TITLE: Phase II Dual-Cohort Study Evaluating the Effects of Pembrolizumab in the Presence of Gut Microbiota Modulation with EDP1503 in Advanced Melanoma Naïve or Refractory to Anti-PD1 Antibody.

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This study is being conducted by institutional members of the Personalized Cancer Care Consortium (PCCC), as well as additional sites.

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1.0 TRIAL SUMMARY

Abbreviated Title	Phase 2 study of Pembrolizumab + EDP1503
Trial Phase	*Phase 2*
Clinical Indication	Melanoma
Trial Type	Interventional
Type of control	Internal
Route of administration	IV and oral
Trial Blinding	NA
Treatment Groups	Anti-PD1 Treatment naïve and refractory melanoma
Number of trial subjects	Minimum: 55, Maximum: 70
Estimated enrollment period	*18 – 26 months*
Estimated duration of trial	*50 months*
Duration of Participation	Up to 2 years
Estimated average length of treatment per patient	3 months

2.0 TRIAL DESIGN

2.1 Trial Design

Phase II study observing the effect of the combination of anti-Programed Death 1 (anti-PD1) antibody (pembrolizumab) and gut microbial manipulation with EDP1503 in advanced melanoma. The study will include 2 cohorts of patients with melanoma including

1. Naïve to anti-PD1 antibody treatment (Cohort 1) and

2. Refractory to anti-PD1 antibody treatment (Cohort 2).

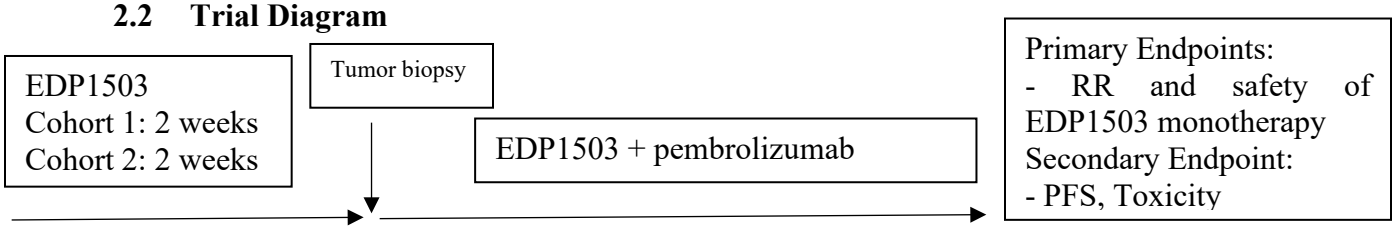
The study will involve initial administration of EDP1503 for a run-in period followed thereafter by joint administration of daily EDP1503 and treatment with pembrolizumab every 3 weeks. The first three patients enrolled to either cohort will pursue a two week EDP1503 run-in period and will be closely monitored for any toxicities related to EDP1503. The second, third, and fourth patients will not be enrolled until the two week monotherapy period of the preceding patient is complete. The study will pursue primary endpoints in cohorts 1 and 2 as below including

1. Depth of response in naïve patients (Cohort 1) as well as the

2. RECIST response rate to pembrolizumab in PD1 previously treated patients (Cohort 2) in the presence of gut microbiota manipulation.

Other secondary clinical parameters such as progression-free survival and safety will additionally be investigated. The study will also provide the opportunity to investigate blood, tumor and stool based factors on response as well as the effect of EDP1503 on the local tumor and systemic immune environment.

2.2 Trial Diagram



Treatment Groups		
Cohort 1	Melanoma	No prior PD1 exposure
Cohort 2	Melanoma	Progression with prior PD1 antibody treatment

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To assess the depth of response (Cohort 1) or RECIST response rate (Cohort 2) of pembrolizumab in the presence of gut microbial manipulation with EDP1503 in subjects naïve (Cohort 1) or refractory (Cohort 2) to anti-PD1 antibody in cohorts of melanoma.

Hypothesis: The administration of pembrolizumab in the presence of gut microbiota manipulation with EDP1503 will enhance the activity of anti-PD1 antibody treatment.

(2) **Objective**: Assessment of safety and tolerability of EDP1503 during the monotherapy run-in phase

Hypothesis: EDP1503 monotherapy will demonstrate a similar safety/tolerability profile to probiotics such as low grade nausea, vomiting, and abdominal pain.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objective**: Assessment of progression-free survival as measured by RECIST and immune-related response criteria

Hypothesis: The administration of pembrolizumab in the presence of gut microbiota manipulation with EDP1503 will lengthen progression-free survival relative to historical comparison with anti-PD1/L1 antibody monotherapy

(2) **Objective**: Assessment of safety and tolerability of pembrolizumab in the presence of gut microbiota manipulation with EDP1503.

Hypothesis: The administration of pembrolizumab in the presence of gut microbiota manipulation with EDP1503 will demonstrate a similar safety/tolerability profile to pembrolizumab alone

3.3 Exploratory Objective

(1) **Objective**: Assessment of whether the administration of EDP1503 alters immune cell phenotypes in the tumor microenvironment.

Hypothesis: Oral administration of EDP1503 will result in changes in the immune cell phenotypes in the tumor microenvironment

(2) **Objective**: Assessment of fecal material for bacterial contents

Hypothesis: Oral administration of EDP1503 will result in increased level of EDP1503 in the fecal contents

(3) **Objective:** Biospecimen collection throughout the study including microbiota contents, peripheral blood and tumor tissue.

Hypothesis: Oral administration of EDP1503 will result in changes in immune cell phenotypes detectable in the systemic circulation

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). The structure of

murine PD-1 has been resolved. 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 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). 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. Keytruda™ (pembrolizumab) has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

Bifidobacteria are a gram positive, anaerobic, non-spore forming, rod shaped genus of bacteria (Weber et al 2015). They are naturally found in the colon of healthy humans and animals throughout early life and into adulthood (O'Callaghan et al 2016). Bifidobacterium are frequently used in foods and supplements in North America and Europe and these strains in food products have been designated 'Generally Regarded As Safe' (GRAS, 21 CFR 170; FDA GRAS Notices) or 'Qualified Presumption of Safety' (QPS) Status (EFSA) along with many other lactic acid producing bacteria. These bacteria are found in fermented foods, yogurts, supplements, and other foodstuffs at levels between $1x10^9$- $1x10^{11}$ CFU/serving (FDA GRAS Notices, EFSA Journal 2013). These strains are selected to impart beneficial health effects on the host and positively impact the host gut microbiota composition, metabolism, and general health without adverse changes to the gut (O'Callaghan et al 2016). Many of them are originally isolated from healthy hosts and are ubiquitous in the diet and the healthy large

intestine of humans soon after birth throughout life. They have been evaluated in 100s of clinical trials (854 references on PubMed of "clinical trials Bifidobacterium") in subjects ranging from preterm infants to elderly adults with minimal adverse events.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochures for pembrolizumab and EDP1503 regarding Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Blockade of the Programmed Death-1 (PD1) receptor by pembrolizumab has demonstrated remarkable clinical activity in patients with advanced melanoma showing a response rate in a randomized phase III trial of 33%[1]. However, 67% of patients do not respond and approximately 25% have disease progression as best overall response. Consideration then should be given to manipulations that would modify the immune microenvironment and facilitate clinical benefit for a greater fraction of patients.

Although multiple pharmacologic approaches to augment the efficacy of pembrolizumab have been proposed, these have to date have focused on targeting the tumor using anti-neoplastic agents or other immune-modifying drugs. We have uncovered evidence that characteristics of the host also are critical, including the germline genetic makeup and also a major environmental factor, which is the composition of the commensal microbiota.

Cancer has at times been described as a disease of the larger meta-organism which emphasizes the interplay between both between cancer and the immune system but additionally between the microbiota that modulate the host immune response. Commensal organisms are essential to the normal development of the immune system both for innate and adaptive immunity, with pattern recognition receptors mediating processes throughout the body including metabolism, morphogenesis, and homeostasis[2]. Gut microbiota especially have been identified as essential in mucosal and tumor immunity, regulating both local and systemic inflammation responses. Disruption of the gut microbiota via antibiotics (or germ-free mice) has been associated with decreased responsiveness of murine tumors to immunotherapies and chemotherapies[3]. Regulation of tumor-infiltrating myeloid-derived cells is proposed as a potential mechanism to facilitate this.

Our group has previously described the T cell-inflamed tumor microenvironment as a potential predictive biomarker for response to multiple immunotherapies including therapeutic vaccines, anti-CTLA-4, and anti-PD-1/PD-L1 antibodies[4-10]. Our analysis of the tumor microenvironment in patients with melanoma suggests that approximately 35-50% of cases show evidence of spontaneous priming of anti-tumor T cells leading to migration of CD8+ effector T cells into tumor sites. This phenotype has been designated the T cell-inflamed tumor microenvironment and is characterized by expression of T cell markers, chemokines for T cell recruitment, and transcripts indicative of type I IFN signaling[5,6,8].

Similar features of the immune response have been proposed to impact on the management of infection and cancer including modulation by commensal microbiota, development of chronic immune responses and regulatory mechanisms to prevent collateral tissue damage[11]. We and others have proposed that manipulation of the microbiome has the potential ability to modulate the anti-tumor immune response and promote the development of an inflamed tumor microenvironment[12,13].

To assess the effect of microbial composition on the immune response to melanoma, our group compared the growth of subcutaneous B16 melanoma in genetically identical C57BL/6 mice derived from two different facilities which have been shown to differ in their commensal microbes (Jackson laboratories and Taconic farms)[14]. We found that B16.SIY tumors exhibited an increased growth rate in mice derived from Taconic ($p < 0.02$), which was associated with reduced induction of tumor specific IFN-g-producing CD8+ T cells ($p < 0.009$). A markedly increased number of SIY antigen-specific CD8 + T cells was found to accumulate within the tumor microenvironment in Jackson versus Taconic mice ($p < 0.03$). Strikingly, the differences in tumor growth, endogenous T cell priming, and SIY antigen-specific T cell infiltration were ablated in animals cohoused for at least two weeks prior to injection of tumor cells, consistent with a microbiota-derived effect. We thus asked whether transfer of Jackson fecal material into Taconic mice by oral gavage prior to tumor inoculation was sufficient to confer protective anti-tumor responses. Indeed, Taconic mice that received Jackson feces exhibit dramatically slower tumor growth ($p < 0.015$) and increased numbers of SIY antigen-specific CD8+ T cells at the tumor site, compared to Taconic mice that received Taconic feces.

We additionally assessed whether transfer of microbial contents impacted on the therapeutic efficacy of anti-PDL1 antibody in these systems. Similar to what was observed above, transfer of fecal contents from Jackson mice to Taconic mice significantly boosted the efficacy of anti-PDL1 antibody while transfer of Taconic fecal contents to Jackson mice had no significant impact.

Thereafter we have pursued a detailed investigation of the microbial contents of the fecal material from Jackson mice as compared with Taconic. In concert with colleagues at the Argonne National Laboratory, 16S ribosomal sequencing identified a small number of bacterial species that were significantly different between these strains. The top candidate was the genus Bifidobacterium. Using commercial sources, we obtained a probiotic consisting of only Bifidobacterium species for preclinical experimentation. In fact, administration of two weekly doses of Bifidobacterium was sufficient to exert anti-tumor activity in C57BL/6 mice from Taconic, which synergized with anti-PD-L1 mAb to cause complete tumor elimination in most animals. After an intensive screening process, Evelo Biosciences identified a strain of Bifidobacterium animalis lactis with consistently high performance in tumor isograft assays including B16 melanoma, and manufactured this strain under GMP into a drug product known as EDP1503.

These data provide evidence implicating the microbiota in shaping anti-tumor immunity and provide a rationale for a clinical trial evaluating the efficacy of pembrolizumab in the presence of gut microbiota manipulation with EDP1503.

In summary, our data suggest that the intestinal microbiota have a significant impact on anti-tumor immunity and see the potential of specific microorganisms to modify the commensal environment of the host and enhance the anti-tumor effect of anti-PD-1. We therefore propose a phase II study evaluating the efficacy of pembrolizumab in the presence of gut microbiota manipulation with EDP1503 in subjects with advanced melanoma who are either naïve or refractory to anti-PD1 antibody treatment.

4.2.2 Rationale for Dose Selection/Regimen/Modification

Pembrolizumab
An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. 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 MK-3475 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 1 and 2 of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 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 MK-3475 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. MK-3475 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 MK-3475 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.

The landscape of adjuvant therapy options for high risk cutaneous melanoma is in a state of flux and treatments that recently formed the backbone of systemic therapy for metastatic disease are now being used earlier. Whereas interferon-α and more recently ipilimumab were standards of care (PMID: 23042723, PMID: 27717298), patients with high risk stage III melanoma now have standard of care options with combination BRAF-MEK inhibition and anti-PD1 antibody. In a phase III study of dabrafenib and trametinib compared with placebos, estimated 3 year relapse-free survival was 58% in the treatment group versus 39% for placebo with the hazard ratio for relapse or death of 0.47 (95% confidence interval [CI], 0.39 to 0.58; P<0.001) (PMID: 28891408). In a phase III study of nivolumab compared with ipilimumab relapse free survival at 12 months was 71% with nivolumab as compared with 61% for ipilimumab with a hazard ratio for relapse or death of 0.65 (97.56% CI, 0.51 to 0.83; P<0.001) (PMID: 28891423). Given these data many patients diagnosed with advanced disease will have previously been treated with PD1 based therapy and approaches that augment systemic immunity to facilitate clinical response in the setting of resistance are of high priority.

EDP1503

Amounts of Bifidobacterium consumed by humans in various commercially available Bifidobacterium-based probiotics range from 1 $x10^9$ to 3.6 $x10^{12}$ CFU[15,16] The Bifidobacterium capsule in this study will contain >7.5$x10^{10}$ CFU/capsule and will be taken at an amount of 2 capsules bid for a total of 4 capsules per day, half in the morning and half in the evening.

Foods containing Bifidobacterium have been consumed by millions of people largely with a lack of side effects. Minor adverse events include bloating, flatus, loose stool, and mild gastrointestinal discomfort. In extremely rare cases, lactic acid bacteria based probiotics have been identified as the cause of bacteremia but generally pose far less threat than one's own gastrointestinal flora[17,18]. Patients who have experienced Bifidobacterium bacteremia are usually immunocompromised and severely sick[19]. In a majority of these cases, antibiotic

administration resulted in a patient recovery owing to Bifidobacteria's sensitivity to a broad panel of antibiotics[19,20]. Despite the well tolerated nature of oral Bifidobacterium, to avoid unnecessary risk, patients who are immunocompromised will be excluded from this study. For subjects who develop a concomitant illness during the trial which requires antibiotic therapy the Bifidobacterium or placebo therapy will be halted until at least 2 days after the cessation of antibiotic therapy.

EDP1503 is a cloned strain of *Bifidobacterium animalis* spp. *lactis*. The final clone was selected after two rounds of cloning to ensure strain purity. *Bifidobacterium animalis* spp. *lactis* is classified as Generally Regarded As Safe (GRAS) in the United States.

EDP1503 drug substance (DS) is manufactured under current Good Manufacturing Practice (GMP) conditions by standard industrial scale fermentation, followed by centrifugation cell separation and freeze-drying together with cryoprotectants. The DS is then milled into a fine powder, packaged into heat-sealed foil pouch and stored at 2-8 ºC.

EDP1503 drug product (DP) formulation is a blend of freeze-dried powder of *Bifidobacterium animalis* spp. *lactis* (DS) and excipients (e.g. Avicel PH200LM, Magnesium Stearate, and Aerosil). The formulated blend is filled into size 0 hard HPMC (hydroxypropylmethylcellulose) capsules, banded, and enterically coated. The enteric coating is designed for duodenal release (pH ≥ 5.5). The manufactured capsules are filled into an air-tight rigid plastic pot and vacuum sealed in a foil pouch. The finished drug product is stored at 2-8ºC.

4.2.3 Rationale for Endpoints

Here we propose a clinical trial testing the hypothesis that the administration of pembrolizumab in the presence of gut microbiota manipulation with EDP1503 will increase the depth of response in patients who are naïve to PD1 antibody and may induce response in patients who have previously progressed on anti-PD1 antibody. In addition the trial will test the hypothesis that EDP1503, as monotherapy, is safe and that any adverse effects are consistent with historical observations of probiotic therapy, such as mild and transient GI disturbance.

The rationale for the primary endpoints in each cohort stem from the intersection of trial feasibility, meaningful clinical patient outcomes and the early nature of investigation surrounding the impact of the microbiome on anti-tumor immunity. Sample size calculations for analysis of a substantial improvement in the response rate in the PD1 naïve population make the analysis of standard RECIST response rate not feasible in a modestly sized phase II study. Randomized designs were also considered though were not supported by the pharmaceutical sponsor in the modestly sized phase II setting. Simultaneously it has been recognized in the melanoma community that quality of response to treatment is particularly meaningful as patients who have deep responses rarely recur[21-23]. In fact multiple groups are proposing randomized studies to investigate early discontinuation of PD1 after maximum response while the Canadian government has launched such a trial[24]. In this context the primary end point for cohort 1 was chosen as deep response (> 80%) as this level of response has been observed consistently in approximately 40% of patients in combination clinical trials of the anti-PD1 antibody (nivolumab) in combination with anti-CTLA4 antibody

(ipilimumab). This deep response rate has been published in the New England Journal of Medicine four times across the phase I CA209-004[25], phase II CHECKMATE-069[26] and phase III CHECKMATE-067 studies[27,28]. In cohort 2 the primary endpoint is improvement in RECIST response as is commonly used. In this refractory cohort no responses are normally expected thus facilitating a straightforward Simon two-stage design.

Prior investigations regarding the modulation of the gut flora in preclinical melanoma models demonstrated that this effect restored partial tumor control however the addition of PDL1 antibody completely ablated tumors. Through our correlative analysis, we hope to propose a model by which we can suggest which patients would benefit from pembrolizumab alone or pembrolizumab in the presence of gut microbiota manipulation with EDP1503 or other probiotic.

4.2.3.1 Efficacy Endpoints

The primary efficacy objective of this trial is to evaluate the anti-tumor activity of pembrolizumab in the presence of gut microbiota manipulation with EDP1503 in subjects with advanced melanoma who are naïve or refractory to anti-PD1 antibody.

RECIST as assessed by the investigator will be used as the primary response rate efficacy endpoint however irRC will also be followed. RECIST will also be used by the local site to determine eligibility and make treatment decisions. The primary efficacy endpoint is response rate based on RECIST. Secondary endpoints are listed in section 3.2.

Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses which may be functionally anergic. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST criteria may not provide a complete response assessment of immunotherapeutic agents such as pembrolizumab therefore the irRC have been developed to encompass this.

Broadly, irRC includes the concept of treatment beyond progression, if radiologic imaging shows PD, tumor assessment should be repeated $\geq$ 4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms progressive disease, subjects will be discontinued from trial therapy. In determining whether or not the tumor burden has increased or decreased, Investigators should consider all target lesions as well as non-target lesions.

In subjects who have initial evidence of radiological PD, it is at the discretion of the treating physician whether to continue a subject on study treatment until repeat imaging is obtained a minimum of 4 weeks later. This decision should be based on the clinical judgment of the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms indicating disease progression

- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

When feasible, subjects should not be discontinued until progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects that are deemed clinically unstable are not required to have repeat imaging for confirmation of progressive disease.

4.2.3.2 Biomarker Research

Our group has previously described the T cell-inflamed tumor microenvironment as a potential predictive biomarker for response to multiple immunotherapies including therapeutic vaccines, anti-CTLA-4, and anti-PD-1/PD-L1 antibodies[4-10]. Our analysis of the tumor microenvironment in patients with melanoma suggests that approximately 35-50% of cases show evidence of spontaneous priming of anti-tumor T cells leading to migration of CD8+ effector T cells into tumor sites. This phenotype has been designated the T cell-inflamed tumor microenvironment and is characterized by expression of T cell markers, chemokines for T cell recruitment, and transcripts indicative of type I IFN signaling[5,6,8].

In addition to response prediction, this gene signature is also potentially useful in identifying those that will not respond. Patients harboring non-inflamed tumors therefore require treatment strategies which may modulate the immune response to facilitate the influx of tumor infiltrating lymphocytes (TIL) and conversion from a non-inflamed to an inflamed tumor.

Beyond the tumor microenvironment, aspects of the host (patient) and commensal environment may also have an essential impact on immunity in response to both radiation and anti-PD1 antibody. Host factors of interest regarding the immune response include circulating immune subsets as well as germline DNA sequence and polymorphisms. Further, a growing literature supports a role for commensal microbiota in the immune response to infection and cancer including development of acute versus chronic immune responses and regulatory mechanisms to prevent collateral tissue damage[11]. We and others have proposed that manipulation of the microbiome has the potential ability to modulate the anti-tumor immune response and promote the development of an inflamed tumor microenvironment[12,13].

Considering this background, we propose to collect peripheral blood for serum, peripheral blood mononuclear cells, germline DNA, as well as fecal samples throughout treatment. **Baseline tumor tissue prior to treatment and/or fresh tumor biopsy at baseline and at after EDP1503 run-in are required on all patients**. Diagnostic tissue from prior biopsy is allowed if the tissue was obtained by core or surgical biopsy (FNA is not acceptable) and no intervening systemic treatment has been administered. Peripheral blood for serum and blood mononuclear cells will be taken at the beginning and end of the run in period when subjects are only taking EDP1503 to evaluate any immediate effects on these biomarkers. Tumor

biopsy will be required at the end of the second week of EDP1503 run-in (prior to starting pembrolizumab) in patient's naïve to anti-PD1 (cohort 1) unless considered not clinically feasible. These biopsies will be analyzed to evaluate the effect of the EDP1503 on the tumor microenvironment.

Fecal samples will be analyzed by a variety of methods to determine quantitatively if there is a correlation between specific features of the host microbiome and/ or its manipulation with the effect on the systemic and tumor biomarkers. Analysis may include microbial culturing and nucleic acid based evaluation. Micro-organisms cultured from patient fecal samples may be stored and studied in preclinical models with the intent of learning more about the interaction between the microbiome and host immune responses.

Additionally, it is requested that any patients who undergo tumor biopsy as part of their standard care during the study have any available tissue forwarded for analysis in comparison of pre-administration and on-/post-treatment tumor microenvironmental factors.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

1. Advanced, unresectable or metastatic melanoma

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.

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

3. Have measurable disease based on RECIST 1.1.

4. Be naïve to exposure in the metastatic setting to PD1/L1 antibody for cohort 1 but have had exposure to PD1/L1 (or PD1/L1 combination therapy) in cohort 2. Prior exposure to CTLA4 antibody in the metastatic setting is not allowed for cohort 1 though exposure in the adjuvant setting is allowed for either cohort. To be eligible for cohort 2, and considered refractory to PD1/L1, a patient must have had a restaging exam showing progressive disease at least 90 days following initiation of anti-PD1/L1 as prior therapy.

    a. Adjuvant therapy with BRAF-MEK, PD1 or CTLA4 based therapy is allowed. Prior adjuvant BRAF-MEK therapy will fulfill treatment requirement in the metastatic setting. Patients who experience progression of disease during adjuvant PD1 therapy or within 6 months of completing adjuvant PD1 therapy will be considered refractory and thus eligible for cohort 1. Patients with progression to active metastatic disease more than 6 months following completion of adjuvant PD1 therapy will be eligible for cohort 1.

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

6. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 days of study initiation.

Table 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
[a]Creatinine clearance should be calculated per institutional standard.	

7. 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.

8. 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.

9. 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.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. For cohort 2: Has BRAF mutant disease but has not yet received treatment with RAF/MEK inhibitors.

    - This criteria can be met via adjuvant treatment with BRAF-MEK inhibitors

2. 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 2 weeks of the first dose.

3. Is currently taking Bifidobacterium based probiotics or is taking pre/pro-biotics regularly.

4. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose.

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

6. Hypersensitivity to pembrolizumab or any of its excipients.

7. Has had a prior anti-cancer monoclonal antibody (mAb) within 3 weeks prior to study Day 1 (excluding anti-PD1 antibodies such as pembrolizumab or nivolumab in cohort 2) or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

8. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

9. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or *in situ* cervical cancer.

10. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to start of study. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

11. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

12. Has known history of, or any evidence of active, non-infectious pneumonitis.

13. Has an active infection requiring antibiotic therapy or has received a course of antibiotics within the previous 2 weeks of starting study treatment.

14. 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.

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

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

17. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

18. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

19. Has received a live vaccine within 30 days of planned start of study therapy.

    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.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 2

Table 2 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Dietary Supplement	**Amount**	**Frequency**	**Route**	**Consumption period**
EDP1503	>$7.5x10^{10}$ CFU/capsule	2 capsules BID	Oral	Daily through the protocol study period

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

5.2.1.2 Dose Modification (Escalation/Titration/Other)

Adverse events (both non-serious and serious) associated with pembrolizumab (or in combination with EDP1503) 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 and EDP1503 must be withheld for drug-related toxicities and severe or life-threatening AEs. Supportive care guidelines, including use of corticosteroids are described below.

All Grade 3-5 adverse events considered possibly, probably or definitely related to study agent(s) must be discussed with the Lead Principal Investigator. All treating clinicians involved in the protocol are welcomed to discuss complicated cases at any time with the Lead Principal Investigator:

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Table 3 Dose Modification Guidelines (for both Pembrolizumab and EDP1503) for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] 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.verse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Lead Principal Investigator. The reason for interruption should be documented in the patient's study record.

If a subject's study treatment has been interrupted for more than 2 doses of pembrolizumab, the investigator must contact the Lead Principal Investigator to review the subject's condition in order to resume the treatment.

If toxicities develop requiring treatment hold of pembrolizumab then EDP1503 supplement should be held as well. If toxicities not characteristic to pembrolizumab are present and the treating investigator believes the patient is deriving clinical benefit, pembrolizumab can be continued while holding EDP1503.

5.2.2 Timing of Dose Administration

All trial treatments and dietary supplements will be administered on an outpatient basis.

An initial EDP1503 administration period of two weeks in cohort 1 and one week in cohort 2 will precede dosing with pembrolizumab and will continue to be administered orally daily for the duration of the study. An exception to this for cohort 2 patients will include any patient who is among the first three patients enrolled who will have a 2 week run in to monitor for EDP1503 toxicities especially.

Pembrolizumab 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) and may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

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).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution. The Pharmacy Manual provides instruction for storage of EDP1503.

5.2.3 Trial Blinding/Masking

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

5.3 Randomization or Treatment Allocation

There is no randomization.

5.4 Stratification

There is no stratification.

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 treating investigator should discuss any questions regarding this with the Lead Principal 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 treating investigator in keeping with the community standards of medical care. All concomitant medication will be recorded 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 recorded.

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 not specified in this protocol
- Other dietary probiotics and prebiotics
- Investigational agents other than pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after discussion between the investigator and Principal Investigator.
    - 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 Lead Principal Investigator.
- Antibiotic therapies may be required by some participants during the study. EDP1503 should not be held during antibiotic therapy unless deemed necessary by the treating investigator. If there are continued gastrointestinal symptoms or the treating

investigator feels the subject is not fully recovered then the restart can be delayed for a maximum of 2 weeks.

- **Antibiotic choice**: Different classes of antibiotics have been shown to disrupt the microbiome and potentially interfere with normal host immune function to varying degrees. If equally indicated amoxicillin is the preferred antibiotic as it minimally alters gut flora. Clindamycin is the most detrimental antibiotic and should be avoided if possible (Zaura et al 2015). Beta-lactams (other than amoxicillin) and fluoroquinolones broadly should be avoided if possible as well (Derosa et al 2017).

  Citations

- Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MPM, Rashid MU, Weintraub A, Nord CE, Savell A, Hu Y, Coates AR, Hubank M, Spratt DA, Wilson M, Keijser BJF, Crielaard W. 2015. Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. mBio6(6):e01693-15.

- Derosa et al 2017. Impact of antibiotics on outcome in patients with metastatic renal cell carcinoma treated with immune checkpoint inhibitors. Poster Abstract 462. J Clin Oncol 35, 2017

Subjects who, in the assessment by the treating investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial or in the case of antibiotics the Bifidobacteria components of the study should be temporarily stopped. Subjects may receive other medications that the treating 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 adverse events with potential immunologic etiology are outlined below. 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 treating investigator determines the events to be related to pembrolizumab and/or EDP1503.

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 below). Refer to Section 5.2.1 for dose modification.

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

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience 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. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis,** that persists greater than 3 days, administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis,** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

    - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
        - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic**:
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis**:
    - For **Grade 2** events, treat with corticosteroids.
    - For **Grade 3-4** events, treat with systemic corticosteroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  Table 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 3 Infusion Reaction Treatment 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 (MK-3475) 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 for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids 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.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

- **Management of Stevens-Johnson syndrome and Toxic Epidermal Necrolysis:**

  To date, approximately 11,000 patients in clinical trials and 27,000 patients in the post-marketing setting have been treated with KEYTRUDA®. One fatal case of SJS in a clinical trial and one fatal case of TEN in the post-marketing setting have been reported in patients treated with KEYTRUDA®. Including these cases, there have been 8 cases of SJS (6 in clinical trials, and 2 post-marketing) and 2 cases of TEN (both post-marketing) all of which were serious.

  - For signs or symptoms of SJS or TEN, withhold KEYTRUDA® and refer the patient for specialized care for assessment and treatment.

  - If SJS or TEN is confirmed, permanently discontinue KEYTRUDA®.

- **Management of Immune-mediated myocarditis:**

  A total of 6 cases of myocarditis have been reported in patients treated with KEYTRUDA® in clinical trials or in an expanded access program. There was 1 fatal case reported in a clinical trial.

  - For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies, and administer corticosteroids as appropriate.

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

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 Principal Investigator and to Merck without delay and within 24 hours to the Principal Investigator 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 treating 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 Lead Principal Investigator. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Lead Principal Investigator 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 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 Principal Investigator 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.

Definition of Dose Limiting Toxicity: Any toxicity of >=Grade 3, any autoimmune toxicity >CTCAE Gr 2; DLT observation period is for the length of monotherapy for each cohort.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved.

- 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
- 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, whichever is later.

  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.

- Administrative reasons

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). Subjects who discontinue for reasons other than progressive disease 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 by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.8.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the 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. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation.

5.9 Subject Replacement Strategy

5.10 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 study drug

In the event of Merck or Evelo decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

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	EDP1503 run-in[b]	1	2	3	4	To be repeated beyond 8 cycles				Discon	Safety Follow-up	Follow Up Visits In Absence of Progression[c] Every 12 weeks post discon
							5	6	7	8			
Scheduling Window (Days):	-21 to -1	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X	X
Pembrolizumab Administration			X	X	X	X	X	X	X	X			
Review Adverse Events	X	X[g]	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test – Urine or Serum β-HCG	X												
CBC with Differential	X	X	X	X	X	X	X	X	X	X	X	X	
Comprehensive Serum Chemistry Panel	X	X	X	X	X	X	X	X	X	X	X	X	
Urinalysis	X												
TSH (with reflexive T3, FT4 as indicated)	X		X	X	X	X	X	X	X	X	X	X	

Trial Period:	Screening Phase	Treatment Cycles[a]									End of Treatment	Post-Treatment	
							To be repeated beyond 8 cycles						
Treatment Cycle/Title:	Study Screening	EDP1503 run-in[b]	1	2	3	4	5	6	7	8	Discon	Safety Follow-up	Follow Up Visits In Absence of Progression[c] Every 12 weeks post discon
Scheduling Window (Days):	-21 to -1	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	
Tumor Imaging (window includes all of cycle following drug administration)	X					X				X			
Archival or Newly Obtained Tissue Collection[d]	X[d]		X[d]										
Correlative Studies Blood Collection[e]	X	X[h]	X[f]			X					X		
Fecal Sample	X		X			X					X	X	

a – Treatment cycles = 21 days

b – EDP1503 alone run-in for two weeks (14 days) in cohort 1 and one week (7 days) in cohort two and continuous thereafter with pembrolizumab. An exception to this for cohort 2 patients will include any patient who is among the first three patients enrolled who will have a 2 week run in to monitor for EDP1503 toxicities especially. Cohort 2 cannot initiate the one week run-in until 3 patients have cleared two week EDP1503 run. Pembrolizumab will be administered on D1 of every cycle. EDP1503 will be taken po (2 capsules bid) for all 21 days of every cycle.

c – Patients who discontinue treatment without progression will have follow up visits for up to 2 years following drug discontinuation

d – Archival tissue for newly diagnosed metastatic melanoma is allowed in cohort 1. Fresh biopsies are otherwise required. Second biopsy at the end of the EDP1503 run-in period after at least 10 days in cohort 1 and at least 4 days in cohort 2.

e – Samples to be collected for correlative blood analysis are detailed in section 8.1.1.11

f – Sample to be taken prior to the initiation of pembrolizumab therapy

g - Nursing assessment for adverse events at 48 hours which can be completed by telephone.

h – Research cytokines, blood culture, whole blood

7.0 REGISTRATION PROCEDURES

7.1 General Guidelines

Prior to registration and any study-specific evaluations being performed, all patients must have given written informed consent for the study and must have completed the pre-treatment evaluations. Patients must meet all of the eligibility requirements listed in Section 3. Eligible patients will be entered on study centrally by the University of Chicago multi-site coordinator. All sites should call the coordinator at (773) 834-1746 or PhaseIICRA@medicine.bsd.uchicago.edu to verify availability of a slot.

7.2 Registration Process

When a potential patient has been identified, notify the CRA via phone or email to ensure a reservation on the study ((773) 834-1746 or PhaseIICRA@medicine.bsd.uchicago.edu). Reservations for potential subjects will only be held for subjects who have signed consent for that particular study.

When registering a subject, the following must occur:

- Confirm that the institution has a current IRB approval letter for the correct version of protocol/consent and has an annual update on file, if appropriate.

- Submit all required materials (Eligibility Checklist, Source documentation, & signed consent form) to confirm eligibility and required pre-study procedures to the CRA a minimum of 48 hours prior to the subject's scheduled therapy start date.

- Source documentation includes copies of all original documents that support each inclusion/exclusion criteria. The eligibility checklist does not serve as source documentation but rather as a checklist that original source documentation exists for each criterion.

- Communicate with the CRA to ensure all necessary supporting source documents are received and the potential subject is eligible to start treatment on schedule. If there are questions about eligibility, the CRA will discuss it with the PI. PI may clarify, but not overturn, eligibility criteria.

- Affiliate sites must confirm registration of subjects by obtaining a subject study ID number from the CRA via phone, fax or email.

- If a subject does not start on the scheduled day 1 treatment date, promptly inform the CRA as the delay in start may deem the subject ineligible and/or require further or repeat testing to ensure eligibility.

- The date the patient receives treatment for the first time will be considered the patient's "OnStudy Date." The patient's subject ID will be assigned and a confirmation of registration will be issued by the CRA on this date. Subjects that sign consent and do not go "OnStudy" will be recorded in the database with the date they signed consent

and the reason for not going "OnStudy" (e.g., Ineligible, Screen Failure or Withdrawn Consent).

8.0 TRIAL PROCEDURES

8.1 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 treating investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the investigator 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.

8.1.1 Administrative Procedures

8.1.1.1 Informed Consent

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

8.1.1.1.1 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/ERC's approval/favorable opinion 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/ERC requirements, applicable laws and local regulations.

8.1.1.2 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.

8.1.1.3 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.

8.1.1.4 Prior and Concomitant Medications Review

8.1.1.4.1 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.

8.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded.

8.1.1.5 Disease Details and Treatments

8.1.1.5.1 Disease Details

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

8.1.1.5.2 Prior Treatment Details

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

8.1.1.5.3 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.

8.1.1.6 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 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

8.1.1.7 Physical Exam

The investigator or qualified designee will perform a physical exam at protocol specified time points as per the Trial Flow Chart (Section 6.0). Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening.

8.1.1.8 Vital Signs

The investigator or qualified designee will take vital signs 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.

8.1.1.9 Eastern Cooperative Oncology Group (ECOG) Performance Scale

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

8.1.1.10 Tumor Imaging and Assessment of Disease

Baseline and restaging radiologic imaging will be performed to encompass all sites of metastatic disease. This will generally include at least CT CAP though the specific imaging study can be changed by the treating investigator depending on patient circumstances. Radiologic imaging modality should be consistent throughout the study for response assessment. For patients who have known bone metastases at baseline, bone scan should additionally be pursued as standard of care for disease assessment.

8.1.1.11 Tumor Tissue Collection and Correlative Studies Blood Sampling

The following tables outline the requirements for tissue and blood banking. These will be collected and stored for batched future analysis.

Biospecimen type	Collection Time Point	Number of samples per patient

Fecal sample	Pre administration of EDP1503 Following run in of EDP1503 At dose 4 of treatment with pembrolizumab At discontinuation visit 30 days after cessation of EDP1503	10 samples (2 samples each from 5 time points)
A paraffin-embedded tissue block of metastatic tumor taken before initiation of treatment.	Pre-treatment: obtained prior treatment on protocol	1 historical sample
Paraffin-embedded tissue block of metastatic tumor taken before initiation of treatment (if available).	Pre-treatment: obtained prior treatment on protocol	1 sample
Paraffin-embedded tissue block of metastatic tumor taken after EDP1503 run in. Where feasible this should be taken from the same metastatic or primary site as the initial biopsy.	Pre-treatment: obtained after the EDP1503 run in	1 sample
Peripheral Blood Mononuclear Cells: ~60 ml of anti-coagulated whole blood in green top tube	Pre-administration of EDP1503: 8-10 tubes taken within 21 days prior to EDP1503 administration. 8-10 tubes taken at the following time points: (1) at day of dose 1 pre-administration of pembrolizumab (2) dose 4 pembrolizumab (3) at time of progressive disease.	Up to 40 samples total over 4 time points
Whole blood for germline DNA: 8-10 mL of whole blood in PAXgene tube	Within 21 days prior to administration of EDP1503	1 sample
Cytokines	Pre administration of EDP1503	4 samples

	Following approximately 48 hours of EDP1503 Following run in of EDP1503 At dose 4 of treatment with pembrolizumab	
Blood Culture	Pre administration of EDP1503 Following run in of EDP1503	2 samples
EDP1503 PCR	Pre administration of EDP1503 Following run in of EDP1503	2 samples

Biospecimens will be shipped and stored in the University of Chicago Human Immune Monitoring Core Facility as below with the exception of one stool sample from each time point which will be sent to Evelo Biosciences.

Human Immune Monitoring Core:

Contact: Yuanyuan Zha
University of Chicago - Human Immune Monitoring Core
910 E. 58th St. MKL057
Chicago, IL 60637
yzha1@bsd.uchicago.edu
Phone: 773-702-4812

Ship human microbiome sample kits to*:

Evelo Biosciences
Contact: Dr Maria Sizova
620 Memorial Drive, 5th Floor
Cambridge, MA 02139
maria@evelobio.com

*A pre-labeled box and shipping label will be provided.

8.1.2 Laboratory Procedures/Assessments

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 14 days prior to EDP1503 administration. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	Thyroid stimulating hormone (TSH)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Total thriiodothyronine (T3) *(If TSH abnormal)*
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Free thyroxine (T4) *(If TSH abnormal)*
Absolute Neutrophil Count	Carbon Dioxide	results are noted	
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Potassium		
	Sodium		
	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 urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.			

8.1.3 Other Procedures

8.1.3.1 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. 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. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit and then proceed to the Follow-Up Period of the study.

8.1.3.2 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (90 ± 7 days) by radiologic imaging to monitor disease status. 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. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab will move from the follow-up phase to the Second Course Phase when they experience disease progression.

8.1.3.3 Second Course Phase (Retreatment Period)

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:

- **Either**
    - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
        - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy
        - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

        OR
    - Had SD, PR or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.1.2
- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.
- 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.
- 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.
- 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 re-treated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year. EDP1503 will also be restarted pending availability of supplement supply.

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

8.2 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 of the Merck's product, 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.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

All adverse events will be recorded from first day of study drug administration through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets.

8.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Principal Investigator, Merck and Evelo

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). No specific information is available on the treatment of overdose of pembrolizumab. 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.

Overdose of EDP1503 will be defined as more than 6 capsules in a day. There is no known safety issue surrounding this however pill counts will be documented to confirm appropriate dosing. Appropriate supportive treatment should be provided if clinically indicated. Overdose of EDP1503 does not require report as a serious adverse event but will be reported as an adverse event.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product 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 of site knowledge of the event to the UC CCC Cancer Clinical Trials Office and Lead Principal Investigator and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

8.2.2 Reporting of Pregnancy and Lactation to the Principal Investigator 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), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant 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 of site knowledge of the event to the UC CCC Cancer Clinical Trials Office and Lead Principal Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

8.2.3 Immediate Reporting of Adverse Events to the Principal Investigator and to Merck

8.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

Refer to Table 6 for additional details regarding each of the above criteria.

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 that occurs to any subject from first day of study drug administration through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck or Evelo product, must be reported within 24 hours of site knowledge of the event to the UC CCC

Cancer Clinical Trials Office and Lead Principal Investigator and within 2 working days to Merck and Sarah Cannon Development Innovation Safety Department who will hold the Global Safety Database for EDP1503.

Non-serious Events of Clinical Interest will be forwarded to Merck and Sarah Cannon Development Innovation Safety Department and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the UC CCC Cancer Clinical Trials Office and Principal Investigator and to Merck and Evelo.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220 and to Sarah Cannon Development Innovation Safety Department facsimile number 1-866-807-4325.

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

8.2.3.2 Unexpected Events

Unexpected events are those not listed at the observed specificity or severity in the protocol, informed consent, investigator brochure, or FDA-approved package insert. An event is considered unexpected if it is listed as occurring within the class of drugs or otherwise expected from the drug's pharmacological properties but which has not been previously observed with this specific investigational agent.

8.2.3.3 Adverse Reactions

An adverse event is considered to be an adverse reaction if there is evidence to suggest a causal relationship to the study agent. This may include a single occurrence of an event strongly associated with drug exposure (e.g. Stevens-Johnson Syndrome), one or more occurrence of an event otherwise uncommon is the study population, or an aggregate analysis of specific events occurring at greater than expected frequency.

8.2.3.4 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours of site knowledge of the event to the UC CCC Cancer Clinical Trials Office and Lead Principal Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220) and Sarah Cannon Development Innovation Safety Department.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1, that is not associated with clinical symptoms or abnormal laboratory results.

2. 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.

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

8.2.4 Evaluating Adverse Events

An investigator will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5. 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.

Table 6 Evaluating Adverse Events

An investigator, will evaluate all adverse events as to:

v5 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 Merck product 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 Merck product 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 Lead Principal Investigator within 24 hours of site knowledge of the event and to Merck and Sarah Cannon Development Innovation Safety Department 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 of site knowledge of the event to the Lead Principal Investigator and to Merck and Sarah Cannon Development Innovation Safety Department 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 study agent(s) to be discontinued?	
Relationship to Merck or Evelo Product	Did study agent(s) cause the adverse event? The determination of the likelihood that Merck product 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 study agent(s) and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely study agent(s) caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to study agent(s) 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 study agent(s)? 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 Merck or Evelo Product (continued)	**The following components are to be used to assess the relationship between the study agent(s) and the AE: (continued)**	
	Dechallenge	Was study agent(s) 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 pembrolizumab; or (3) the trial is a single-dose drug trial); or (4) pembrolizumab is only used one time.)
	Rechallenge	Was the subject re-exposed to study agent(s) 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) pembrolizumab is only used one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY STUDY AGENT(S), OR IF REEXPOSURE TO STUDY AGENT(S) POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE LEAD PRINCIPAL INVESTIGATOR 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 study agent(s) or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator 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 study agent(s) relationship).**
Yes, there is a reasonable possibility of study agent(s) relationship.		There is evidence of exposure to study agent(s). The temporal sequence of the AE onset relative to the administration of study agent(s) is reasonable. The AE is more likely explained by study agent(s)than by another cause.
No, there is not a reasonable possibility of study agent(s) relationship		Subject did not receive the study agent(s) OR temporal sequence of the AE onset relative to administration of study agent(s) is not reasonable OR the AE is more likely explained by another cause than the study agent(s). (Also entered for a subject with overdose without an associated AE.)

8.2.5 Serious Adverse Event Reporting to the Coordinating Center

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

For reporting SUSARs, reporting will comply with CFR 312.32.

All serious adverse events and all Events of Clinical Interest that have been specified to require expedited reporting occurring on this study require expedited reporting to the University of Chicago Comprehensive Cancer Center (UC CCC). The responsible Research Nurse or other designated individual at the treating site should report the SAE to the Lead Principal Investigator and the Cancer Clinical Trials Office (CCTO) within 24 hours of site knowledge of the event. Reports should be made using the 'Serious Event Report' Form. Please scan and send via email (preferred) or fax to the following:

University of Chicago Phase II CRA General:
PhaseIICRA@medicine.bsd.uchicago.edu
Phone: 773-834-1746
Fax: 773-702-4889

UC CCC Cancer Clinical Trials Office Quality Assurance:
qaccto@bsd.uchicago.edu

All unexpected adverse reactions must be reported to the IND holder so that the University of Chicago CCTO can inform the FDA. The responsible Research Nurse or other designated individual at the treating site should provide a complete written report using the FDA MedWatch 3500A form. The completed form should be sent to the CCTO at qaccto@bsd.uchicago.edu and to the Phase II CRA at PhaseIICRA@medicine.bsd.uchicago.edu within the specified timelines below regardless of whether all information regarding the event is available. If applicable, a follow-up report should be provided to the CCTO if additional information on the event becomes available.

Participating sites should not forward any adverse event reports directly to the FDA. The CCTO will report all events to the FDA as per the current FDA guidelines.

> *Fatal or Life-threatening Events*: within 4 calendar days from treating investigator knowledge of the event
>
> *All Other Reportable Events:* within 10 calendar days of treating investigator knowledge of the event

All serious adverse events should also be reported to the local IRB of record according to their policies and procedures.

8.2.6 Serious Adverse Event Reporting by the Coordinating Center

The designated UC CCC Regulatory Manager will notify all participating sites of all unexpected and serious adverse reactions that occur on this clinical trial and which are reported to the FDA and/or UC Institutional Review Board (IRB). When reported to the FDA, a copy of the completed Form 3500A (MedWatch) will be provided to the responsible Regulatory Manager by the CCTO IND Coordinator for distribution to all participating sites.

8.2.7 Serious Adverse Event Reporting to Merck

Any serious adverse event reported to the UC CCC, whether or not related to Merck product, must also be reported within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

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

The University of Chicago coordinating center staff will be responsible for reporting all events to Merck.

8.2.8 Serious Adverse Event Reporting to Evelo (Sarah Cannon Safety Department)

Any serious adverse event reported to the UC CCC, whether or not related to Evelo product, must also be reported within 2 working days to Sarah Cannon Development Innovation Safety Department.

Non-serious Events of Clinical Interest will be forwarded to Sarah Cannon Development Innovation Safety Department and will be handled in the same manner as SAEs.

SAE reports and any other relevant safety information are to be forwarded to Sarah Cannon Development Innovation Safety Department.

Safety Dept. Fax #: 1-866-807-4325

Safety Dept. Email: CANN.SAE@SCRI-Innovations.com

The University of Chicago coordinating center staff will be responsible for reporting all events to Sarah Cannon Development Innovation Safety Department.

9.0 DATA REPORTING

Data reporting will be performed utilizing the eVelos electronic data capture system. The University of Chicago CRA will provide you with the applicable user registration information.

All required data must be recorded in the eVelos database at the completion of each cycle. AEs are to be entered in real time. SAEs are to be entered on the Serious Event Form within 24 hours of the site's knowledge of the event and sent via email (preferred) or fax to the

University of Chicago (PhaseIICRA@medicine.bsd.uchicago.edu or qaccto@bsd.uchicago.edu; Fax: 773-702-4889). All case report forms must be completed by designated study personnel. Each screened (consented) patient is to be entered into eVelos within 48 hours of patient registration. In addition to direct data entry, sites may be required to provide supporting source documentation. Source records are original documents, data, and records (e.g., medical records, raw data collection forms, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. Each site will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each subject enrolled in this clinical trial. Source records must be adequate to reconstruct all data transcribed onto the case report form.

10.0 STATISTICAL ANALYSIS PLAN

10.1 Statistical Analysis Plan

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF.
Safety	All participants who take at least 1 dose of study intervention.
Evaluable	All participants and who had no important protocol deviations affecting efficacy variables.

Hypothesis: Modulation of the gut microbiota with EDP1503 will augment the depth of response or response rate in patients with advanced melanoma who are naïve or refractory to anti-PD1 antibody.

The study will have two cohorts of subjects including:
1. Subjects with advanced melanoma who are naïve to anti-PD1 antibody.
2. Subjects with advanced melanoma who are refractory to anti-PD1 antibody.

Cohort one will employ a single stage design while cohort two will employ a Simon two stage design in which subjects will be treated with pembrolizumab in combination with EDP1503.

Cohort 1: Subjects with melanoma who are naive to anti-PD1 antibody:
Given the robust activity of pembrolizumab in patients with melanoma naïve to anti-PD1 monotherapy, we have proposed **"deep response" (>80% tumor reduction) as the primary response endpoint in the frontline setting for cohort 1.** This was based on the experience described of anti-PD1 antibody (nivolumab) in combination with anti-CTLA4 antibody (ipilimumab) in which approximately 40% of patients had >80% tumor reduction as best overall response[25,29-31]. Utilizing this response endpoint as opposed to the RECIST response definition of 30% tumor shrinkage will allow the sample size to be reduced and thus facilitate a more rapid completion of the trial.

The co-primary efficacy endpoint will be rate of deep response by RECIST. Given the deep response rate of approximately 5% to monotherapy anti-PD1 antibody, we will consider a response rate of 0.05 as not promising, and a response rate of 0.20 as promising. The type I error rate is set at 0.1 and the type II error rate at 0.1. Given that pembrolizumab is a clinical standard of care, a single stage accrual design will be employed including 35 patients. If 4 or more deep responses are observed in 35 total subjects the combination would be deemed of substantial interest in patients with PD1 treatment naïve melanoma, given that the EDP1503 safety is also established (co-primary objective). A chi-squared test will be performed for the responder rate, and a 95% confidence interval may be produced, if deemed appropriate.

For the secondary efficacy objectives, progression-free survival (PFS) will be estimated by the Kaplan-Meier method. PFS will be defined as the time from first dose of either drug until disease progression or death from any cause. Surviving subjects without progression will be censored as of the date of the last negative examination.

Adverse events will be tabulated by type, severity, and attribution. Immune responses, as assessed at baseline, week 6, and week 12 will be analyzed by mixed effects regression for longitudinal data. Baseline and post-treatment values will also be compared between responders and non-responders. Microbial data will be analyzed descriptively, pooling the data with that from subjects in other studies.

Cohort 2 For subjects with melanoma who are refractory to anti-PD1 antibody:

The primary efficacy endpoint of cohort 2 will be RECIST response rate. Given that response would not be expected in subjects with prior progression on anti-PD1, we will consider a response rate of 0.05 as not promising and 0.20 as promising. The type I error rate is set at 0.1 and the type II error rate at 0.1. A total of 35 subjects would be proposed. In an optimal Simon two stage design, 1 response in 20 subjects would be required in the first stage and 4 or more responses observed in 35 total subjects the combination would be deemed of substantial interest in the treatment of patients with PD1 refractory melanoma, given that the EDP1503 safety is also established (co-primary objective). A chi-squared test will be performed for the responder rate, and a 95% confidence interval may be produced, if deemed appropriate. However if any responses are observed detailed investigation of changes in the microbiome and tumor microenvironment will be indicated. The probability of stopping this cohort early is 42% if the true response rate is only 0.05.

Description of secondary and correlative endpoints will be the same as cohort 1.

Hypothesis: EDP1503 monotherapy will demonstrate a similar safety and tolerability profile to probiotics though may be associated with low grade nausea, vomiting, and abdominal pain.

The study will have two cohorts of subjects with a monotherapy run-in with EDP1503 as described earlier. There will be 2 analyses of safety across this study:

1. EDP1503 monotherapy run-in
2. Overall safety for the combination of pembrolizumab and EDP1503

All safety analyses will be performed on the Safety Population.

The co-primary endpoint for this study is the incidence of SAEs and AEs during the monotherapy run-in with ED the monotherapy period with EDP1503.

All AEs will be coded using the latest available version of the Medical Dictionary for Regulatory Activities (MedDRA) and categorized by intensity (mild/moderate/severe).
Treatment-emergent AEs and SAEs will be listed and summarized by treatment period and participant population, system organ class and preferred term.

Definition of Dose Limiting Toxicity: Any toxicity of >=Grade 3, any autoimmune toxicity >CTCAE Gr 2; DLT observation period is for the length of monotherapy for each cohort. The first three patients enrolled will successively complete their 2 week DLT period prior to initiation of the subsequent patients.

Safety laboratory measurements: These variables will be listed and summarized by treatment group and participant population.

Statistical analysis of tumor biopsies

Monotherapy with pembrolizumab is associated with an approximately 40% response rate in therapy naive patients. Morphologic predictors of response include expression of PDL-1, an interferon gamma target indicative of an active inflammatory response to tumor, and the presence of tumor infiltrating CD8 T cells, dendritic cells, and NK cells of particular phenotypes. The therapeutic hypothesis of EDP1503 is that it will either induce or augment an antitumor inflammatory response, and/or will induce production of neoantigen presenting MHC class I in the tumor.

The primary goal of the biopsy analysis is to determine whether the tumor has sustained an infiltration by innate and adaptive immune cells, and/or increased tumoral expression of antigen expression apparatus such as components of MHC Class I, consistent with the therapeutic hypothesis. The secondary goal is to determine whether either the baseline level of inflammatory infiltrate or the level induced by EDP1503 treatment is predictive of immediate an extended response to the combination of EDP1503 with pembrolizumab.

The primary assays for analysis of the paired biopsies are the Nanostring IO 360 assay, an extensively validated transcript profiling assay used for semi-quantitative cell enumeration and transcriptional response in biopsies (Danaher *et al*.), as well as immunofluoresence based direct enumeration of immune cells and their spatial co-localization.

The primary hypothesis for statistical analysis of the paired biopsies is to accept or reject the hypothesis that the tumor inflammation signature (TIS, or T cell-inflamed GEP), a function calculated from the abundance of 18 transcripts detected in the sample (Table below), has changed in the tumor due to therapy. This function is highly correlated with pembrolizumab treatment outcome (Ayers *et al*.).

Table: 18 component transcripts of the TIS score

TIGIT	PD-L1	PSMB10	STAT1
CD27	CXCR6	IDO1	HLADRB1
CD8A	CMKLR1	CXCL9	HLAE
PD-L2	NKG7	HLADQA1	
LAG3	CCl5	CD276	

The variance of baseline data due to sampling variation is provided by published studies on the variability of the TIS due to sampling (Walldren *et al*.) From the dataset of multiple samplings of a baseline sample, a ratio of paired baseline TIS scores (n=85) was calculated, median (IQR): 0.9 (.25). To determine whether a difference in TIS score from day 14 to baseline is statistically significant from replicate TIS measurements, a 95% Bca confidence interval was constructed from 10000 bootstrap replicates using the ratios of paired baseline TIS scores, (0.81,1.31). Based on this estimate, a TIS ratio score > 1.31 would suggest a significant result at the 95% confidence level.

Association of the TIS with response rate (either deep response of > 80% in Cohort 1 or RECIST response rate in Cohort 2) and PFS will be analyzed following the established methods of (Ayers *et al*.).

Several outcomes may be envisaged:

- EDP1503 causes increase in TIS in a subset of patients and that increase is associated with an increase in RR and PFS.
- Baseline TIS is associated with PFS, and changes induced by EDP1503 treatment are not associated with changes in RR and PFS.
- EDP1503 does not affect TIS in any patient.

These hypotheses will be explored using linear mixed effects models fitted to immunological parameters and related composite scores to determine associations of rate of change with treatment outcome, following the strategy of Ribas *et al*.

We will also perform additional exploratory analyses.

In addition to the TIS, the IO360 panel provides for the individual semi-quantitative enumeration of multiple cell types including NK, DC, and T cells, which will be enumerated in pre and post treatment biopsies.

The immunofluorescense assays will additionally provide quantitation of immune cell types. The ability to sample multiple portions of each pre and post treatment sample with IF will allow for high-dimensional resolution of group differences determined by Wilcoxon rank-sum test. These results will be cross correlated with the Nanostring analysis, and the quality of the correlation compared to Ribas *et al*.

References:

Danaher P *et al*. Gene expression markers of Tumor Infiltrating Lymphocytes. JITC 5:18-33 (2017)

Ayers *et al*. IFNg related mRNA profile predicts clinical response to PD-1 blockade. JCI 127(8):2930-2940 (2017)

Walldren *et al*. Development and analytical performance of a molecular diagnostic for anti-PD1 response on the nCounter Dx Analysis System. ASCO 2016, Abstract 3034.

Ribas A *et al*. Oncolytic Virtherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. Cell 170, 1109-1119 (2017)

10.2 Sample Size and Expected Accrual

The study will be conducted through the University of Chicago Personal Cancer Care Consortium which includes both sophisticated private practices as well as collaborating academic centers. Patient accrual through this mechanism would be projected to be 3 subjects per month. Cohort 1 and Cohort 2 will enroll 35 evaluable patients each.

See section 10.1 for the description of the sample size.

11.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

11.1 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.

Clinical Supplies will be provided by Merck and Evelo as summarized in Table 7.

Table 7 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

Product Name & Potency	Dosage Form
EDP1503 >7.5x10^{10} organisms/capsule	Enteric capsule size 0 for oral delivery

11.2 Packaging and Labeling Information

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

11.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Principal Investigator 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.

11.4 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.

11.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

12.0 ADMINISTRATIVE AND REGULATORY DETAILS

12.1 Institutional Review Board (IRB) Approval and Consent

Unless otherwise specified, each participating institution must obtain its own IRB approval. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

12.2 Food and Drug Administration (FDA) Approval

This study will be conducted under an IND held by Dr. Randy Sweis at the University of Chicago. The University of Chicago CCTO will be responsible for facilitating all communications with the FDA on behalf of the IND holder. Participating sites should not communicate directly with the FDA.

12.3 Required Documentation

Prior to the selection of a study site that is not a full member of the Personalized Cancer Care Consortium, the audit and trial oversight processes for the site must be reviewed and approved by the UC CCC Clinical Research Advisory Committee.

Before the study can be initiated at any site, the following documentation must be provided to the Cancer Clinical Trials Office (CCTO) at the University of Chicago Comprehensive Cancer Center.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Investigational drug accountability standard operating procedures

Additionally, before the study can be initiated at any site, the required executed research contract/subcontract must be on file with the University of Chicago.

12.4 Data and Safety Monitoring

This study will be remotely monitored by the designated University of Chicago Clinical Research Associate (CRA) in accordance with the University of Chicago, Section of Hematology/Oncology standard operating procedure titled Monitoring of Multi-Institutional Investigator Initiated Clinical Trials.

Prior to subject recruitment, and unless otherwise specified, a participating site will undergo a Site Initiation Teleconference to be conducted by the designated University of Chicago research team. The site's principal investigator and his or her study staff must attend the site initiation meeting.

Monitoring will be conducted to verify the following:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Compliance with regulations
- Submission of required source documents

Participating sites will also undergo a site close-out teleconference upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and to ensure that the site Investigator is aware of his/her ongoing responsibilities.

Unless otherwise specified, this protocol will undergo weekly review at the multi-institutional data and safety monitoring teleconference as per procedures specified by the UC CCC NCI-approved Data and Safety Monitoring Plan. The conference will review:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

Protocol deviations are to be documented using the Protocol Deviation Form and sent via email to PhaseIICRA@medicine.bsd.uchicago.edu. Deviations that are considered major because they impact subject safety or alter the risk/benefit ratio, compromise the integrity of the study data, and/or affect subjects' willingness to participate in the study must be reported within 7 days. Please contact the University of Chicago CRA (PhaseIICRA@medicine.bsd.uchicago.edu) if you have questions about how to report deviations. All major protocol deviations should also be reported to the local IRB of record according to their policies and procedures.

12.5 Auditing

In addition to the clinical monitoring procedures, the University of Chicago Comprehensive Cancer Center will perform routine Quality Assurance Audits of investigator-initiated clinical

trials as described in the NCI-approved UC CCC DSM Plan. Audits provide assurance that trials are conducted and study data are collected, documented and reported in compliance with the protocol. Further, quality assurance audits ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements. The audit will review subjects enrolled at the University of Chicago in accordance with audit procedures specified in the UC CCC Data and Safety Monitoring plan. For institutions who are formal members of the Personalized Cancer Care Consortium (PCCC), the UC CCC will conduct on site quality assurance audits on average every two years during the enrollment and treatment phase of the study.

Auditing procedures for participating sites that are not full members of the PCCC must be specified and approved by the UC CCC Clinical Research Advisory Committee. In general, for sites that are not full members of the PCCC, auditing responsibility will be delegated to the participating center, with the annual audit report forwarded to the University of Chicago for review.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the University of Chicago Cancer Clinical Trials Office and Regulatory Manager that such a request has been made.

12.6 Amendments to the Protocol

All modifications to the protocol, consent form, and/or questionnaires will be submitted to the University of Chicago IRB for review and approval. A list of the proposed modifications or amendments to the protocol and/or an explanation of the need of these modifications will be submitted, along with a revised protocol incorporating the modifications. Only the Study Lead PI can authorize any modifications, amendments, or termination of the protocol. Once a protocol amendment has been approved by the University of Chicago IRB, the Regulatory Manager will send the amended protocol and consent form (if applicable) to the affiliate institutions electronically. Upon receipt of the packet the affiliate institution is expected to do the following:

- The affiliate must reply to the email from the Regulatory Manager indicating that the amendment was received by the institution and that it will be submitted to the local IRB.

- The amendment should be submitted to the affiliate institution's IRB as soon as possible after receipt. The amendment **must** be IRB approved by the institution **within 3 months** from the date that it was received.

- **The University of Chicago version date and/or amendment number must appear on the affiliate consent form and on the affiliate IRB approval letter.** The version dates can be found on the footer of every page of the protocol and consent form. The amendment number can be found on the University of Chicago IRB amendment approval letter that is sent with the protocol/amendment mailing.

The IRB approval for the amendment and the amended consent form (if amended consent is necessary) for the affiliate institution must be sent to the designated UC Regulatory Manager as soon as it is received.

12.7 Annual IRB Renewals, Continuing Review and Final Reports

A continuing review of the protocol will be completed by the University of Chicago IRB and the participating institutions' IRBs at least once a year for the duration of the study. The annual IRB renewal approvals for participating institutions should be forwarded promptly to the Regulatory Manager. If the institution's IRB requires a new version of the consent form with the annual renewal, the consent form should be included with the renewal letter.

12.8 Record Retention

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

12.9 Obligations of Study Site Investigators

The Study Site Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Study Site Principal Investigator is responsible for personally overseeing the treatment of all study patients. He/she must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Study Site Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the CRFs. Periodically, monitoring visits or audits will be conducted and he/she must provide access to original records to permit verification of proper entry of data.

13.0 APPENDICES

13.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

13.2 Common Terminology Criteria for Adverse Events V5 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

13.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

In addition, volumetric analysis will be explored by central review for response assessment.

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14.0

CRITERIA FOR irRECIST

Tumor Burden **(Baseline and New)**	**Non-Target Lesions** **(Baseline and New)**	**Response**
Disappearance of non-nodal lesions. All pathologic lymph nodes < 10 mm (short axis)	Disappearance of non-nodal lesions. All pathologic lymph nodes < 10 mm (short axis)	irCR[a]
≥ 30% decrease from baseline	Any	irPR[a]
≥ 20% increase from nadir and at least 5 mm	Any	irPD[a]
Neither sufficient decrease to qualify for PR, nor sufficient increase to qualify for PD	Any	irSD
Disappearance of all non-nodal lesions. All pathologic lymph nodes < 10 mm	Any other than disappearance of all non-nodal lesions and reduction of pathologic lymph nodes < 10 mm	irPR[a]

Not all evaluated[b]	Any	irNE

a Selection as best response requires confirmation by 2 consecutive measurements at least 4 weeks apart.

b If some lesions are measured, response may be inferred from available measurements. For example, growth in evaluated target lesions may be sufficient for irPD regardless of status of non-evaluated lesions.

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