AM MALE**

In the past 8 weeks have you:

7. Donated blood, platelets or plasma? **YES / NO**
8. Had any vaccinations or other shots? **YES / NO**
9. Had contact with someone who had a smallpox vaccination? **YES / NO**

In the past 3 months have you:

10. Used any antibiotics? **YES / NO**
11. Had any diarrhea? **YES / NO**
12. Had any vomiting? **YES / NO**
13. Had any food poisoning? **YES / NO**
14. Had any fevers? **YES / NO**
15. Been hospitalized (long enough to stay overnight)? **YES / NO**
16. Been seen in an emergency room or urgent care visit? **YES / NO**

In the past 12 months have you

17. Had a blood transfusion? **YES / NO**
18. Had a transplant such as organ, tissue, or bone marrow? **YES / NO**
19. Had a graft such as bone or skin? **YES / NO**
20. Come into contact with someone else's blood? **YES / NO**
21. Had an accidental needle-stick? **YES / NO**
22. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus? **YES / NO**
23. Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex? **YES / NO**
24. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything not prescribed by their doctor? **YES / NO**
25. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates? **YES / NO**
26. Had sexual contact with a person who has hepatitis? **YES / NO**
27. Lived with a person who has hepatitis? **YES / NO**
28. Had a tattoo? **YES / NO**
29. Had ear or body piercing? **YES / NO**
30. Had or been treated for syphilis or gonorrhea? **YES / NO**
31. Been in juvenile detention, lockup, jail, or prison for more than 72 hours? **YES / NO**

In the past three years have you

32. Been outside the United States or Canada? **YES / NO**

From 1980 through 1996,

33. Did you spend time that adds up to three (3) months or more in the United Kingdom? (Northern Ireland, Wales, Scotland, England [sometimes called Great Britain])? **YES / NO**
34. Were you a member of the U.S. military, a civilian military employee, or a dependent of a member of the U.S. Military? **YES / NO**

From 1980 to the present, did you

35. Spend time that adds up to five (5) years or more in Europe? **YES / NO**
36. Receive a blood transfusion in the United Kingdom or France? **YES / NO**

From 1977 to the present, have you:

37. Received money, drugs, or other payment for sex? **YES / NO**
38. Male donors: had sexual contact with another male, even once? **YES / NO / I AM FEMALE**

COVID-19 Related Exposure

39. Have you at any time been diagnosed with laboratory-confirmed SARS-CoV-2 infection?
40. Have you at any time experienced symptoms of COVID-19 (e.g., fever, cough, shortness of breath) not explained adequately by another diagnosis?
41. Have you been exposed to a suspected or confirmed case of COVID-19 or SARS-CoV-2 infection?

Have you EVER:

42. Had *C. difficile* infection previously? **YES / NO**
43. Had a positive test for the HIV/AIDS virus? **YES / NO**
44. Used needles to take drugs, steroids, or anything not prescribed by your doctor? **YES / NO**
45. Used clotting factor concentrates? **YES / NO**
46. Had hepatitis? **YES / NO**
47. Had malaria? **YES / NO**
48. Had Chagas' disease? **YES / NO**
49. Had babesiosis? **YES / NO**
50. Received a dura mater (or brain covering) graft? **YES / NO**
51. Had any type of cancer, including leukemia? **YES / NO**
52. Had parents, brothers, sisters, or children diagnosed with cancer? **YES / NO**
53. Had any problems with your heart or lungs? **YES / NO**
54. Had a bleeding condition or a blood disease? **YES / NO**
55. Had sexual contact with anyone who was born in or lived in Africa? **YES / NO**
56. Been in Africa? **YES / NO**
57. Been in the Caribbean? **YES / NO**
58. Been in Central or South America (anywhere south of the US Border including Mexico)? **YES / NO**
59. Been elsewhere outside the US? **YES / NO**
60. Had relatives who had Creutzfeldt-Jakob disease? **YES / NO**
61. Been previously tested for HIV or viral hepatitis? **YES / NO**
62. Tested positive for MRSA (methicillin resistant *Staphylococcus aureus*), VRE (vancomycin resistant enterococcus) or other resistant bacteria? **YES / NO**
63. Had diarrhea lasting for longer than 2 weeks? **YES / NO**
64. Had irritable bowel syndrome? **YES / NO**
65. Had chronic constipation? **YES / NO**
66. Had chronic pain? **YES / NO**

A physician will review this form to determine if you can be scheduled for a screening visit with an infectious disease physician for additional questions. If you answer in the positive to questions 39-41, you will be referred to an infectious disease physician for additional evaluation. If you are eligible based on this visit, you will be scheduled for laboratory testing. If you are eligible and willing to participate as a potential stool donor, you will be asked to do the following:

1) Schedule a screening visit with a physician in Oakland at the Falk Medical Building
2) Have blood tests and stool tests collected for testing
3) Donate stool either in person or off-site on the morning of the scheduled procedure.

Please mail this form to:

ATTN: Diwakar Davar, MD and Hassane Zarour, MD
UPCI Fecal Transplant Program
Hillman Cancer Center, UPCI Research Pavilion
5117 Center Avenue
Suite 1.32d

Appendix 2: FMT Recipient (and Donor) Laboratory Testing

Required stool and serologic testing is detailed below.

Both donors and recipients will provide stool samples after signing ICF.

Serologic tests (blood, stool, nasal swabs) in donors will be performed per Section 4.2.3. (Figure 4).

Blood	Stool	Nasal
#HIV antibody $Hepatitis A total Ab #Hepatitis B surface Ab, core Ab, surface Ag #Hepatitis C Ab #Syphilis screening serology Strongyloides stercoralis Ab #Entamoeba histolytica Ab #HTLV-1 and HTLV-2 Ab *CMV IgG *EBV AB panel *HSV-1 IgG *HSV-2 IgG *HHV-6 IgG *JC virus Ab	@Bacteria • Campylobacter • Clostridium difficile • E.coli H7 • Helicobacter pylori • Salmonella • Shigella • Vibrio • Yersinia @Viruses • Rotavirus • Adenovirus • Norovirus • SARS-CoV-2 @Fungi/Parasites • Giardia • Cryptosporidia • Ova/parasites #MDRO • Vancomycin Resitant Enterococci (VRE) • Carbapenum Resitant Enterobacteriaceae (CRE) • Extended Spectrum Beat-Lactamase producing bacteria (ESBLs)	*MRSA %SARS-CoV-2 (will be done 2 weeks before)

% if positive, ineligible to donate FMT and will result in reflex referral to Infectious Disease Physician.

- Determination of "SARS-CoV-2 negative" status will be made on specimens obtained at two timepoints: 1st sample collected at least 14 days before stool sample donation, and a 2nd sample collected at least 14 days after stool sample donation.

- As the turnaround of the test is 48-72 hours, the stool sample will be released for FMT use no sooner than 48-72 hours after 2nd test results and donor is deemed "SARS-CoV-2 negative".

- Donors who were excluded based on based on screening and/or testing as described in 3.1 and 4.1.1-4.1.2 above will not be allowed to donate stool specimens for use in this study at this time. This is subject to subsequent revision should stool-specific SARS-CoV-2 testing become available.

#if positive, ineligible to donate FMT

*if positive, eligible to receive FMT from seropositive or negative donors (i.e. donors who are similarly positive or who test negative).

@if positive, must be repeated and be tested negative prior to determination of eligibility (NOTE: exceptions may be made in the case of asymptomatic recipients who test positive **AFTER** consultation with PI).

$if positive, must be followed by Hepatitis A IgG and IgM.

- For donors: IgM positive donors are ineligible to donate. IgG positive donors are eligible to donate.
- For recipients: IgM/G status does not affect ability to receive FMT.

Appendix 3: Dietary History Questionnaire for Microbiome Sampling

Version date 1/17/19

Dietary History Questionnaire

This is a food frequency questionnaire (FFQ) modified for patients receiving immune checkpoint blockade (ICB) therapy. This FFQ is modified from the publically available Diet History Questionnaire (DHQ) developed by staff at the Risk Factor Assessment Branch (RFAB) of the National Institutes of Health. This FFQ consists of a finite list of foods and beverages with response categories to indicate usual frequency of consumption over the time period queried.

This FFQ is designed to be completed every time you visit receive treatment or are in to see your physician. It can either be done at home or at each study visit.

Demographics

First Name _____

Last Name _____

Sex _____

Current Medications (including supplements).

For this question, please report the names and dosages of all prescription medications you take. Please include supplements and any as-needed (prescription or over the counter) medicines you take at least weekly.

Current Medications (including supplements and vitamins).

Visits

For this question, please indicate today's date and your weight in pounds (lbs).

Date _____

Weight (in lbs) _____

Beverages

What beverages do you drink?

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

Please quantify intake in 8oz terms. Please choose only one answer for each question.

Do you drink beverages?

- No
- Yes

Do you drink fruit and/or vegetable juices?

- No
- Yes

How often do you drink fruit juice?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

How often do you drink vegetable juice?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

How often do you drink other fruit/vegetable juice?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Do you drink milk or milk-based beverages?
- No
- Yes

How often do you drink milk including flavored milk (chocolate, strawberry etc.)?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day

- 4-5 times per day
- 6 or more times per day

How often do you drink milk based meal replacements (Boost, Ensure, Carnation Instant Breakfast)??

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Do you drink coffee and/or tea?

- No
- Yes

How often do you drink coffee and/or tea?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Do you typically add sugar (or sugar equivalents such as Equal, Splenda etc.) to your coffee and/or tea?

- No
- Yes

How much sugar (or sugar equivalents such as Equal, Splenda etc.) do you add?

- 1 sachet
- 2 sacets
- 3 or more sachets

Do you typically add milk (or milk equivalents such as half-and-half, creamer etc.) to your coffee and/or tea?

- No
- Yes

How much milk (or milk equivalents such as half-and-half, creamer etc.) do you add?

- 1 sachet

- 2 sacets
- 3 or more sachets

Do you drink soda/pop or other carbonated beverages?
- No
- Yes

How often do you drink soda/pop or other carbonated beverages (including diet soda)?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Do you drink sports/energy drinks (including diet variety) such as Gatorade, Powerade etc.?
- No
- Yes

How often do you drink sports/energy drinks (including diet variety) such as Gatorade, Powerade etc.?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Do you drink alcohol (including beer, spirits, wine)?
- No
- Yes

How often do you drink beer?
- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day

- 6 or more times per day

How often do you drink wine?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

How often do you drink mixed drinks and/or liquor?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Fruits

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

Please quantify intake in terms of servings. The following are examples of 1 SERVING of fruit: 1 piece of medium fresh fruit (apple, orange etc); OR 2 pieces of small fresh fruit (apricot, plum etc); 1/2 cup of tinned/frozen fruit (berries, tinned peaches etc); OR 1 handful of dried fruit (raisins, trail mix).

Please choose only one answer for each question.

Do you consume fruits?

- No
- Yes

For fruit, which of the following categories of fruit do you typically consume?

- Apples
- Berries (raspberries, strawberries, blueberries)
- Citrus fruits (orange, grapefruit, clementines)
- Stone fruits (peaches, apricots)
- Tropical fruits (bananas, pineapple)
- Melons (watermelon, honeydew)
- Grapes

How often do you consume a serving of fruit?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Vegetables

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.
Please quantify intake in terms of servings. The following are examples of 1 SERVING of vegetables: 1 medium vegetable (potatoe, carrot, yam, etc.); OR 1/2 cup cooked vegetable (broccoli, peas, spinach etc.); OR 1 cup salad).
Please choose only one answer for each question.

Do you consume vegetables?

- No
- Yes

For vegatables, which of the following categories of vegetables do you typically consume?

- Cruciferous vegetables (broccoli, brussel sprouts)
- Leafy greens
- Marrow vegetables (cucumber, zucchini)
- Root vegetables (potatoes, sweet potatoes)
- Stem vegetables (asparagus)

How often do you consume a serving of vegetables?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Legumes

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.
Please quantify intake in terms of servings. The following are examples of 1 SERVING of legumes: 3/4 cup cooked lentils (green, red lentils etc.), 3/4 cup cooked beans (kidney beans, white beans, chickpeas, baked beans, split beans, etc.).
Please choose only one answer for each question.

Do you consume legumes or pulses?

- No
- Yes

How often do you consume a serving of legumes?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Cereals & Breads

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.
Please quantify intake in terms of servings. The following are examples of 1 SERVING of breads and/or cereals: 1/2 cup cooked oatmeal (or porridge, cream of wheat etc); OR 1/2 cup cereal or muesli; OR 1 slice whole-grain bread; OR 2 slices white bread).
Please choose only one answer for each question.

Do you consume breads and cereals?

- No
- Yes

For bread, do you typically consume white or whole-grain bread?

- White
- Whole grain

How often do you consume a serving of breads and/or cereals?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week

- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Rice & Pastas

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

Please quantify intake in terms of servings. The following are examples of 1 SERVING of rice or pasta: 1/2 cup cooked brown rice or whole-wheat pasta (or quinoa etc.); OR 1 cup cooked white rice or regular pasta).

Please choose only one answer for each question.

Do you consume rice and/or pasta?

- No
- Yes

For rice, do you typically consume white, brown rice or rice alternatives (quinoa etc.)?

- White
- Brown
- Quinoa

For pasta, do you typically consume regular or whole-wheat pasta?

- Regular
- Whole-wheat

How often do you consume a serving of rice or pasta (including alternatives such as quinoa etc.)?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Meat & Poultry

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

Please quantify intake in terms of servings. The following are examples of 1 SERVING of meat and/or poultry: 1 3-oz serving; OR 1 chicken breast; OR 1/2 cup ground meat or poultry).
Please choose only one answer for each question.

Do you consume meat and/or poultry?

- No
- Yes

If you consume meat and/or poultry, what is your primary method of cooking?

- Grilling
- Pan or shallow frying
- Deep-frying
- Baking
- Other

How often do you consume a serving of chicken or other poultry (1 chicken breast or equivalent)?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

How often do you consume a serving of pork, beef or other meat (1 3-oz pork chop, 1 5-oz patty, etc.)?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Fish & Shellfish

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

Please quantify intake in terms of servings. The following are examples of 1 SERVING of fish and/or shellfish: 1 3-oz serving of fish; OR 1 4-oz serving of shrimp; OR 3/4-lb cooked lobster or crab).
Please choose only one answer for each question.

Do you consume fish and/or shellfish?

- No
- Yes

If you consume fish and/or shellfish, what is your primary method of cooking?

- Grilling
- Pan or shallow frying
- Deep-frying
- Baking
- Other

How often do you consume a serving of fish and/or shellfish?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Eggs

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.
Please quantify intake in terms of servings. The following are examples of 1 SERVING of eggs: 1 egg; OR 3 egg whites; OR 1 cup egg substitute.
Please choose only one answer for each question.

Do you consume eggs?

- No
- Yes

If you consume eggs, do you typically consume whole eggs or egg products (egg whites, and egg yolks in egg whites frozen, refrigerated liquid, or dried forms)?

- Whole eggs including yolks
- Egg whites
- Egg substitutes

If you consume eggs, what is your primary method of cooking?

- Grilling
- Pan or shallow frying
- Deep-frying
- Baking
- Other

How often do you consume a serving of eggs?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Meat Alternatives

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.
Please quantify intake in terms of servings. The following is an example of 1 SERVING of meat alternatives: 1/2 cup raw/cooked tofu (or alternative).
Please choose only one answer for each question.

Do you consume meat alternatives (tempeh, tofu, seitan, gluten, etc)?

- No
- Yes

How often do you consume a serving of meat alternatives (tempeh, tofu, seitan, gluten, etc)?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Yoghurt & Cheese

For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.

Please quantify intake in terms of servings. The following are examples of 1 SERVING of yoghurt and/or cheese: 1 cup yoghurt/cheese; 8 fluid oz yoghurt/cheese).
Please choose only one answer for each question.

Do you consume yoghurt and/or cheese?

- No
- Yes

If you consume yoghurt and/or cheese, what type do you typically eat?

- Whole fat cheese or yoghurt
- Low fat cheese or yoghurt
- 2% cheese or yoghurt
- Non-fat cheese or yoghurt
- Cottage cheese

How often do you consume a serving of yoghurt and/or cheese?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Sweets & Desserts
For this question, please estimate the frequency and quantity of the item in question. Frequency is estimated as: 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day, 4-5 times per day, 6 or more times per day.
Please quantify intake in terms of servings. The following are examples of 1 SERVING of dessert: 1 oz serving; OR 100g serving; OR 1 2" brownie; OR 1/2 cup ice-cream.
Please choose only one answer for each question.

Do you consume sweets, baked goods and/or desserts?

- No
- Yes

If you consume sweets, baked goods and/or desserts, what type do you typically eat?

- Ice-cream
- Cakes and cookies and brownies
- Muffins
- Pies
- Doughnuts

How often do you consume a serving of sweets, baked goods and/or desserts?

- <1 time per week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Appendix 4: Memory Card for Patients while on Study

This is a questionnaire to facilitate self-monitoring of symptoms that may be related to the fecal microbiome transplant (FMT) and/or pembrolizumab therapy that you have received. You should review this questionnaire daily while on study. If you do develop symptoms, particularly if these symptoms are persistent, please bring this to the attention of the investigator and your study nurse. This questionnaire will be administered at each study visit.

Memory Card					
Adverse Event	**1**	**2**	**3**	**4**	**5**
Abdominal discomfort	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; limiting instrumental ADL	Severe discomfort; limiting self care ADL	N/A	N/A
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	N/A
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	N/A
Fever	38.0-39.0 degrees C (100.4-102.2 degrees F)	>39.0-40.0 degrees C (102.3-104.0 degrees F)	>40.0 degrees C (> 104.0degrees F) for ≤24 hrs	>40.0 degrees C (> 104.0degrees F) for >24 hrs	N/A
Flatulence	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; limiting instrumental ADL	Severe discomfort; limiting self care ADL	N/A	N/A
Vomiting	1 - 2 episodes (separated by 5minutes) in 24 hrs	3 - 5 episodes (separated by 5minutes) in 24 hrs	≥6 episodes (separated by 5minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	N/A

Appendix 5: Dose Modifications for Pembrolizumab for Immune-Related Adverse Events

Adverse events (AEs) associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids, and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, or skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided here.

Table 5-1: Dose Modification Guidelines for Drug-related Adverse Events: Pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

irAE	CTCAE Grade	Action Taken with Pembrolizumab	irAE Management with Corticosteroid and/or Other Therapies	Monitoring and Follow-Up
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor subjects for signs and symptoms of pneumonitis. Evaluate subjects with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment. Add prophylactic antibiotics for opportunistic
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		

				infections
Diarrhea/colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor subjects for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). Subjects with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. Subjects with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. Consider infliximab 5 mg/kg for treatment of severe immune-related colitis that does not respond within 1 week to high-dose steroids.
	Grade 4	Permanently discontinue		
AST/ALT elevation or increased bilirubin	Grade 2	Withhold	Administer corticosteroids (initial dose of prednisone 0.5-1 mg/kg or equivalent)	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to

			followed by taper.	baseline or is stable).
	Grade 3 or 4	Permanently discontinue	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper.	
TIDM or hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia **associated with** evidence of β-islet failure	Withhold	Initiate insulin replacement therapy for subjects with T1DM. Administer antihyperglycemic in subjects with hyperglycemia.	Monitor subjects for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated.	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency).
	Grade 3 or 4	Withhold or permanently discontinue		
Hyperthyroidism	Grade 2	Withhold	Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate.	Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[a, b]		
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care.	Monitor for signs and symptoms of thyroid disorders.

Nephritis and renal dysfunction	Grade 2	Withhold	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor changes of renal function.
	Grade 3 or 4	Permanently discontinue		
Rash	Grade 1 or 2	Continue	Manage with topical steroids with or without drug interruption	
	Grade 3[c]	Withhold until toxicity resolves to Grade 0-1	Administer corticosteroids (initial dose of prednisone 1-2 mg/kg or equivalent) followed by taper.	Restart pembrolizumab at same dose if rash is mild and assessed as Grade 3 based only on body surface area and resolves without oral steroids. If oral steroids are required, or if rash is severe, decrease by 1 dose level once resolved to Grade 0-1.
	Grade 4	Permanently discontinue		
Amylase and/or lipase increased.	Grade 3 or less	May continue treatment with approval of PI or medical monitor		Permanently discontinue if clinical signs and symptoms of pancreatitis develop (abdominal pain, nausea, vomiting).
	Grade 4	Withhold until toxicity resolves to Grade 0-1[d].		
Myocarditis	Grade 1 or 2	Hold therapy till toxicity resolves to Grade 0	Admit patient and start high-dose corticosteroids.	Intensive care unit admission; consider other immunosuppressants if the patient's condition deteriorates.
	Grade 3 or 4	Permanently discontinue		
Neuropathy	Grade 2	Toxicity resolves to ≤ Grade 1 or		

		baseline within 4 weeks, restart at same dose. Toxicity does not resolve to ≤ Grade 1 within 4 weeks, reduce dose 1 dose level.		
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Grade 3, or intolerable/ persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids.	Ensure adequate evaluation to confirm etiology or exclude other causes.
	Grade 4 or recurrent Grade 3.	Permanently discontinue		

AE = adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GI = gastrointestinal; irAE = immune-related adverse event; IV = intravenous; T1DM = Type 1 diabetes mellitus.

[a]Decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

[b]For subjects with Grade 3 or 4 immune-related endocrinopathy where pembrolizumab must be withheld, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

[c]Participants with Grade 3 rash in the absence of desquamation, with no mucosal involvement, not requiring systemic steroids, and resolving to Grade 1 within 14 days do not have to withhold study treatment.

[d]If Grade 4 amylase and/or lipase increase is asymptomatic and abdominal imaging suggests no pathology, study treatment dosing may continue with medical monitor approval.

Appendix 6: Toxicity Management of Infusion Reactions Related to Pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion reactions, including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab-associated infusion reaction are provided in Table 8-1.

Table 6-1: Infusion Reaction Management Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration		

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