SUMMARY OF CHANGES

HCC 18-087 Protocol Amendment #7

Old Protocol Date: 2-19-2021

New Protocol Date: 5-20-2021

#	Section	Page(s)	Change
1.	Entire document	All	Updated version date
2.	5.2.2. Dose Modifications and Toxicity Management	32-36	Change: Table 3 was replaced with an updated table. Justification: Table 3 was updated due to pembrolizumab IB version 20 and per Merck recommendation.

SPONSOR: Robert Edwards, MD

TITLE: Systemic Immune Checkpoint Blockade and Intraperitoneal Chemo-Immunotherapy in Recurrent Ovarian Cancer

IND NUMBER: 140618

TABLE OF CONTENTS

1.0 TRIAL SUMMARY

Abbreviated Title	**Systemic Immune Checkpoint Blockade and Intraperitoneal Chemo-Immunotherapy in Recurrent Ovarian Cancer**
Trial Phase	*Phase II*
Clinical Indication	Recurrent Platinum Sensitive Ovarian Cancer Patients
Trial Type	Single arm efficacy trial
Type of control	Historical comparison control, no control arm
Route of administration	Intravenous Pembrolizumab, Intraperitoneal Cisplatin, Intraperitoneal Rintatolimod
Trial Blinding	No blinding
Treatment Groups	Single arm
Number of trial participants	45
Estimated enrollment period	*2018-2019*
Estimated duration of trial	*5 years*
Duration of Participation	4 years
Estimated average length of treatment per patient	31 weeks

2.0 TRIAL DESIGN

2.1 Trial Design

This is a phase II single arm efficacy/safety trial that will evaluate the effectiveness of combining intensive locoregional intraperitoneal (IP) chemoimmunotherapy of cisplatin with IP rintatolimod (TLR-3 agonist) and IV infusion of the checkpoint inhibitor pembrolizumab (IVP) for patients with recurrent platinum-sensitive ovarian cancer (OC).

Patient cohort

This trial will enroll up to 45 patients; eligibility criteria include patients at first or second recurrence previously diagnosed with platinum-sensitive ovarian/fallopian tube carcinoma or primary peritoneal carcinomatosis. Platinum-sensitive OC is defined as patients with a treatment-free interval greater than 6 months. Patients must have at least one peritoneal lesion, measurable by CT/MRI scan. Patients will be allowed if RECIST 1.1 criteria is met for measurable retroperitoneal lesions if there also is the presence of peritoneal lesions during laparoscopy. The lesion should be large enough for biopsy and resection, ideally to yield greater than 2 grams of tumor for immunoassays. If biopsy is insufficient or not feasible during laparoscopy, patients may be allowed to proceed at the discretion of the PI if there are otherwise measurable lesions. All epithelial invasive histologies are included in this phase II design.

Patients will receive a total of six treatment cycles, at 3-week intervals (Schema 1, Section 2.2). We will use an IP neoadjuvant approach, followed by interval cytoreduction (usually laparoscopically) of residual tumor. The decision to perform interval cytoreductive surgery is at

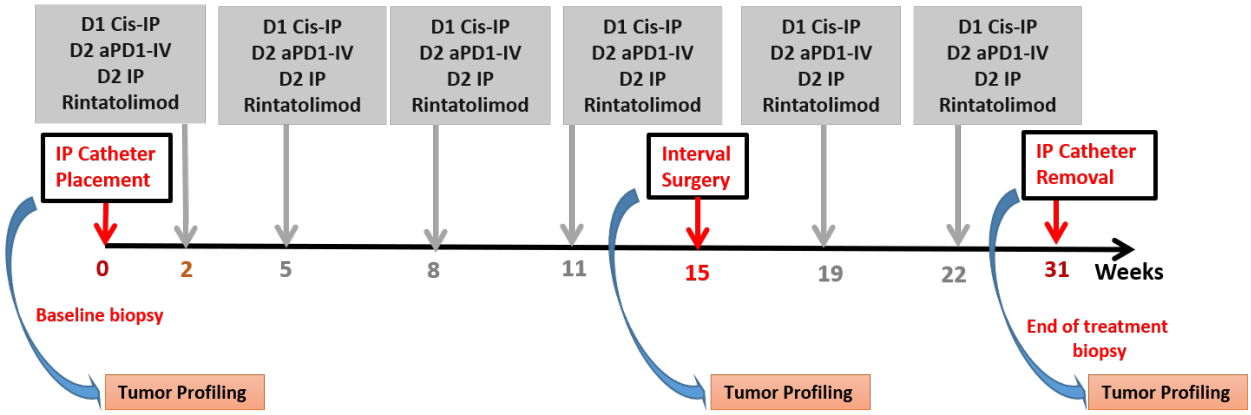
the discretion of the treating physician. Cytoreduction will occur approximately 4 weeks after the fourth treatment cycle. Post-surgery we will consolidate with 2 additional courses of same chemo-immunotherapy regimen. Catheter will be removed 12 weeks after the last treatment. All surgical procedures, if done laparoscopically, are outpatient and will yield up to three serial biopsies of the tumor sites: 1) at catheter placement; 2) at interval cytoreduction which consists of removal of any visible tumor sites and the site biopsied initially whether tumor is present or not; 3) at catheter removal, when site of first tumor biopsy will be re-biopsied for pathologic response.

We will also obtain serial IP fluid samples, to be collected each time the patient is treated IP and the day after IP therapy. Collection of tumor and IP samples are further detailed below.

Safety Endpoints

Subject safety and tolerability will be monitored continuously using Bayesian methods as detailed in statistical analysis section (Section 8).

2.2 Trial Diagram



Schema 1. Trial design and treatment schedule diagram. Horizontal line represents time in weeks. The schedule for blood, tumor tissue and IP fluid collection is detailed in the text.

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:**

This phase II trial will consist of up to 45 evaluable patients to specifically define the clinical efficacy and impact on immunologic efficacy endpoints of IP cisplatin/rintatolimod and IV pembrolizumab. Primary clinical efficacy endpoint will be objective response rate by RECIST

1.1 criteria at 13 weeks (CT imaging at 7 weeks and 13 weeks) as compared to historical controls. The trial schema encapsulates a trial design similar to our recent phase I SPORE trial (UPCI 11-128/NCT02615574, Edwards - Clinical PI; Kalinski- Translational PI) in which intraperitoneal cisplatin and rintatolimod was well tolerated and induced favorable immune response on interim analysis.

The strategy of combining pembrolizumab with IP cisplatin/rintatolimod is to induce cell death and cell damage of the peritoneal tumor implants with chemotherapy followed by locoregional and systemic modulation of innate and adaptive immune effectors to further promote immune clearance of remaining tumor.

Objective/Pathologic Response

Imaging will be performed at baseline, two weeks after cycle 2 and cycle 4 for objective responses by RECIST 1.1 criteria. Imaging will again be performed 4-6 weeks after completion adjuvant chemotherapy for baseline post treatment. Between 2 months after successful completion of the trial, the IP port will be removed laparoscopically. During the IP port removal, a second-look treatment effect evaluation and biopsy for pathologic response will be performed.

Disease Progression

Progression during trial will be assessed by iRECIST. If there is confirmed progression prior to interval surgery, patients will not undergo interval surgery, just IP port removal and biopsy after confirmed progression by iRECIST. Patients will be followed for 3 years or until recurrence after completing treatment to determine time to progression (TTP), ratio of progression-free interval (PFI) as compared to their initial PFI, and survival will be a secondary efficacy endpoint. Follow-up data will be used to estimate progression-free and overall survival at 3 years.

Biological Endpoints

We will characterize the immune response changes in each patient as measured by the total number of tumor-infiltrating CD3+CD8+ T cells (TILs) with the baseline evaluation of initial laparoscopic tumor biopsy when IP catheter is placed compared to interval surgical samples and laparoscopic biopsy at time of IP catheter removal. Changes in the frequency of T cells in the peritoneal washes at each cycle will also be evaluated. Since measurements of intra-patient changes in intra-tumoral CD8+ T cells before and after chemotherapy with chemokine modulation (in solid fraction of the tumor), as the primary endpoint of efficacy, were perceived as less reliable due to the difficulties in the longitudinal sampling of "representative" parts of tumor when comparing the original tumor biopsy to the resected tumors, and likely heterogeneous impact of chemotherapy on different parts of the tumors (resulting in the variations in immune infiltrates), such solid tumor evaluations will be performed as correlative studies and compared with the immune cell changes in the peritoneal fluid (which composition has also been shown to be predictive for the long-term outcomes in OC[1]. Frequent flushing of

the peritoneal catheter is standard of care at our institution to maintain catheter patency and assess for locoregional toxicity. It also allows collection of peritoneal samples.

Hypothesis:

We postulate that a treatment regimen that combines induction of locoregional immunogenic cell death using cisplatin and immune modulation through TLR-3 activation combined with immune checkpoint blockade via systemic anti-PD-1 which induces increased TILs and an improved anti-tumor T cell response. To test this hypothesis, we propose to:

1. Perform phase II clinical trial of combination IP cisplatin/rintatolimod chemoimmunotherapy plus IV pembrolizumab with efficacy clinical endpoints.

2. Identify the impact of combination treatment on the tumor immune microenvironment. This aim will evaluate the ability of the treatment to promote TIL accumulation, modulate biomarkers of immune exhaustion, and induce genes associated with cytotoxic anti-tumor immunity.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) Objective:

Toxicity Endpoints

In addition to clinical and immunologic efficacy, a secondary objective is further monitoring of patient safety and adverse events. Provision is made to suspend the trial if unexpected and serious toxicities occur as outlined in study procedures section. Anticipated toxicities such as renal toxicity or locoregional toxicities of IP therapy will be monitored weekly. Patients will also be closely monitored for immune related toxicities (pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, and infusion related toxicities). Dose adjustments and schedule adjustments will be made according to guidelines for key toxicity parameters, outlined below in study procedures section.

3.3 Exploratory Objective

(1) **Objective:**

Correlative Studies

This represents the laboratory portion of our project and will test the hypothesis that IP administration of cisplatin and rintatolimod will result in expansion of type 1 immunity (CTL, NK-, and Th1 cells) in the peritoneal fluid and resected residual tumor mass of OC patients, an effect further increased by anti-PD1 immune checkpoint blockade.

<u>Sample collection</u>

Blood: Up to 30cc of blood will be collected serially in purple top (K2 EDTA) tubes, in conjunction with IP washes, at catheter placement and removal, during interval cytoreduction (decision to proceed with interval cytoreduction is at the discretion of the treating physician), and at each point where the intraperitoneal catheter is accessed for IP infusion (as further detailed below). Additionally, 10 cc of blood will be collected in a cell free DNA tube at IP port placement, interval surgery, and IP port removal.

Peritoneal cells and fluid: Peritoneal cells and fluid will be collected through the IP catheter during catheter flushes, necessary to maintain patency. Collection will occur at catheter placement and removal, and each time the catheter is accessed for IP infusion.

At each time point we will collect, whenever present, any spontaneously outflowing liquid (*IP fluid*). If there is 5 mL or greater return of IP Fluid, 2 mL will be sent for cell count and differential in a purple top (K2 EDTA) tube. If the sample does not yield a return then it is not sent for cell count and differential. We will also perform a "peritoneal wash", by injecting through the catheter 50 cc of sterile saline, followed by collection of the "peritoneal wash" fluid (*IP wash*, average of 8 cc expected). The dilution factor of the harvested cells (and soluble factors) in the IP wash or IP fluid will be evaluated by measuring the albumin concentration.

We will collect IP wash/fluid at days 1 (before treatment), 2 (before IV pembrolizumab, at end of IV pembrolizumab but before IP rintatolimod begins, and at the end of IP rintatolimod) , and 3 of cycles 1-6. IP wash/fluid will also be collected at IP port placement, interval surgery and IP port removal.

Tumor tissue: Tumor biopsy (typically obtained laparoscopically) will be collected at baseline (at catheter insertion), after four treatment cycles, during cytoreductive surgery, and at time of IP port removal if feasible. The primary tumor biopsy site will be marked by a surgical clip so it can be assessed by biopsy at subsequent laparoscopic surgery.

Sample preparation

Tumors will be immediately processed for FACS and genomic profiling, or they will be dissociated and frozen viably in 10% DMSO-containing cryopreservation medium. For FACS and genomic profiling, the tumor will be minced into 1mm pieces and single cells dissociated with liberase. A portion of the tumor tissue will also be placed in formalin, followed by paraffin embedding. FFPE blocks will be used for immunohistochemistry (IHC).

IP cells and supernatants will be separately cryopreserved, until ready to use.

Using these clinical specimens, we propose to:

3.3a. Identify longitudinal phenotypic changes of immune cell subsets in the blood and tumor microenvironment (tumor tissue, IP fluid, and IP wash).

These experiments will test the hypothesis that treatment increases the accumulation of activated effector cytotoxic, granzyme B^{high} CD8 T and of CD4 $Tbet^{high}$ Th1 cells, while also monitoring the accumulation of immune suppressive cells CD4+FOXP3+ Tregs and/or

MDSCs (CD11b+CD33+DRlow/-). T cell exhaustion markers (PD1, PDL1, LAG3, TIM3, EOMES etc.) [1,2] will also be monitored in the blood, tumor, and peritoneal fluid/washes via multicolor flow cytometry. Flow cytometry assays will be performed by Dr. Vlad's lab (Co-PI) at the Magee-Womens Research Institute (MWRI) Flow Core. Anda Vlad serves as the MWRI Flow Core Director.

In parallel with T cell immunity, we will monitor changes in humoral anti-tumor immunity, focusing primarily on antibodies to MUC1 and MUC16 (CA125) tumor antigens. We postulate that increase in new IgG1 tumor antigen specific responses are indicative of Th1 immunity and positively correlate with cytotoxic anti-tumor responses [3]. Antibody responses will be tested using commercial ELISA kits, as per our previously published results [4].

Tumor immunoscore will measure in situ immune infiltrates [5], by recording the type, density, and location of immune cells within the tumor samples. Using IHC we will focus on CD3+ T cells, CD8+ cytotoxic T cells (CTL), and CD45RO+ memory T cells, which are typically associated with a longer disease-free survival.

Tissue PDL1 expression will be determined by IHC using the anti-PDL1 clone 22C3 IHC detection assay and will instruct us on the frequency of PDL1 positive cells and their tumor vs stroma distribution.

All these T cell and tumor cell biomarkers will be further validated through orthogonal approaches (NanoString, RNAseq), as routinely employed by us (Fig. 1, 4, and 5) and as further described below.

3.3b. Measure systemic and locoregional changes in immune modulatory chemokines and cytokines.

We will focus on the relative expression of effector to suppressor cytokines and chemokines, in serum/plasma and IP wash/fluid. We postulate that treatment will induce increased effector cytokines (consistent with Th1/CTL responses) and reduce secretion of suppressive chemokines. Among the effector cytokines/chemokines we will measure IFNγ and IFNγ and IFNγ–induced cytokines, like CXCL9, CXCL10, CXCL12, IL-12p70, IL-18, and IL-23. Among the suppressor cytokines, we will focus on IL-10 and TGBF-β. Selection of these candidates is guided by our preliminary data, especially from studies on rintatolimod, either alone or in combination with cisplatin (NCT02432378) and from in vivo studies in mice treated with anti-PDL1 [6]. All measurements will use ELISA arrays as previously employed by us [4,7,8].

3.3c. Perform transcriptomic analyses to identify treatment-induced changes in tumor inflammation.

The effect of therapy can be best appreciated when blood and all key components of the tumor are simultaneously examined in a longitudinal approach. In addition to the focused analyses described above (in 3.3a and 3.3b), using defined phenotypic and functional markers of immune cell subsets in blood, tumor, and IP fluid/wash, we will also perform RNAseq which will provide information of the whole tumor transcriptome, including genes used for the above

phenotypic profiles. Additional markers identified at the RNA level will be further confirmed at the protein level, via flow cytometry, IHC, Western Blot or ELISA, depending on availability of detection antibodies.

All genomic profiling assays will be guided by expertise from Adrian Lee, PhD co-investigator on this project. Bioinformatic analyses will be performed with guidance from George Tseng, PhD, Co-investigator on this project.

Note: Given the sensitivity of genomic assays and their requirement for little biological material (100ng RNA or DNA), all clinical specimens (blood, tumor, IP cells) will be processed first for RNA/DNA extraction. Remaining material will be used for live cell assays (like flow cytometry, for example), which typically require increased cell numbers.

RNA/DNA banked from these samples will be used in future ancillary genomic studies, to be separately conducted. For example, longitudinal profiling of the tumor mutanome would lead to a phylogenetic analysis of the evolution of tumor somatic mutations, and their potential for neoepitope formation. Analysis of Vβ/Jβ usage landscape (ImmunoSeq) will assess changes in T cell receptor (TCR) clonality during treatment, and whether these changes correlate with clinical outcome. Such studies have the potential to identify changes in tumor heterogeneity during combination therapy. They will also teach us if and how "cold" OC tumors (low neoantigen load and low TILs) become "inflamed" (more mutated, higher TILs) during combination therapy.

3.3d. Perform mechanistic studies in vitro in human cells and cell lines and in vivo in mice to validate effector (or resistance) mechanisms triggered by combination therapy.

These experiments are designed to further dissect in vitro and in vivo the mechanisms responsible for the positive (or negative) effect of each of the three drugs used in our clinical trial and to assess their potential synergistic (or antagonistic) interactions.

For example, our preliminary data from mouse studies and early emerging data from patients in UPCI 11-128/NCT02615574 suggests that one of the pathways triggered by both cisplatin and rintatolimod involves DDX58/RIG-I and TRAIL. In addition, we identified that cisplatin-induced PDL1 up-regulation occurs in part via an activated Kras/Mek/Erk pathway. Results from this trial will further instruct us on the involvement of these pathways which we can subsequently dissect using pharmaceutical targeting with agonists and antagonists (for TRAIL, RIG-I etc.) or using OC cell lines with constitutive or conditional mutations in several oncogenic/tumor suppressor pathways, including Kras (Table A).

	Cell line nomenclature	Originating mouse genotype	Cell line genetic traits
OSE-derived cell lines	MOSE	$MUC1^{+/-}$	Wild type Kras and Pten
	M**K**OSE	$MUC1^{+/-}$LSL-$Kras^{G12D/+}$	Silenced oncogenic KrasG12D

	M**K**OSE-AdCre	MUC1+/-LSL-Kras$^{G12D/+}$	Active oncogenic KrasG12D
	MOSE-**p53**	MUC1$^{+/-}$	CRISPR/Cas9 edited p53 loss
	M**P**OSE	MUC1$^{+/-}$Pten$^{loxP/loxP}$	Silenced Pten deletion
	M**P**OSE-AdCre	MUC1$^{+/-}$Pten$^{loxP/loxP}$	Pten deletion
	M**KP**-T	MUC1$^{+/-}$LSL-Kras$^{G12D/+}$ Pten$^{loxP/loxP}$	Kras LOH and Pten deletion
Tumor-derived cell lines	2F8 and 2F8cis	MUC1$^{+/-}$LSL-Kras$^{G12D/+}$ Pten$^{loxP/loxP}$	Kras LOH and Pten deletion
	M**KP**-Liver	MUC1$^{+/-}$LSL-Kras$^{G12D/+}$ Pten$^{loxP/loxP}$	Oncogenic KrasG12D and Pten deletion
	M**KP**-Lung	MUC1$^{+/-}$LSL-Kras$^{G12D/+}$ Pten$^{loxP/loxP}$	Oncogenic KrasG12D and Pten deletion

Table A. Genetic traits of our novel murine OC cell lines, to be used in this project. Bold letters denote genetic modifications in either Kras (K), Pten (P) or both (KP). Letter M denotes presence of MUC1 transgene. Top rows: OSE-derived cell lines, immortalized and transformed as per Roby et al [9]. MOSE-p53 cells are defective in p53, post CRISPR/Cas9 editing of MOSE cells. Bottom rows: tumor-derived cell lines, which have been passaged until stable proliferation rate was obtained. Clone 2F8 was obtained via limiting dilution of MKP-T cells [8]. Clone 2F8cis are cisplatin-resistant derivatives of 2F8 cells. Until recently, preclinical modeling of OC largely relied on two murine cell lines- ID8 and IG10- derived from ovarian surface epithelium (OSE) [9]. We (the Vlad Lab) recently developed the largest collection of new murine cell lines with well-defined genetic traits (Table A and reference [8].

In addition to cell lines derived from mice with endometrioid (Kras and Pten driven tumors- [8], we have recently generated a CRSPR/Cas9 engineered, p53-deleted OC cell line that triggers aggressive IP tumors with high grade serous histology.

Anticipated results, potential pitfalls, and alternative approaches:

Completion of this project will allow us to identify signals of efficacy based on changes in tumor dimensions between first and second surgery as measured at laparoscopy. It will test whether treatment promotes CTL infiltration into the tumor microenvironment, and measure the magnitude and duration of this effect.

We expect to see responses in platinum-sensitive disease so the nature of infiltrate in the residual tumor site will provide histologic evidence of inflammatory cell death. We will also assess the safety profile of the novel IP cisplatin/rintatolimod and IV pembrolizumab treatment combination.

We hypothesize that the dose of 200 mg of pembrolizumab, most widely used in current trials will also be well tolerated here.

We acknowledge that measurements of intra-patient changes in intra-tumoral CD8+ T cells (in the solid fraction of the tumor) before and after chemo-immunotherapy will be challenging, due to difficulties in longitudinal sampling of tumor when comparing the original biopsy to the resected tumors.

However, we note that our experience is in line with (or better than) a recent report from Tozzi et al [10], regarding increased feasibility, efficacy, and low morbidity of the laparoscopic resection during peritoneal debulking surgery. Two thirds of our patients in the UPCI 11-128/NCT02615574 study had completed interval debulking. In cases when laparoscopy is not possible, a mini-laparotomy (1-day hospital stay) will be performed.

We are also aware of the likely heterogeneous impact of chemotherapy on different parts of the tumors (resulting in variations in immune infiltrates). To mitigate this, TIL evaluations will be performed as correlative studies and compared with the immune cell changes in the peritoneal fluid tumor-associated lymphocytes, which have also been shown to be predictive for the long-term outcomes in OC [11].

In the eventuality that cellularity of peritoneal fluid is low, we will prioritize the sample processing for RNA extraction and RNAseq (instead of flow cytometry). This would allow us to measure the complete gene profiling of peritoneal resident cells and identify treatment-induced gene signatures. Based on our experience with 11-128, and given that 100 ng RNA would suffice for genomic platforms, we are confident that enough material will be obtained at each sample collection time point.

4.0 BACKGROUND & RATIONALE

4.1 Background

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator brochure.

The therapeutic advancements in OC have been partly attributed to intensified locoregional methods such as the combination of surgery and intraperitoneal (IP) infusion of chemotherapy, which remains the front-line standard of care after optimal surgery [12]. There is consistent, statistically significant evidence of the survival benefit of intraperitoneal chemotherapy [1,13] although controversies do persist, and the optimal schedule and regimen remains to be determined (Walker et al, SGO 2017- NCT01167712 /GOG 252). New immunotherapy agents targeting immune activation and modulation pathways are making major impacts on survival for several types of solid cancers, including OC [14,15]. Therefore, new treatment paradigms that focus

on how to combine IP chemotherapy with immunotherapy will allow surgeons to gain insights into how these new agents may be integrated into the current standard of care and be used strategically, in sequence or in combination. The field of immuno-oncology has experienced remarkable growth in the use of monoclonal antibodies targeting immunomodulatory signals called "immune checkpoints". These checkpoint pathways are up-regulated on tumor cells and tumor infiltrating lymphocytes (TILs). Immune checkpoint inhibitor therapies have been approved by the FDA as single agents in melanoma, squamous cell lung cancer, uroepithelial cancer, and HPV-induced cancers of the head and neck. The most effective checkpoint inhibitor class targets the programmed death-1 (PD-1) receptor and its ligands (PDL-1/2). Expression of PD-1 is found on activated TILs while the ligands (PDL-1/2) are expressed on tumor and immune suppressive cells in the microenvironment.

We propose here a new phase II clinical trial to modulate the tumor environment using IP cisplatin in combination with IP rintatolimod (Ampligen®, AIM ImmunoTech, Inc.) and systemic (IV) anti-PD-1 (aPD1, pembrolizumab, Merck).

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [16]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes 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 (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma [17,18].

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 immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [19,20].

The structure of murine PD-1 has been resolved [21]. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail 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, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [20,22-24]. The mechanism by which PD-1 down-modulates T-cell responses is

similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [25,26]. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in OC.

4.1.2 Preclinical and Clinical Trial Data and Rationale

Solid evidence to date demonstrates that the immune system plays an important role in the development and progression of OC, and can influence the disease outcome. Early studies reported that CD3+ TILs were present in 55% of OC and the presence of TILs was correlated with improved clinical prognosis [27]. BRCA1-deficient tumors exhibit higher neoantigen load and have particularly high numbers of TILs, partly explaining the superior outcome in these patients [28,29]. Improved prognosis is mostly attributable to cytotoxic CD8+ TILs, while intra-tumoral Tregs, macrophages, and immature myeloid cells appear to confer a worse prognosis [11,30]. Understanding OC as an immunogenic tumor supports efforts to harness immunotherapeutic strategies to further improve prognosis. The challenge remains to determine which patients will benefit from immunotherapies and to identify how immunotherapy should be incorporated into the OC standard of care. In addition, most of the studies targeting the tumor microenvironment have primarily focused on the primary tumor, and much less is known about recurrent disease. Despite an initial response to standard treatment with aggressive surgical debulking and platinum/taxane drug combination, ovarian tumors invariably recur and patients ultimately die of platinum-resistant disease.

This project focuses on the tumor microenvironment in recurrent disease. Our approach stems from (i) significant expertise of the Principal Investigator (PI) in IP chemotherapy and IP immunotherapy with immune modulators [31-34], (ii) our recent results from a phase I trial using cisplatin/rintatolimod combination in recurrent OC, (NCT02432378); and (iii) significant preclinical data from co-investigators on the in vivo efficacy of cisplatin/aPD1 combination in our new OC animal models [6].

<u>Intraperitoneal administration of immune modulators provides clinical benefit for OC patients.</u>

We have had a long-standing interest in using locoregional immune modulation of OC, primarily via IP administration of chemokines that support robust anti-tumor T cell immunity [33-38]. Our early studies came from a phase II clinical trial with IP recombinant IL-2, administered in weekly infusions of 6 x 10^5 IU/m2 [34]. Thirty-one subjects with platinum-resistant or platinum-refractory disease sequentially entered the study and clinical responses were surgically confirmed in 24 patients. Our results show that the IP regimen was generally well tolerated. Of the 24 patients assessed for response, there were 6 (4 complete, 2 partial) responses for an overall response rate of 25.0%. We also found significant associations between changes in CD3 counts and survival and between IFNγ-secreting CD8 T cells survival. This study provides important evidence for the use of IP IL-2 in platinum-resistant OC and identifies several immune correlates of survival. *Our current approach builds on these encouraging results using locoregional immune modulation of the tumor microenvironment*.

<u>Multiplex profiling identifies distinct local and systemic alterations during intraperitoneal chemotherapy for ovarian cancer.</u>

Despite evidence supporting IP chemotherapy, there is a poor understanding of the molecular mechanism(s) leading to the survival advantage. We focus here on sampling the tumor microenvironment to assess tumor involution through analysis of dynamic biomarkers. Throughout the years, we have conducted a number of phase I and phase II trials in which we used IP catheters to collect peritoneal fluid for pharmacokinetic and immunokinetic studies [39-41]. In our most recent study we analyzed a total of 30 whole blood, 12 peritoneal fluid (PF), and 20 peritoneal wash (PW) samples, from women enrolled in GOG 252 [32]. Samples were acquired prior to each of the first three chemotherapy cycles. Using the NanoString multiplex profiling platforms we assessed expression changes in miRNA and immune inflammatory genes isolated from systemic (blood) or locoregional (peritoneal) samples. Our results demonstrate that the first round of chemotherapy triggered fewer differentially expressed (DE) immune genes compared to the second cycle of chemotherapy and that several of the DE genes are involved in the NK signaling pathway. *Expertise with multiplex profiling (Nanostring, RNAseq, flow cytometry etc.) and biomarker analysis, gained from this and other studies will be employed in the current proposal, focused on capturing treatment-induced changes in the tumor microenvironment.*

<u>Immune checkpoint blockade alone has limited efficacy in ovarian cancer, raising the need for combination therapies</u>.

Our recent data from in vivo treatment with ICB shows that anti-PDL1 increases survival in an aggressive OC preclinical model. Using tissue from primary orthotopic tumors (triggered in conditional Cre-loxP mice) we developed a vast collection of murine OC cell lines [8]. Intraperitoneal injection of one of these cell lines (2F8) leads to numerous abdominal tumor implants, mirroring aggressive recurrent OC. Weekly IP injection of anti-PDL1 antibody prolongs survival, increases CD8 TILs, and induces expression of genes associated with anti-tumor cytotoxic immune responses in the 2F8 tumor model (Fig.1) [6]. Although significant, the in vivo ICB efficacy is not complete, raising the need for new combinatorial approaches.

Several clinical trials to date have tested the in vivo efficacy of PD1/PDL1 inhibition with blocking antibodies, including nivolumab (anti-PD1), pembrolizumab (anti-PD1) and avelumab (anti-PDL1). Overall, the efficacy reported in these OC trials has been more modest than seen in some other cancers, including melanoma, lung, and head and neck cancers. However, in all currently published trials, immune checkpoint inhibitors have been administered as monotherapy, in heavily pretreated patients with recurrent disease.

The goal in our current studies is to further increase efficacy of immune checkpoint blockade through a novel combination therapy.

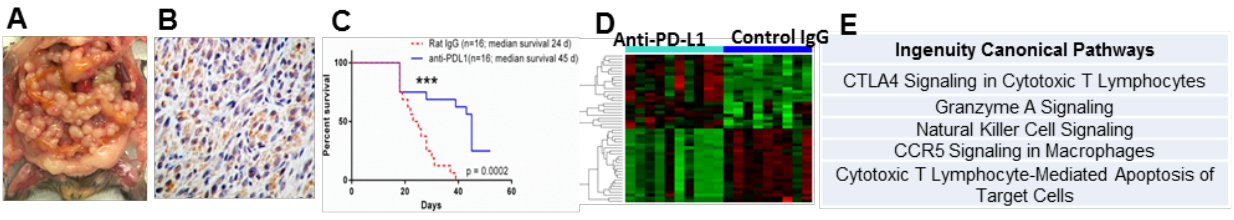


Fig. 1 In vivo efficacy of anti-PDL1 against 2F8 ovarian mouse tumors. (A). Mice challenged IP with 0.8 million 2F8 syngeneic, murine OC cells develop wide spread peritoneal carcinomatosis, mirroring late stage disease. (B) Mouse

PDL1 expression (IHC) in 2F8 tumors at necropsy. (C) Tumor-bearing mice, treated IP with 200 µg anti-PDL1 every two weeks, starting at day 21 post tumor graft (n=16 mice, median survival 45 days), have increased survival (blue line) compared to control mice (red dotted line) receiving isotype control rat IgG (n=16 mice, median survival 24 days) (p=0.0002). (D) NanoString analysis (n=511 immune gene probes) revealed differentially expressed genes induced by the PDL1 blockade compared to isotype control treated mice. (E) Top pathways triggered by PDL1, using Ingenuity Pathway analyses.

<u>Cisplatin is immunogenic, supporting the rationale for chemo-immunotherapy combination regimens</u>

Several ongoing phase II single-arm studies are evaluating chemotherapy in combination with PD-1/PD-L1 blockade (NCT02520154, NCT02520154, and NCT02766582, https://clinicaltrials.gov/). Results from these studies, all using systemic (IV) drug administration will begin to define how to best fit immune checkpoint inhibitors into the treatment of OC. *Our clinical trial is the first one to combine a locoregional (IP) chemo-immune modulation regimen with systemic anti-PD1.*

We have chosen cisplatin as the cytotoxic platform for our IP delivery system based on the fact that cisplatin has a longer dwell time in the peritoneal cavity and is more active in OC than the taxanes [42,43]. In addition, many of our patients have likely received front-line carboplatin and are thus less likely to develop hypersensitivity to secondary cisplatin (compared to re-exposure to carboplatin), particularly given the proposed combination with immunogenic agents.

Both clinical and translational studies indicate that at least part of the antitumor efficacy of platinum chemotherapeutics may be due to immune potentiating mechanisms. Nevertheless, some of the immune modulatory effects can lead to immune resistance, for example via immune checkpoint up-regulation.

We tested the effect of cisplatin on several human OC cell lines that mirror the spectrum of drug susceptibility seen in patients with recurrent disease. Platinum-sensitive (OVCA432) and moderately platinum-resistant (OVCA420) cells express low surface PDL1 at baseline. Exposure to IC50 cisplatin triggers PDL1 up-regulation in a time-dependent manner, as detected by flow cytometry and Western blot (Fig. 2.) and a similar, time and dose dependent increase in frequency of PDL1 positive cells was observed in both cell lines (data not shown). Using murine cell lines with conditional mutations in three major oncogenic/tumor suppressor pathways (p53, Kras, Pten), we established that cisplatin-induced PDL1 up-regulation occurs mostly via the Kras/Mek/Erk pathway (Grabosch, Vlad, Edwards et al; in preparation). These results demonstrate that *cisplatin triggers an innate (tumor cell-intrinsic) immune resistance mechanism and provide the rationale for combination with PD1/PDL1 blockade.*

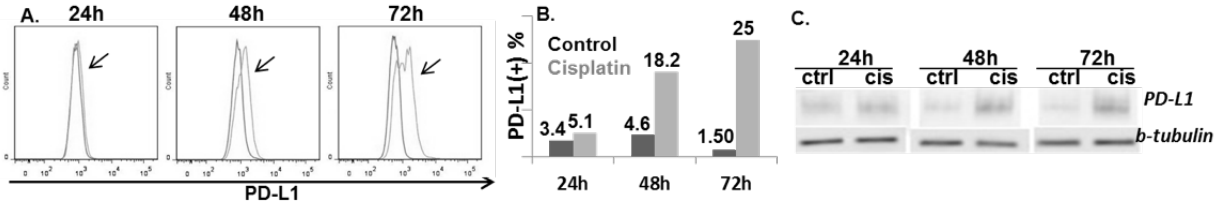


Fig. 2. Cisplatin-induced PDL1 upregulation in OVCA432 human OC cell line. (A) Cells were exposed to IC50 cisplatin in vitro and cell surface PDL1 was monitored in time, via flow cytometry. Untreated cells were used as controls. (B) Percentage of PDL1 positive cells in cisplatin treated and untreated cells, in time. Means of two independent experiments are shown. (C) PDL1 detection by Western blot.

To expand our mechanistic understanding of cisplatin-induced immune modulation, we employed new OC animal models recently developed in our lab. The 2F8cis cells have been derived from 2F8 cells via continuous exposure in vitro, to progressively increasing amounts of cisplatin, until becoming moderately resistant to cisplatin (Fig. 3). Intraperitoneal injection of 2F8 and 2F8cis triggers widespread peritoneal carcinomatosis. Similarly to OVCA432 and OVCA420, cisplatin also induces PDL1 up-regulation in 2F8 and 2F8cis cells, as detected by flow cytometry.

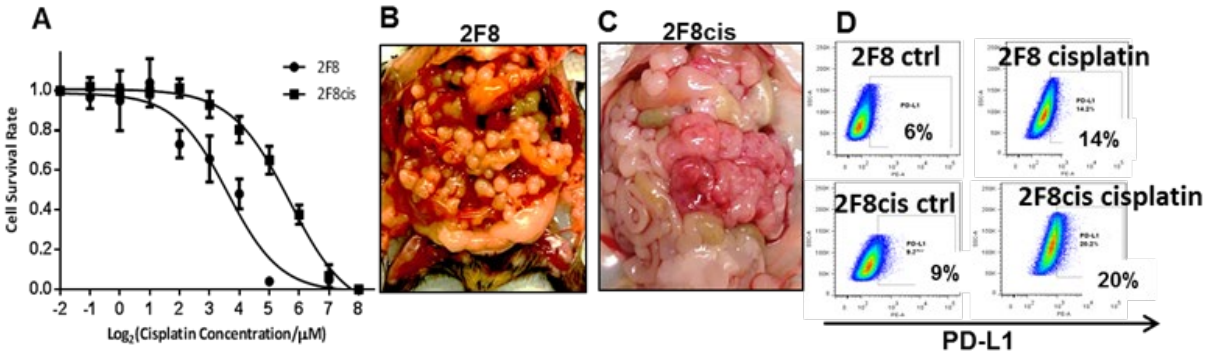


Fig. 3. (A). Cisplatin dose response curves for 2F8 and its moderately resistant derivative 2F8cis cells. (B) Both cell lines trigger aggressive peritoneal carcinomatosis in syngeneic hosts. (C) Exposure to cisplatin IC50 triggers increase in PDL1, as detected by flow cytometry.

In addition to increased tumor PDL1 expression in vitro, we observed that exposure of tumor-bearing mice to cisplatin in vivo induces an increase in tumor T cell infiltration, suggesting that tumors exposed to cisplatin become more immunogenic in vivo. To further understand this pro-inflammatory effect, we performed RNAseq on 2F8 and 2F8cis cells treated for 72h with IC50 cisplatin, and identified all differentially expressed genes. As reference, we used cells exposed to (1000 IU) IFNα, a potent immune modulator and inducer of immune genes, including PDL1 [44].

Our results demonstrate that there is a partial overlap between the cisplatin-induced and IFNα-induced DE genes in 2F8 and 2F8cis tumor cells, and that some of the common genes can contribute to tumor immunogenicity (Fig. 4B). Pathway analyses show that one of the pathways comprising the overlapping genes is the antigen presentation pathway (Fig. 4C). In line with this finding, in vitro exposure to cisplatin, triggers up-regulation and translocation at the plasma membrane of calreticulin, a pro-phagocytic "eat-me" signal [45](Fig. 4D). Additionally, cisplatin treatment also increases MHC I levels (Fig. 4E), augmenting the potential to be recognized by tumor-reactive CD8+ T cells.

Overall, these results suggest that exposure to cisplatin has dual potential: it increases tumor immunogenicity and recognition by CD8 TILs while also triggering immune evasion through PDL1 up-regulation either directly (through tumor cell-intrinsic mechanisms) or indirectly, via adaptive immune resistance due to IFNα–secreting TILs. *These findings support our rationale for the cisplatin/immune checkpoint blockade treatment combination proposed here.*

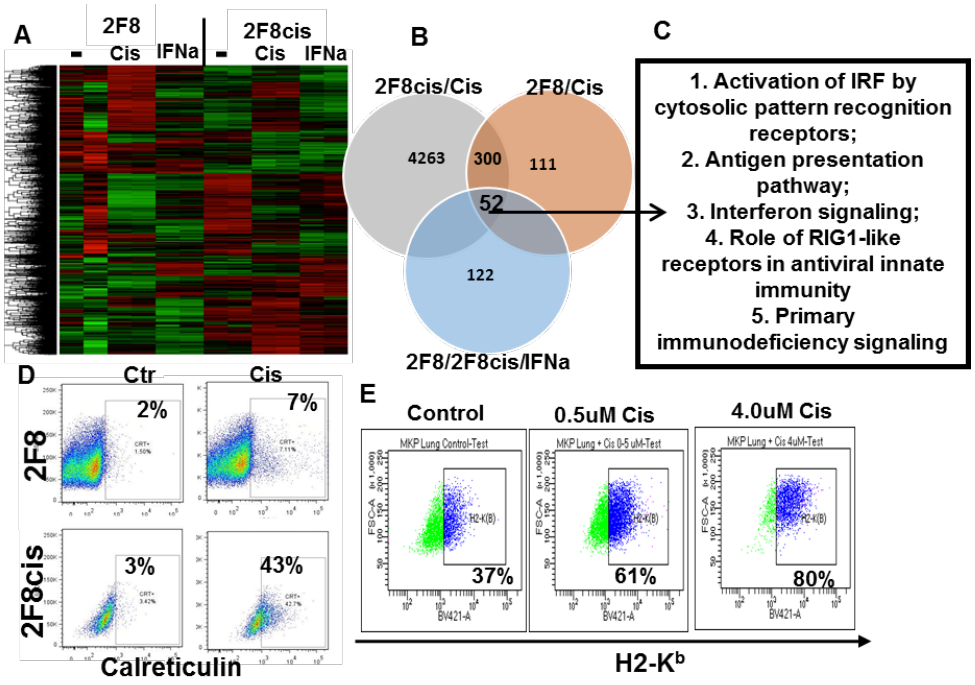


Fig. 4. Cisplatin-induced changes on immune gene expression in 2F8 and 2F8cis cells. (A) Heat map showing relative gene expression (RNAseq) in 2F8 and 2F8cis cells upon exposure for 72 h to IC50 cisplatin (Cis) or IFNα. Untreated (-) cells were kept as control. (B) All DE genes in each treatment group vs. control were identified. The circles in the Venn diagram represent DE genes in cisplatin treated vs control 2F8cis cells (grey), cisplatin treated vs control 2F8 cells (orange). The blue circle represents genes by IFNα in both 2F8 and 2F8cis. The genes common to all group comparisons (n=52, arrow) were used for Ingenuity Pathway Analysis. (C) Top 5 pathways commonly induced by cisplatin and IFNα. (D, E) Exposure to IC50 cisplatin for 72h increases calreticulin cell surface expression. (E) Dose effect of cisplatin on cell surface MHC-I expression (H2-Kb).

<u>Rintatolimod has potentiating immune modulatory properties</u>

Rintatolimod (Poly I:Poly C12U, Ampligen®) is a synthetic double-stranded ribonucleic acid (dsRNA) in which uridylic acid (U) substitution in the cytidylic chain creates a region of nonhydrogen bonding in the molecular configuration. Rintatolimod is an activator of toll-like receptor 3 (TLR3) and exerts at least 3 interrelated activities (in vivo and in vitro): 1) immunomodulatory activity, 2) antiviral activity against RNA and DNA viruses, and 3) tumor cell antiproliferative (antineoplastic) activity. Rintatolimod can directly activate 2 dsRNA-dependent enzymes, 2',5' oligoadenylate synthetase and p68 protein kinase which have been associated with antiviral and antitumor activity [46]. Clinical experience with rintatolimod in cancer therapy totals over 80 individuals. Evidence to date demonstrates that rintatolimod is generally well tolerated with no evidence of hematologic, liver, or renal toxicity noted previously with other dsRNAs. Side effects have usually consisted of occasional mild fatigue or flu-like symptoms [46].

Our preliminary results demonstrate that rintatolimod alone can act as an immune modulator by increasing MHC-I and calreticulin expression on tumor cells (data not shown, Ross et al, unpublished), supporting its suitability for combination treatment. In addition, we have shown that rintatolimod, in combination with cisplatin and IFNα, increases the CD8+ T cell density and

CD8/Treg ratio in tumor bearing mice. These results have provided the preclinical evidence for our ongoing phase I clinical trial UPCI 11-128/NCT02615574 and support its use in this project.

Rintatolimod will be provided by AIM ImmunoTech Inc., as indicated in the attached letter of authorization.

Cisplatin/rintatolimod IP combination is well tolerated in patients

Based on our preclinical results, we recently designed and implemented an investigator initiated phase I clinical trial using IP cisplatin in combination with IP rintatolimod, and IP IFN alpha in recurrent OC (UPCI 11-128/ NCT02432378, Edwards -Clinical PI; Kalinski- Translational PI). This trial, which is about to be completed, is being conducted at Magee-Womens Hospital (MWH), as part of the University of Pittsburgh Cancer Institute/ Roswell Park Cancer Institute Ovarian Cancer SPORE (NIH P50 CA159981).

The treatment regimen in the 11-128 trial combines IP cisplatin (50mg/m2) followed 24h later by IP rintatolimod (200mg) and one week later by IP IFN alpha. Patients in tier 1 received only IP cisplatin/rintatolimod. Patients in tiers 2, 3, and 4 also received IFNα at 2, 6, and 18 million units (MU), respectively. All safety cohorts additionally received celecoxib 200-400mg/day.

Our interim analysis from this small (9 patient) phase I dose escalation trial demonstrates that clinically, the cisplatin/rintatolimod combination (tier 1) was well tolerated. Local inflammatory effects are being evaluated in the biomarker analysis but some degree of fibrosis was observed.

However, following administration of IFNα (in tiers 2, 3, and 4) we have noted markedly increased, diffused fibrosis, with less impact on the tumor sites, by gross examination. Based on the observed toxicity, and given preliminary data in support of immune checkpoint blockade, we are removing here IFNα in favor of systemic anti-PD1 (IV pembrolizumab).

We have recently performed a preliminary analysis of samples isolated from two of the three patients in tier 1, who received cisplatin/rintatolimod only (no IFNα). We obtained IP wash cells via the IP catheter, as further explained below, at three consecutive time points: during cycle 2 before cisplatin infusion (C2D1), 24 h after cisplatin, but before rintatolimod infusion (C2D2), and 4 days after cisplatin (C2D4). RNA extracted from IP wash cells was used for NanoString analyses of 770 immune genes. Heatmap of DE genes shows treatment-induced changes across the three time points (Fig. 5A). Cisplatin clearly triggered up-regulation of several genes, as seen by the difference between C2D1 and C2D2. The change in most of the treatment-induced genes reverted by day 4 (C2D2 vs C2D4). Notably, many of the genes up-regulated early (between C2D1 and C2D2) overlap with those we experimentally measured by RNAseq upon cell exposure of tumor cells to cisplatin in vitro (Fig. 5B and data not shown). Although the IP wash cells obtained from patients are heterogeneous and consist of a mixture of tumor cells, fibroblast, and immune cells, many of the up-regulated immune genes (TNFSF10/TRAIL, DDX58/RIG-I, IFIT1, CXCL10) replicate the changes seen in mouse OC cells and are consistent with interferon-driven, type 1 immunity (Fig.4). We also observed evidence for genes that decrease during treatment, including MUC1 and PRAME tumor antigens [8,47-49], suggestive of anti-tumor effects.

Once completed, these studies will instruct us on the best timing for the IP sample collection protocol and allow selection of immune biomarkers.

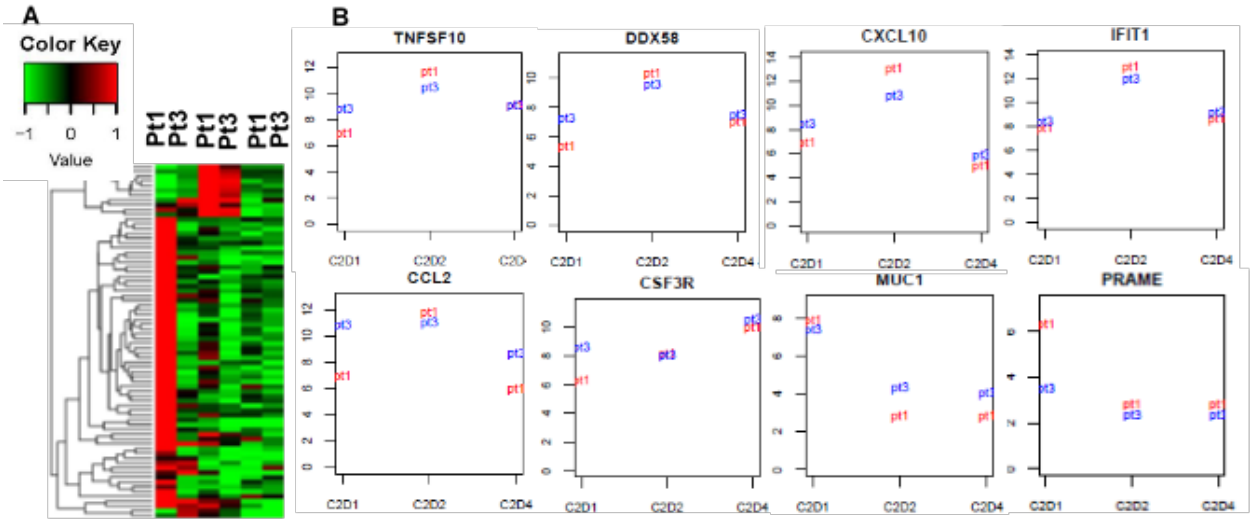


Fig. 5. Locoregional gene expression changes in two patients (Pt 1and Pt3) enrolled in the Phase 1 clinical trial UPCI 11-128/ NCT02432378. The patients were part of tier 1, IP cisplatin/rintatolimod combination. A. Heatmap showing relative gene expression of differentially expressed immune genes, in which red indicates high gene expression and green indicates low gene expression. RNA was extracted from IP wash cells from patients 1 and 3. The cells were acquired at three consecutive time points during cycle 2 (C2) of treatment: cycle, 2 day 1 (C2D1, before cisplatin infusion); cycle 2, day 2 (C2D2, one day after cisplatin, but before rintatolimod infusion); cycle 2, day 4 (C2D4, 4 days after cisplatin infusion). B. Representation of expression change over time in patient 1 (pt1, red) and patient 3 (pt3, blue) for selected differentially expressed immune genes. Y-axis is the log2 normalized counts, X-axis indicates the three time points, C2D1, C2D2, C2D4 from left to right, respectively.

In sum, our preclinical studies and recent clinical trial data suggest that IP cisplatin/rintatolimod is well tolerated and may amplify the therapeutic effects of the current standard of care.

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

4.1.3 Justification for Dose

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W),
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and

- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2262 participants were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer, and classical Hodgkin Lymphoma, confirming 200 mg Q3W as the appropriate dose independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics, and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

The planned dose of cisplatin is part of our institutional standard of care for intraperitoneal delivery of cisplatin in the recurrent setting, all patients will receive 4 neoadjuvant cycles (1 cycle = 21 days) 50 mg/m^2 cisplatin administered over 1 hour (capped at 100 mg total dose per infusion), on day 1 (Monday) of cycles 1, 2, 3, 4 and up to 2 adjuvant cycles of intraperitoneal chemotherapy.

As detailed in the above section 1.2, our phase I clinical trial using IP cisplatin in combination with IP rintatolimod, and IP IFNα in recurrent OC (UPCI 11-128/ NCT02432378, Edwards - Clinical PI; Kalinski- Translational PI) was well tolerated in the IP cisplatin and IP rintatolimod safety cohort. The addition of IP IFNα safety cohort was noted to have markedly increased, diffused fibrosis, with less impact on the tumor sites, by gross examination. Based on the observed toxicity, and given our supporting preliminary data in section 1.2, we are removing here IFNα in favor of systemic anti-PD1 (IV pembrolizumab) with IP cisplatin and IP rinatolimod. This trial,

which is about to be completed, is being conducted at Magee-Womens Hospital (MWH), as part of the University of Pittsburgh Cancer Institute/ Roswell Park Cancer Institute Ovarian Cancer SPORE (NIH P50 CA159981).

5.0 METHODOLOGY

5.1 Study Population

Patients with recurrent platinum sensitive OC.

5.1.1 Participant Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Patients must be at least 18 years of age on the day of signing informed consent.

2. Patients must have first or second peritoneal recurrence of epithelial adenocarcinoma or carcinosarcoma of ovarian, tubal or peritoneal origin:

    a. Histologic documentation of the original primary tumor is required via the pathology report.

    b. Original tumor blocks from the primary diagnosis will be requested by our study pathologist at Magee-Women's Hospital of UPMC Cancer Centers if the patient did not have their initial surgery at Magee. Original tumor blocks may be reviewed after registration (informed consent and enrollment). Tumor block should be held until study is completed.

3. Patients must have completed prior platinum-based therapy. Response can be complete or partial if it otherwise meets platinum sensitive criteria, see below.

4. Patients must be platinum-sensitive, defined as having a progression free interval (PFI) of more than 6 months (180 days) from any platinum therapy. Patients are allowed to have had other lines of therapy since last platinum if PFI after platinum therapy meets platinum sensitive criteria.

5. Patients must have measurable disease in the peritoneal cavity, measurable per RECIST 1.1 criteria:

    a. A mass with a length of 1.0 cm or greater and/or

    b. A lymph node with a length of 1.5 cm or greater in the shortest axis.

6. Patients must be a reasonable candidate for laparoscopy and IP platinum regimen with no prior evidence of clinically significant intra-abdominal adhesions, persistent abdominal wall infections, renal toxicity or bowel obstruction.

7. Patients of childbearing potential must:

    a. Have a negative pregnancy test prior to the study entry.

    b. Must discontinue breastfeeding prior to the first date of treatment on this study if applicable.

    c. Agree to follow the contraceptive guidance in Appendix 3 during the treatment period and for at least 120 days after the last dose of study treatment.

8. Patients must agree to the protocol designated clinical monitoring to receive the study regimens.

9. The participant provides written informed consent for the trial.

10. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Evaluation of ECOG is to be performed within 28 days prior to the date of allocation/randomization.

11. Have adequate organ function as defined in the following table (Table 1). Specimens must be collected within 28days prior to the start of study treatment.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/μL
Platelets	≥100 000/μL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Creatinine OR Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	

Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal. [a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks. [b] Creatinine clearance (CrCl) should be calculated per institutional standard. Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.	

5.1.2 Participant Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. A WOCBP who has a positive urine pregnancy test within 72 hours prior to infusion of treatment regimen (see Appendix 3). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. Note: in the event that 72 hours have elapsed between the screening pregnancy test and the first dose of study treatment, another pregnancy test (urine or serum) must be performed and must be negative in order for subject to start receiving study medication

2. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (e.g., CTLA-4, OX-40, CD137).

3. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks prior to allocation. Any daily oral agent requires only a 2 week washout.

    - Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy may be eligible.

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

4. Has received prior radiotherapy within 2 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.

5. Patients with previous pelvic radiation therapy.

6. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

7. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment.

    - Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

8. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug.

9. Patients with tumors of low malignant potential, except ovarian pseudomyxomas, or with no peritoneal disease.

10. Concurrent malignancy or malignancy within 3 years prior to starting study drug, with the exception of adequately treated basal or squamous cell carcinoma, non- melanomatous skin cancer or curatively resected cervical cancer or per physician discretion that the previous cancer was adequately treated with curative intent and unlikely to recur (the study PI must concur with this determination).
11. Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, i.e. without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of study treatment.

12. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.

13. Has a known allergy to cisplatin chemotherapy. Patients with carboplatin allergy may be included if they tolerate a test dose of IV cisplatin given in monitored floor conditions.

14. 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 (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

15. Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.

16. Has an active infection requiring systemic therapy.

17. Has a known history of Human Immunodeficiency Virus (HIV). Note: No HIV testing is required unless mandated by local health authority.

18. Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA qualitative is detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required unless mandated by local health authority.

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

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

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

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

Inclusion of Women and Minorities

All patients on this trial are women. The University of Pittsburgh will not exclude potential patients from participating in this or any study solely on the basis of ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire epithelial OC population treated.

Subject Entry and Registration

When a suitable candidate has been obtained for protocol entry, the following steps will be taken:

An approved consent form and authorization permitting release of personal health information must be signed by the patient or guardian. Current FDA, NCI, and institutional regulations concerning informed consent will be followed.

All eligibility requirements indicated in Section 5 must be satisfied.

Subject Eligibility Checklist must be completed.

5.1.3 Lifestyle Restrictions

No restrictions. The patient must be able to comply with trial visits/testing.

5.1.3.1 Meals and Dietary Restrictions

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

5.1.3.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Refer to Appendix 3 for approved methods of contraception.

5.1.4 Pregnancy

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab, the participant will be immediately discontinued from study treatment. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Merck within 2 working days if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck.

5.1.5 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, participants who are breast-feeding are not eligible for enrollment.

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	Use
Cisplatin	50mg/m²	Q3W	Intraperitoneal	Day 1 of each 3 week cycle	Standard of care
Rintatolimod	200mg	Q3W	Intraperitoneal	Day 2 of each 3 week cycle	Experimental

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 2 of each 3 week cycle	Experimental

Intraperitoneal Chemotherapy (Institutional Standard of Care)

Catheter placement and diagnostic biopsy

All patients, as part of the clinical care for their disease, will have IP catheters (such as 14-French Bard fenestrated catheter with a subcutaneous reservoir) implanted to allow neoadjuvant and adjuvant chemotherapy and will undergo laparoscopic surgery to obtain tumor biopsies, place the catheter, assess the suitability for IP therapies, and obtain base line peritoneal washes using the same procedure as will be used during IP therapy described below.

The tumor biopsy will be used to confirm tumor histology. If multiple tumor sites exist, multiple samples may be harvested and disease distribution will be noted in the operative note.

The catheter will be subject to standard catheter care based on institutional guidelines.

5.2.1 Timing of Dose Administration

Trial treatment should be administered on day 1 and 2 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Cisplatin administration

As a part of our institutional standard of care, all patients will receive 4 neoadjuvant cycles (1 cycle = 21 days) 50 mg/m^2 cisplatin administered over 1 hour (capped at 100 mg total dose per infusion), on day 1 (Monday) of cycles 1, 2, 3, and 4, and up to 2 adjuvant cycles of IPC.

Prehydration for cisplatin

Five hundred to one thousand milliliters of 0.9% sodium chloride will be given IV to prehydrate prior to infusion of cisplatin IP. Post IPC hydration with an additional liter of 0.9% sodium chloride will also occur.

Premedications for cisplatin

Benadryl 50mg IV on Day 1

Palonosetron (Aloxi) 0.25 mg IV X 1 dose

Fosaprepitant (Emend) 150 mg IV in Total Volume 150 mL 0.9% sodium chloride to infuse over 20 minutes

Note: The above premedications may be adjusted at the investigator/sub-investigator's discretion based upon institutional guidelines and the patient's medical status.

Intraperitoneal cisplatin administration

Cisplatin is generally given into an established, semi-permanent access device implanted with a catheter attached to a port in the peritoneal cavity. Five hundred milliliters to one liter of cisplatin containing fluid is infused per institutional standards, no less than 45 minutes, followed by an additional 500 to 1000 mL of 0.9% sodium chloride, where feasible, to a total of 2000 mL IP ensuring adequate distribution of the fluid. Ideally, a total of two liters of fluid is instilled into the abdominal cavity if tolerated.

Cisplatin will be administered on day 1 (Monday) of each 21-day cycle by IP infusion at 50 mg/m^2 over 1 hour. Each patient will receive 4 neoadjuvant cycles and 2 adjuvant cycles.

No attempt will be made to retrieve the infusate. However, if a large amount of ascites is present, ascites may be drained by paracentesis or accessed by port prior to instillation of the drug.

Post hydration after cisplatin

One liter of 0.9% sodium chloride will be given intravenously. If fluid overload is of concern, patient may be treated with Lasix or Mannitol as needed to maintain urine output.

Pembrolizumab administration

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion on day 2 (Tuesday) of cycles 1-4 of neoadjuvant chemotherapy and adjuvant cycles 5-6.

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.

Rintatolimod administration

Similar to our other phase I protocol (SPORE trial of IPCKM dosage determination, NCT02615574, Edwards - Clinical PI; Kalinski- Translational PI), all patients will receive IP rintatolimod for 4 neoadjuvant cycles (1-4), 200 mg administered over 1-2 hours, and 2 adjuvant cycles (cycles 5-6).

Rintatolimod will be administered into an established, semi-permanent access device implanted with a catheter attached to a port in the peritoneal cavity 30 minutes after pembrolizumab. Administered on day 2 (Tuesday) of each cycle by IP infusion at 200mg over 1-2 hours. Each patient will receive infusion for cycles 1-4 of neoadjuvant chemotherapy and adjuvant cycles 5-6.

5.2.2. Dose Modifications and Toxicity Management

The following toxicities, if they occur during Cycle 1 and are felt to be related to the protocol treatment, are considered to be dose-limiting toxicities (DLT) and are based on the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0:

a) Any death not clearly due to the underlying disease or extraneous causes
b) Any AE leading to a dose delay of greater than 14 days in initiation of cycle 2 will be considered a DLT
c) A DLT is specifically defined as any of the following toxicities that occur related to any of the study interventions (cisplatin, rintatolimod, pembrolizumab) or the combination (as assessed by the Investigator) that occurs during the first cycle of treatment:
    1. Grade 3 renal toxicity
    2. Grade 2 or greater bronchospasm
    3. Grade 2 or greater allergic reaction or generalized urticaria
    4. Grade 2 or greater autoimmune reaction
    5. Grade 3 injection site reactions
    6. Grade 3 anaphylaxis
    7. Grade 2 pneumonitis
    8. ≥ Grade 3 hematologic toxicities lasting more than 48 hours, including neutropenia, thrombocytopenia and hemorrhage
    9. ≥ Grade 3 non-hematologic toxicities
    10. Grade 3 neurological symptoms
    11. Fever > 41°C, uncontrolled for over 4 hours in the absence of a medical cause
    12. Grade 3 diarrhea, Grade 3 skin toxicity, or Grade 3 liver function test increase
    13. Evaluation of liver toxicity as defined by Hy's Law:
        - The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST
        - Total Bilirubin>2xULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
        - No other reason can be found to explain the combination of increased ALT/AST and total bilirubin

A Dose Modification and toxicity management <u>for immune-related AEs associated with pembrolizumab</u>

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

Table 3 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Study intervention must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last study intervention treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever)

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
	Recurrent Grade 3 or Grade 4	Permanently discontinue		and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
	associated with evidence of β-cell failure		• Administer antihyperglycemic in participants with hyperglycemia	
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal [d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed. [e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).				

Dose modification and toxicity management of infusion-reactions related to pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4 Pembrolizumab Infusion Reaction Dose modification and 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 participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** 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 participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 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 participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of Pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).

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: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

Dose modification of cisplatin

All patients who discontinue treatment early, whether due to progression, non-compliance, or toxicity, will be asked to participate in a follow-up visit to monitor their safety. Subjects reporting mild or moderate adverse events (CTCAE v5 grades 1-2) after the first or second cycle may continue on the protocol therapy as long as the adverse event has resolved to ≤ Grade 1 by the time they are due for their next cycle. In case of repetitive mild to moderate adverse effects observed in a patient, at the discretion of the Investigator/Sub-investigator, the next adjuvant dose of IP treatment can be reduced by one step in each consecutive cycle of adjuvant treatment, compared to the intended/initially tolerated dose. Cisplatin dose reduction may be maintained at the modified dose for subsequent cycles at the discretion of the treating physician and Principal Investigator. Cisplatin dose reduction is at the discretion of the PI and treating physician for clear cisplatin induced toxicity and dose adjustment. Treatment is at the discretion of the PI and treating physician. Cisplatin dosing may be reduced 20% for renal function impairment based on an increase of serum creatinine greater than 20% from baseline. Additional increases in creatinine that persists at the next cycle will require removal from study.

Dose modification of rintatolimod

There will be no dose adjustment of rintatolimod as no specific toxicity has been identified to date.

5.3 Randomization or Treatment Allocation

Single arm phase II study, no randomization

5.4 Stratification

No planned 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 final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician.

5.5.1 Acceptable Concomitant Medications

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

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

5.5.2 Prohibited Concomitant Medications

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the Investigator considers necessary for a participant's welfare may be

administered at the discretion of the Investigator in keeping with the community standards of medical care.

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation from study therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician. However, the decision to continue the participant on study treatment requires the mutual agreement of the investigator, the Sponsor, and the participant.

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

5.5.3 Rescue Medications & Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 5.2.2, [Table 3]. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to [Table 3] in Section 5.2.2 for guidelines regarding dose modification and supportive care.

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

5.6 Participant Withdrawal/Discontinuation Criteria

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 7.1.4 – Other Procedures

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment
- Confirmed radiographic disease progression outlined in Section 7.1.2.6
- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment
- Significant side effects (such as persistent fever [≥ 101°F (38.3°C) beyond 48 hours after study treatment], catheter infection, or bowel obstruction). Note: Subjects with loss of peritoneal catheter function may choose to have the catheter replaced with up to a 4-week treatment delay
- Unacceptable adverse experiences as described in Section 5.2.2.
- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, placed the participant at unnecessary risk from continued administration of study treatment.
- The participant has a confirmed positive serum pregnancy test
- Noncompliance with study treatment or procedure requirements
- Recurrent Grade 2 pneumonitis
- Discontinuation of treatment may be considered for participants who have attained a confirmed complete response (CR) and have been treated for at least 6 cycles (at least 22 weeks), receiving at least 2 cycles of the combination including 2 doses of pembrolizumab and at least 80% of the planned doses of IP Cisplatin/IP Rintatolimod beyond the date when the initial CR was declared.
- The participant is lost to follow-up
- Administrative reasons

5.7 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 participants
4. Plans to modify or discontinue the development of the study drug

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

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Parameter/ Week	**Pre-Treatment[A]**	**2**	**5**	**7**	**8**	**11**	**13**	**15-16**	**19**	**22**	Safety Follow-up	**31[H] IP Cath removal**	**Survival Follow-Up**
Cycle		**1**	**2**		**3**	**4**			**5**	**6**	**4-6 weeks Post Treatment End**	**End of Study**	**Every 12 weeks (+/- 4)**
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Baseline Pregnancy Test – see Appendix 3	X												
Baseline TSH, T3/T4	X												
Demographics & Medical History	X												
Prior and Commitment Medication Review	X	X	X		X	X	X		X	X	X		
Full Physical Examination [B]	X						X[#]						
Directed Physical Examination		X[I]	X		X	X			X	X	X		
Vital signs[C]	X	X	X		X	X	X[#]		X	X	X		
Height and Weight	X												
ECOG Performance Status	X	X[I]	X		X	X	X[#]		X	X	X		

Parameter/ Week	**Pre-Treatment[A]**	**2**	**5**	**7**	**8**	**11**	**13**	**15-16**	**19**	**22**	Safety Follow-up	**31[H] IP Cath removal**	**Survival Follow-Up**
Cycle		**1**	**2**		**3**	**4**			**5**	**6**	**4-6 weeks Post Treatment End**	**End of Study**	**Every 12 weeks (+/- 4)**
Adverse Events			X		X	X	X[#]		X	X	X		
CBC with differential, Mag, Phos, LDH, uric acid, CA-125, and CMP[D,]	X	X[I]	X		X	X	X		X	X	X		
Urine analysis	X	X	X		X	X	X		X	X	X		
PT/INR and aPTT	X												
EKG	X	X[I]											
Tumor size CT imaging	X*			X*			X*				X		X*
IP catheter and tumor biopsy	X											X	
Tumor debulking								X					
Cisplatin (IP)		X	X		X	X			X	X			
IP Rintatolimod		X	X		X	X			X	X			
IV Pembrolizumab		X	X		X	X			X	X			
Peritoneal washes [E]	X[G]	X	X		X	X		X	X	X	X	X	

Parameter/ Week	**Pre-Treatment[A]**	**2**	**5**	**7**	**8**	**11**	**13**	**15-16**	**19**	**22**	Safety Follow-up	**31[H] IP Cath removal**	**Survival Follow-Up**
Cycle		**1**	**2**		**3**	**4**			**5**	**6**	**4-6 weeks Post Treatment End**	**End of Study**	**Every 12 weeks (+/- 4)**
Blood (30-40 cc) drawn for in vitro assays [F]	X	X	X		X	X		X	X	X	X	X	
Survival Status **													X

A. 28 day screening period to C1D1 unless otherwise noted.

B: Physical examination will be performed by the attending physician or fellow/APP (advanced practice providers).

C: Vital signs (temperature, blood pressure, pulse rate, and respiratory rate) will be taken prior to the administration of each dose of trial treatment.

D: Glucose, BUN, creatinine, sodium, potassium, chloride, CO_2, calcium, total protein, albumin, alkaline phosphatase, AST, ALT, total bilirubin,

E: Peritoneal washes will be collected before IP cisplatin (day 1), before IV pembrolizumab (day 2), before IP rintatolimod (after pembrolizumab, day 2), 1 hour after IP rintatolimod (day 2), the day after IV pembrolizumab/IP rintatolimod (day 3), and day of surgery (peritoneal flushes assist in maintaining peritoneal catheter patency). 2ml sample will be sent for cell count and differential on days 1, 2, 3, at the time of port placement, interval debulking, and port removal if there is greater than 5mL return. Cell count and differential will be collected in a purple top tube.

F: Blood samples (30cc) will be collected within 2 hours (+/- 15min) before IP cisplatin, before IV pembrolizumab, the day after IV pembrolizumab/IP rintatolimod and on day of surgery. For blood collected at procedures (IP port placement, interval surgery, and IP port removal) an additional 10cc will be collected in cell free DNA tubes. Research blood samples are to be collected in a purple top tube.

G: Post-op visit after IP port placement. IP ports are routinely checked for leaking by infusing 500 mL into the port. An attempt to retrieve IP fluid/ ascites will be attempted at each visit.

H: Patients will be considered off study at catheter removal. If catheter is not removed, then patients will be off study following the day 3 catheter wash after the last adjuvant cycle of chemotherapy.

I: C1D1 labs, and EKG do not need to be repeated if done within 28 days of C1D1. For subsequent cycles, labs/testing should within 2 business days.

Activities will occur at this time point, prior to debulking surgery.

* Imaging will be performed as a part of standard care, after cycle 2 and 4 (week 7 and 13) and 4-6 weeks after completion of therapy, which is a CT imaging of chest/abdomen/pelvis with IV/PO contrast.

** Patients will be followed for survival for a period of until death, withdrawal of consent, or the end of the trial, whichever occurs first. This follow up may be done by medical record or phone.

After placement of IP port, ideal start time is days 1-14 after port placement with no more than 21 day window unless wound infection or complications precluding start of treatment. To be approved by PI. Additionally, there is a window of ± 3 days available for scheduling/rescheduling the remainder of treatments and/or procedures at the discretion of the Sub-investigator, and as discussed with the Investigator if a course is missed or a subject's treatment and/or testing day(s) need to be rescheduled due to the subject's inability to comply with the study calendar (this includes but is not limited to: hospitalizations, business, vacation plans, travel from long distances for study treatment, in advance of the scheduled date to allow ready access to the result(s), reduce financial burden on the subject [i.e. non-UPMC insurance coverage] or reduce travel inconvenience, illness, transportation issues, holidays, family emergencies, etc.).

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

Given lack of information regarding safety and tolerability of this combination regimen, the first 3 patients should be staggered (second patient should not be able to begin treatment until 3 weeks after the first patient has begun the treatment, i.e. completed cycle 1, similarly third patient should not begin treatment until second patient has completed cycle 1). Moreover, patients should be observed for 3 weeks for DLTs

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to participant 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 participant. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

Consent must be documented by the participant's dated signature or by the participant'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 participant before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the participant must receive the IRB/ERC's approval/favorable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant'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 participant's dated signature or by the participant'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 regulations and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

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

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the participant during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a participant 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 participant will move into survival follow-up.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each participant to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (see Appendix 2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

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

7.1.2.2 Full Physical Exam

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

7.1.2.3 Directed Physical Exam

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

7.1.2.4 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.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Appendix 1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

Tumor imaging is strongly preferred to be acquired by computed tomography (CT). For the abdomen and pelvis, contrast-enhanced magnetic resonance imaging (MRI) may be used when CT with iodinated contrast is contraindicated, or when local practice mandates it. MRI is the strongly preferred modality for imaging the brain. The same imaging technique regarding modality, ideally the same scanner, and the use of contrast should be used in a participant throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the assessment of response or progression based on imaging.

7.1.2.6.1 Initial Tumor Imaging

Initial tumor imaging will be performed as part of routine clinical management and must be performed within 28 days prior to the date of IP port placement. Routine imaging includes oral and IV contrasted CT of the chest/abdomen/pelvis. The site study team must review screening images to confirm the participant has measurable disease per RECIST 1.1.

7.1.2.6.1 Tumor Imaging During the Study

The first on-study imaging assessment should be performed at 7 weeks (49 days ±7 days) from the IP port placement, again at 13 weeks (91 days ±7 days) prior to interval surgery, after 4-6 weeks after treatment completion. Subsequent tumor imaging should be performed every 12 weeks (84 days ±7 days) or more frequently if clinically indicated. After 31 weeks (245 days ±7 days), participants who remain on treatment will have imaging performed every 12 weeks (84 days ±7 days). Imaging timing should follow calendar days and should not be adjusted for delays in cycle starts. Imaging should continue to be performed until disease progression is identified by the Investigator.

Objective response should be confirmed by a repeat imaging assessment. Tumor imaging to confirm PR or CR should be performed at least 4 weeks after the first indication of a response is observed. Participants will then return to regular scheduled imaging every 12 weeks, starting with the next scheduled imaging time point. Participants who receive additional imaging for confirmation do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point.

Per iRECIST (Section 9.2.1.6), disease progression should be confirmed by the site 4 to 8 weeks after first radiologic evidence of PD in clinically stable participants. Participants who have unconfirmed disease progression may continue on treatment at the discretion of the Investigator until progression is confirmed by the site provided they have met the conditions detailed in Section 9.2.1.6. Participants who receive confirmatory imaging do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point, if clinically stable. Participants who have confirmed disease progression by iRECIST, as assessed by the site, will discontinue study treatment. Exceptions are detailed in Section 9.2.1.6.

7.1.2.6.2 End of Treatment and Follow-up Tumor Imaging

In participants who discontinue study treatment, tumor imaging should be performed at the time of treatment discontinuation (±4-week window). If previous imaging was obtained within 4 weeks prior to the date of discontinuation, then imaging at treatment discontinuation is not mandatory. In participants who discontinue study treatment due to documented disease progression and the Investigator elects not to implement iRECIST, this is the final required tumor imaging.

In participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using the same imaging schedule used while on treatment (every 12 weeks in Year 1 or every 12 weeks after Year 1) to monitor disease status until the start of a new anticancer treatment, disease progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.2.6.3 RECIST 1.1 Assessment of Disease

RECIST 1.1 will be used as the primary measure for assessment of tumor response, date of disease progression, and as a basis for all protocol guidelines related to disease status (eg, discontinuation of study treatment). Although RECIST 1.1 references a maximum of 5 target lesions in total and 2 per organ, the Sponsor allows a maximum of 10 target lesions in total and 5 per organ, if clinically relevant to enable a broader sampling of tumor burden.

7.1.2.6.4 iRECIST Assessment of Disease

iRECIST is based on RECIST 1.1, but adapted to account for the unique tumor response seen with immunotherapeutic drugs. When clinically stable, participants should not be discontinued until progression is confirmed by the Investigator, working with local radiology, according to the rules below. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some participants can have a transient tumor flare in the first few months after the start of immunotherapy, and then experience subsequent disease response.

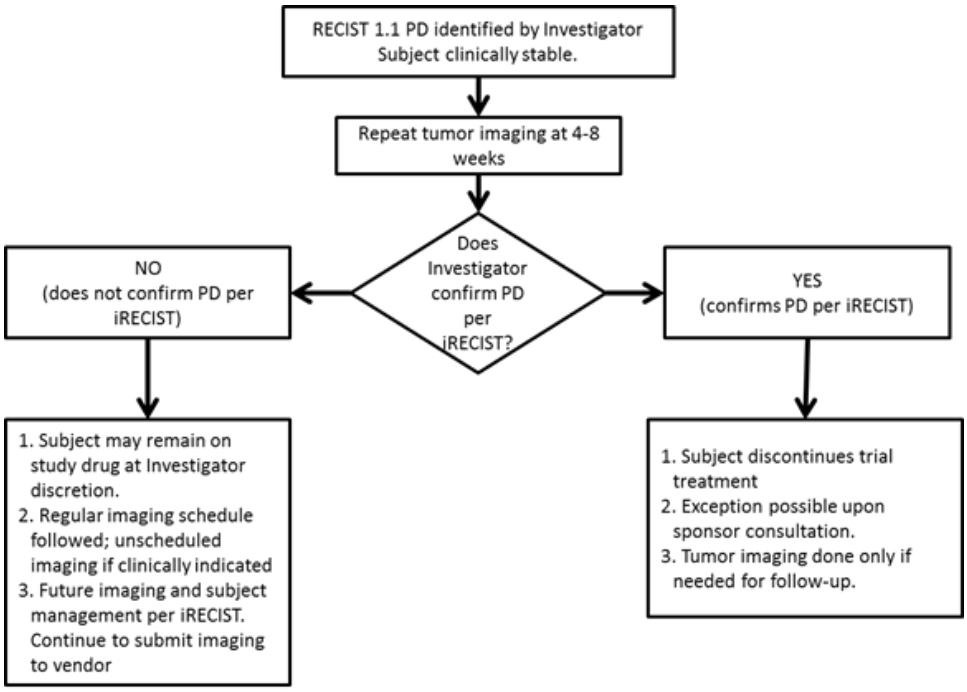
A description of the adaptations and iRECIST process is provided in Appendix 4, with additional detail in the iRECIST publication [50]. iRECIST will be used by the Investigator to assess tumor response or progression, and make treatment decisions to remove or continue on protocol.

Table 5 Imaging and Treatment after First Radiologic Evidence of Progressive Disease

	Clinically Stable		**Clinically Unstable**	
	Imaging	**Treatment**	**Imaging**	**Treatment**
First radiologic evidence of PD by RECIST 1.1	Repeat imaging at 4 to 8 weeks to confirm PD.	May continue study treatment at the Investigator's discretion while awaiting confirmatory tumor imaging by site by iRECIST.	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only.	Discontinue treatment
Repeat tumor imaging confirms PD (iCPD) by iRECIST per Investigator assessment	No additional imaging required.	Discontinue treatment (exception is possible upon consultation with Sponsor).	No additional imaging required.	Not applicable
Repeat tumor imaging shows iUPD by iRECIST per Investigator assessment	Repeat imaging at 4 to 8 weeks to confirm PD. May occur at next regularly scheduled imaging visit.	Continue study treatment at the Investigator's discretion.	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only.	Discontinue treatment
Repeat tumor imaging shows iSD, iPR, or iCR by iRECIST per Investigator assessment.	Continue regularly scheduled imaging assessments.	Continue study treatment at the Investigator's discretion.	Continue regularly scheduled imaging assessments.	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion. Next tumor image should occur according to the regular imaging schedule.

iCPD = iRECIST confirmed progressive disease; iCR = iRECIST complete response; iRECIST = modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics; iSD = iRECIST stable disease; iUPD = iRECIST unconfirmed progressive disease; PD = progressive disease; RECIST 1.1 = Response Evaluation Criteria in Solid Tumors 1.1..

Figure 1: Imaging and Treatment for Clinically Stable Participants after First Radiologic Evidence of PD Assessed by the Investigator



7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Sample collection

Blood: Up to 30 cc of blood will be collected serially, in conjunction with IP washes, at catheter placement and removal, during interval cytoreduction (if performed at the discretion of the treating physician), and at each point where the intraperitoneal catheter is accessed for IP infusion (as further detailed below).

Peritoneal cells and fluid: Peritoneal cells and fluid will be collected through the IP catheter during catheter flushes, necessary to maintain patency. Collection will occur at catheter placement and removal, and each time the catheter is accessed for IP infusion.

At each time point we will collect, whenever present, any spontaneously outflowing liquid (*IP fluid*). We will also perform a "peritoneal wash", by injecting through the catheter 50 cc of sterile saline, followed by collection of the "peritoneal wash" fluid (*IP wash*, average of 8 cc expected). The dilution factor of the harvested cells (and soluble factors) in the IP wash or IP fluid will be evaluated by measuring the albumin concentration.

We will collect IP wash/fluid at days 1, 2, and 3 of cycles 1-6.

Tumor tissue: Tumor biopsy (typically obtained laparoscopically) will be collected at baseline (at catheter insertion) and again after four treatment cycles, and at time of IP port removal if feasible. The primary tumor biopsy site will be marked by a surgical clip so it can be assessed by biopsy at subsequent laparoscopic surgery.

7.1.3 Laboratory Procedures/Assessments

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

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 6.

Table 6 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	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	T4
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		
	Creatinine		
	CA-125		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in your region.

Laboratory tests for screening should be performed within 28 days prior to the first dose of treatment. 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.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a participant discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. After discontinuing treatment following assessment of CR, these participants should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.3.2).

7.1.4.2 Blinding/Unblinding

Unblinded

7.1.5 Visit Requirements

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

7.1.5.1 Screening

Patients will be screened by the research staff with approval by PI.

7.1.5.2 Treatment Period

After placement of IP port and start of cycle 1 Day 1 until removal at 31 weeks.

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 4-6 weeks after the last dose of study treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Participants with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-cancer therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

7.1.5.3.2 Follow-up Visits

Participants who discontinue study treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (84 ± 14 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-cancer therapy, disease progression, death, end of the study. Information regarding post-study anti-cancer treatment will be collected if new treatment is initiated.

7.1.5.3.3 Survival Follow-up

Participants who experience confirmed disease progression or start a new anticancer therapy, will move into the Survival Follow-Up Phase and will be obtained from the medical record or contacted by telephone every 12 weeks (+/- 4 weeks) to assess for survival status until death, withdrawal of consent, or the end of the trial, whichever occurs first.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation participant 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.

All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event cause the

participant to be excluded from the study, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

● All AEs from the time of treatment allocation/randomization through 30 days following cessation of study treatment must be collected by the investigator.

● All AEs meeting serious criteria, from the time of treatment allocation/randomization through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be recorded by the investigator.

● All pregnancies and exposure during breastfeeding, from the time of treatment allocation/randomization through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.

● Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify Merck.

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

For purposes of this study, 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. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") 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 to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

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

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

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

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

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

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

7.2.3.1 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 another important medical event

- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Refer to Table 7 for additional details regarding each of the above criteria.

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

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to Merck Global Safety.

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

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

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

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates

new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, 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.

7.2.4 Evaluating Adverse Events

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

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 7 Evaluating Adverse Events

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

V5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the participant, 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 participant taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days..	

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

7.2.5 Sponsor Responsibility for Reporting Adverse Events

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

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

Objectives

This trial will evaluate the anti-tumor efficacy of pembrolizumab in conjunction with IP cisplatin/rintatolimod. In addition to immunologic efficacy, a second objective is further monitoring of patient safety and adverse events. Provision is made to suspend the trial if unexpected and serious toxicities occur or if dose limiting toxicities (DLTs) from the list below in excess of 30% are observed:

a) Any death not clearly due to the underlying disease or extraneous causes
b) Any AE leading to a dose delay of greater than 14 days in initiation of cycle 2 will be considered a DLT
c) A DLT is specifically defined as any of the following toxicities that occur related to any of the study interventions (cisplatin, rintatolimod, pembrolizumab) or the combination (as assessed by the Investigator) that occurs during the first cycle of treatment:
    1. Grade 3 renal toxicity
    2. Grade 2 or greater bronchospasm
    3. Grade 2 or greater allergic reaction or generalized urticaria
    4. Grade 2 or greater autoimmune reaction
    5. Grade 3 injection site reactions
    6. Grade 3 anaphylaxis
    7. Grade 2 pneumonitis
    8. ≥ Grade 3 hematologic toxicities lasting more than 48 hours, including neutropenia, thrombocytopenia and hemorrhage
    9. ≥ Grade 3 non-hematologic toxicities
    10. Grade 3 neurological symptoms
    11. Fever > 41°C, uncontrolled for over 4 hours in the absence of a medical cause
    12. Grade 3 diarrhea, Grade 3 skin toxicity, or Grade 3 liver function test increase
    13. Evaluation of liver toxicity as defined by Hy's Law:
        - The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST
        - Total Bilirubin>2xULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

Efficacy Study Design and Endpoints

The primary clinical endpoint is objective response. A secondary, translational endpoint is increase in the CD8 TIL infiltration in tumor and/or IP washes (pre-treatment vs post-treatment),

The primary endpoint is the objective response rate (rate of CR+PR by RECIST 1.1) at 13 weeks. Based on Ten Tije and Wils et al, the intent-to-treat objective response rate for IP cisplatin alone in platinum-sensitive recurrent ovarian cancer was approximately 45% [51]. An increase from 45% to 65% among response-evaluable patients would be of sufficient interest to warrant further study of the addition of rintatolimod and pembrolizumab in the presence of an acceptable safety profile. An optimal Simon two stage design with $\alpha = \beta = .10$ has been selected that will require 20 patients in the first stage and 25 in the second. In the first stage, 12 patients must have a response (PR or CR) in order to continue to the second stage. If the trial continues to the second stage and accrues 25 more patients and there are 25 or more patients with an objective response among the total of 45 treated, the study will be considered successful at demonstrating increase efficacy compared to cisplatin alone. The probability of early termination if the response rate is 45% is .59.

Continuous Monitoring of RLTs during the Study

Subject safety will be monitored continuously using Bayesian methods. If the posterior probability is .80 or greater that 30% or more of treated subjects experience a treatment related RLT as defined above, the RLT rate would be unacceptable and the study will be suspended pending review by the DSMC. This posterior probability will be calculated from the study's accumulating data and a weakly informative prior distribution. If π denotes a random variable representing the proportion of subjects who will experience a treatment related RLT, we assume π has a beta distribution with parameters a = 1.2 and b = 2.8 which assumes a mean of 30%. The table below describes the number of RLTs per number of treated patients that would trigger suspension. The table also shows the posterior probability that the RLT rate exceeds 33% and the binomial probability associated with the decision for an assumed 33% RLT rate.

Table 8: Number of RLTs per Number of Patients Treated Needed to Trigger Suspension

Subjects	*Treatment-Related RLTs*	*PP(π > 30%)**	*Pr($X \geq r \mid p = .30$)*
6	*4*	*.903*	*.070*
9	*5*	*.904*	*.099*
12	*6*	*.890*	*.118*
15	*7*	*.880*	*.131*
17	*8*	*.903*	*.105*
20	*9*	*.896*	*.113*
23	*10*	*.891*	*.197*
26	*11*	*.886*	*.125*
29	*12*	*.883*	*.129*
32	*13*	*.880*	*.133*

35	*14*	*.877*	*.135*
38	*15*	*.876*	*.137*
41	*16*	*.874*	*.138*
44	*17*	*.873*	*.139*

**π is the RLT rate. The minimum acceptable upper bound of a treatment-related RLT is 30%. PP(π > 30%) is the posterior probability that the RLT rate exceeds this 30% upper bound. This posterior probability of an RLT is calculated from the prior distribution, the number of subjects treated and the observed number of treatment-related RLTs.*

Using these assumptions, the trial will be suspended if:

4 subjects experience treatment related RLT among the first 6 subjects enrolled or,

5 subjects experience treatment related RLT among the first 9 subjects enrolled or,

6 subjects experience treatment related RLT among the first 12 enrolled etc.

8.2 Statistical Analysis Plan

Proposed Data Analysis Primary Objectives

Objective response rate and progression-free based on RECIST 1.1 criteria (RC) will be estimated with 90% confidence intervals. The objective response rate (ORR) will be estimated by the proportion of subjects with the best response of complete response (CR), or partial response (PR) by RC, with corresponding exact 90% confidence limits being reported.

Proposed Data Analysis Secondary Analysis

The progression-free survival (PFS) will be measured from the initial date of the study treatment to the date of documented tumor progression using RC, or the date of death in the absence of tumor progression. PFS will be estimated by the Kaplan-Meier method with Greenwood 90% confidence intervals

Within-patient changes in CD8+ cells will be measured in tumor tissue and in peritoneal fluid at weeks 1,8, and 12. Mixed linear models will be used to estimate changes from baseline.

An assessment of the association among immune parameters, and ORR will be conducted. Endpoints for analysis include (CTL, NK- and Th1 cells) in the peritoneal fluid and resected residual tumor mass The panel of immune parameters will be tested for association with objective response with the Jonckheere-Terpstra test for trend by ordering response 1-4 for PD, SD, PR and CR, respectively. Significant association will be adjusted for initial tumor grade, and the interval between initial diagnosis of OC and protocol accrual. To guard against false positive results, the estimated false discovery rate will be calculated by the method of Benjamini and Hochberg (J. R. Statist. Soc. B Vol 57, No 1, 289-300, 1995).

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

Pembrolizumab will be provided by Merck as summarized in Table 8.

Table 8 Product Descriptions

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

9.2 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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

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

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

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the participants 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.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Quality Control and Quality Assurance

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

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

10.2 Data Handling and Record-Keeping

Investigators must enter the information required by the protocol on to the protocol specific case Report Forms (CRFs) which will be stored in the site's electronic database. Only investigators and clinical research staff will have access to this data base which is locked.

The investigator (i.e., the study site principal investigator) will maintain records in accordance with Good Clinical Practice. All research files will be retained in a secured locked facility for the maximum period required following termination of the research by the IRB.

10.3 Institutional Review Board (IRB) Approval

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

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

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

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Investigator's decision to modify the previously accepted clinical protocol:

- for a Phase 1 clinical study: The Sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to the Phase 1 clinical protocol that significantly affects the safety of the subjects. For changes that do not affect critical safety assessments, the revisions to the clinical protocol will be addressed in the Annual Report to the IND.

- for Phase 2 and 3 clinical studies: The Sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to a Phase 2 or Phase 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of Phase 2 and 3 clinical protocol changes requiring the submission of a Protocol Amendment include:

  - O Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.

  - O Any significant change in the design of the protocol (such as the addition or deletion of a control group).

  - O The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor the safety of the investigational drug.

10.3.1 Ethical and Scientific Conduct of the Clinical Study

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

Any change or addition to this protocol requires a written protocol amendment that must be reviewed by Merck and the investigator. IRB approval is required before implementation a copy of the written approval of the IRB must be provided to Merck.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Merck in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Merck must be notified and the IRB at the center must be informed immediately.

10.4 Subject Informed Consent

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

The investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled patients are adequately addressed and that the patients are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the investigator will obtain the informed consent of enrolled patients for continued participation in the clinical study.

11.0 REFERENCES

1. Alberts DS, Markman M, Armstrong D, et al: Intraperitoneal therapy for stage III ovarian cancer: a therapy whose time has come! Journal of clinical oncology : official journal of the American Society of Clinical Oncology 20:3944-6, 2002
2. Wherry EJ, Kurachi M: Molecular and cellular insights into T cell exhaustion. Nature reviews. Immunology 15:486-99, 2015
3. Luborsky JL, Barua A, Shatavi SV, et al: Anti-tumor antibodies in ovarian cancer. Am J Reprod Immunol 54:55-62, 2005
4. Budiu RA, Mantia-Smaldone G, Elishaev E, et al: Soluble MUC1 and serum MUC1-specific antibodies are potential prognostic biomarkers for platinum-resistant ovarian cancer. Cancer immunology, immunotherapy : CII 60:975-84, 2011
5. Mlecnik B, Bindea G, Angell HK, et al: Integrative Analyses of Colorectal Cancer Show Immunoscore Is a Stronger Predictor of Patient Survival Than Microsatellite Instability. Immunity 44:698-711, 2016
6. Mony JT, Zhang L, Ma T, et al: Anti-PD-L1 prolongs survival and triggers T cell but not humoral anti-tumor immune responses in a human MUC1-expressing preclinical ovarian cancer model. Cancer immunology, immunotherapy : CII 64:1095-108, 2015
7. Budiu RA, Elishaev E, Brozick J, et al: Immunobiology of human mucin 1 in a preclinical ovarian tumor model. Oncogene 32:3664-75, 2013
8. Zhang L, Ma T, Brozick J, et al: Effects of Kras activation and Pten deletion alone or in combination on MUC1 biology and epithelial-to-mesenchymal transition in ovarian cancer. Oncogene 35:5010-20, 2016
9. Roby KF, Taylor CC, Sweetwood JP, et al: Development of a syngeneic mouse model for events related to ovarian cancer. Carcinogenesis 21:585-91, 2000
10. Tozzi R, Gubbala K, Majd HS, et al: Interval Laparoscopic En-Bloc Resection of the Pelvis (L-EnBRP) in patients with stage IIIC-IV ovarian cancer: Description of the technique and surgical outcomes. Gynecologic oncology 142:477-83, 2016
11. Curiel TJ, Coukos G, Zou L, et al: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 10:942-9, 2004
12. Bowtell DD, Bohm S, Ahmed AA, et al: Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nature reviews. Cancer 15:668-79, 2015
13. Armstrong DK, Bundy B, Wenzel L, et al: Intraperitoneal cisplatin and paclitaxel in ovarian cancer. The New England journal of medicine 354:34-43, 2006
14. Emens LA, Ascierto PA, Darcy PK, et al: Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape. European Journal of Cancer 81:116-129
15. Suryawanshi S, Vlad AM, Lin HM, et al: Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 19:1213-24, 2013

16. Disis ML: Immune regulation of cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 28:4531-8, 2010

17. Dudley ME, Wunderlich JR, Yang JC, et al: Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 23:2346-57, 2005

18. Hunder NN, Wallen H, Cao J, et al: Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. The New England journal of medicine 358:2698-703, 2008

19. Greenwald RJ, Freeman GJ, Sharpe AH: The B7 family revisited. Annual review of immunology 23:515-48, 2005

20. Okazaki T, Maeda A, Nishimura H, et al: PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proceedings of the National Academy of Sciences of the United States of America 98:13866-71, 2001

21. Zhang X, Schwartz JC, Guo X, et al: Structural and functional analysis of the costimulatory receptor programmed death-1. Immunity 20:337-47, 2004

22. Chemnitz JM, Parry RV, Nichols KE, et al: SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. Journal of immunology 173:945-54, 2004

23. Riley JL: PD-1 signaling in primary T cells. Immunological reviews 229:114-25, 2009

24. Sheppard KA, Fitz LJ, Lee JM, et al: PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS letters 574:37-41, 2004

25. Francisco LM, Sage PT, Sharpe AH: The PD-1 pathway in tolerance and autoimmunity. Immunological reviews 236:219-42, 2010

26. Parry RV, Chemnitz JM, Frauwirth KA, et al: CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Molecular and cellular biology 25:9543-53, 2005

27. Zhang L, Conejo-Garcia JR, Katsaros D, et al: Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. The New England journal of medicine 348:203-13, 2003

28. Clarke B, Tinker AV, Lee CH, et al: Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. Mod Pathol 22:393-402, 2009

29. McAlpine JN, Porter H, Kobel M, et al: BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. Mod Pathol 25:740-750, 2012

30. Cui TX, Kryczek I, Zhao L, et al: Myeloid derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. Immunity 39:10.1016/j.immuni.2013.08.025, 2013

31. Edwards RP, Gooding W, Lembersky BC, et al: Comparison of toxicity and survival following intraperitoneal recombinant interleukin-2 for persistent ovarian cancer after

platinum: twenty-four-hour versus 7-day infusion. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 15:3399-407, 1997

32. Grabosch S, Tseng G, Edwards RP, et al: Multiplex profiling identifies distinct local and systemic alterations during intraperitoneal chemotherapy for ovarian cancer: An NRG Oncology/Gynecologic Oncology Group Study. Gynecologic oncology 146:137-145, 2017

33. Mantia-Smaldone GM, Edwards RP, Vlad AM: Targeted treatment of recurrent platinum-resistant ovarian cancer: current and emerging therapies. Cancer management and research 3:25-38, 2011

34. Vlad AM, Budiu RA, Lenzner DE, et al: A phase II trial of intraperitoneal interleukin-2 in patients with platinum-resistant or platinum-refractory ovarian cancer. Cancer immunology, immunotherapy : CII 59:293-301, 2010

35. Kuss I, Rabinowich H, Gooding W, et al: Expression of zeta in T cells prior to interleukin-2 therapy as a predictor of response and survival in patients with ovarian carcinoma. Cancer Biother Radiopharm 17:631-40, 2002

36. Lenzi R, Edwards R, June C, et al: Phase II study of intraperitoneal recombinant interleukin-12 (rhIL-12) in patients with peritoneal carcinomatosis (residual disease < 1 cm) associated with ovarian cancer or primary peritoneal carcinoma. J Transl Med 5:66, 2007

37. Taylor DD, Edwards RP, Case CR, et al: Modulation of CD3-zeta as a marker of clinical response to IL-2 therapy in ovarian cancer patients. Gynecologic oncology 94:54-60, 2004

38. Wong JL, Berk E, Edwards RP, et al: IL-18-primed helper NK cells collaborate with dendritic cells to promote recruitment of effector CD8+ T cells to the tumor microenvironment. Cancer Res 73:4653-62, 2013

39. Lesnock JL, Richard SD, Zorn KK, et al: Completion of intraperitoneal chemotherapy in advanced ovarian cancer and catheter-related complications. Gynecologic oncology 116:345-50, 2010

40. Naumann RW, Sukumvanich P, Edwards RP: Practice patterns of intraperitoneal chemotherapy in women with ovarian cancer. Gynecologic oncology 114:37-41, 2009

41. Richard SD, Sukumvanich P, Lesnock JL, et al: Intraperitoneal catheter leads to prolongation of the time to normalization of serum CA125 levels. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 20:932-5, 2010

42. Feldman AL, Libutti SK, Pingpank JF, et al: Analysis of factors associated with outcome in patients with malignant peritoneal mesothelioma undergoing surgical debulking and intraperitoneal chemotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 21:4560-7, 2003

43. Goodman MD, McPartland S, Detelich D, et al: Chemotherapy for intraperitoneal use: a review of hyperthermic intraperitoneal chemotherapy and early post-operative intraperitoneal chemotherapy. J Gastrointest Oncol 7:45-57, 2016

44. Parker BS, Rautela J, Hertzog PJ: Antitumour actions of interferons: implications for cancer therapy. Nature reviews. Cancer 16:131-44, 2016

45. Feng M, Chen JY, Weissman-Tsukamoto R, et al: Macrophages eat cancer cells using their own calreticulin as a guide: Roles of TLR and Btk. Proceedings of the National Academy of Sciences 112:2145-2150, 2015

46. Mitchell WM: Efficacy of rintatolimod in the treatment of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Expert Rev Clin Pharmacol 9:755-70, 2016

47. Salmaninejad A, Zamani MR, Pourvahedi M, et al: Cancer/Testis Antigens: Expression, Regulation, Tumor Invasion, and Use in Immunotherapy of Cancers. Immunol Invest 45:619-40, 2016

48. Vlad AM, Finn OJ: Glycoprotein tumor antigens for immunotherapy of breast cancer. Breast disease 20:73-9, 2004

49. Vlad AM, Kettel JC, Alajez NM, et al: MUC1 immunobiology: from discovery to clinical applications. Advances in immunology 82:249-93, 2004

50. Seymour L, Bogaerts J, Perrone A, et al: iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. The Lancet. Oncology 18:e143-e152, 2017

51. ten Tije BJ, Wils J: Intraperitoneal cisplatin in the treatment of refractory or recurrent advanced ovarian carcinoma. Oncology 49:442-4, 1992

12.0 APPENDICES

Appendix 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.

Appendix 2: Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

Appendix 3: Contraceptive Guidance and Pregnancy Testing.

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 10 during the protocol-defined time frame in Section X.

Table 10 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen- containing) hormonal contraception [b, c] ○ Oral

○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception [b, c] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen- only contraceptive implant [b, c] ● Intrauterine hormone-releasing system (IUS) [b] ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e. when used consistently and correctly). b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least 120 after the last dose of study treatment. c) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test.

Following initiation of treatment, pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected; at the time points specified in the Schedule of Activities, and as required locally.

Pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.

Appendix 4: Description of the iRECIST Process for Assessment of Disease Progression

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

In participants who show evidence of radiological PD by RECIST 1.1 the Investigator will decide whether to continue a participant on study treatment until repeat imaging is obtained (using iRECIST for participant management (see Table 5 and Figures 1 and 3). This decision by the Investigator should be based on the participant's overall clinical condition.

Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia, radiation, or other palliative care

Any participant deemed clinically unstable should be discontinued from study treatment at site-assessed first radiologic evidence of PD, and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the Investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirm PD by iRECIST, per Investigator assessment. I

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥ 20% and ≥ 5 mm from nadir
    - Please note: the iRECIST publication uses the terminology "sum of measurements", but "sum of diameters" will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including iUPD (unconfirmed progressive disease) and iCPD (confirmed progressive disease). For purposes of iRECIST assessment, the first visit showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated, and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the participant will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening
    - For target lesions, worsening is a further increase in the sum of diameters of ≥ 5 mm, compared to any prior iUPD time point
    - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
    - For new lesions, worsening is any of these:
        - An increase in the new lesion sum of diameters by ≥ 5 mm from a prior iUPD time point
        - Visible growth of new non-target lesions
        - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND
- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the scan on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation scan proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Imaging

If repeat imaging does not confirm PD per iRECIST, as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, participants will be discontinued from study treatment.

NOTE: If a participant has confirmed radiographic progression (iCPD) as defined above, but the participant is achieving a clinically meaningful benefit, an exception to continue study treatment may be considered. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 6.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (ie, achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions
    - Sum of diameters reaches the PD threshold (≥ 20% and ≥ 5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudo-progression.
- Non-target lesions

  - If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.
  - If non-target lesions had shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.

- New lesions
  - New lesions appear for the first time
  - Additional new lesions appear
  - Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
  - Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, except in one respect. If new lesions occurred at a prior instance of iUPD, and at the confirmatory scan the burden of new lesions has increased from its smallest value (for new target lesions, their sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication [50].