TITLE: A Phase II Evaluation of Pembrolizumab in Combination with IV Bevacizumab and Oral Metronomic Cyclophosphamide in the Treatment of Recurrent Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer

Roswell Park Cancer Institute **NCT#02853318**

Study Number: **I 270715**

Initial Date: **06 November 2015**

Amendment#1: **20 July 2016**

Amendment#2: **25 August 2016**

Amendment #3: **01 August 2017**

Amendment #4: **19 December 2017**

Amendment #5: **22 March 2019**

Amendment #6: **25 April 2019**

Amendment #7: **03 October 2019**

IND Holder: **Roswell Park Cancer Institute**

Principal Investigator: **Emese Zsiros, MD PhD**
Roswell Park Cancer Institute
Elm & Carlton Streets
Buffalo, New York 14263
716-845-8337
Emese.Zsiros@RoswellPark.org

Sponsor : **Roswell Park Cancer Institute**

Industry/Other Supporter: **Merck & Co., Inc.**

Confidentiality Statement

Study Contacts

Name	Institution	Email	Role	Protocol Section
Junko Matsuzaki, PhD	Roswell Park Cancer Institute, Buffalo, NY	Junko.Matsuzaki@RoswellPark.org	Immune Analysis Facility Director	8.8.1 8.9.1,
Anthony Miliotto	Roswell Park Cancer Institute, Buffalo, NY	Anthony.Miliotto@RoswellPark.org	Senior Research Associate	8.8.3
	QualTek Molecular Laboratories, Newtown, PA	MISPsamples@qmlabs.com	IHC for PD-L1 expression	8.11.1
Correlative Sciences Pathology Office (CSPO)	Roswell Park Cancer Institute, Buffalo, NY	Carol.Siegel@RoswellPark.org	Sample storage	8.9.1, 8.9.2
Warren Davis, PhD	Roswell Park Cancer Institute, Buffalo, NY	Warren.Davis@RoswellPark.org	Microbiome sample processing and storage	8.10.2

SYNOPSIS

Title / Phase	A Phase II Evaluation of Pembrolizumab in Combination with IV Bevacizumab and Oral Metronomic Cyclophosphamide in the Treatment of Recurrent Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer.
Roswell Park Cancer Institute Study Number	I 270715
Roswell Park Cancer Institute Investigator	Emese Zsiros, MD PhD
Sponsor	Roswell Park Cancer Institute
Industry/ Other Supporter	Merck & Co., Inc.
Study Drug(s)	Pembrolizumab Bevacizumab Cyclophosphamide
Objectives	**Primary:** • To evaluate improvement in progression-free survival for patients treated with anti-PD1 pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide as compared to a historical control. **Secondary:** • To obtain pilot data on clinical response rates using both RECIST1.1 criteria (Response Evaluation Criteria in Solid Tumors) and immune related response criteria (irRECIST). • To obtain data on changes in tumor microenvironment prior to and subsequent to therapy and, to screen for potential biomarkers to predict clinical benefit • To determine the safety and tolerability of the treatment combination in the study population. • To evaluate overall survival in patients treated with anti-PD1 pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide. • To assess the impact of the combination of anti-PD1 pembrolizumab, IV bevacizumab and oral metronomic cyclophosphamide on anti-tumor immune responses in ovarian cancer.
Study Design	This is an open label Phase II study (with a safety lead-in cohort of n=5) of anti-PD1 antibody pembrolizumab, in combination with IV bevacizumab and oral metronomic cyclophosphamide in patients with recurrent platinum sensitive, resistant or refractory epithelial ovarian, fallopian tube, or primary peritoneal cancer.
Target Accrual and Study Duration	The study will enroll approximately 40 evaluable patients in total. Accrual is expected to take 18 months.
Study Procedures	**Disease Evaluation:** CT at Baseline, after 3rd cycle, after 6th cycle then, after every 6th cycle (or sooner if disease progression) **Adverse Events:** Day 1 of each cycle, end of treatment, safety follow-up.

	Hematology: Baseline, Day 1 of each cycle, end of treatment, safety follow-up **Chemistry:** Baseline, Day 1 of each cycle, end of treatment, safety follow-up **Performance Status:** Baseline, Day 1 of each cycle, end of treatment, post treatment evaluations **Full Physical Examination (including basic neurological examination, vital signs, and body weight):** Baseline, Day 1 Cycle 1, Day 1 of every 3rd cycle, end of treatment, post treatment evaluations **Directed Physical Examination (including basic neurological exam, vital signs, and body weight):** On cycles not requiring a full physical examination (e.g. cycles 2 and 3, cycles 5 and 6) **Vital Signs:** Baseline, Day 1 of each cycle, end of treatment, post treatment evaluations **Pregnancy test**: Within 72 hours prior to receiving first dose of study treatment in patients of child bearing potential **PT/INR and APTT:** Baseline, Cycle 6 Day 1, Cycle 12 Day 1, end of treatment, safety follow-up. **Urinalysis:** Baseline, Cycle 1Day 1, every 2nd cycle after Cycle 1, end of treatment. **UPCR Ratio:** Baseline, Cycle 1Day 1 and, Day 1 of every 2nd cycle. **T3, FT4, and TSH:** Baseline, Cycle 3 Day 1 then Day 1 of every 3rd cycle, end of treatment, safety follow-up **Tissue Biopsy:** Baseline, 2-3 weeks after Cycle 3, end of treatment (optional) **Blood Collection (correlative studies):** Baseline (within 2 weeks prior to treatment initiation), Cycle 2 – Day 1, Cycle 4- Day 1, Cycle 7- Day 1, Cycle 10 –Day 1, and at the end of study treatment or at time of discontinuation (within two weeks after the last treatment cycle is completed) or, at the time of disease progression or for any other reason that requires study treatment termination, whichever occurs sooner. On those patients, who have already completed 10 cycles, the one additional correlative blood sample will be collected at the time of their next cycle. **Human Microbiome collection (correlative studies):** Stool, vaginal and skin samples with correlative questionnaires prior to cycle#1 (within 7 days of starting treatment, preferably on Day 1 of cycle 1 prior to staring chemotherapy), after 3 cycles (within 7 days of starting cycle 4, preferably on Day 1 of cycle 4 prior to starting chemotherapy), Cycle 10 –Day 1 (within 7 days of starting cycle 10, preferably on Day 1 of cycle 10 prior to starting chemotherapy) and at the end of treatment (during the last physical exam at the

	time of study termination, stool sample collection within 7 days of this visit) On those patients, who have already completed 10 cycles, the one additional microbiome sample will be collected at the time of their next cycle. **Study Questionnaires:** • Microbiome Initial Assessment Questionnaire: Cycle 1-Day 1 • Microbiome Collection Questionnaire: Cycle 1-Day 1, Cycle 4-Day 1, Cycle 10 –Day 1 and End of Treatment (or time of discontinuation) • EORTC QLQ-C30 and EORTC QLQ-OV28: Cycle 1-Day 1, Cycle 4-Day 1, Cycle 7 – Day 1, Cycle 10 –Day 1, End of Treatment (or time of discontinuation) • Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF): Cycle 1-Day 1, Cycle 4-Day 1, Cycle 7 – Day 1, Cycle 10 –Day 1, End of Treatment (or time of discontinuation) • On those patients, who have already completed 10 cycles, the additional correlative questionnaires will be collected at the time of their next cycle. **Dermatology:** Recommended skin check at baseline, after Cycle 3 and at end of study treatment – optional for patients
Statistical Analysis	**Sample Size Determination:** The *primary objective* of the Phase II study is to evaluate the progression-free survival (PFS) for patients treated with pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide in women with recurrent/persistent platinum –sensitive, -resistant, or -refractory ovarian, primary peritoneal, or fallopian tube cancers. The median PFS of platinum resistant ovarian cancer patients treated with standard chemotherapy is approximately 3.5 months. In a recent study platinum-resistant ovarian cancer patients treated with a combination of IV bevacizumab and oral metronomic cyclophosphamide had a median PFS and overall survival (OS) of 4.5 and 10.7 months, respectively. In another study, patients with recurrent ovarian cancer treated with bevacizumab plus low-dose metronomic oral cyclophosphamide had a median response duration of 3.9 months. Therefore we would consider a PFS of 7 months or greater to be of interest in this population. Consider a sample size of n=40 patients, with up to 1 year of follow-up, and a maximum drop-out rate of 10%. If the true 7-month PFS rate is 50% (a median PFS of 7 months) for the proposed treatment combination, then a one-sided Wald-type test has approximately an 86.1% chance of resulting in significance. A nominal significance level of 10% is considered. **Safety Analysis:** The frequency of toxicities will be tabulated by grade across all dose levels and cycles. Toxicity rates will be estimated using 90% confidence intervals, which will be obtained

	using Jeffreys prior method. All participants who receive any study treatment will be considered evaluable for toxicity. The overall and progression free survival will be summarized using standard Kaplan-Meier methods, with estimates of median survival and given survival rates will be obtained with 90% confidence intervals. **Safety Lead-in Cohort:** A safety lead-in cohort of n_1=5 patients will enrolled and the safety/tolerability of the combination will be examined after each patient completes at least 3 cycles. If 2 or less patients have experienced a drug related toxicity requiring drug/treatment delay or suspension (as defined in Sections 6.4, 6.5, and 6.7.3), then an additional n_2=35 patients are enrolled to complete the Phase II study. Otherwise, the study will be suspended and the research team will meet to discuss possible safety concerns and will decide what actions to take with respect to study continuation. **Efficacy Analysis**: The overall and progression free survival will be summarized using standard Kaplan-Meier methods, with estimates of median survival and given survival rates will be obtained with 90% confidence intervals. Objective tumor response will be tabulated for all patients that completed at least three cycles of the proposed treatment combination. The response rate will be estimated using 90% confidence intervals, obtained using Jeffrey's prior method. **Correlative Data Analysis:** The blood, tumor, stool, vaginal and skin microbiome samples will be collected as biomarkers and will be reported using the appropriate descriptive statistics depending on clinical outcome. Associations between these markers and PFS will be evaluated using Cox regression models; while associations with tumor response will be evaluated using logistic regression models.

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Participant Name: _____

Medical Record No.: _____

Title: A Phase II Evaluation of Pembrolizumab in Combination with IV Bevacizumab and Oral Metronomic Cyclophosphamide in the Treatment of Recurrent Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Age ≥ 18 years of age on day of signing informed consent	
☐	☐	☐	2. Have an ECOG Performance Status of 0 or 1 (Refer to Appendix A)	
☐	☐	☐	3. Have measurable disease per RECIST 1.1 or irRECIST criteria present.	
☐	☐	☐	4. Participant may have serous, endometrioid, clear cell, mucinous or undifferentiated type of recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer. o Histologic confirmation of the original primary tumor is required via the pathology report.	
☐	☐	☐	5. Participant can be either platinum-sensitive (platinum free interval (PFI) ≥ 6 months prior to recent recurrence) or platinum-resistant (PFI < 6 months prior to recent recurrence. If the participant has a platinum sensitive disease, she may only enroll in this clinical trial if there is a contraindication for her to receive further treatment with platinum-based chemotherapy (such as serious persistent toxicity or severe hypersensitivity to platinum agents or she declines standard of care).	
☐	☐	☐	6. Participant must be willing to undergo core or excisional biopsy of a tumor lesion within 4 weeks (28 days) prior to initiation of treatment on Day 1 and, after 3 cycles of study treatment. Participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Principal Investigator.	
☐	☐	☐	7. Have the following clinical laboratory values: • Absolute neutrophil count (ANC): ≥ 1,500 /mcL • Platelets: ≥ 100,000 / mcL • Hemoglobin: ≥ 9 g/dL or 5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment) • Serum creatinine: ≤1.5 X upper limit of normal (ULN) OR measured or calculated creatinine clearance ≥ 60 mL/min for participant with creatinine levels > 1.5 X institutional ULN (refer to Appendix B). GFR can also be used in place of creatinine or CrCl. • Urine Protein Creatine Ratio (UPCR) < 1 prior to starting cycle 1 (refer to Appendix C). • Serum total bilirubin: ≤ 1.5 X ULN OR direct bilirubin ≤ ULN for participants with total bilirubin levels > 1.5 ULN	

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
			• AST (SGOT) and ALT (SGPT): ≤ 2.5 X ULN OR ≤ 5 X ULN for participants with liver metastases • Albumin: > 2.5 mg/dL • International Normalized Ratio (INR) or Prothrombin Time (PT): ≤1.5 unless participant is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants • Activated Partial Thromboplastin Time (aPTT): ≤1.5 X ULN unless participant is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants	
☐	☐	☐	8. Participants of childbearing potential must have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.	
☐	☐	☐	9. Participants of childbearing potential must be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (participants of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year). Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.	
☐	☐	☐	10. Participant has recovered from toxicities of prior chemotherapy or other therapy (to grade 2 or less).	
☐	☐	☐	11. Participant may have received prior investigational therapy (including immune therapy).	
☐	☐	☐	12. Participant may have received prior hormonal therapy.	
☐	☐	☐	13. Participant may have received bevacizumab (or other antiangiogenic agent) and/or cyclophosphamide in the past.	
☐	☐	☐	14. Participant has had at least 4 weeks of postoperative recovery from surgery prior to enrollment to ensure complete wound healing. Participants with bowel resections at surgery should begin protocol at least 42 days after surgery.	
☐	☐	☐	15. Ability to swallow and retain oral medication.	
☐	☐	☐	16. Participant or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Participant meets all entry criteria: ☐ **Yes** ☐ **No**
If "NO", do not enroll participant in study.

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Participant Name: _____

Medical Record No.: _____

Title: A Phase II Evaluation of Pembrolizumab in Combination with IV Bevacizumab and Oral Metronomic Cyclophosphamide in the Treatment of Recurrent Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer

EXCLUSION CRITERIA				
No	**Yes**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.	
☐	☐	☐	2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment or, is taking any other medication that might affect immune function.	
☐	☐	☐	3. Has a known history of active TB (Bacillus Tuberculosis).	
☐	☐	☐	4. Hypersensitivity to bevacizumab, cyclophosphamide, pembrolizumab or any of its excipients.	
☐	☐	☐	5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.	
☐	☐	☐	6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent. **Note**: Participants with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study. **Note**: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy and, has to be at least 28 days after the surgery.	
☐	☐	☐	7. Has a known additional malignancy that is progressing or requires active treatment: exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or cervical cancer in situ that has undergone potentially curative therapy.	

EXCLUSION CRITERIA				
No	**Yes**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability.	
☐	☐	☐	9. Has active autoimmune disease that has required systemic treatment in the past 6 months (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) is not considered a form of systemic treatment.	
☐	☐	☐	10. Has known history of, or any evidence of active, non-infectious pneumonitis.	
☐	☐	☐	11. Has an active infection requiring systemic therapy.	
☐	☐	☐	12. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the participant's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator.	
☐	☐	☐	13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.	
☐	☐	☐	14. Is pregnant or breastfeeding, or expecting to conceive within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.	
☐	☐	☐	15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.	
☐	☐	☐	16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).	
☐	☐	☐	17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).	
☐	☐	☐	18. Has received a live vaccine within 30 days of planned start of study therapy. **Note**: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.	
☐	☐	☐	19. Active or history of inflammatory bowel disease (colitis, Crohn's), diverticulitis, irritable bowel disease, celiac disease, or other serious, chronic, gastrointestinal conditions associated with diarrhea. Active or history of systemic lupus erythematosus or Wegener's granulomatosis.	

EXCLUSION CRITERIA				
No	**Yes**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	20. Participant has clinical symptoms or signs of partial or complete gastrointestinal obstruction or, requires parenteral hydration and/or nutrition.	
☐	☐	☐	21. Participant requires, or is likely to require, more than a two-week course of corticosteroids for intercurrent illness. Participant must complete the course of corticosteroids 2 weeks before screening to meet eligibility.	
☐	☐	☐	22. Participant has a serious, non- healing wound, ulcer, or bone fracture.	
☐	☐	☐	23. Participant has a clinically significant cardiovascular disease including: • Uncontrolled hypertension, defined as systolic > 150 mmHg or diastolic > 90 mmHg • Myocardial infarction or unstable angina within 6 months prior to enrollment • New York Heart Association (NYHA) Grade II or greater congestive heart failure (refer to Appendix F) • Participant has a grade II or greater peripheral vascular disease • Participant has a clinically significant peripheral artery disease (e.g. those with claudication, within 6 months)	
☐	☐	☐	24. Participant has organ allografts.	
☐	☐	☐	25. Participant is receiving medication(s) that might affect immune function.	
☐	☐	☐	26. Unwilling or unable to follow protocol requirements.	

Investigator Signature: _____ Date: _____

Printed Name of Investigator: _____

TABLE OF CONTENTS

1 Background 20
1.1 Advanced Solid Tumors and Immunotherapy 20
1.2 Antiangiogenic Therapy Improves Immune Therapy 21
1.3 PD-1 and PD-L1 Background 22
1.3.1 PD-1 and PD-L1 Pharmaceutical and Therapeutic Background 22
1.3.2 PD-1 and PD-L1 Blockade in Ovarian Cancer 23
1.4 Pembrolizumab 23
1.4.1 Preclinical and Clinical Studies with Pembrolizumab 23
1.5 Bevacizumab 23
1.5.1 Bevacizumab and Ovarian Cancer 24
1.6 Cyclophosphamide 25
1.6.1 Rational for Combining Bevacizumab with Cyclophosphamide in Ovarian Cancer 25
1.7 Translational Science Background 26
1.7.1 PD-L1 Expression (Integrated Biomarker) 26
1.7.2 Analysis of Tumor Infiltrating Immune cells and Targetable Immune Activating and Immune Inhibitory Genes (Exploratory Biomarker) 26
1.7.3 Neo-antigen Landscape (Exploratory Biomarker) 27
1.7.4 T Cell Receptor (TCR) Repertoires (Exploratory Biomarker) 27
1.7.5 Tumor-Associated Antigen (TAA) Serologic Responses (Exploratory Biomarker) 27
1.7.6 Cytokine and Chemokine Landscape (Exploratory Biomarker) 27
1.7.7 Tumor Associated Lymphocytes in Ascites (Exploratory Biomarker) 27
1.7.8 Human microbiome collection (Exploratory Biomarker) 27
1.8 Risks and/or Benefits 29
1.8.1 Pembrolizumab 29
1.8.2 Bevacizumab 29
1.8.3 Cyclophosphamide 31
2 Rationale 31
2.1 Rationale for Dose Selection/Regimen/Modification for Pembrolizumab 31
2.2 Rationale for Endpoints 32
3 Objectives 33
3.1 Primary Objectives 33
3.2 Secondary Objectives 33
4 Methodology 33
4.1 Study Design 33
4.2 Study Endpoints 34

4.2.1 Primary Endpoints....34
4.2.2 Secondary Endpoints....34
4.2.3 Replacement of Participants....34
4.3 Target Accrual and Study Duration....35
5 Participant Selection....35
5.1 Inclusion Criteria....35
5.2 Exclusion Criteria....37
5.3 Inclusion of Women and Minorities....38
6 Treatment Plan....39
6.1 Dosing and Administration....39
6.1.1 Dosing of Pembrolizumab....40
6.1.2 Dosing of Bevacizumab....40
6.1.3 Dosing of Cyclophosphamide....40
6.2 Timing of Dose Administration....40
6.3 Prohibited Concomitant Therapeutic Modalities....40
6.4 Dose-Limiting Toxicity and Dose Delays for Pembrolizumab....40
6.5 Dose Delays for Bevacizumab....43
6.6 General Concomitant Medications/ Vaccinations....46
6.6.1 Acceptable Concomitant Medications....46
6.6.2 Prohibited Concomitant Medications....46
6.7 Supportive Care Guidelines....47
6.7.1 Pembrolizumab Supportive Care Guidelines....47
6.7.2 Bevacizumab Supportive Care Guidelines....52
6.7.3 Cyclophosphamide Supportive Care Guidelines....52
6.8 Diet/Activity/Other Considerations....52
6.8.1 Diet....52
6.8.2 Contraception....52
6.8.3 Use in Pregnancy....53
6.8.4 Use in Nursing Women....53
6.8.5 Skin Toxicity....53
6.9 Duration of Treatment....54
6.9.1 Assessing for Secondary Cytoreduction and additional tumor biopsies to evaluate for response to treatment....54
6.10 Treatment Discontinuation....54
6.11 Compliance....55
7 Investigational Products....55

7.1 Pembrolizumab 55
7.1.1 Active Substance and Source 55
7.1.2 Drug Shipment 56
7.1.3 Preparation 56
7.1.4 Storage and Stability 56
7.1.5 Handling and Disposal 56
7.1.6 Overdose 57
7.2 Bevacizumab 57
7.3 Cyclophosphamide 57
8 Study Procedures 57
8.1 Administrative Procedures 62
8.1.1 Informed Consent 62
8.1.2 Inclusion/Exclusion Criteria 62
8.1.3 Medical History 62
8.1.4 Prior and Concomitant Medications Review 62
8.1.5 Post-Study Anti-Cancer Therapy Status 62
8.1.6 Survival Follow-Up 63
8.2 Clinical Procedures/Assessments 63
8.2.1 Adverse Event (AE) Monitoring 63
8.2.2 Full Physical Examination 63
8.2.3 Directed Physical Exam 63
8.2.4 Vital Signs 64
8.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale 64
8.3 Laboratory Procedures/Assessments 64
8.4 Efficacy Measurements 64
8.4.1 Tumor Imaging and Assessment of Disease 64
8.5 Tissue Collection, Correlative Blood Sampling and Microbiome Samples 64
8.6 End of Treatment/Withdrawal/Discontinuation 65
8.7 Post-Treatment Visits 65
8.7.1 Safety Follow-Up Visit 65
8.7.2 Follow-up Visits 65
8.7.3 Survival Follow-Up 65
8.8 Collection and Handling of Specimens 66
8.8.1 Blood Sample Collection and Processing for Correlative Studies 66
8.8.2 Tumor Tissue Collection 67
8.8.3 Ascites 67

8.9 Pathology 68
8.9.1 Pre- and On-Treatment Biopsies 68
8.9.2 Pre-Treatment Formalin-Fixed Paraffin-Embedded (FFPE) Biopsy Samples 69
8.10 Human Microbiome collection 69
8.10.1 Assessing quality of life and emotional well-being and lifestyle using the EORTC QLQ-C30, QLQ-OV28, MFSI-SF and Microbiome questionnaires 69
8.10.2 Microbiome collection time points 70
8.10.3 Skin microbiome collection 71
8.10.4 Vaginal sample collection technique and specimen handling 72
8.10.5 Fecal microbiome collection 72
8.11 Planned Correlative Studies 73
8.11.1 Assessment of dynamic changes in tumor microenvironment prior to and subsequent to therapy 73
8.11.2 Exploration of peripheral blood biomarkers as early predictors of clinical benefit 74
8.11.3 Exploration of genetic predictors of response 75
8.11.4 Exploration of human microbiome as a predictor of clinical response and its association with potential side effects 76
8.11.5 Assessment of Biospecimens and Clinical data 77
9 Efficacy evaluations 77
9.1 Baseline 77
9.1.1 Measurable Lesion Definitions and Target Lesion Selection 77
9.1.2 Non-measurable Lesion Definitions 78
9.1.3 Target and Non-Target Lymph Node Lesion Definitions 78
9.1.4 Non-Target Lesion Selection 78
9.1.5 Bone Lesions 78
9.1.6 Cystic and Necrotic Lesions as Target Lesions 79
9.1.7 Lesions with Prior Local Treatment 79
9.1.8 No Disease at Baseline 79
9.2 Follow-Up 79
9.2.1 Recording of Target and New Measurable Lesion Measurements 79
9.2.2 Definition of New Measurable Lesions 79
9.2.3 Non-Target Lesion Assessment 80
9.2.4 New Non-Measurable Lesions Definition and Assessment 80
9.2.5 irRECIST Overall Tumor Assessments 80
9.2.6 Confirmation Measurement 81
9.3 Guidelines for Evaluation of Measurable Disease 81
10 Safety Evaluation 82

10.1 Adverse Events 82
10.1.1 Definition 82
10.1.1.1 Diagnosis Versus Signs and Symptoms 83
10.1.1.3 Abnormal Laboratory Values 83
10.1.1.4 Preexisting Medical Conditions (Baseline Conditions) 83
10.1.2 Grading and Relationship to Drug 84
10.1.3 Reporting Adverse Events 88
10.2 Events of Clinical Interest (ECIs) 88
10.3 Serious Adverse Events 90
10.3.1 Definition 90
10.3.2 Reporting Serious Adverse Events 90
10.3.3 Reporting Events of Clinical Interest (ECIs) 91
10.3.4 Reporting of Overdose 91
10.3.5 Reporting of Pregnancy and Lactation 91
10.4 Follow-Up for Serious Adverse Events 91
10.5 Follow-Up for Events of Clinical Interest 91
10.6 Unanticipated Problems 92
10.6.1 Definition 92
10.6.2 Reporting Unanticipated Problems 92
10.7 FDA Reporting 93
11 Data and Safety Monitoring 93
12 Statistical Methodology 94
12.1 Sample Size Determination 94
12.2 Demographics and Baseline Characteristics 95
12.3 Phase II Analysis 95
12.3.1 Safety Lead-in Cohort 95
12.3.2 Primary Objective 96
12.3.3 Secondary Objectives 96
12.4 Interim Analysis and Criteria for Early Termination of the Study 97
13 Ethical and Regulatory Standards 97
13.1 Ethical Principles 97
13.2 Informed Consent 97
14 Study Responsibilities 98
14.1 Data Collection 98
14.2 Maintenance of Study Documents 98
15 Administrative Rules 99

15.1 Revisions to the Protocol 99
15.2 Termination of the Study 99
15.3 Confidentiality 99
16 Appendices 100
17 References....126

IN-TEXT TABLES

Table 1 Study Treatment Regimen 39
Table 2 Pembrolizumab: Dose Modification Guidelines for Drug-Related Adverse Events ..41
Table 3 Infusion Reaction Treatment Guidelines 51
Table 4 Schedule of Procedures and Observations 58
Table 5 Analysis of Tumor Microenvironment 73
Table 6 Analysis of Peripheral Blood Biomarkers 75
Table 7 Analysis of Genetic Predictors 75
Table 8 Guidelines for Routine Adverse Event Reporting for Phase 2 Studies (Regardless of Expectedness) 88
Table 9 Events of Clinical Interest 88

APPENDICES

Appendix A ECOG Performance Status Scores 101
Appendix B Calculation for Creatinine Clearance 102
Appendix C Procedure for Obtaining a Urine Protein Creatinine Ratio (UPCR) 103
Appendix D Laboratory Tests 104
Appendix E Medication Diary 105
Appendix F NYHA Functional Classification System 107
Appendix G EORTC QLQ-C30 108
Appendix H EORTC OLQ-OV28 110
Appendix I Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) 112
Appendix J Microbiome Collection Questionnaire 113
Appendix K Microbiome Initial Assessment Questionnaire 117
Appendix L Collection Instructions: Skin 119
Appendix M Collection Instructions: Vaginal 120
Appendix N Collection Instructions: Stool 121

1 BACKGROUND

1.1 Advanced Solid Tumors and Immunotherapy

Despite advances in surgery and chemotherapy over the past two decades, epithelial ovarian cancer (EOC) is still the leading cause of death from gynecologic cancer in the United States. Most ovarian cancer patients present with advance stage disease at the time of the diagnosis, and although most of them respond to first-line chemotherapy, these responses are not durable and the majority of these women will die of their disease. The clinical course of ovarian cancer patients is marked by periods of remission and relapse of sequentially shortening duration until chemotherapy resistance develops or significant toxicity occurs. Second line chemotherapies achieve very limited clinical benefit; therefore, there is an enormous unmet need for the development of novel therapies.

Recent scientific evidence demonstrated that EOC is an immunogenic tumor that can be recognized by the host immune system, and the increased infiltration of cytotoxic T cells in tumor islets correlates with significantly longer survival (1, 2), while increased numbers of immunosuppressive cells such as $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) or B7-H4 expressing tumor macrophages predict poor survival (2-4). T cell responses to multiple antigens overexpressed by ovarian cancers, such as folate receptor α (FRα), New York-esophageal-1 (NY-ESO-1), p53, human epidermal growth factor receptor 2 (Her2)/neu, survivin, sperm surface protein Sp17, WT1, MUC1, melanoma associated antigen-3 (MAGE3), human telomerase reverse transcriptase (hTERT) and insulin-like growth factor binding protein-2 have been described in several studies, which further supports the rationale for immunotherapy in ovarian cancer (5-7). The success of immunotherapy however heavily depends on the homing ability of cytotoxic T lymphocytes (CTLs) into tumors, which is a complex multistep process involving adhesive interactions with vascular cells and migration within the stroma. Much of this process is regulated by tumor endothelial cells (EC) and chemotactic gradients within the tumor microenvironment (8, 9). Even in case of successful CTL infiltration, antitumor immunity is counterbalanced by an immune-suppressive microenvironment, constituted in part by tumor cells, Tregs and tolerance-inducing myeloid cells.

Harnessing the immune system to treat cancer is the major goal of immunotherapy. Although the identification of several tumor-specific antigens in ovarian cancer has provided the foundation for designing successful immunotherapies, immune cells still have to overcome the immune escape of the tumor or cancer immunoediting. The mechanisms for immunomodulation (therapeutic intervention aimed at modifying the body's immune response) in ovarian cancer include activation of professional antigen-presenting cells (APCs) by engaging costimulatory receptors (such as CD40), activation of effector T lymphocytes by immunostimulatory monoclonal antibodies (mAb), improving effector T cell homing by normalizing tumor vasculature and finally depletion of Tregs or immunosuppressive machineries. All of these components are essential to achieve meaningful clinical outcome

Thus, a renewed emphasis on immunotherapy has emerged over the last several years with the development and testing of a novel class of immunotherapeutic agents called checkpoint inhibitors. The use of immune checkpoint inhibitors, which work by targeting molecules that serve as checks and balances in the regulation of immune responses, might be a promising avenue of immunotherapeutic research in ovarian cancer. Checkpoint inhibitor

immunotherapy is still in early-phase testing for ovarian cancer. Understanding the pivotal role of the tumor microenvironment in suppressing anticancer immunity, the unique adverse effects profiles of these agents, and the exploration of combinatorial treatment regimens will ultimately lead to enhance the efficacy of ovarian cancer immunotherapies and improved patient care (10).

1.2 Antiangiogenic Therapy Improves Immune Therapy

Targeting tumor angiogenesis is an important complementary approach to improve outcomes of patients with solid tumors (11). Tumor neovasculature is characterized by disruption of intercellular adhesion between EC and disorganization of supportive pericytes, which results in increased permeability and effusion formation (ascites in ovarian cancer) as well as in release of tumor cells into lymphatic and vascular spaces (12, 13). Currently the VEGF pathway is the most widely studied angiogenic pathway in carcinogenesis, and is comprised of VEGF-A (also known as VEGF) and the two receptor tyrosine kinases, VEGFR1 and VEGFR2 (14). VEGF was first recognized as an adverse factor in ovarian cancer in the 1990s as patients with increased VEGF expression in early stage ovarian cancer were shown to have significantly worse clinical outcome (15).

Since then we understand that tumor EC often use VEGF to establish a substantial barrier to limit T cell infiltration as VEGF deregulates vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) on EC, thus impedes T cell extravasation (16, 17). Blockade of VEGF has been shown to increase the amount of T cell infiltration in tumors, thus dismantling this tumor endothelial barrier is crucial to improve T cell trafficking to tumor microenvironment (18).

Besides modulating lymphocyte endothelial trafficking VEGF also has profound effects on immune regulatory cell function, specifically inhibiting DC maturation and antigen presentation via VEGFR-1 (19-22) VEGF blockade was shown to restore DC function and enhance immunotherapy (11, 23).

Also in recent studies selective expression of the death mediator Fas ligand (FasL, also called CD95L) was described in the vasculature of human and mouse solid tumors but not in normal vasculature (24, 25). In these tumors, FasL expression was associated with scarce CTL infiltration and a predominance of Tregs (24). Tumor-derived VEGF-A, interleukin 10 (IL-10) and prostaglandin E_2 (PGE_2) cooperatively induced FasL expression on tumor EC, which was shown to kill effector CTLs but not Tregs because of their higher levels of c-FLIP expression (26). In mice pharmacologic and genetic inhibition of VEGF-A (with bevacizumab) produced a marked increase in the influx of tumor-rejecting $CD8^+$ CTLs over $FoxP3^+$ Tregs that was dependent on attenuation of FasL expression and led to CD8-dependent tumor growth suppression (26).

Current anti-angiogenic therapy using bevacizumab, or other second-generation multitargeted receptor tyrosine kinase inhibitors (RTKIs) such as sunitinib,(27-29) sorafenib (30, 31), or pazopanib (32), improves PFS for a period of time in most types of cancer, but the anti-tumor effects of these drugs are short-lived. Most patients who initially respond will eventually develop drug resistance, tumor recurrence, or metastases indicating that anti-angiogenic therapy alone are not sufficient to prevent tumor progression and cancer metastasis in a long run (33-37). Their modest efficacy is most likely a result of redundant and complementary angiogenic pathways, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and other pathway that compensate for VEGF blockade and allow angiogenesis to

occur despite anti-VEGF treatment (37) . Hypoxia induced by anti-angiogenesis therapy also modifies some of the signaling pathways in tumor and stromal cells, which could further contribute to resistance to therapy (38). In the clinical setting improvements achieved in PFS do not translate to OS benefits for the majority of cancer patients, thus combination of antiangiogenic drugs are necessary to enhance their efficacy.

1.3 PD-1 and PD-L1 Background

1.3.1 PD-1 and PD-L1 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors: Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma

(MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

1.3.2 PD-1 and PD-L1 Blockade in Ovarian Cancer

Programmed cell death 1 and its ligand (PD-1-PD-L1) is expressed in many ovarian cancer patients and showed the closest relation to unfavorable prognosis among other immunosuppressive molecules that have been tested in ovarian cancer (39). Checkpoint blocking antibodies, such as those directed against cytotoxic T-lymphocyte antigen 4 (CTLA-4) and PD-1, have demonstrated significant recent promise in the treatment of an expanding list of malignancies, and each plays a non-redundant role in modulating immune responses (40). CTLA-4 attenuates the early activation of naïve and memory T cells, while PD-1 is primarily involved in modulating T cell activity in peripheral tissues via interaction with its ligands, PD-L1 and PD-L2 (40, 41). The blockade of PD-1 or PD-L1 was shown to enhance T-cell function and tumor cell lysis in the tumor microenvironment (42), however has limited effect as a monotherapy as peripheral tolerance is also highly depended on the on tumor endothelial death barrier and CTL infiltration to tumor microenvironment. Thus efforts to further enhance the efficacy of immune checkpoint blockade through rational treatment combinations are needed.

1.4 Pembrolizumab

Pembrolizumab (formerly MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

Upon administration, pembrolizumab binds to PD-1, an inhibitory signaling receptor expressed on the surface of activated T cells, and blocks the binding to and activation of PD-1 by its ligands, which results in the activation of T-cell-mediated immune responses against tumor cells. The ligands for PD-1 include PD-L1, which is expressed on antigen presenting cells (APCs) and overexpressed on certain cancer cells, and PD-L2, which is primarily expressed on APCs. Activated PD-1 negatively regulates T-cell activation through the suppression of the PI3K/Akt pathway.

Pembrolizumab (Keytruda™) has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

1.4.1 Preclinical and Clinical Studies with Pembrolizumab

A detailed discussion of the preclinical and clinical pharmacology, pharmacokinetics, and toxicology of pembrolizumab can be found in the Investigator's Brochure.

1.5 Bevacizumab

Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody that neutralizes VEGF and blocks its signal transduction through both VEGFR-1 and VEGFR-2 as demonstrated by the inhibition of VEGF-induced cell proliferation, survival permeability, nitric oxide production, migration and tissue factor production (43).

The FDA approved bevacizumab as a first-line treatment for patients with unresectable metastatic colorectal cancer in 2004, after a randomized double-blind phase III clinical trial

demonstrated significant survival benefit in those patients treated with the addition of bevacizumab to irinotecan, 5-fluorouracil, and leucovorin (44).

1.5.1 Bevacizumab and Ovarian Cancer

In early phase II trials in ovarian cancer patients bevacizumab yielded single-agent activity beyond that was seen in colorectal or lung cancer (45). The median PFS was 4.7 months, which was statistically superior ($p < .0001$) to median PFS of 1.8 months for a historical cohort of more than 300 similar patients entered onto previous GOG phase II trials of inactive cytotoxic agents. Clinical response rate was 18% (5% complete response) with median response duration of 10.5 months. An additional 55% had stable disease. Single-agent bevacizumab was well tolerated with no hematologic events of grade 3 or higher (45).

To date, four phase III randomized clinical trials testing bevacizumab in ovarian cancer have been published.

In later clinical trials the use of bevacizumab added significant clinical benefit when combined with standard chemotherapy in four randomized, double-blind, phase III trials, both as front-line treatment (GOG-0218 and ICON7) and in patients with recurrent disease (OCEANS and AURELIA) (46, 47).

In two pivotal, well designed, phase III, clinical trials (GOG-0218 and ICON7) in women with advanced epithelial ovarian, fallopian tube or primary peritoneal cancer, first-line treatment with bevacizumab in combination with standard chemotherapy (carboplatin plus paclitaxel) followed by maintenance treatment with bevacizumab alone significantly prolonged progression-free survival (by 3.8 months in GOG-0218 in the concurrent and maintenance arm and by 5.4 months in ICON-7 in patient with high risk for recurrence) relative to standard chemotherapy. Overall survival did not differ significantly between patients receiving standard chemotherapy plus placebo maintenance and those receiving concurrent plus maintenance bevacizumab in any of these trials – in GOG-0218 median OS 39.3 vs 39.7 and 38.7 months vs ICON-7 58.6 vs. 58.0 months respectively (47-49).

OCEANS (50) was a randomized phase III trial testing the combination of carboplatin and gemcitabine with or without the addition of bevacizumab given concurrently and then as maintenance until progression in recurrent platinum-sensitive EOC. PFS for the bevacizumab arm was 12.4 months (vs. 8.4 months in the placebo group; $p < .001$). By the final analysis, however, there was no difference in overall survival (OS; 33.6 vs. 32.9 months). Of note, nearly all of the women in both arms went on to receive additional therapy, including bevacizumab.

In the AURELIA trial (51), bevacizumab was combined with the investigator's choice of single-agent chemotherapy (pegylated liposomal doxorubicin, weekly paclitaxel, or topotecan), in platinum-resistant EOC. The addition of bevacizumab improved PFS (6.7 vs. 3.4 months) and response rate, but there was no significant difference in OS (16.6 vs. 13.3 months; $p < .17$). Crossover to single-agent bevacizumab was permitted after progression with chemotherapy alone. AURELIA was the first phase III trial to show a benefit with combination therapy in the platinum-resistant population and is the basis for the drug FDA approval in EOC.

Bevacizumab was recently FDA approved for use in combination with single-agent chemotherapy for platinum-resistant disease; however, its optimal role in this cancer remains unclear. Although bevacizumab has activity in many patients with EOC, trials have identified

neither the optimal setting for treatment nor a predictive biomarker. The lack of a proven OS survival benefit does not necessarily speak against the drug's efficacy and the median survival in EOC is longer than in many other solid tumors; therefore, OS data can be confounded by subsequent lines of therapy.

A detailed discussion of the preclinical pharmacology, pharmacokinetics, and toxicology of bevacizumab can be found in the Package Insert (http://medlibrary.org/lib/rx/meds/avastin-1/).

1.6 Cyclophosphamide

Besides the normalization of tumor vasculature to improve CTL homing, alteration of Tregs is also crucial in regulating anticancer immune responses. The increased presence of Tregs is associated with poor prognosis in ovarian cancer, while the ability to selective eliminate these cells has been repeatedly shown to bolster immune responses in preclinical cancer models. Tregs not only suppress cognate T cell responses but also blunt the innate arm of immunity, inhibiting NK cell proliferation and effector functions through the capacity to down regulate NKG2D level on these cells, thus associated with poor prognosis in cancer patients (52).

Cyclophosphamide is widely available, inexpensive and has multiple immune-modifying properties. Oral metronomic cyclophosphamide and VEGF blockade have independently been reported to selectively reduce the subset of immunosuppressive Tregs in vivo (53, 54). Metronomic chemotherapy refers to the close, regular administration of a chemotherapeutic drug at relatively low (non-toxic) doses, over prolonged periods, with no extended drug-free break periods (53, 55).

Oral administration of metronomic cyclophosphamide in advanced cancer patients induces a profound and selective reduction of circulating Tregs, associated with a suppression of their inhibitory functions on conventional T cells and NK cells leading to a restoration of peripheral T cell proliferation and innate killing activities (56). Cyclophosphamide also interferes with homeostatic proliferation of Tregs, increases their susceptibility to apoptosis, and impairs Treg functionality by decreasing the expression of suppression markers, such as glucocorticoid-induced TNF receptor–related protein (GITR) and FoxP3 (56-58).

Furthermore it was also shown that high-avidity tumor specific $CD8^+$ T cells are not deleted in the periphery with the use of cyclophosphamide, instead, these high-avidity T cells can be recruited and activated to provide a potent antitumor immune response if Tregs are inhibited, which further validates the addition of cyclophosphamide to enhance immunotherapy (59).

Therefore, metronomic regimen of cyclophosphamide does not only affect tumor angiogenesis but also strongly curtails immunosuppressive regulatory T cells; favoring a better control of tumor progression, thus represent a critical immuno-intervention in the oncologist's armamentarium.

1.6.1 Rational for Combining Bevacizumab with Cyclophosphamide in Ovarian Cancer

As already discussed above, bevacizumab mainly has clinical benefit in patients who have advanced metastatic disease only or primarily when it is combined with conventional chemotherapy. The exact mechanisms for the chemo-enhancing effects of bevacizumab are still unknown, but this has been investigated along with other antiangiogenic drugs that could be combined with traditional chemotherapy or immunomodulating agents to improve clinical response (60).

Treatment combinations of metronomic chemotherapy and an antiangiogenic drug have moved into phase II clinical trial testing with particularly encouraging results thus far reported in metastatic breast and recurrent ovarian cancer. Oral chemotherapy drugs such as cyclophosphamide and methotrexate are the main chemotherapeutics used for such trials.

There are already several studies in ovarian cancer which showed that the combination of IV bevacizumab (15 mg/kg every 3 weeks) and low dose metronomic oral cyclophosphamide (50 mg) is an effective, well-tolerated chemotherapy regimen in heavily pretreated patients with recurrent ovarian carcinoma and this combination significantly improved PFS and OS in responders (61, 62). Response rates were more favorable to the rates reported for similar patients receiving other commonly used second-line chemotherapeutic agents or just bevacizumab or cyclophosphamide alone, showing an objective response rate between 40.5-53.5% with approximately 10% of patients in each series demonstrating a complete response (61, 63-65). The reported severe adverse events in these studies were around 1-1.5%, the regimen was overall very well tolerated with only about 10% of patients encountering a side effect that required discontinuing treatment (46, 61, 63, 65). Also the median PFS reported in these studies in patients with recurrent ovarian cancer ranged from 3.9 months to 5.0 months (61, 63-65).

Therefore the combination of bevacizumab and low dose metronomic oral cyclophosphamide is active in recurrent ovarian cancer with improved clinical outcomes; hence the combination of these drugs is warranted.

1.7 Translational Science Background

Correlative translational studies for biomarkers will be performed depending on clinical response and initial mRNA analysis using NanoString from blood samples and tumor tissue biopsies. The list of potential biomarkers tested in genomics, immunohistochemistry, flow cytometry, serology, microbiome analysis and Luminex assay will be determined by the Principal Investigator (PI) after the initial analysis of clinical response and NanoString results.

1.7.1 PD-L1 Expression (Integrated Biomarker)

Patients with tumors expressing of PD-L1 have shown improved clinical benefit from therapies targeting the PD-1/PD-L1 pathway (42, 66). However, the utility of PD-L1 expression as a predictive biomarker has been debated as it is highly dynamic and can be up-regulated in response to immune activating factors. In this trial, pre- and post-treatment tumor formalin-fixed, paraffin-embedded (FFPE) tissue will be evaluated by immunohistochemistry (IHC) for expression of PD-L1 as a predictive biomarker in this patient population.

1.7.2 Analysis of Tumor Infiltrating Immune cells and Targetable Immune Activating and Immune Inhibitory Genes (Exploratory Biomarker)

Presence of immune cells in tumor microenvironment has been shown to be predictive of response to immunotherapies. Assessment of the immune subsets in the tumors will help to establish predictive markers on the basis of pre-treatment phenotype as well to determine whether clinical efficacy correlates with the degree of infiltration of specific immune cell subsets. As such, pre- and post-treatment FFPE will be evaluated for the presence of TILs and immune markers determined by the PI. Examples of such immune checkpoint markers include4-1BB, OX40, GITR, CD40, ICOS and PD-L1, IDO, B7-H3, B7-H4, LAG3, TIM-3, PD-1, CTLA-

4, VISTA, and BTLA. Thus, depending on NanoString results and tissue availability, these markers potentially will be tested on pre and post-treatment biopsy samples (67).

1.7.3 Neo-antigen Landscape (Exploratory Biomarker)

A recent study by Snyder at al. used next-generation whole exome sequencing to characterize neo-antigen landscape in patients with malignant melanoma and defined particular neo-epitope signatures that were highly predictive of clinical benefit from anti-CTLA-4 therapy in patients with malignant melanoma (68). To determine whether specific tumor genetic determinants, including neo-epitope signatures influence the response to either treatment, depending on tissue availability and clinical response, DNA isolated from tumor samples may be processed for whole exome analysis to assess for specific driver mutations and for potential neo-antigens.

1.7.4 T Cell Receptor (TCR) Repertoires (Exploratory Biomarker)

Since cancer-testis (CT) antigens may not be present in all patients, but the analysis of overall changes in TCR repertoires during therapy may help to determine whether certain dominant T cell clones emerge or persist in response to therapy. Recently, Cha et al, reported that maintenance of specific T cell clonotypes in blood of patients with advanced melanoma treated with ipilimumab was associated with improved survival (69). Similar TCR repertoire studies were performed in tumors at MSKCC in the setting of a clinical trial in patients with early stage breast cancer treated with neoadjuvant cryoablation of breast lesion and ipilimumab, where combination therapy demonstrated most significant increase in intra-tumoral T cell clones(70). Thus, depending on tissue availability and clinical response, in this trial DNA may be used for TCR repertoire analysis and deep sequencing of TCR CDR3 regions.

1.7.5 Tumor-Associated Antigen (TAA) Serologic Responses (Exploratory Biomarker)

Expression of CT antigens in a large percentage of gynecologic malignancies has been reported in multiple studies. Monitoring for early responses to CT antigens and TAA such as NY-ESO1, MAGE 4, SOX2, and p53 may help identify the patients that are more likely to derive benefit from therapy. As such, pre- and post-treatment serum may be used for assessment of TAA serologic responses.

1.7.6 Cytokine and Chemokine Landscape (Exploratory Biomarker)

Cytokines and chemokines augment or dampen immune response by activating/exhausting certain subset of immune cells and by governing the migration of immune and cancer cells. Depending on clinical response and NanoString results – Luminex assay will be used to monitor the changes in the cytokine and chemokine landscape during the treatment.

1.7.7 Tumor Associated Lymphocytes in Ascites (Exploratory Biomarker)

Patients who have ascites at any time point during the clinical trial will be offered to undergo paracentesis for symptom relief. Ascites from these patients will be collected and later analyzed for presence of immune cells and inflammatory markers.

1.7.8 Human microbiome collection (Exploratory Biomarker)

The human microbiome plays an essential role in digestion and nutrient absorption, epithelial homeostasis, angiogenesis, and in the proper function of nervous and immune systems (71, 72).

Disruption of the microbiome could lead to chronic inflammation, altered immune responses and carcinogenesis (73, 74). Most recent research shows that there is a complex reciprocal modulation between gut microbiota and the human brain, which involves interaction of the central nervous system, sympathetic and parasympathetic branches of the autonomic nervous system, the enteric nervous system, and elements of the neuroendocrine and neuroimmune systems. For example, some microbiota synthesize neurotransmitters directly (e.g., gamma-amino butyric acid) while many modulate the synthesis of neurotransmitters, such as serotonin, dopamine and norepinephrine, and brain-derived neurotropic factor (75). Microbiotic abnormalities have been linked to reductions in neurotransmitter levels, cognitive deficits, anxiety, depression, chronic pain and fatigue (76, 77).

The composition of the microbiota also regulates the levels of tryptophan in the systemic circulation as well levels and nature of tryptophan catabolites (TRYCATs). These catabolites influence epithelial barrier integrity and the presence of an inflammatory or tolerogenic environment in the intestine and beyond (78). The composition of the microbiota also determines the levels and ratios of short chain fatty acids, such as butyrate and propionate, which are key energy source for colonocytes (79). Dysbiosis (altered microbiome) can lead to reduced levels of butyrate, and therefore may have adverse effects on epithelial barrier integrity, increase bacterial translocation into the systemic circulation, effects energy homeostasis, and the T helper 17/regulatory/T cell balance. (75, 79).

Increasing evidence suggests that different gut, skin, and vaginal microbiota profiles may be related to the etiology of certain gynecological cancers, such as cervical cancer, uterine cancer, and ovarian cancer (80). Patients' severity of side effects and response to certain cancer treatments can also be impacted by gut microbiome composition (73). In a recent study by Vetizou *et al.* it was shown that anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota and the authors showed the key role for *Bacteroidales* in the immunostimulatory effects of CTLA-4 blockade both in mice and patients (81). In another recent landmark paper by Sivan *et al.* (82) was shown that *Bifidobacterium* as associated with the antitumor effects and oral administration of Bifidobacterium alone improved tumor control to the same degree as PD-L1-specific antibody therapy, and combination treatment nearly abolished tumor outgrowth. Thus, the authors suggested that manipulating the microbiota may modulate cancer immunotherapy (82).

Skin reactions are the most common immune-related side effects of checkpoint inhibitor therapy affecting approximately one third of patients on PD-1 inhibitor therapy (83). Currently, no tools are available to identify individuals at highest risk of cutaneous side effects prior to therapy, although changes in the skin microbiome have been shown in several immune mediated skin diseases, such as atopic dermatitis, psoriasis and vitiligo (84, 85). There is no published data on microbiome composition in patients with ovarian cancer, particularly in patients with recurrent ovarian cancer receiving treatment with a check point inhibitor. Thus, in parallel with the other above mentioned translational studies, we will collect pre-, on- and post-treatment skin, vaginal and fecal samples from patients participating in this clinical trial to characterize how the microbiomes of these parts of the body relate to disease severity and treatment response. We will study associations of the cutaneous microbiome and the presentation of skin eruptions during the intervention. Moreover, we will correlate efficacy of therapy with the presence of specific cutaneous side effects and the microbiome composition in ovarian cancer patients. If there are sufficient tumor samples from the core biopsies, we will test the microbiome composition in the

tumor microenvironment as well. We will also ask our patients to complete surveys to assess their emotional well-being, social functioning, and energy/fatigue level as well as a basic survey on their nutrition and lifestyle in order to gather a more comprehensive data on the interaction of microbiome and central nervous and immune systems.

1.8 Risks and/or Benefits

1.8.1 Pembrolizumab

The most common adverse reactions (reported in ≥ 20% of patients) to pembrolizumab (KEYTRUDA®) include fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia and, diarrhea. Refer to package insert for additional information (http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/125514lbl.pdf).

Pembrolizumab is generally well tolerated and demonstrates a favorable safety profile in comparison to chemotherapy. Pembrolizumab is an immunomodulatory agent, and based on this mechanism of action, immune mediated adverse events are of primary concern. Important identified risks for pembrolizumab are of an immune mediate nature, including: pneumonitis, colitis, thyroid disorders (hypothyroidism/ hyperthyroidism), hepatitis, hypophysitis, Type I diabetes mellitus, uveitis, and nephritis.

After a recent review of data, events newly characterized as identified risks also include pancreatitis, myositis, and severe skin reaction; these are included in the reference safety information (refer to IB).

The majority of immune-mediated adverse events were mild to moderate in severity, were manageable with appropriate care, and rarely required discontinuation of therapy. Further details around frequency, reporting, and management of immune-related adverse events (irAEs) are described in the Investigator's Brochure. In addition to the previously noted identified risks, infusion-related reactions are a risk but are not considered immune mediated see Section 10.2).

1.8.2 Bevacizumab

As VEGF plays an important role in normal physiologic processes – such as stabilization of damaged endothelia and wound healing – thus the inhibition of VEGF carries a unique toxicity profile (86). Bevacizumab in general is well tolerated in all patients. The most common toxicities associated with bevacizumab includes mucosal bleeding, hypertension (HTN), proteinuria and wound healing complications, however these adverse events are generally manageable (87). New-onset HTN and exacerbation of existing HTN are the most commonly reported adverse events attributable to bevacizumab. In randomized trials, the incidence of grade 3/4 HTN in bevacizumab-treated patients ranged from 3.0% to 14.8% compared with 0% to 2.0% for controls (88).

Bevacizumab related proteinuria is typically mild in severity and non-dose-limiting, but it can rarely escalate to nephrotic syndrome. The incidence of grade 3/4 proteinuria in bevacizumab-receiving arms in meta-analysis of 1850 patients treated in seven randomized trials reported statistically significant relative risks of proteinuria equal to 1.4 with low-dose bevacizumab and 2.2 at higher doses (88).

The delay of wound healing, wound dehiscence, fistula, and abscess has been reported in patients receiving bevacizumab. Because of the long half-life of bevacizumab (~ 20 days), the risk of

wound-healing complications persist even after its discontinuation (86). A pooled analysis of two randomized metastatic colorectal cancer trials found no difference in the rate of wound-healing complications in bevacizumab-treated patients and controls, when bevacizumab was given at least 28 days following major surgery; however patients who underwent major surgery while receiving bevacizumab had a wound-healing complication rate of 13% compared to 3.4% in controls (89).

GOG-0218 and ICON7 did not show increased wound-healing complications in women with ovarian cancer, although most participants were not surgical candidates (45, 90). GOG-0218 protocol also delayed the administration of bevacizumab or placebo until the second cycle of chemotherapy, regardless of the timing of primary debulking surgery. In the Bevacizumab Regimens' Investigation of Treatment Effects (BRiTE) registry, 622 patients had surgery after starting bevacizumab therapy; 23 (3.7%) wound-healing complications were reported, occurring more often in patients undergoing major abdominal surgeries < 60 days following the last bevacizumab dose (89, 91). According to the BRiTE registry (91) and another study by Scappaticci (89), the optimal interval between the last bevacizumab dose administered and subsequent major surgery is generally considered to be 60 days.

The previously reported rare life threatening complication related to bevacizumab treatment in non-gynecologic tumors included hemorrhagic complications such as gastrointestinal (GI) perforations (2.4%) and arterial and venous thromboembolic events (ATE) (3%) (Genentech BioOncology, 2006). Of these, GI perforations have been observed in ovarian cancer in 5.4% of the patients (87). Identification of clinical risk factors for bevacizumab-related GI perforations minimizes the incidence of this adverse event as the majority of events occurred in patients with at least one of the following characteristics: acute diverticulitis, intra-abdominal abscess, GI obstruction, tumor at the GI perforation site, carcinomatosis, or prior radiation therapy (86).

Venous thromboembolism (VTE) has been reported in many bevacizumab trials, VTE incidence rates in bevacizumab-treated patients that were not statistically different from those seen in controls. The incidence of VTE in phase II ovarian studies was relatively low (2% to 3%) (45).

The rates of both **arterial thromboembolism** and **hemorrhage** are increased in patients receiving bevacizumab therapy. Under normal conditions, VEGF mediates the repair of endothelial surfaces that have sustained damage secondary to cardiovascular disease and other microangiopathies (92). This results in exposed subendothelial tissues that initiate the clotting cascade and subsequent clot formation. The underlying prothrombotic state characteristic of cancer patients might exacerbate this process. Though somewhat counterintuitive, the mechanism leading to hemorrhage might also result from a lack of endothelial repair in areas where subendothelial tissues are violated by pathophysiologic processes that may or may not be related to the malignancy.

Hemorrhage: The incidence of hemorrhage is increased with bevacizumab therapy. Epistaxis is common, occurring in 20-40% of patients, but it is generally mild and rarely requires medical intervention. Life-threatening and fatal hemorrhagic events have been observed in bevacizumab studies and included pulmonary hemorrhage, CNS bleeding and gastrointestinal (GI) bleeding. In a phase 2 study in non-small cell lung cancer, 6 cases of life-threatening hemoptysis or hematemesis were reported among 66 patients treated with bevacizumab and chemotherapy; 4 of

these events were fatal In the pivotal phase 3 trial in advanced colorectal cancer, the rate of GI hemorrhage (all grades) was 24% in the IFL/bevacizumab arm compared to 6% in the IFL arm; grade 3-4 hemorrhage was 3.1% for IFL/bevacizumab and 2.5% for IFL. Serious GI hemorrhage has also been observed in clinical trials with bevacizumab in patients with pancreatic cancer or varices treated with bevacizumab.

The incidence of **arterial thromboembolic (ATE)** events – such as transient ischemic attacks, cerebral infarction, unstable angina, troponin elevation, and acute myocardial infarction – has been reported to be slightly higher in bevacizumab-treated patients compared to controls (4.4% vs 1.9%) (93, 94). Risk factors for ATE identified in this analysis included age ≥ 65 years, male sex, and history of ATE. Ovarian cancer studies reported similarly low incidences of ATE (0% to 3%) (86, 87, 94).

All patients on bevacizumab should be considered at risk for ATEs, and extra caution should be exercised when prescribing bevacizumab to those > 65 years old with a history of ATE or conditions which predispose them to ATE. There is no modification, aside from discontinuation of therapy, known to be more effective than standard medical treatment for bevacizumab-associated ATE events. As with all bevacizumab-related adverse events, the continuation of bevacizumab in specific situations in which bevacizumab benefit was either greater than the severity of toxicity or underlying predisposing factors were reversible (e.g., cardiac revascularization) might be considered, though overall prognosis might preclude such risks.

Reversible posterior leukoencephalopathy syndrome (RPLS) is a rare neurological disorder that has been reported in association with HTN, eclampsia, and various states of immunosuppression due to organ transplant, chemotherapy – including bevacizumab, or autoimmune disorders. RPLS can present with headache, seizure, lethargy, confusion, blindness, and other visual and neurologic disturbances, which can occur at any interval following bevacizumab administration. The mechanistic link between bevacizumab and RPLS is unknown, but RPLS not associated with bevacizumab involves loss of cerebral vascular autoregulation, disruption of the cerebral tissue/capillary interface, and vasogenic edema. There is no specific treatment for RPLS outside of confirming the diagnosis, providing supportive care, which includes aggressive management of HTN, and discontinuing bevacizumab therapy.

In summary, bevacizumab is the first biologic therapy targeted at tumor pathophysiology to show significant activity in ovarian cancer with acceptable added toxicity. The adverse events associated with bevacizumab, however, are specific to its biologic effects and are potentially serious, even fatal.

1.8.3 Cyclophosphamide

Common adverse reactions to cyclophosphamide include neutropenia, febrile neutropenia, fever, alopecia, nausea, vomiting and, diarrhea. Refer to package insert for additional information (http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/040745Orig1s003lbl.pdf).

2 RATIONALE

2.1 Rationale for Dose Selection/Regimen/Modification for Pembrolizumab

An open-label Phase I trial (KEYNOTE 001) is completed, evaluating the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three

dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date.

Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

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

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

The above data indicate that the proposed fixed dose regimen for pembrolizumab of 200mg Q3W in the current study is similar to the 10 mg/kg Q2W dose regimen, with regard to efficacy and tolerability (refer to Investigator's Brochure, section 5.4.1.3: Overall Adverse Events: P102, A Phase Ib Study of Pembrolizumab in Patients with Advanced Solid Tumors).

2.2 Rationale for Endpoints

According to the design schema the estimated sample size is 40 patients overall. Noting that the median PFS of platinum-resistant ovarian cancer patients treated with standard chemotherapy is 3.5 months, we would consider a PFS of 7 months or greater to be of interest in this population. We expect that no more than 30% of patients treated with standard chemotherapy would achieve 7 months of PFS. Therefore, we will test the null hypothesis that the PFS response to): Pembrolizumab 200 mg IV + bevacizumab 15 mg/kg every 3 weeks IV + cyclophosphamide 50 mg po every day combination therapy is equal to 30% versus the one-sided hypothesis that it is greater than 30%. Please see section 8.0 for more detailed information on statistical analysis.

3 OBJECTIVES

3.1 Primary Objectives

- To evaluate improvement in progression-free survival for patients treated with anti-PD1 pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide as compared to a historical control.

3.2 Secondary Objectives

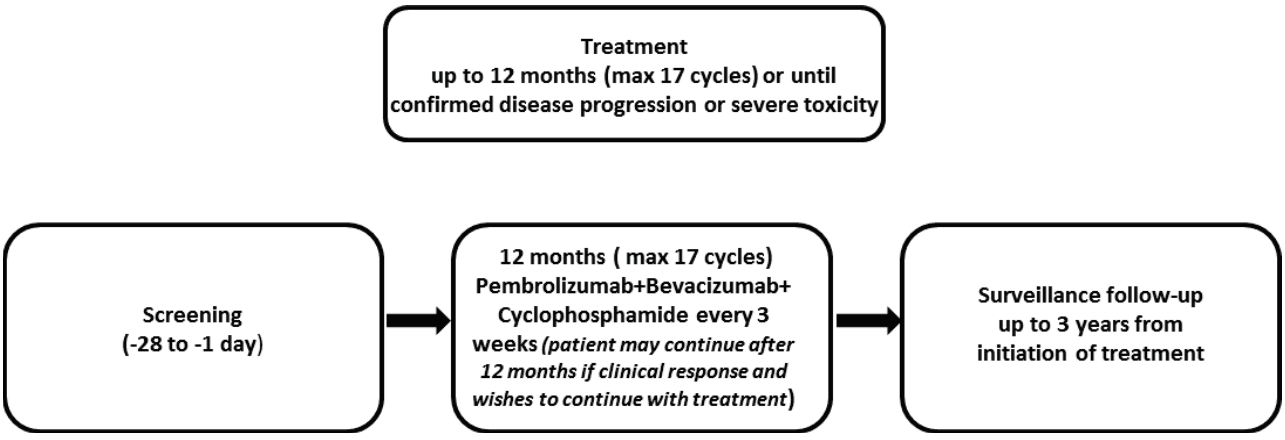
- To obtain pilot data on clinical response rates using both RECIST1.1 criteria (Response Evaluation Criteria in Solid Tumors) and immune related response criteria (irRECIST).
- To obtain data on changes in tumor microenvironment prior to and subsequent to therapy and, to screen for potential biomarkers to predict clinical benefit.
- To determine the safety and tolerability of the treatment combination in the study population.
- To evaluate overall survival in patients treated with anti-PD1 pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide.
- To assess the impact of the combination of anti-PD1 pembrolizumab, IV bevacizumab and oral metronomic cyclophosphamide on anti-tumor immune responses in ovarian cancer.

4 METHODOLOGY

4.1 Study Design

This is an open label Phase II study of anti-PD1 antibody pembrolizumab, in combination with IV bevacizumab and oral metronomic cyclophosphamide in patients with recurrent platinum sensitive, resistant or refractory epithelial ovarian, fallopian tube, or primary peritoneal cancer. The study will include an initial safety lead-in cohort of 5 patients. The estimated total sample size is n=40. The study schema is depicted in Figure 1.

Figure 1 Trial Diagram



As shown in Figure 1, each cycle consists of 3 weeks. Pembrolizumab with bevacizumab will be administered every 3 weeks IV, while cyclophosphamide will be given continuously orally every day.

Dosing regimen: Pembrolizumab 200 mg IV + bevacizumab 15 mg/kg every 3 weeks IV + cyclophosphamide 50 mg po every day up to 12 months (maximum of 17 cycles) or until disease progression, or until development of unacceptable toxicity, whichever occurs sooner.

Safety Lead-in Cohort: There will be an initial safety lead-in cohort of 5 patients. The safety/tolerability of the combination will be examined after each patient completes at least 3 cycles (i.e., the first 9 weeks of therapy). If 2 or less patients have experienced a drug related toxicity requiring drug/treatment delay or suspension (as defined in Sections 6.4, 6.5, and 6.7.3), then an additional 35 patients will be enrolled to complete the Phase II study. Otherwise, the study will be suspended and the research team will meet to discuss possible safety concerns and will decide what actions to take with respect to study continuation.

Assessing response to treatment: Tumor response will be assessed after the 3rd and 6th cycles then after every 6 cycles by investigator-assessed clinical exam and immune-related response criteria (irRECIST) by independent, central, blinded radiographic review. Conventional RECIST 1.1 will also be documented during the trial, however RECIST 1.1 will not be used to determine disease progression, as it has been shown in prior clinical trials using Pembrolizumab in large number of patients, that conventional RECIST might underestimate the benefit of pembrolizumab in approximately 15% of patients; thus using irRECIST could permit treatment beyond initial progression per RECIST v1.1 and prevent premature cessation of treatment (95-97).

If, after 12 months of treatment the patient remains without any evidence of disease progression (has stable disease, partial or complete response), they will be offered to continue with the treatment until disease progression or until treatment is tolerated without severe (grade 3 or 4) toxicity that would require drug discontinuation.

Note: If the patient has good clinical response and chooses to remain on the treatment beyond 12 months, we will continue to collect the same clinical data as we were during the first 12 months, as these laboratory tests and imaging studies are standard of care when patients are receiving chemotherapy for ovarian cancer.

4.2 Study Endpoints

4.2.1 Primary Endpoints

- The hazard of having a disease progression or dying (PFS endpoint) by treatment.

4.2.2 Secondary Endpoints

- The frequency and duration of objective tumor response within 6 months of study entry as assessed by immune-related response criteria (irRECIST).
- The duration of overall survival (OS).
- The frequency and severity of adverse events, as assessed by CTCAE.

4.2.3 Replacement of Participants

Any participant, who discontinues the study prior to completion of at least 4 cycles of therapy (prior to CT scan after cycle 3 to assess for clinical response) for reasons other than treatment related toxicity, will be excluded from the analyses and replaced.

4.3 Target Accrual and Study Duration

The study will enroll approximately 40 evaluable patients in total (includes a safety lead-in cohort of n=5). Accrual is expected to take 18 months.

Study treatment is for 12 months (or a maximum of 17 cycles), with a follow-up period of up to 3 years from initiation of study treatment; for a total study duration of approximately 5 years.

5 PARTICIPANT SELECTION

All participants will sign an informed consent prior to study related tests. All participants will meet the inclusion and exclusion criteria summarized in Section 5.1 and Section 5.2.

5.1 Inclusion Criteria

To be included in this study, participants must meet the following criteria:

1. Age ≥ 18 years of age on day of signing informed consent.
2. Have an ECOG Performance Status of 0 or 1 (refer to Appendix A**)**.
3. Have measurable disease per RECIST 1.1 or irRECIST criteria present.
4. Participant may have serous, endometrioid, clear cell, mucinous or undifferentiated type of recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer.
    - Histologic confirmation of the original primary tumor is required via the pathology report.
5. Participant can be either platinum-sensitive (platinum free interval (PFI) ≥ 6 months prior to recent recurrence) or platinum-resistant (PFI < 6 months prior to recent recurrence). If the participant has a platinum sensitive disease, she may only enroll in this clinical trial if there is a contraindication for her to receive further treatment with platinum-based chemotherapy (such as serious persistent toxicity or severe hypersensitivity to platinum agents or she declines standard of care).
6. Participant must be willing to undergo core or excisional biopsy of a tumor lesion within 4 weeks (28 days) prior to initiation of treatment on Day 1and, after 3 cycles of study treatment. Participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Principal Investigator.
7. Have the following clinical laboratory values:
    - Absolute neutrophil count (ANC): ≥ 1,500 /mcL
    - Platelets: ≥ 100,000 / mcL
    - Hemoglobin: ≥ 9 g/dL or 5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)

- Serum creatinine: ≤1.5 X upper limit of normal (ULN) ***OR*** measured or calculated creatinine clearance ≥ 60 mL/min for participant with creatinine levels > 1.5 X institutional ULN (refer to Appendix B). GFR can also be used in place of creatinine or CrCl.
- Urine Protein Creatine Ratio (UPCR) < 1 prior to enrollment (refer to Appendix C).
- Serum total bilirubin: ≤ 1.5 X ULN ***OR*** direct bilirubin ≤ ULN for participants with total bilirubin levels > 1.5 ULN
- AST (SGOT) and ALT (SGPT): ≤ 2.5 X ULN ***OR*** ≤ 5 X ULN for participants with liver metastases
- Albumin: > 2.5 mg/dL
- International Normalized Ratio (INR) or Prothrombin Time (PT): ≤1.5 unless participant is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
- Activated Partial Thromboplastin Time (aPTT): ≤1.5 X ULN unless participant is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

8. Participants of childbearing potential must have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
9. Participants of childbearing potential must be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (participants of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year). Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.
10. Participant has recovered from toxicities of prior chemotherapy or other therapy (to grade 2 or less).
11. Participant may have received prior investigational therapy (including immune therapy).
12. Participant may have received prior hormonal therapy.
13. Participant may have received bevacizumab (or other antiangiogenic agent) and/or cyclophosphamide in the past.
14. Participant has had at least 4 weeks of postoperative recovery from surgery prior to enrollment to ensure complete wound healing. Participants with bowel resections at surgery should begin protocol at least 42 days after surgery.
15. Ability to swallow and retain oral medication.
16. Participant or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.

5.2 Exclusion Criteria

Participants will be excluded from this study for the following:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment or, is taking any other medication that might affect immune function.
3. Has a known history of active TB (Bacillus Tuberculosis).
4. Hypersensitivity to bevacizumab, cyclophosphamide, pembrolizumab or any of its excipients.
5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Participants with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study
    - Note: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy and, has to be at least 28 days after the surgery
7. Has a known additional malignancy that is progressing or requires active treatment: exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or cervical cancer in situ that has undergone potentially curative therapy.
8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
9. Has active autoimmune disease that has required systemic treatment in the past 6 months (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) is not considered a form of systemic treatment.
10. Has known history of, or any evidence of active, non-infectious pneumonitis.
11. Has an active infection requiring systemic therapy.
12. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the participant's participation

for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator.

13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
14. Is pregnant or breastfeeding, or expecting to conceive within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
18. Has received a live vaccine within 30 days of planned start of study therapy.
    - Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
19. Active or history of inflammatory bowel disease (colitis, Crohn's), diverticulitis, irritable bowel disease, celiac disease, or other serious, chronic, gastrointestinal conditions associated with diarrhea. Active or history of systemic lupus erythematosus or Wegener's granulomatosis.
20. Participant has clinical symptoms or signs of partial or complete gastrointestinal obstruction or, requires parenteral hydration and/or nutrition.
21. Participant requires, or is likely to require, more than a two - week course of corticosteroids for intercurrent illness. Participant must complete the course of corticosteroids 2 weeks before screening to meet eligibility.
22. Participant has a serious, non- healing wound, ulcer, or bone fracture.
23. Participant has a clinically significant cardiovascular disease including:
    - Uncontrolled hypertension, defined as systolic > 150 mmHg or diastolic > 90 mmHg
    - Myocardial infarction or unstable angina within 6 months prior to enrollment
    - New York Heart Association (NYHA) Grade II or greater congestive heart failure (refer to Appendix F)
    - Participant has a grade II or greater peripheral vascular disease
    - Participant has a clinically significant peripheral artery disease (e.g. those with claudication, within 6 months)
24. Participant has organ allografts.
25. Participant is receiving medication(s) that might affect immune function.
26. Unwilling or unable to follow protocol requirements.

5.3 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this study.

6 TREATMENT PLAN

6.1 Dosing and Administration

Duration of treatment is 12 months (with a maximum of 17 cycles) or until disease progression or development of unacceptable toxicity, whichever occurs sooner, or any severe toxicity that requires drug discontinuation.

If after 12 months of treatment, a patient remains without any evidence of disease progression (has stable disease, partial or complete response), they will be offered to continue with the treatment until disease progression or until treatment is tolerated without severe (grade 3 or 4) toxicity that requires drug discontinuation.

***Each cycle consists of 3 weeks*:** Pembrolizumab with bevacizumab will be administered every 3 weeks IV, while cyclophosphamide will be given continuously orally every day. Pembrolizumab will be administered first, followed by bevacizumab.

Initially, a safety lead-in cohort of n=5 patients will be enrolled under the proposed study treatment regimen (as outlined in Table 1) and the safety/tolerability of the combination will be examined after each patient completes at least 3 cycles. At that time, the study team will decide what action to take, e.g., continue the study as is, make changes to the study with regard to treatment or dose modifications, or discontinue the study.

If 2 or less patients have experienced a drug related toxicity requiring drug/treatment delay or suspension (as defined in Sections 6.4, 6.5, and 6.7.3), then an additional 35 patients will be enrolled to complete the Phase II study. Otherwise, the study will be suspended and the research team will meet to discuss possible safety concerns and will decide what actions to take with respect to study continuation.

The rationale for selection of doses to be used in this trial is provided in Section 2.1.

Reported adverse events (AEs) and potential risks are described in Section 1.8. Appropriate dose modifications are described in Section 6.4

The treatment regimen to be used in this trial is outlined below (**Table 1**):

Table 1 Study Treatment Regimen

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental
Bevacizumab	15 mg/kg	Q3W	IV infusion	Day 1 of each 3 week cycle	Standard
Cyclophosphamide	50 mg	Q.D.	p.o.	Day 1 to day 21 continuously	Standard

In case of significant toxicity, evaluation will be performed to determine which drug could have been responsible for the adverse reaction and also to exclude other possible causes.

If the adverse reaction is attributed to pembrolizumab, drug will be either withheld or permanently discontinued per Section 6.4

If the adverse reaction is attributed to bevacizumab or cyclophosphamide, drug will be either withheld or permanently discontinued per Section 6.5

Note: *There are no dose reductions for pembrolizumab, bevacizumab or, cyclophosphamide.*

6.1.1 Dosing of Pembrolizumab

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

Refer to the *Pharmacy Manual: Pembrolizumab (MK-3475)* for specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

6.1.2 Dosing of Bevacizumab

Bevacizumab will be administered at 15 mg/kg IV every 3 weeks (following pembrolizumab infusion), infused over 30 min (first time infusion over 90 min, second time infusion over 60 min, then every subsequent infusion for 30 min). Bevacizumab dosing will be based on the participant's actual body weight for each treatment cycle.

6.1.3 Dosing of Cyclophosphamide

50 mg orally: daily during each 21 day cycle.

Patient will be instructed to swallow cyclophosphamide capsules whole, without cutting, chewing or crushing. Patient should take the capsule in the morning, it can be taken with or without food, and patient is recommended to drink 2-3 quarts of fluid/day.

There will be no dose reductions for cyclophosphamide.

6.2 Timing of Dose Administration

Trial treatment will be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed in Table 4 (Schedule of Procedures and Observations). 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.

6.3 Prohibited Concomitant Therapeutic Modalities

Prior to documented disease progression, the following therapeutic modalities are prohibited:

1. Anti-neoplastic therapy not otherwise specified in the current protocol, including cytotoxic, biologic, hormonal, or radiation therapy, regardless of indication (treatment of measurable disease or consolidation therapy).

6.4 Dose-Limiting Toxicity and Dose Delays for Pembrolizumab

There will be no dose reductions for pembrolizumab.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-

related toxicities and severe or life-threatening AEs (to include as events only if toxicities are considered related, probably related, or possibly related to the drug) as per **Table 2** below. Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0).

A **dose limiting toxicity** (DLT) will be defined as any ≥ grade 3 clinically significant toxicity, which is deemed to be probably or possibly treatment related and, occurs within the first 9 weeks of therapy (9 weeks = 3 cycles).

See **Section 6.7.1** and *Event of Clinical Interest Guidance Document* for supportive care guidelines, including use of corticosteroids.

Table 2 Pembrolizumab: Dose Modification Guidelines for Drug-Related Adverse Events

General instructions:

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

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 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) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation
	Grade 4	Permanently discontinue		

				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 / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 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 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 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		

All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		
1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. **NOTE:** For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).				

6.5 Dose Delays for Bevacizumab

There will be no dose reduction for bevacizumab. Treatment should be interrupted or discontinued for certain adverse events, as described below:

- **Hypertension**

Patients receiving bevacizumab should be monitored prior to each dose with measurement of blood pressure. Medication classes used for management of patients with Grade 2 or 3 hypertension receiving bevacizumab included angiotensin-converting enzyme inhibitors, beta blockers, diuretics, and calcium channel blockers, but preferably should be a beta blocking agent, if no contraindications. The use of anxiolytics in conjunction with specific anti-hypertensive agents is not prohibited. The goal for blood pressure control should be consistent with general medical practice guidelines (i.e. < 150/90 mmHg in general and < 130/80 mmHg for patients with diabetes).

- For asymptomatic grade 2 or grade 3 hypertension (systolic > 150 mm Hg or diastolic > 90 without any concern for life-threatening consequences) treating physician may administer antihypertensive (and/or anxiolytic) agent in the office and proceed with treatment if blood pressure lowers to <150/90 - per physicians' discretion. Patient will be asked to monitor BPs regularly at home and follow up with primary care physician within a week.
- For symptomatic grade 2 or grade 3 hypertension or for grade 4 hypertension (BPs that are concerning for life-threatening consequences), hold bevacizumab treatment (and pembrolizumab, but patient can continue with oral cyclophosphamide) up to 1 week, (see below), administer antihypertensive therapy as clinically needed and arrange appropriate follow up/admission for blood pressure control.
- During the period of combination chemotherapy with bevacizumab, if hypertension becomes controlled (under 150/90 during the majority of the time)

and symptomatic hypertension has resolved by one week after holding treatment, continue all therapy.

- During the period of combination chemotherapy with bevacizumab, if hypertension remains persistently uncontrolled on one or several antihypertensive agents taken appropriately or remains symptomatic one week after holding treatment, the next treatment cycle should contain pembrolizumab and cyclophosphamide only, if applicable, as otherwise indicated in the protocol, with bevacizumab omitted.
- If uncontrolled or symptomatic hypertension has not resolved by three weeks after holding treatment with bevacizumab (despite patient taking antihypertensive agents appropriately) , treatment with bevacizumab should be discontinued for the remainder of the study, but patient may continue with pembrolizumab and oral cyclophosphamide

- **Proteinuria**

Patients receiving bevacizumab should be monitored by urine analysis for urine protein: creatinine (UPC) ratio prior to every other dose of bevacizumab. If,

- UPC ratio ≤ 3.5: Continue bevacizumab
- UPC ratio > 3.5: Hold bevacizumab until UPC ratio recovers to < 3.5. If therapy is held for > 2 months due to proteinuria, discontinue bevacizumab
- Grade 4 or nephrotic syndrome: Discontinue bevacizumab

- **Hemorrhage**

Bevacizumab will be discontinued in patients with CTCAE Grade 3 hemorrhage and receiving full-dose anticoagulation. For all other patients with CTCAE Grade 3 hemorrhage, bevacizumab should be held until ALL of the following criteria are met:

- bleeding has resolved
- blood hemoglobin level is stable
- there is no bleeding diathesis that would increase the risk of therapy
- there is no anatomical or pathologic condition that can increase the risk of hemorrhage recurrence

Patients who experience delay of resolution according to the above criteria for >3 weeks, recurrence of Grade 3 hemorrhage, or any CTCAE Grade 4 hemorrhage will be taken off bevacizumab therapy.

- **Thrombosis**

Arterial Thrombosis: Bevacizumab will be discontinued for ≥ CTCAE Grade 3 arterial thrombotic events (including cerebrovascular ischemia, transient ischemic attack, cardiac ischemia/infarction, peripheral or visceral arterial ischemia) or CTCAE Grade 2 arterial thrombotic events new or worsened since beginning bevacizumab therapy.

Venous Thrombosis: Treatment with bevacizumab will be held for CTCAE Grade 3 or asymptomatic CTCAE Grade 4 (including pulmonary embolism) venous thrombosis. For

patients on therapeutic anticoagulation, PT INR or PTT (whichever appropriate) should be monitored closely during bevacizumab therapy.

- The participant must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin (or other anticoagulant) or on stable dose of heparin prior to restarting treatment.
- The participant must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels).
- The subject must not have had hemorrhagic events while on study.
- The patient is benefiting from treatment (no evidence of disease progression). Patients with symptomatic Grade 4 thromboembolic events after study enrollment but prior to course 2 should be managed according to the above guidelines above.

However, patients with symptomatic CTCAE Grade 4 after receiving any bevacizumab, or recurrent/worsening venous thromboembolic events after resumption of bevacizumab treatment will be taken off bevacizumab therapy.

- **Coagulopathy**

Bevacizumab should be held if the coagulation parameters are higher than the intended therapeutic range or for coagulopathy as follows:

- For CTCAE Grade 3 or 4 coagulopathy: hold treatment, until PT/PTT resolve to Grade 1.
- For patients with PT/INR > therapeutic range while on therapeutic warfarin, treatment with bevacizumab/placebo will be held until PT/INR is within the therapeutic range.

Patients experiencing treatment delay > three weeks because of failure to meet the above criteria will be taken off bevacizumab therapy.

- **Wound Disruption/Bowel Perforation, Fistula, or GI Leak**

Treatment with bevacizumab will be modified in the event of wound disruption requiring medical or surgical intervention, bowel perforation or fistula (including tracheal-esophageal fistula) as follows:

- In the event of superficial wound separations healing by secondary intention with no evidence of fascial dehiscence or infection, therapy with bevacizumab may be initiated with weekly wound examinations until complete closure. After initiation of bevacizumab, bevacizumab will be discontinued for any new event, regardless of Grade.

- **Intestinal Obstruction**

- Bevacizumab will be held for occurrence of CTCAE Grade 3 toxicity, until resolution to ≤CTCAE Grade 1 and will be permanently discontinued for occurrence of CTCAE Grade 4 toxicity.

Since the development of intestinal obstruction could be a result of cancer progression, the investigator should take steps to evaluate such patients for the possibility of disease

progression, using clinical, laboratory and radiographic information as clinically indicated; in the event of disease progression, all protocol-directed therapy would be discontinued.

- **Treatment Guidelines for Reversible Posterior Leukoencephalopathy Syndrome (RPLS)**
    - Bevacizumab should be held in patients with symptoms/signs suggestive of RPLS, pending work-up and management, including control of blood pressure. Bevacizumab should be discontinued upon diagnosis of RPLS.

Note: Resumption of bevacizumab may be considered in patients who have documented benefit from the agent, provided that RPLS was mild and has completely resolved clinically and radiographically within 2-4 weeks; decision to resume bevacizumab in these patients must be discussed with the Principal Investigator and approved by Merck.

6.6 General Concomitant Medications/ Vaccinations

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

6.6.1 Acceptable Concomitant Medications

All treatments that the principal 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 medications ongoing within 28 days before the first dose of trial treatment as well as any that will be discontinued within 1 week prior to the first dose of study drug will be recorded on the CRF. This is to include all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. Assessment of concomitant medications occurs at the beginning of every treatment cycle during the trial period. Any changes that occur during the trial period (e.g., drug dosage, frequency, route, date) will be included on the CRF.

Concomitant medications administered after 30 days after the last dose of trial treatment will be recorded for SAEs and ECIs as defined in Section 10.3**Section 10.2,** respectively.

Participants may be pretreated for nausea and vomiting with appropriate anti-emetics.

6.6.2 Prohibited Concomitant Medications

Participants are prohibited from receiving the following therapies during the Baseline period and Treatment Phase of the study:

- 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 trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with Merck.

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 trial. Participants may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria (Section 5.2) describes other medications which are prohibited in this trial.

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

6.7 Supportive Care Guidelines

6.7.1 Pembrolizumab Supportive Care Guidelines

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

Note: If after the evaluation the event is determined not to be related, the investigator is instructed to follow the *ECI Document* reporting guidance but does not need to follow the treatment guidance (as outlined in the *ECI Guidance Document*).

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the *ECI Guidance Document*.

- **Pneumonitis**
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
- Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**

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

  - All participants who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For Grade 2 diarrhea/colitis that persists greater than 3 days, administer oral corticosteroids.
  - For Grade 3 or 4 diarrhea/ colitis that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.

  When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 Diabetes Mellitus or ≥ Grade 3 Hyperglycemia**

  For Type 1 diabetes mellitus (TIDM), if new onset, including diabetic ketoacidosis (DKA) ***or,*** Grade 3-Grade 4 hyperglycemia if associated with ketosis (ketonuria) or metabolic acidosis (DKA):

  - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria
  - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

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

- **Hyperthyroidism or Hypothyroidism**

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

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):

        - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hematologic**
    - For **Grade 2** events, treat with corticosteroids, if indicated.
    - For **Grade 3-4** events, treat with systemic or oral corticosteroids, as appropriate.
- **Hepatic**
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous glucocorticosteroids for 24 to 48 hours.

  When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

  In addition, the event must be reported as a Drug Induced Liver Injury (DILI) ECI, if the patient meets the laboratory criteria for potential DILI defined as:

    - An elevated alanine transaminase (ALT) or aspartate transaminase (AST) lab value that is greater than or equal to three times (3X) the upper limit of normal (ULN) and
    - An elevated total bilirubin lab value that is greater than or equal to two times (2X) ULN and, at the same time, an alkaline phosphatase (ALP) lab value that is less than 2X ULN,
    - As a result of within-protocol-specific testing or, unscheduled testing.
- **Neurologic**
    - For **Grade 2** events, consider treatment with corticosteroids, as appropriate.
    - For **Grade 3-4** events, treat with systemic corticosteroids. If condition worsens, consider IVIG or other immunosuppressive therapies, as per local guidelines.

  When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Ocular**
    - For **Grade 2** events, treat with topical steroids, such as 1% prednisolone acetate suspension and iridocyclitics.

- For **Grade 3-4** events, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.

  When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Skin**
  - For **Grade 2** events, symptomatic treatment with topical glucocorticosteroids or urea-containing creams, in combination with oral anti-pruritics.
  - For **Grade 3-4** events, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

  NOTE: Refer to the *ECI Guidance Document* (Section 3.9.1: Immediate Evaluation for Potential Skin ECIs) for detailed evaluation and reporting requirements.

- **Other Events of Clinical Interest (ECIs)**

  The following AEs, regardless of grade, are considered ECIs and should be reported to Merck (see **Section 10.3.3**):

  - Myocarditis
  - Pericarditis
  - Pancreatitis
  - Any additional Grade 3 or higher event which the investigator considers to be immune-related.
    - For **Grade 2** events, or **Grade 1** events that do not improve with symptomatic treatment: Systemic corticosteroids may be indicated.
    - For **Grade 3-4** events, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Infusion Reactions**

  Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. **Table 3** shows treatment guidelines for participants who experience an infusion reaction associated with administration of pembrolizumab.

Table 3 Infusion Reaction Treatment Guidelines

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

***Note*:** Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.

6.7.2 Bevacizumab Supportive Care Guidelines

If an infusion-related adverse reaction occurs, the patient should be premedicated for the next course; however, the infusion time for bevacizumab may not be decreased for the next infusion. If the next infusion is well tolerated with pre-medication, the infusion time for the next dose may then be decreased by 30 ± 10 minutes as long as the patient continues to be pre-medicated. If a patient experiences an infusion-associated adverse event with the 60-minute infusion, all subsequent doses should be given over 90 ± 15 minutes. Similarly, if a patient experiences an infusion-associated adverse event with the 30-minute infusion, all subsequent doses should be given over 60 ± 10 minutes.

6.7.2.1 Suggested Prophylaxis in Event of Prior Bevacizumab Infusion Reaction

In the event of a prior bevacizumab hypersensitivity reaction, subsequent infusions should be delivered over 90 minutes, and the following prophylactic regimen is recommended upon re-exposure:

1. H1 blocker (diphenhydramine 25-50 mg IVP or orally one hour prior to injection; or an equivalent dose of an alternate H1 blocker, such as loratadine 10 mg or fexofenadine 60 mg).

2. H2 blocker (famotidine 20 mg IVP or orally one hour prior to injection; or an equivalent dose of an alternate H2 blocker).

3. Dexamethasone (10 mg administered PO 12 and 6 hours prior to bevacizumab injection). Recommended only for hypersensitivity reaction.

6.7.3 Cyclophosphamide Supportive Care Guidelines

- Oral cyclophosphamide will be held in patients with Grade 3 or higher hematologic, gastrointestinal, hepatic, genitourinary, neurologic, cardiac or pulmonary toxicities until patient recovers to at least grade 1 toxicity level.
- In case of Grade 3 or higher neurologic, cardiac or pulmonary toxicity that is attributed to cyclophosphamide treatment, drug will be discontinued permanently

6.8 Diet/Activity/Other Considerations

6.8.1 Diet

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

6.8.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the

duration of the study. The two birth control methods can be either: two barrier methods or, a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy. The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

6.8.3 Use in Pregnancy

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

The 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.

6.8.4 Use in Nursing Women

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

6.8.5 Skin Toxicity

Skin reactions are the most common immune-related side effects of checkpoint inhibitor therapy affecting approximately one third of patients on PD-1 inhibitor therapy (83). Currently, no tools are available to identify individuals at highest risk of cutaneous side effects prior to therapy, although changes in the skin microbiome have been shown in several immune mediated skin diseases, such as atopic dermatitis, psoriasis and vitiligo (84, 85).

Prior to starting on study treatment (and after Cycle 3 and at end of treatment or disease progression), participants will be encouraged to have a skin check performed through the Dermatology Department. RPCI Dermatology will schedule appointments at a time that is convenient for the participant (without a wait time) and will monitor their skin for potential cutaneous side effects and will provide preventive/management skin care.

In addition, if any cutaneous symptoms or side effects are noted, participants will be offered immediate follow up with dermatology, particularly if the participant has grade 3-4 skin toxicity.

6.9 Duration of Treatment

Participants may remain on study and continue to receive treatment (12 months with a maximum of 17 cycles) in the absence of disease progression, unacceptable toxicity or withdrawal from study, intercurrent illness that prevents further administration of treatment, participant demonstrates an inability/refusal to comply with oral medication regime or, participant withdraws from study.

If patient has good clinical response after 12 months of treatment without any severe adverse event, she may be offered to continue with the treatment after discussing this with her provider and the Principal Investigator.

Note: If the patient has good clinical response and chooses to remain on the treatment beyond 12 months, we will continue to collect the same clinical data as we were during the first 12 months, as these laboratory tests and imaging studies are standard of care when patients are receiving chemotherapy for ovarian cancer

6.9.1 Assessing for Secondary Cytoreduction and additional tumor biopsies to evaluate for response to treatment

Patients who have stable disease or showed progression on this clinical trial may be offered to undergo PET CT with biopsy of non-responsive tumor lesions to evaluate for viable tumor tissue and for translational studies or could be considered for secondary cytoreduction, if surgerycould provide additional survival benefit. The goal of secondary cytoreduction is to achieve complete resection, which has been shown to increase survival in this patient population. Tumor tissue harvested during secondary cytoreduction will be used for translational studies and also to evaluate for response to treatment by assessing for viable/necrotic tissue. Patients who have found to have mostly necrotic tumor tissue (thus clear clinical benefit from treatment), will be offered to stay on the trial, even if per prior imaging they met criteria for disease progression, as the reliability of CT scans determining disease progression in this patient population has not been reported. Survival data from these patients will be censored based on true progression (new viable tumor tissue growth on CT scan or on biopsy/surgery) and there treatment course will be further described in future publications.

6.10 Treatment Discontinuation

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the participant's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Toxicity; treatment related or unrelated
- Investigator judgment

  - The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance
- Participant voluntary withdrawal
  - A participant may withdraw from the study at any time, for any reason. If a participant discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Participant lost to follow-up
- Sponsor decision.
- Intercurrent illness that prevents further administration of treatment.
- The participant has a confirmed positive serum pregnancy test.

6.11 Compliance

A medication diary (Appendix E) will be provided to monitor participant compliance for oral cyclophosphamide.

As most studies show that adherence to long-term oral drug therapies are around 40-50%, we will consider a 60% of compliance with oral Cytoxan adequate. We will monitor adherence by patient self-report, pill diaries as well as rates of prescription refills. If patient is below the 60% compliance (which will be assessed each 3 weeks) – she will be asked to increase compliance with the next cycle. If she fails to comply in more than 2 cycles (>6 weeks) without any medical reason, she will be replaced at any point in the study.

7 INVESTIGATIONAL PRODUCTS

7.1 Pembrolizumab

Pembrolizumab (KEYTRUDA®) is a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is an IgG4 kappa immunoglobulin with an approximate molecular weight of 149 kDa.

7.1.1 Active Substance and Source

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

Pembrolizumab lyophilized powder for injection is a sterile, preservative-free, white- to off-white lyophilized powder in single-use vials. Each vial is reconstituted and diluted for intravenous infusion. Each 2 mL of reconstituted solution contains 50 mg of pembrolizumab and is formulated in L-histidine (3.1 mg), polysorbate 80 (0.4 mg), and sucrose (140 mg); may contain hydrochloric acid/sodium hydroxide to adjust pH to 5.5.

Pembrolizumab solution for injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution that requires dilution for intravenous infusion. Each vial contains 100 mg of pembrolizumab in 4 mL of solution. Each 1 mL of solution contains 25 mg

of pembrolizumab and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

7.1.2 Drug Shipment

Pembrolizumab will be provided by Merck & Co., Inc. and shipped to the participating site, and will be labeled in accordance with regulatory requirements.

The date of receipt and the amount of drug received will be documented. Drug shipment records will be retained by the investigational pharmacist or designee.

7.1.3 Preparation

Pembrolizumab Powder for Solution for Infusion, 50 mg/vial, is reconstituted with sterile water for injection prior to use.

The lyophilized DP after reconstitution with sterile water for injection and the liquid DP are clear to opalescent solutions, essentially free of visible particles. The reconstituted lyophilized product and the liquid product are intended for intravenous (IV) administration. The reconstituted DP solution or the liquid DP can be further diluted with normal saline or 5% dextrose in the concentration range of 1 to 10 mg/mL in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be used immediately to prepare the infusion solution in the IV bag, and the infusion solution should be administered immediately. Diluted pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion. In addition, IV bags can be stored at 2° to 8°C for up to a cumulative time of 20 hours. This recommendation is based on up to 24 hours of room temperature and up to 24 hours of refrigerated stability data of diluted pembrolizumab solutions in the IV bags.

Please refer to *Pharmacy Manual: Pembrolizumab (MK-3475)* for more detailed preparation instructions.

7.1.4 Storage and Stability

The Investigator or designate will be responsible for ensuring that the investigational product is securely maintained in a locked, limited-access facility, and in accordance with the applicable regulatory requirements.

Pembrolizumab for injection (lyophilized powder): Store vials under refrigeration at 2°C to 8°C (36°F to 46°F).

Pembrolizumab injection (solution): Store vials under refrigeration at 2°C to 8°C (36°F to 46°F) in original carton to protect from light. Do not freeze. Do not shake.

Drug storage temperature will be maintained and recorded, as applicable.

7.1.5 Handling and Disposal

All products dispensed will be recorded on a product accountability record. Records of product lot numbers and dates received will be entered on a product accountability form. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

All unused and/or partially used investigational drug will be destroyed according to standard practices after properly accounting for the dispensing. Partially used vials of study drug will not be re-used for other participants.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

7.1.6 Overdose

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab.

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

7.2 Bevacizumab

Please refer to package insert. Use of biosimilar per Institute standard is allowed.

7.3 Cyclophosphamide

Please refer to package insert.

8 STUDY PROCEDURES

Eligibility of each participant will be established prior to enrollment.

Informed consent MUST be completed prior to receiving any study related procedures.

The individual trial procedures are described in detail below and summarized in Table 4.

All on-study visit procedures are allowed a window of ± 3 days unless otherwise noted (Note: It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator).

Unless otherwise defined in the written protocol text, all procedures/assessments will be conducted in accordance with RPCI Clinical Research Services Standard Operating Procedures.

Table 4 Schedule of Procedures and Observations

Trial Period:	Screening Phase	Treatment Cycles[1]									End of Treatment[2] OR At time of Discontinuation	Post-Treatment		
Treatment Cycle/Title:	Baseline[3]	1	2	3	4	To be repeated beyond 8 cycles						Safety Follow-up[4]	Follow Up Visits[5]	Survival Follow-Up[6]
						5	6	7	8	10				
Administrative Procedures														
Demographics and Medical History	X	X	X	X	X	X	X	X	X	X	X	X		
Prior and Concomitant Medication Review	X[7]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]		
Trial Treatment Administration[9]		X	X	X	X	X	X	X	X	X				
Post-study anticancer therapy status[10]											X	X		
Survival Status											X	X		X
Clinical Procedures/Assessments														
Review Adverse Events[11]		X	X	X	X	X	X	X	X	X	X	X	X	
Full Physical Examination[12]; including vital signs and body weight	X	X			X			X		X	X	X	X	
Directed Physical Examination including vital signs and body weight			X	X		X	X		X					
ECOG Performance Status[13]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Laboratory Procedures/Assessments: Refer to Appendix D														
Pregnancy Test – Urine or Serum β-HCG	X[14]													
PT/INR and aPTT	X						X				X	X		
Hematology[15]	X	X	X	X	X	X	X	X	X	X	X	X		
Chemistry[15]	X	X	X	X	X	X	X	X	X	X	X	X		
Urinalysis[15](every other	X	X		X		X		X		X	X			

Trial Period:	Screening Phase	Treatment Cycles[1]									End of Treatment[2] OR At time of Discontinuation	Post-Treatment		
Treatment Cycle/Title:	Baseline[3]	1	2	3	4	To be repeated beyond 8 cycles						Safety Follow-up[4]	Follow Up Visits[5]	Survival Follow-Up[6]
						5	6	7	8	10				
cycle)														
Urine sample for Urine Protein Creatinine Ratio [16]	X	X		X		X		X		X				
T3, FT4 and TSH [17]	X			X			X				X	X		
Efficacy Measurements														
Imaging: CT (chest, abdomen, and pelvis)[18]	X			X			X				X		X	
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood														
Newly Obtained (or Archival) Tissue Collection[19]	X			X							X			
Correlative Studies Blood Collection[20]	X[21]		X		X			X		X[29]	X			
Ascites Collection [22]														
Dermatology visit (will be recommended to all patients at RPCI) [23]	X				X						X			
Human microbiome collection (stool, vaginal and skin samples) [24]		X[25]			X[26]					X[28]	X[27]			
Microbiome Initial Assessment Questionnaire		X												
Microbiome Collection Questionnaire		X			X					X[28]	X			
Quality of Life Questionnaires: EORTC QLQ-C30, QLQ-OV28, MFSI-SF		X			X			X		X[29]	X			

Trial Period:	Screening Phase	Treatment Cycles[1]									End of Treatment[2] OR At time of Discontinuation	Post-Treatment		
Treatment Cycle/Title:	Baseline[3]	1	2	3	4	To be repeated beyond 8 cycles						Safety Follow-up[4]	Follow Up Visits[5]	Survival Follow-Up[6]
						5	6	7	8	10				
1	1. Each cycle is 3 weeks: pembrolizumab + bevacizumab will be administered on Day 1 of each 3 week cycle and, cyclophosphamide will be taken (po) daily. Treatment will be for 12 months (with ***a maximum of 17 cycles***) or until disease progression or development of unacceptable toxicity (whichever occurs sooner) or, any severe toxicity (e.g. Grade 3 - Grade 4 toxicity requiring drug discontinuation). ***Note***: After Cycle I Day 1, all on-study visit procedures are allowed a window of ± 3 days unless otherwise noted. 2. When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation 3. Performed within 28 days prior to first dose of study medication. 4. Safety Follow-Up Visit should be conducted approximately 30 days (± 3 days) after the last dose of trial treatment, or until resolution of any drug-related toxicity or before the initiation of a new anti-cancer treatment, whichever comes first. 5. Participants who discontinue trial treatment for a reason *other than disease progression* will move into the Follow-Up Phase: assessments every 3 months, for 1 year, following end of treatment then, every 6 months (Section 8.7.2). 6. Once a participant experiences *confirmed disease progression or starts a new anti-cancer therapy*: Telephone contact every 12 weeks (± 1 week: up to 3 years from initiation of treatment) to assess for survival status until death, withdrawal of consent, or the end of study, whichever occurs first (Section 8.7.3). 7. List any medications that are ongoing, or that will be discontinued, within 1 week prior to first dose of study drug. 8. List any ongoing medications with dose changes, as applicable. 9. Trial treatment is administered on Day 1 of each cycle after all procedures/assessments have been completed. Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle (following Cycle 1) due to administrative reasons. 10. Review all new anti-neoplastic therapy initiated after the last dose of trial treatment (Section 8.1.5) 11. All AEs of unknown etiology associated with pembrolizumab, bevacizumab and cyclophosphamide exposure should be evaluated to determine if it is possibly an ECI of a potentially immunologic etiology (termed immune-related adverse events, or irAEs). Please refer to Section 10.2, as well as the separate Event of Clinical Interest Guidance Document regarding the identification, evaluation and management of potential irAEs. 12. .On study treatment days (prior to administration of study treatment), the Full Physical Examination will include basic neurological examination, vital signs and, body weight: height collected at baseline only. 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. 13. At baseline, prior to the administration of each dose of trial treatment and discontinuation of trial treatment: (Appendix A) 14. Within 72 hours prior to first study treatment. If the urine test is positive, or cannot be confirmed as negative, a serum pregnancy test will be required. 15. Refer to Appendix D.													

Trial Period:	Screening Phase	Treatment Cycles[1]									End of Treatment[2] OR At time of Discontinuation	Post-Treatment		
Treatment Cycle/Title:	Baseline[3]	1	2	3	4	To be repeated beyond 8 cycles						Safety Follow-up[4]	Follow Up Visits[5]	Survival Follow-Up[6]
						5	6	7	8	10				

16. Baseline, Cycle 1 Day 1 and, Day 1 of every 2nd cycle. ***Note***: For the purpose of this study, participant must have a Urine Protein Creatine Ratio (UPCR) < 1 prior to enrollment and < 3.5 for any subsequent cycles. Refer to Appendix C
17. Every 3 cycles
18. To be obtained within 30 days of trial start, then after the 3rd and 6th cycles. If patient remains on the regimen longer than that, CT will be performed after every 6th cycle, or sooner if there is clinical suspicion for disease progression. Refer to Section 8.4.1
19. Baseline, 2-3 weeks after Cycle 3, end of treatment (optional). See Section 8.9.1 and Section 8.9.2 for processing and handling.
20. Peripheral blood samples will be collected at baseline (within 2 weeks prior to start of study treatment), Cycle 2 – Day 1, Cycle 4 – Day 1, Cycle 7 –Day 1 and at the end of study treatment or at time of discontinuation (within two weeks after the last cycle is completed or, at the time of progression; or for any other reason that requires study termination, whichever occurs sooner. ***Note*** *for samples collected while participant is receiving study treatment*: Blood is to be collected prior to pembrolizumab administration – it is not required to hold cyclophosphamide dosing until after sample collection. Refer to Section 8.8.1 (Blood Collection Sampling and Processing)
21. Note : Within 2 weeks prior to start of study treatment
22. If a patient has ascites at any time during the study period and is willing to undergo paracentesis, or wishes to undergo paracentesis for symptom relief. This is optional for the patient. Refer to Section 8.8.3for collection, processing and, handling
23. Dermatology visit will be recommended to all patients at RPCI prior to starting treatment, after 3 cycles and, at time of progression to monitor cutaneous side effects. In addition to this, if any cutaneous symptoms or side effects are noted, they will be offered immediate follow up with dermatology, particularly if patient has grade 3-4 skin toxicity (see Section 6.8.5).
24. Stool collection within 24 hours of next study visit. If not possible, then at the time of visit via digital rectal exam. If not able to collect via either method, then within next 7 days via home collection per patient. Telephone contact to remind patient about stool collection and to review instructions for vaginal and skin microbiome collection 3-5 days prior to study visit by the clinical research coordinator.
25. Cycle 1-Day 1 prior to starting chemotherapy is preferred (or within 7 days prior to start of treatment)
26. Day 1-Cycle 4 prior to starting chemotherapy is preferred (or within 7 days prior to starting Cycle 4)
27. Samples collected during the last physical examination at the time of study termination (stool sample collection within 7 days of visit)
28. On those patients, who have already completed 10 cycles, the one additional correlative blood, microbiome and questionnaires will be collected at the time of their next cycle. At the discretion of the investigator, selected patients may be asked to bring an additional stool samples for translational studies

8.1 Administrative Procedures

8.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial: Informed consent ***MUST*** be completed prior to receiving any study related procedures.

8.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial: The eligibility of each participant will be established prior to study enrollment.

8.1.3 Medical History

8.1.3.1 Disease Details

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

8.1.3.2 Prior Treatment Details

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

8.1.4 Prior and Concomitant Medications Review

8.1.4.1 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 subject has enrolled in this study will be recorded separately and not listed as a prior medication.

8.1.4.2 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 10.1.

8.1.5 Post-Study 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 subject will move into survival follow-up.

8.1.6 Survival Follow-Up

Once a participant experiences confirmed disease progression or starts a new anti-cancer therapy, the participant moves into the survival follow-up phase and should be contacted by telephone every 12 weeks (up to 3 years from initiation of treatment) to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

8.2 Clinical Procedures/Assessments

8.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Schedule of Procedures and Observations 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 4 (see **Section 10.1.2**). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

All AEs of unknown etiology associated with pembrolizumab, bevacizumab and cyclophosphamide exposure should be evaluated to determine if it is possibly an ECI of a potentially immunologic etiology (termed immune-related adverse events, or irAEs). Please refer to **Section 10.2**, as well as the separate *Event of Clinical Interest Guidance Document* regarding the identification, evaluation and management of potential irAEs.

8.2.2 Full Physical Examination

The investigator or qualified designee will perform a complete physical exam (including basic neurological, vital signs and body weight) during the baseline screening period (if clinically indicated, a neuro exam will be performed). Height will be collected at baseline only. Clinically significant abnormal findings should be recorded as medical history.

8.2.3 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 (to include basic neurological, vital signs and body weight) as clinically indicated prior to trial treatment administration.

8.2.4 Vital Signs

The investigator or qualified designee will take vital signs (temperature, heart rate, respiratory rate, blood pressure) at baseline, prior to the administration of each dose of trial treatment and at treatment discontinuation.

8.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (Appendix A) at baseline screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment..

8.3 Laboratory Procedures/Assessments

Details regarding the timing of specific laboratory procedures/assessments to be performed in this trial are provided in Table 4. The laboratory tests for hematology, chemistry, urinalysis, and others are specified in Appendix D.

8.4 Efficacy Measurements

8.4.1 Tumor Imaging and Assessment of Disease

Radiographic tumor measurements should be obtained via contrast CT of the chest, abdomen and pelvis. Immune related response criteria (irRECIST) (96) will be used to assess disease at baseline and subsequent assessments.

CT needs to be obtained within 30 days of trial start, then after the 3rd and 6th cycles. If patient remains in the regimen longer than that, CT will be performed after every 6th cycles or sooner if there is clinical suspicion for disease progression.

In addition to irRECIST, RECIST 1.1 (98) measurements will also be used for disease assessment.

8.5 Tissue Collection, Correlative Blood Sampling and Microbiome Samples

Mandatory research tumor tissue core biopsies are obtained at two time points: prior to initiating study treatment and after 3 cycles of study treatment (refer to **Section 8.8.2** for processing and handling). If patient remains on the treatment much longer, at the time of progression she will be offered to undergo another CT guided biopsy for research purposes, however this is not mandatory.

Mandatory research blood samples are obtained at five time points: Baseline (within 2 weeks prior to initiating study treatment), Cycle 2 - Day 1, Cycle 4 – Day 1, Cycle 7 – Day 1, Cycle 10 –Day 1 and at the end of study treatment or at time of discontinuation (within 2 weeks after the last treatment cycle is completed) or, at the time of disease progression or for any other reason that requires study treatment termination, whichever occurs sooner (refer to **Section 8.8.1** for processing and handling).

Mandatory stool, vaginal, skin microbiome collection as well as correlative study questionnaires prior to cycle#1 (within 7 days of starting treatment, preferably on D1 of cycle 1 prior to staring chemotherapy), after 3 cycles (within 7 days of starting cycle 4, preferably on D1 of cycle 4 prior to starting chemotherapy and at Cycle 10 – Day 1), at the end of treatment (during the last physical exam at the time of study termination, stool sample collection within 7 days of this visit) (Refer to Section 8.10).

On those patients, who have already completed 10 cycles, the one additional correlative blood, microbiome and questionnaires will be collected at the time of their next cycle.

8.6 End of Treatment/Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation.

Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in **Section 8.2**: Assessing and Recording Adverse Events.

8.7 Post-Treatment Visits

8.7.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first.

All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first.

SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

8.7.2 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 3 months by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 6 months (up to 3 years from initiation of treatment).

Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression or, death.

8.7.3 Survival Follow-Up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12

weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

8.8 Collection and Handling of Specimens

8.8.1 Blood Sample Collection and Processing for Correlative Studies

Peripheral blood samples will be collected prior to starting treatment (within 2 weeks) and, prior to study treatment administration after the 1st cycle (Day 1-Cycle 2), the 3rd cycle (Day 1-Cycle 4) and the 6th cycle (Day 1-Cycle 7) and at 2 weeks after ending study treatment (e.g., at 12 months or, at the time of progression; or any other reason that requires study termination, whichever occurs sooner).

Blood samples will be collected via venipuncture for Correlative Studies Analysis (see Section 8.11):

Samples will be collected using two, 10 mL green-top tubes and one, 10 mL red-top tube, two lavender-top tubes and one PAXgene RNA tube for each time point listed below:

- Baseline (within 2 weeks prior to start of treatment)
- Cycle 2 – Day 1
- Cycle 4 - Day 1
- Cycle 7 – Day 1
- Cycle 10 –Day 1
- End of Treatment or at time of discontinuation (within 2 weeks after the last treatment cycle is completed) or, at the time of disease progression or for any other reason that requires study treatment termination, whichever occurs sooner.
- On those patients, who have already completed 10 cycles, the one additional correlative blood sample will be collected at the time of their next cycle.

Note *for samples collected while participant is receiving study treatment*: Blood is to be collected prior to pembrolizumab administration – it is not required to hold cyclophosphamide dosing until after sample collection.

All of the above blood samples will be sent at ambient temperature for processing, and/or to be stored frozen, to the Center for Immunotherapy at Roswell Park Cancer Institute for further studies:

Roswell Park Cancer Institute
Immune Analysis Facility Shared Resource
Cancer Cell Center, 4th Floor, Room 416
Elm and Carlton Streets
Buffalo, NY 14263
Telephone #: (716) 845-8459
Junko.Matsuzaki@RoswellPark.org

Note: All laboratories housing research samples need to maintain current, study-specific **Temperature Logs** and **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

8.8.2 Tumor Tissue Collection

Mandatory research tumor tissue core biopsies are obtained at two time points: prior to initiating study treatment and after 3 cycles of study treatment. If patient remains on the treatment much longer at the time of progression she will be offered to undergo another CT guided biopsy for research purposes, however this is not mandatory.

Pre-treatment tumor biopsy needs to be obtained within 4 weeks (28 days) prior to treatment start. (Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Principal Investigator).

On-treatment CT guided tumor biopsies will be done after 3 cycles of treatment (between week #8- #10), even if patient has no signs of disease progression and wishes to remain on the regimen. If patient completes 12 months of treatment and has measurable lesion but wishes to continue with treatment, repeat CT guided biopsy will be offered.

Ovarian adenocarcinoma tissue biopsy (before and after treatment) will be collected by needle core biopsy A minimum of 7 cores will be obtained (preferably 1 cm or more in length).

8.8.3 Ascites

If a patient has ascites at any time during the study period and is willing to undergo paracentesis, or wishes to undergo paracentesis for symptom relief, it is recommended to obtain a minimum of 200 cc (and up to 500 cc) by paracentesis in order to yield an adequate amount of cells for analysis.

Lower or higher volumes are acceptable. Note: The higher the volume of ascites fluid submitted, the better the yield of Tumor Ascites Lymphocytes (TALs).

Ascites fluid will be shipped on wet ice, the same day as collection, to:

Roswell Park Cancer Institute
Immune Analysis Facility Shared Resource
Cancer Cell Center, 4th Floor, Room 403
Elm and Carlton Streets
Buffalo, NY 14263
Telephone #: (716) 845-4199
Anthony.Miliotto@RoswellPark.org

The laboratory at the IAF at RPCI will process the ascites and bank for batch analysis. Samples will be processed at RPCI to obtain cell-free ascites (supernatant following centrifugation). The cell pellet will be utilized for isolation of TALs.

Note: All laboratories housing research samples need to maintain current, study-specific **Temperature Logs** and **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

8.9 Pathology

8.9.1 Pre- and On-Treatment Biopsies

Ovarian adenocarcinoma tissue biopsy before and after treatment will be collected by needle core biopsy [participants for whom newly-obtained biopsy samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Principal Investigator. Seven cores will be obtained, preferably 1 cm or more in length. Adequate specimen must be collected and divided into 5 different aliquots. These specimens in order of priority include tissue collected and:

1) 2 cores: Fixed in formalin for 8 to 24 hours and processed as a paraffin embedded block (FFPE block) for subsequent immunohistochemistry correlative studies
2) 1 core: Placed in RPMI with 5% FBS on wet ice (flow cytometry specimen) for immediate processing to isolate infiltrating immune cells; tissue will be used for subsequent flow cytometry sorting of tumor and subpopulations of hematopoietic cells
3) 3 cores (each cores will be stored separately): Snap frozen in cryovials in liquid nitrogen (RNA specimen) for subsequent RNA isolation
4) 1 core (separately): Snap-frozen in cryovials in liquid nitrogen (DNA specimen) for subsequent DNA analysis

For **needle core biopsies** the following is the minimum amount of tissue for each of the above listed specimens and each specimen is listed in the order of priority if less than a full complement of 7 cores can be procured:

1) FFPE block - 2 needle cores not less than an 18 gauge needle and not less than 1 cm in length
2) Flow cytometry specimen - 1 needle core not less than an 18 gauge needle and not less than 1 cm in length
3) RNA specimen – minimum of 3 needle cores not less than an 18 gauge needle and not less than 1 cm in length
4) DNA specimen. minimum of 1 needle core biopsy not less than an 18 gauge needle and not less than 1 cm in length for DNA analysis

* Core biopsy tissue samples are to be labeled with a study-specific ID number, clinical study number, protocol time point and, protocol day.

Tissue in RPMI with 5% FBS will be sent same day to Junko Matsuzaki laboratory for additional processing and analysis. Snap frozen tissue may be batched in CSPO (S-636, GBSB) and will be sent to Dr. Zsiros' lab upon request:

Center for Immunotherapy at Roswell Park Cancer Institute for further studies:

Roswell Park Cancer Institute
Immune Analysis Facility Shared Resource
Cancer Cell Center, 4th Floor, Room 416
Elm and Carlton Streets
Buffalo, NY 14263
Telephone #: (716) 845-8459
Junko.Matsuzaki@RoswellPark.org

Core biopsy tissue that is fixed in formalin will be delivered to: RPCI Paraffin Core Facility, GBSB S-601, Telephone number: 845-3006. The finished blocks will be batched in CSPO (S-636, GBSB) and will be sent to Dr. Zsiros' lab upon request.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

8.9.2 Pre-Treatment Formalin-Fixed Paraffin-Embedded (FFPE) Biopsy Samples

For patients whom a new biopsy is contraindicated, the following sections of tissue from the most recent (must be within 28 days prior to start of study treatment) neoplastic tissue biopsy that exists in the Paraffin Archive in the Department of Pathology (or outside institution) will be collected:

1. Eight unstained sections cut at 4 microns on plus glass slides.
2. One H&E stained section

Slides will be forwarded from CSPO to Dr. Zsiros' laboratory in the Center for Immunotherapy (see above for full contact information) upon request.

8.10 Human Microbiome collection

8.10.1 Assessing quality of life and emotional well-being and lifestyle using the EORTC QLQ-C30, QLQ-OV28, MFSI-SF and Microbiome questionnaires

EORTC QLQ-C30 (Appendix G) has been developed to assess the quality of life of cancer patients. It is supplemented by disease-specific modules such as breast, lung, head & neck and, ovarian (QLQ-OV28: Appendix H). The Multidimensional Fatigue Symptom Inventory-Short

Form (MFSI-SF: Appendix I) gathers information on emotional well-being, social functioning, energy/fatigue and physical functioning. All of these questionnaires have been well validated in large number of studies and have been widely used in cancer patients.

The Microbiome Initial Assessment Questionnaire (Appendix K) and the Microbiome Collection Questionnaire (Appendix J) were specifically designed for patients enrolling in this study in order to broaden the information gathered on nutrition and basic lifestyle habits that have been shown to have an impact on human microbiome composition.

At every collection visit the clinical research coordinator will administer the following questionnaires to assess the patient's quality of life, stress/fatigue level as well as basic lifestyle information:

1. **EORTC QLQ-C30 and EORTC QLQ-OV28**
2. **Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF)**
3. **Microbiome Collection Questionnaire**

The **Microbiome Initial Assessment Questionnaire** will be completed only once, prior to cycle #1.

EORTC QLQ-C30, EORTC QLQ-OV28 and Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) will be collected on Cycle 7 Day – 1, Cycle 10 Day 1 and at the end of study as well to assess quality of life during the study. The Microbiome Collection Questionnaire will be collected each time there is microbiome sampling (see Section 8.10.2)

Paper questionnaires will be collected by the clinical research coordinator on the day of the patient's visit (see below Microbiome collection time points) and will be kept in a secured, locked-up study folder in the clinic. Prior to analysis, the PI will request that a database be built (either in EXPeRT or REDCAP) and the data from the paper questionnaires collected throughout the trial be entered into the database by the CRC for analysis.

8.10.2 Microbiome collection time points

All collections (skin, vaginal sampling and stool collection) will be performed at the following time points:

1. Prior to cycle#1 (within 7 days of starting treatment, preferably on D1 of cycle 1 prior to staring chemotherapy)
2. After 3 cycles (within 7 days of starting cycle 4, preferably on D1 of cycle 4 prior to starting chemotherapy)
3. After 9 cycles, (within 7 days of starting cycle 10, preferably on D1 of cycle 10 prior to starting chemotherapy)
4. At the end of treatment (during the last physical exam at the time of study termination, stool sample collection within 7 days of this visit)\

On those patients, who have already completed 10 cycles, the one additional microbiome sample will be collected at the time of their next cycle.

Prior to each time point the clinical research coordinator will call the patient on the phone (3-5 days prior) to remind her to follow the written instructions given to her prior to collection and to answer any questions the patient may have.

All microbiome samples will be labeled with the participant's MR number, participant's initials, participant's study number, clinical study number, protocol time point, and protocol day. Unless otherwise indicated all samples will be sent to DBBR for de-identification, storage and future DNA extraction.

Roswell Park Cancer Institute
DBBR Laboratory
GBSB Bldg. 7th Floor, Rm. 726 via Tube station # 86
Attn: Study Number – I 270715 (RQ-006865)
Elm & Carlton Streets
Buffalo, NY 14263
Tel: 716-845-1036
Fax: 716-845-1350

warren.davis@roswellpark.org

8.10.3 Skin microbiome collection

Prior to each collection patients will be instructed by the clinical research coordinator. The participant will also receive written instructions (Appendix L) to follow the guidelines for optimal collection. Prior to each collection visit patients will be asked:

- to abstain from using antibiotic containing soaps and washes for 1 week
- to abstain from topical antibiotics for 1 week – unless patient was specifically instructed to use antibiotics
- not to swim in pool, use hot tubs or tan for 3 days prior to sample collection
- to abstain from using moisturizers and cosmetics or other topical products and washing sampled skin areas for 24 hours

8.10.3.1 Skin microbiome collection sites

Skin samples for microbiome analysis will be collected from the following sites:

- bilateral volar forearms
- bilateral inguinal crease
- bilateral upper back

8.10.3.2 Skin microbiome collection technique and specimen handling:

A standard kit put together by DBBR for skin microbiome collection will be sent and stored at the gynecologic clinic by the clinical research coordinator.

Swab-scrape-swab technique will be used, as described by Oh et al (99). If a skin eruption is present, an area closest to the planned collection site that is unaffected by the cutaneous eruption will be sampled.

Skin microbiome collection will be done prior to cycle #1, cycle #4, cycle #10 and at the time of study termination by clinician and/or research nurse, as described above. Labeled tubes will be sent to DBBR for de-identification, storage and future DNA extraction and genetic analysis depending on patients' clinical response.

8.10.3.3 Skin checkups and monitoring for cutaneous side effects

Given the high frequency of dermatologic side effects with immune check point inhibitors, we will encourage all patients to undergo a dermatological examination before the initiation of the study and after cycle #3 and at the end of the study. Any skin lesion will be documented and followed up for changes by a dermatologist. In addition to this, if any cutaneous symptoms or side effects are noted, the patient will be offered immediate follow up with dermatology, particularly if patient has grade 3-4 skin toxicity.

8.10.4 Vaginal sample collection technique and specimen handling

Prior to collecting the vaginal samples patients will be given written instructions (Appendix M) during her prior visit to not to use any vaginal lubricants/cream/medications or perform vaginal douching 3 days prior to her visit.

A standard kit put together by DBBR for vaginal microbiome collection will be used. Vaginal samples will be collected during sterile speculum exam with no lubrication use (water is permitted) posterior fornix and lateral fornices with a swab swirled around. Two separate collections will be performed. One collection will be obtained for Gram staining and one for bacterial genomic DNA extraction. The first vaginal collection of vaginal fluid will be used for genomic DNA extraction following Roswell Park Cancer Institute's internal protocol on vaginal microbiome collection . It will be sent to DBBR immediately for de-identification, storage and future DNA extraction. The second collection will be a standard vaginal culture and will be sent to the lab for Gram-stain as vaginal culture.

8.10.5 Fecal microbiome collection

The stool specimen will be self-collected by participants. Patients will be provided with a fecal sample collection kit. Patients will be provided with written instructions (Appendix N), and study staff will explain how to use the kits to collect fecal samples in the privacy of their own homes prior to scheduled clinic visits. We will instruct the participants to collect stool specimens within 24 h before each sampling visit and bring them to the visits. If patient is not able to provide us the sample with home collection or enough sample for analysis, we will perform stool sample collection during the routine rectovaginal exam at that time of their study visit. If not

successful with stool sample collection sample with either of these methods (home collection or during office visit), we will ask the patient to collect the sample within the following 7 days and coordinate the sample handing with the clinical research coordinator (sample to be either brought to the clinic or mailed to the clinic in a kit provided).

Collected stool samples will be sent to DBBR for de-identification, storage and future DNA extraction.

Stored stool samples in DBBR will be sent out to commercial entities without PHI for stool metabolite analysis per PI's decision.

Stool samples can be used for fecal microbiota transfer to animals under approved IACUC protocols.

8.11 Planned Correlative Studies

NOTE: This section contains a comprehensive list of proposed correlative studies, however depending on the clinical response of patients, available tumor tissue and budget, the PI will select the most appropriate translational studies to obtain the most comprehensive analysis on the collected specimens (See comment in Section 1.7).

8.11.1 Assessment of dynamic changes in tumor microenvironment prior to and subsequent to therapy

Pre-treatment and on-treatment tumor CT guided biopsy tissue will be processed for RNA isolation and evaluated for gene RNASeq. PI may use any prior/and or future banked tissue, blood, ascites and microbiome specimen on all enrolled patient for translational research purposes.

Transcriptional findings will be validated by immunohistochemistry/ immunofluorescence for expression of PD-L1 as a predictive biomarker and presence of various infiltrating immune cell subsets, including CD8 cells, $CD4^+$ effector cells ($FoxP3^-$), $CD4^+$ regulatory T cells ($FoxP3^+$), and myeloid cells. Immunohistochemistry for PD-L1 expression will be performed by QualTek Laboratories, the rest of the staining will be done at Roswell Park Cancer Institute. Depending on tissue availability, tumors will be assessed for expression of immune inhibitory receptors and proteins (such as indoleamine dioxygenase (IDO), B7-H3, B7-H4, LAG3, TIM3, PD-1, CTLA-4, VISTA, and BTLA), and immune-activating receptors (such as 4-1BB (CD137), OX40, GITR, CD40, and ICOS). DNA extracted from tumors will be sent for deep sequencing of T cell receptor V beta chain to determine repertoire composition.

Refer to **Table 5** for planned sample analysis methods for changes in the tumor microenvironment pre- and post-treatment.

Table 5 Analysis of Tumor Microenvironment

Biomarker	Approach	Parameters*
Immune cell subsets	RNASeq+/- IHC**	CD3, CD4, CD8, FOXP3, CD56, CD14, CD33, CD19, CD11c, CD103

Targetable immune activating genes	RNASeq+/- IHC*	4-1BB, OX40, GITR, CD40, and ICOS
Targetable immune inhibitory genes	RNASeq+/- IHC*	PD-L1, IDO, B7-H3, B7-H4, LAG3, TIM-3, PD-1, CTLA-4, VISTA, and BTLA
Neo-antigen Landscape	Whole-exome sequencing of DNA	Mutation load, driver mutations, neo-antigens
Cancer-testis antigens	RNASeq+/- IHC*	e.g. NY-ESO-1, MAGE A4, etc.
T cell receptor repertoire	Adaptive Biotechnologies Immunoseq	T cell density, TCR clonality, clonal overlap.

*Additional parameters will be added from the panel, depending on the results from the initial few patients
**Validation by IHC will depend on tissue availability

The following will be shipped to Tyler Curiel at University of Texas, San Antonio:

- De-identified IHC slides for PDL1 and PDL2 staining
- Qualtek data
- Nanostring digital spatial imaging data on tumor microenvironment
- RNA and whole exome sequencing data

All data and slides will be sent in a blinded fashion and are not to include any PHI. Only the data on clinical efficacy will be shared with his team.

Tyler Curiel
Cancer Therapy and Research Center
7979 Wurzbach Rd # 600,
San Antonio, TX 78229
curielt@uthscsa.edu

8.11.2 Exploration of peripheral blood biomarkers as early predictors of clinical benefit

Pre-treatment and on-treatment/post-treatment samples will be analyzed by NanoString platform and by flow cytometry to study the effects of treatment on various peripheral blood immune cell subsets. PBMC isolated from whole blood will be processed for multicolor flow cytometry analyses looking at different cell subsets such as $CD4^+$, $CD8^+$, NK, NKT, and regulatory T cells ($CD25^+FoxP3^+$), markers of T cell activation (CD25, CD62L, HLA-DR, CD150, ki67, granzyme B, ICOS, CD137, OX40, GITR), inhibition/exhaustion (PD-1, LAG3, TIM3, CD160), and percentages of myeloid-derived suppressor cells (MDSCs) (HLA-DR, CD33, CD14, CD15, CD3, CD19, CD56, CD16). DNA extracted from PBMC would be sent for deep sequencing of T cell receptor V beta chain to determine repertoire composition.

Serum cytokines and chemokines will be measured to evaluate for evidence of Th1/Th2/Th17 or other type of immune response (ELISA).

Refer to **Table 6** for planned analyses of tentative peripheral blood biomarkers.

Table 6 Analysis of Peripheral Blood Biomarkers

Biomarker	Approach	Parameters*
Transcriptional profile*	RNASeq	Markers related to immune activation and inhibition, as discussed in text
T cell activation	Flow cytometry	CD25, CD62L, HLA-DR, CD150, ki67, granzyme B, ICOS, CD137, OX40, GITR
T cell exhaustion	Flow cytometry	PD-1, LAG3, TIM3, CD160
MDSC	Flow cytometry	HLA-DR, CD33, CD14, CD15, CD3, CD19, CD56, CD16
T cell receptor repertoire	Adaptive Biotechnologies Immunoseq	T cell density, TCR clonality, clonal overlap.
Inflammatory Cytokines	Flow Cytometry: HCYTMAG-60K-PX41 | MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel	sCD40L, EGF, Eotaxin/CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF

*Due to potential differences in PBMC processing amongst different sites (if additional sites are used), transcriptional profiling of freshly frozen buffy coats will be used to get a broad standardized assessment of the immune changes in the peripheral blood.

8.11.3 Exploration of genetic predictors of response

To determine whether specific tumor genetic determinants influence the response to treatment, DNA isolated from archived tumor samples may be processed for whole exome analysis to assess for specific driver mutations and for potential neoantigens using appropriate bioinformatics algorithms developed at MSKCC.

Refer to **Table 7** for tentative planned genomic analyses.

Table 7 Analysis of Genetic Predictors

Biomarker	Approach	Parameters
Genetic drivers	Whole exome sequencing	Presence of mutations/alterations in a panel of known oncogenes/tumor suppressors
Neoantigen signature	Whole exome sequencing	Presence of immunogenic epitopes based on prediction algorithms

8.11.4 Exploration of human microbiome as a predictor of clinical response and its association with potential side effects

Depending on clinical results and patient's side effects, bacterial DNA will be extracted from fecal, skin and vaginal samples using standard DNA extraction methods per Roswell Park Cancer Institute microbiome protocol. Collected clinical questionnaires will be used to interpret the final analyzed microbiome data. The microbial community will be assessed using 16S rRNA profiling of the V4 variable region via MiSeq next generation sequencing in the RPCI Genomics Shared Resource. Prior to sequencing, DNA with be amplified with PCR and barcoded primers so that amplicons can be pooled for analysis. Following sequencing, sequences will be screened and assigned to samples using the barcodes, and all failed sequence reads, low-quality sequence ends and tags will be removed and sequences will be depleted of any non-bacterial ribosome sequences and chimeras using the Black Box Chimera Check software B2C2 (described and available at http://www.researchandtesting.com/B2C2.html for free). Sequences will be imported into ARB and aligned to a 16S rRNA curated database of sequences representative of the gut, skin and vaginal microbiomes from curated databases.

Cleaned sequences will be aligned in MOTHUR. Sequences will be imported into ARB and phylogenetic relationships of the aligned 16S rRNA gene sequences will be inferred using maximum likelihood from a reference tree generated using RAXML in ARB (version 100, www.arb-silva.de) and placer. Phylogenetic trees will be constructed from approximately 10,000 16S rRNA sequences using the weighted UniFrac metric that can detect differences in how many organisms from each lineage are present, as well as detecting differences in which organisms are present. A distance matrix based on the UniFrac metric will be exported and used in multivariate analysis to explain the variation in the microbial communities. As an alternative analysis, we will also test other distance-based metrics to optimize characterization the microbial communities' qPCR of 16S rRNA genes using standard techniques and established primers will be used to estimate the copy number for different microbial groups. The lowest quantifiable 16S rRNA gene copy number estimate for bacterial groups is as follows: Eubacteria: 700; Lactobacteriaceae: 400; Enterobacteriaceae: 250; Bacteroidaceae: 450; Clostridium Cluster XIVa: 1000 copies. Assuming that the bacterial biomass of the gut is 1011 cells/g and that there is at minimum one 16S rRNA gene copy per cell, the detection limit is very low.

Sequences will be clustered into operational taxonomic units (OTU) at 97% similarity based on the average neighbor-joining algorithm and classified using a naïve Bayesian classifier implementation in MOTHUR. After classification, sequences will be assigned to phylum and genus-level phylotypes, after which alpha (diversity within an individual) and beta (diversity between individuals) diversity will be calculated. Alpha diversity will be measured with the inverse Simpson index and beta diversity as the Theta YC distance metric from UniFrac.

- In microbial communities, 16S rRNA genes are usually not normally distributed. Therefore, bacterial communities will be analyzed using NMS, an approach that has been widely used in ecological community data analysis and is based on a distance metric between samples (i.e., Euclidean, Jenson-Shannon Divergence, or UniFrac). NMS analysis is an iterative, non-parametric ordination technique that searches for the best

position of the different microbial populations, measured as 16S rRNA genes, in multi-dimensions that minimizes the stress of the multiple dimensional configurations of samples. A Monte Carlo test will be used to assess whether the final solution that minimizes stress is significantly different from the solution produced by random chance. NMS provides composite scores of 16S rRNA genes that can be used in regression analysis. In the context of the current study design, this means that each person has a microbial community fingerprint signature. This approach is defined as a composite measure of microbial community composition.

8.11.5 Assessment of Biospecimens and Clinical data

Biospecimens, clinical efficacy, basic clinical information, and toxicity data could be shared with other academic institutions and commercial entities without PHI with whom Roswell Park Cancer Institute has an MTA in place with in order to enhance biomarker/translational research.

9 EFFICACY EVALUATIONS

Tumor response will be assessed after the 3rd and 6th cycles then after every 6 cycles by immune-related response criteria (irRECIST) (96, 100) by independent, central, blinded radiographic review. Conventional RECIST 1.1 (98) will also be documented during the trial, however RECIST 1.1 will not be used to determine disease progression, as it has been shown in prior clinical trials using Pembrolizumab in large number of patients, that conventional RECIST might underestimate the benefit of pembrolizumab in approximately 15% of patients; thus using irRECIST could permit treatment beyond initial progression per RECIST v1.1 and prevent premature cessation of treatment (95-97).

The irRECIST will be used for tumor response assessment at time of continuing review and will be used for IRB reporting and formal analysis.

9.1 Baseline

9.1.1 Measurable Lesion Definitions and Target Lesion Selection

- All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, will be identified as target lesions and recorded and measured at baseline
- Measurable lesions must be accurately measured in at least one dimension with a minimum size of:
    - 10 mm in the longest diameter by CT or MRI scan (or no less than double the slice thickness) for non- nodal lesions and ≥ 15 mm in short axis for nodal lesions.
    - 10 mm caliber measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
    - 20 mm by chest X-ray

- A sum of the longest diameter (short axis for lymph nodes) of all target lesions will be calculated and reported as the baseline sum diameters. This will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

9.1.2 Non-measurable Lesion Definitions

- Non-target lesions will include:
    - Measurable lesions not selected as target lesions
    - All sites of non-measurable disease, such as neoplastic masses that are too small to measure because their longest uninterrupted diameter is < 10 mm (or < two times the axial slice thickness), i.e., the longest perpendicular diameter is ≥ 10 and < 15 mm.
    - Other types of lesions that are confidently felt to represent neoplastic tissue, but are difficult to measure in a reproducible manner. These include bone metastases, leptomeningeal metastases, malignant ascites, pleural or pericardial effusions, ascites, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, ill-defined abdominal masses, skin lesions, etc.
- All lesions or sites of disease not recorded as target lesions (e.g., small lesions and non-measurable lesions) should be identified as non-target lesions and indicated as present in the source documents at baseline. There is no limit to the number of non-target lesions that can be recorded at baseline. The general location will also be documented on the images, drawing a regularly-shaped Region of Interest.
- Measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation.

9.1.3 Target and Non-Target Lymph Node Lesion Definitions

- To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

9.1.4 Non-Target Lesion Selection

- All lesions or sites of disease not recorded as target lesions should be recorded as non-target lesions at baseline. There is no limit to the number on non-target lesions that can be recorded at baseline.

9.1.5 Bone Lesions

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

- Regardless of the imaging modality blastic bone lesions will not be selected as target lesions. Lytic or mixed lytic-blastic lesions with a measurable soft tissue component ≥ 10 mm can be selected as target lesions.

9.1.6 Cystic and Necrotic Lesions as Target Lesions

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- Lesions that are partially cystic or necrotic can be selected as target lesions.
- The longest diameter of such a lesion will be added to the ***Total Measured Tumor Burden*** (TMTB) of all target lesions at baseline.

If other lesions with a non-liquid/non-necrotic component are present, those should be preferred.

9.1.7 Lesions with Prior Local Treatment

- During target lesion selection the radiologist will consider information on the anatomical sites of previous intervention (e.g. previous irradiation, RF-ablation, TACE, surgery, etc.).
- Lesions undergoing prior intervention will not be selected as target lesions unless there has been a demonstration of progress in the lesion.

9.1.8 No Disease at Baseline

- If a patient has no measurable and no non-measurable disease at baseline the radiologist will assign '***No Disease***' (irND) as the overall tumor assessment for any available follow-up time-points unless new measurable lesions are identified and contribute to the TMTB.

9.2 Follow-Up

9.2.1 Recording of Target and New Measurable Lesion Measurements

- The longest diameters of non-nodal target and new non-nodal measurable lesions, and short axes of nodal target and new nodal measurable lesions will be recorded. Together they determine the ***Total Measured Tumor Burden (TMTB)*** at follow-up.

9.2.2 Definition of New Measurable Lesions

- In order to be selected as new measurable lesions (≤ 2 lesions per organ, ≤ 5 lesions total, per time-point), new lesions must meet criteria as defined for baseline target lesion selection and meet the same minimum size requirements of 10 mm in long diameter and minimum 15 mm in short axis for new measurable lymph nodes. New measurable lesions shall be prioritized according to size, and the largest lesions shall be selected as new measured lesions.

9.2.3 Non-Target Lesion Assessment

- The RECIST 1.1 definitions (98) for the assessment of non-target lesions apply (i.e., measurements of the non-target lesions will not be performed, but the presence or absence of each should be noted throughout follow-up and evaluation).
- The response of non-target lesions primarily contributes to the overall response assessments of irCR and irNon-CR/Non-PD (irNN).
- Non-target lesions do not affect irPR and irSD assessments.
- Only a massive and unequivocal worsening of non-target lesions alone, even without progress in the TMTB is indicative of irPD.

9.2.4 New Non-Measurable Lesions Definition and Assessment

- All new lesions not selected as new measurable lesions are considered new non-measurable lesions and are followed qualitatively.
- Only a massive and unequivocal progression of new non-measurable lesions leads to an overall assessment of irPD for the time-point.
- Persisting new non- measurable lesions prevent irCR.

9.2.5 irRECIST Overall Tumor Assessments

The irRECIST overall tumor assessment is based on the ***TMTB*** (total measured tumor burden) of measured target and new lesions, non-target lesion assessment and new non-measurable lesions.

- **irCR:** Complete disappearance of all measurable and non-measurable lesions. Lymph nodes must decrease to < 10 mm in short axis.
- **irPR:** Decrease of ≥ 30% in TMTB relative to baseline, non-target lesions are irNN, and no unequivocal progression of new non-measurable lesions.
- **irSD:** Failure to meet criteria for irCR or irPR in the absence of irPD.
- **irNN:** No target disease was identified at baseline and at follow-up the patient fails to meet criteria for irCR or irPD.
- **irPD:** Minimum 20% increase and minimum 5 mm absolute increase in TMTB compared to nadir, or irPD for non-target or new non-measurable lesions. Confirmation of progression is recommended minimum 6 weeks after the first irPD assessment.
- **irNE:** Used in exceptional cases where insufficient data exists.
- **irND:** In adjuvant setting when no disease is detected.

New Lesions: the presence of new lesion(s) does not define progression. The measurements of the new lesion(s) are included in the sum of the measurements (the sum of the measurements = the sum of the longest diameters of all target lesions and new lesions, if any).

9.2.6 Confirmation Measurement

A confirmatory assessment is required no less than 6 weeks after an irPR or irCR is deemed by irRECIST (96)

9.3 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. . The preferred imaging modality for this clinical trial is conventional CT scan using IV contrast.

- **Clinical Lesions:** Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- **Chest x-ray:** Chest x-ray is not accurate in assessment of lesion size in the lung and should not be used as a method of measurement. Conventional chest CT is the preferred imaging modality to evaluate response to treatment.
- **Conventional CT and MRI:** This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans) however, conventional CT scan is the preferred modality to determine response to treatment.

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the irRECIST or RECIST 1.1 guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be

used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

- **Ultrasound:** Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.
- **Endoscopy, Laparoscopy:** The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.
- **Tumor Markers:** Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.
- **Cytology, Histology:** These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10 SAFETY EVALUATION

10.1 Adverse Events

10.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of ‘unrelated’, ‘unlikely’, ‘possible’, ‘probable’, or ‘definite’).

An AE is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

10.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

10.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

10.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

10.1.1.4 Preexisting Medical Conditions (Baseline Conditions)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on

an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

10.1.1.5 Overdose

Refer to **Section 7.1.6** for a definition of pembrolizumab overdose.

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

10.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 4.

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.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or

concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness. An investigator, who is a qualified physician, will evaluate all adverse events using the following guidelines:

V4.0 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 subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. 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.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the 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 the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the 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 the 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)**	
	De-challenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive de-challenge. If no, this is a negative de-challenge. (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 Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the 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) Merck 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 THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR 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 the 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 a Merck product relationship).**	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.	
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

10.1.3 Reporting Adverse Events

Table 8 Guidelines for Routine Adverse Event Reporting for Phase 2 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Routine AEs occurring from the time the consent form is signed until 30 days after the last intervention, or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

10.2 Events of Clinical Interest (ECIs)

Refer to the separate guidance document entitled "*Event of Clinical Interest Guidance Document*" for detailed instruction regarding identification, evaluation and management of ECIs and immune-related adverse events (irAEs).

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

Any Grade 3 or higher event that the investigator considers to be immune-related should be reported as an ECI regardless of whether the specific event term is in **Table 9** and **reported to Merck within 24 hours** from the time the Investigator/physician is aware of such an occurrence. Adverse events that are both an SAE and an ECI should be reported one time as an SAE only. ECIs will not be indicated in the data base, but will be tracked by the clinical research coordinator in an AE log to facilitate reporting to Merck (refer to *Event of Clinical Interest Guidance Document*, v.5, page 8).

Table 9 Events of Clinical Interest

Pneumonitis (reported as ECI if ≥ Grade 2)		
Acute interstitial pneumonitis	Interstitial lung disease	Pneumonitis
Colitis (reported as ECI if ≥ Grade 2 or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Intestinal Obstruction	Colitis	Colitis microscopic
Enterocolitis	Enterocolitis hemorrhagic	Gastrointestinal perforation
Necrotizing colitis	Diarrhea	

Endocrine (reported as ECI if ≥ Grade 3 or ≥ Grade 2 and resulting in dose modification or use of systemic steroids to treat the AE)		
Adrenal Insufficiency	Hyperthyroidism	Hypophysitis
Hypopituitarism	Hypothyroidism	Thyroid disorder
Thyroiditis	Hyperglycemia, if ≥Grade 3 and associated with ketosis or metabolic acidosis (DKA)	
Endocrine (reported as ECI)		
Type 1 diabetes mellitus (if new onset)		
Hematologic (reported as ECI if ≥ Grade 3 or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Autoimmune hemolytic anemia	Aplastic anemia	Thrombotic Thrombocytopenic Purpura (TTP)
Idiopathic (or immune) Thrombocytopenia Purpura (ITP)	Disseminated Intravascular Coagulation (DIC)	Hemolytic Uremic Syndrome (HUS)
Any Grade 4 anemia regardless of underlying mechanism		
Hepatic (reported as ECI if ≥ Grade 2, or any grade resulting in dose modification or use of systemic steroids to treat the		
Hepatitis	Autoimmune hepatitis	Transaminase elevations (ALT and/or AST)
Infusion Reactions (reported as ECI for any grade)		
Allergic reaction	Anaphylaxis	Cytokine release syndrome
Serum sickness	Infusion reactions	Infusion-like reactions
Neurologic (reported as ECI for any grade)		
Autoimmune neuropathy	Guillain-Barre syndrome	Demyelinating polyneuropathy
Myasthenic syndrome		
Ocular (report as ECI if ≥ Grade 2 or any grade resulting in dose modification or use of systemic steroids to treat the AE)		
Uveitis	Iritis	
Renal (reported as ECI if ≥ Grade 2)		
Nephritis	Nephritis autoimmune	Renal Failure
Renal failure acute	Creatinine elevations (report as ECI if ≥Grade 3 or any grade resulting in dose modification or use of systemic steroids to treat the AE)	
Skin (reported as ECI for any grade)		
Dermatitis exfoliative	Erythema multiforme	Stevens-Johnson syndrome
Toxic epidermal necrolysis		
Skin (reported as ECI if ≥ Grade 3)		
Pruritus	Rash	Rash generalized
Rash maculopapular		
Any rash considered clinically significant in the physician's judgment		
Other (reported as ECI for any grade)		
Myocarditis	Pancreatitis	Pericarditis
Any other Grade 3 event which is considered immune-related by the physician		

Events of clinical interest for this study include:

- An overdose of pembrolizumab that is not associated with clinical symptoms or abnormal laboratory results.
- 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. Guidance for assessment and follow up of these criteria can be found in the *Event of Clinical Interest Guidance Document*.

10.3 Serious Adverse Events

10.3.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does **NOT** include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Is associated with an overdose
- Is a new cancer (that is not a condition of the study)

Additionally, any serious adverse event considered by an investigator who is a qualified physician to be related to the investigational drug, that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph, also must be reported immediately to the Sponsor.

10.3.2 Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 90 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 90 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to **Section 10.6** for details on reporting Unanticipated Problems.

10.3.3 Reporting Events of Clinical Interest (ECIs)

ECIs (both non-serious and serious adverse events) identified in the *Event of Clinical Interest Guidance Document* from the date of first dose of pembrolizumab through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Principal Investigator and within 2 working days to Merck Global Safety, regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

Attn: Worldwide Product Safety

FAX 215 993-1220

10.3.4 Reporting of Overdose

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

10.3.5 Reporting of Pregnancy and Lactation

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a participant (spontaneously reported to them), including the pregnancy of a male participant's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier. All participants and female partners of male participants who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

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

10.4 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

10.5 Follow-Up for Events of Clinical Interest

All related ECIs should be followed to resolution. The Adverse Experience eCRF should be updated with information regarding duration and clinical course of the event. Information

obtained from the consulting specialist, including diagnosis, should be recorded in the appropriate AE fields. Free-text fields should be used to record narrative information:

- Clinical course of the event
- Course of treatment
- Evidence supporting recovery
- Follow-up to the clinical course

Any treatments administered for the event should also be entered in the Concomitant Medication eCRF.

10.6 Unanticipated Problems

10.6.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
  b) The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed **Serious** per **Section 10.3**.

10.6.2 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. The Unanticipated Problem Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Compliance will submit the UP to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Unanticipated Problem Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

10.7 FDA Reporting

When RPCI is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening;

Or, meets **ANY** of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human participants including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to CRSCompliance@RoswellPark.org.

11 DATA AND SAFETY MONITORING

The RPCI Data and Safety Monitoring Board will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMB will review the study annually and will make

recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

12 STATISTICAL METHODOLOGY

The **primary objective** of this study is:

- To evaluate improvement in progression-free survival for patients treated with anti-PD1 pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide as compared to a historical control. Data will be collected to be used for final analysis (Clinical Data Appendix).

The **secondary objectives** of this study are:

- To obtain pilot data on clinical response rates using both RECIST1.1 criteria (Response Evaluation Criteria in Solid Tumors) and immune related response criteria (irRECIST).
- To obtain data on changes in tumor microenvironment prior to and subsequent to therapy and, to screen for potential biomarkers to predict clinical benefit.
- To determine the safety and tolerability of the treatment combination in the study population.
- To evaluate overall survival in patients treated with anti-PD1 pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide.
- To assess the impact of the combination of anti-PD1 pembrolizumab, IV bevacizumab and oral metronomic cyclophosphamide on anti-tumor immune responses in ovarian cancer.

12.1 Sample Size Determination

The *primary objective* of the Phase II study is to evaluate the progression-free survival (PFS) for patients treated with pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide in women with recurrent/persistent platinum –sensitive, -resistant, or -refractory ovarian, primary peritoneal, or fallopian tube cancers. The median PFS of platinum resistant ovarian cancer patients treated with standard chemotherapy is approximately 3.5 months. In a recent study, platinum-resistant ovarian cancer patients treated with a combination of IV bevacizumab and oral metronomic cyclophosphamide (61) a median PFS and overall survival (OS) of 4.5 and 10.7 months, respectively. In another study, patients with recurrent ovarian treated with bevacizumab plus low-dose metronomic oral cyclophosphamide (62) had a median response duration of 3.9 months. Therefore we would consider a PFS of 7 months or greater to be of interest in this population.

Based on this we expect that no more than 30% of patients treated with standard chemotherapy would achieve 7 months of PFS. Therefore, we will test the null hypothesis that the 7-month PFS rate (π) in patients treated with pembrolizumab 200 mg IV + bevacizumab 15 mg/kg every 3 weeks IV + cyclophosphamide 50 mg po every day combination therapy is less than or equal to 30% versus the one-sided hypothesis that it is greater than 30%. That is:

$$H_0: \pi \leq 0.30$$
$$H_A: \pi > 0.30$$

Where π is the 7-month PFS rate.

Consider a sample size of n=40 patients (including 5 patients from the safety cohort), with up to 1 year of follow-up, and a maximum drop-out rate of 10%. If the true 7-month PFS rate is 50% (a median PFS of 7 months) for the proposed treatment combination, then a one-sided Wald-type test has approximately an 86.1% chance of resulting in significance. A nominal significance level of 10% is considered, this would be the probability of erroneously finding a truly ineffective treatment as worthy of further research.

The study will thus enroll 40 evaluable patients in total, with 5 in the safety cohort and an additional 35 to complete the Phase II study. The accrual is expected to take 18 months.

12.2 Demographics and Baseline Characteristics

Descriptive statistics (as appropriate: n, percent, mean, median, min and max) will be used to summarize demographic and baseline characteristics.

12.3 Phase II Analysis

12.3.1 Safety Lead-in Cohort

A safety lead-in cohort of n_1=5 patients will enrolled and the safety/tolerability of the combination will be examined after each patient completes at least 3 cycles. If 2 or less patients have experienced a drug related toxicity requiring drug/treatment delay or suspension (as defined in Sections 6.4, 6.5, and 6.7.3), then an additional n_2=35 patients are enrolled to complete the Phase II study. Otherwise, the study will be suspended and the research team (to include RPCI Phase I committee, Study PI and study statistician) will meet to discuss possible safety concerns and will decide what actions to take with respect to study continuation.

If the true SAE rate of the proposed treatment combination is 0.30, then there is an 83% chance of continuing study enrollment. Additional scenarios are given below:

True Treatment Toxicity Rate	0.1	0.2	0.3	0.4	0.5
Chance of Continuation	0.99	0.94	0.83	0.68	0.50

12.3.2 Primary Objective

The primary endpoint is progression free survival (PFS). The PFS will be treated as bivariate time-to-event data, where an event indicator variable (0=censored, 1=event) is paired with a continuous time variable. Patients who are progression-free and still on treatment at last follow-up or those who drop-out of the study, for any reason, will be considered censored events; while patients who experience disease progression or death from disease will be considered PFS events. The PFS time will be measured as the time from the start of the study treatment until PFS event, last follow-up or drop-out.

The primary analysis will consider the following hypotheses about the 7 month PFS rate ((π)) in women with recurrent/persistent platinum –sensitive, -resistant, or -refractory ovarian, primary peritoneal, or fallopian tube cancers treated with pembrolizumab in combination with IV bevacizumab and oral metronomic cyclophosphamide:

$$H_0: \pi \leq 0.30$$
$$H_A: \pi > 0.30$$

These hypotheses will be assessed using the set of patients whom completed at least 1 cycle treatment or those who stopped the first cycle due to disease progression or toxicity. The PFS will be summarized using standard Kaplan-Meier methods, from which an estimate of the 7-month survival rate and corresponding standard error will be obtained using the methods proposed by Breslow and Day (101). A Wald type test, utilizing these estimates, will be used to evaluate the given hypotheses. These methods have been applied to survival outcomes in similar settings (102). All analysis will be conducted in SAS v9.4 (Cary, NC) at a significance level of 0.10.

12.3.3 Secondary Objectives

The frequency of toxicities, AE's and SAE's, will be tabulated by grade (as appropriate) across all dose levels and cycles. The toxicity, AE and SAE, rates will be estimated using 90% confidence intervals, which will be obtained using Jeffreys prior method. All participants who receive any study treatment will be considered evaluable for these outcomes.

The overall and progression free survival will be summarized using standard Kaplan-Meier methods, with estimates of median survival and given survival rates will be obtained with 90% confidence intervals.

Objective tumor response will be tabulated for all patients that completed at least three cycles of the proposed treatment combination. The response rate will be estimated using 90% confidence intervals, obtained using Jeffreys prior method.

The plasma and tumor biomarkers will be reported using the appropriate descriptive statistics. Associations between these markers and PFS will be evaluated using Cox regression models; while associations with tumor response will be evaluated using logistic regression models.

12.4 Interim Analysis and Criteria for Early Termination of the Study

No additional explicit interim analyses or early termination criteria are planned for the Phase II study. However, the toxicity and safety outcomes will be reviewed regularly through the DSMB.

13 ETHICAL AND REGULATORY STANDARDS

13.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each participant (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the participant is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the participant log and participant records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining participant authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the participant is treated. The clinical trial should be conducted in accordance with the ethical principles embodied in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, consistent with good clinical practice and the applicable regulatory requirements and according to the guidelines in this protocol, including attached appendices.

13.2 Informed Consent

The Investigator (or IRB specified designee) is responsible for obtaining written consent from each participant or the participant's legally authorized representative in accordance with GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the participant according to applicable GCP guidelines, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The participant should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the participant and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the participant file. At any stage, the participant may withdraw from the study and such a decision will not affect any further treatment options.

14 STUDY RESPONSIBILITIES

14.1 Data Collection

Data entry into the database is to be completed in a timely fashion after the participant's clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Source Form, which is handled in an expedited fashion.

Data management activities will be performed using eClinical. eClinical is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the eClinical Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs (via the EXPeRT Module). eClinical is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the Clinical Research Coordinator and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

14.2 Maintenance of Study Documents

Essential documents will be retained per RPCI's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI.

15 ADMINISTRATIVE RULES

15.1 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

15.2 Termination of the Study

It is agreed that, for reasonable cause, either the RPCI Investigators or Merck, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of participants enrolled in the study.

Merck's Clinical Criteria for Early Trial Termination

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

- Quality or quantity of data recording is inaccurate or incomplete
- Poor adherence to protocol and regulatory requirements
- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
- Plans to modify or discontinue the development of the study drug

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

15.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

16 APPENDICES

Appendix A ECOG Performance Status Scores

Description	Status
Fully active, able to carry on all pre-disease performance without restriction.	0
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.	1
Ambulatory and capable of all self-care but unable to carry out any work activities.	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4
Dead	5

Appendix B Calculation for Creatinine Clearance

Cockroft-Gault Equation*

Men: CrCl = [(140-YR) x IBW] / (SCr x 72)

Women: CrCl = 0.85 x [(140-YR) x IBW] / (SCr x 72)

Where:

CrCl is creatine clearance (mL/min)

IBW is ideal body weight (kg)

SCr is serum creatinine (mg/dL)

YR is age (years)

*Cockcroft D, W, Gault M, H, Prediction of Creatinine Clearance from Serum Creatinine. Nephron. 1976; 16 (1):31-41)

Appendix C Procedure for Obtaining a Urine Protein Creatinine Ratio (UPCR)

1. Obtain at least 4 ml of a random urine sample in a sterile container(does not have to be a 24-hour urine)
2. Determine protein concentration (mg/dL)
3. Determine creatinine concentration (mg/dL)
4. Divide #2 by #3 above:

$$UPCR = \frac{protein\ concentration\ (\frac{mg}{dL})}{creatinine\ concentration\ (\frac{mg}{dL})}$$

The UPC directly correlates with the amount of protein excreted in the urine per 24 hr (i.e., a UPC of 1 should be equivalent to 1 g protein in a 24 hr urine collection).

For the purpose of this study, participant must have a Urine Protein Creatine Ratio (UPCR) < 1 prior to enrollment and ≤ 3.5 for any subsequent cycles.

Appendix D Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin (β-hCG)†
Hemoglobin	Alkaline phosphatase	Glucose	PT (INR)
Platelet count	Alanine aminotransferase (ALT)	Protein	aPTT
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Total triiodothyronine (T3)
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Free thyroxine (T4)
Absolute Neutrophil Count	Carbon Dioxide *(CO_2 or bicarbonate)*‡	results are noted	Thyroid stimulating hormone (TSH)
Absolute Lymphocyte Count	Uric Acid	Urine pregnancy test †	
	Calcium	UPCR	Blood for correlative studies
	Chloride		
	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		

If patient has a random blood glucose value ≥ 200 mg/dL: a fasting blood sugar level and Hemoglobin A1C will be obtained within 48 hours and, treatment will be held until the patient is clinically and metabolically stable – this will be determined by the treating physician.

† 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.

Appendix E Medication Diary

Study No.: _____ **Subject's Name: _____**
Drug Name: _____ **Cycle #: _____**
Medical Record No.: _____

Study Medication Calendar for Cyclophosphamide

You should swallow your medication whole: without cutting, chewing, or crushing. The capsule should be taken in the morning (with or without food) and, it is recommended to drink 2-3 quarts of fluid per day. Wash your hands immediately if you touch a broken tablet

Please complete this calendar on a daily basis immediately after you take your capsule. Fill in the date for each day and write the total number of capsules you take each day. Take **1** capsule each day, in the morning (with or without food).

Start Date: _____

Cycle Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date							
Number of capsules taken							
Cycle Day	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Date							
Number of capsules taken							
Cycle Day	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Date							
Number of capsules taken							
Cycle Day	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date							
Number of capsules taken							

Cycle Day	Day 29	Day 30	Day 31
Date			
Number of capsules taken			

Please remember to bring this calendar with you to your next clinic appointment.

Coordinator's Use Only

$$\%\ Compliance = \left(\frac{Number\ of\ Pills\ Taken}{Number\ of\ Pills\ Scheduled}\right) \times 100$$

$$\_\_\_\_\%\ Compliance = \left(\frac{\quad}{\quad}\right) \times 100$$

Subject's Signature: _____ Date: _____

CRC's Signature: _____ Date: _____

Investigator's Signature: _____ Date: _____

Appendix F NYHA Functional Classification System

New York Heart Association (NYHA) Congestive Heart Failure (CHF) Functional Classification System[a]	
Class	**Functional Description**
NYHA Class I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity (e.g., shortness of breath when walking, climbing stairs, etc.).
NYHA Class II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
NYHA Class III	Marked limitations in activity due to symptoms, even during less-than-ordinary activity [e.g., walking short distance (20-100 m)]. Comfortable only at rest.
NYHA Class IV	Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

[a] The Criteria Committee of the New York Heart Association. (1994). *Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels.* (9th ed.). Boston: Little, Brown & Co. pp. 253–256.

Appendix G EORTC QLQ-C30

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: |_|_|_|

Your birthdate (Day, Month, Year): |_|_|_|_|_|_|

Today's date (Day, Month, Year): 31 |_|_|_|_|_|_|

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Appendix H EORTC OLQ-OV28

EORTC QLQ - OV28

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:	Not at All	A Little	Quite a Bit	Very Much
31. Did you have abdominal pain?	1	2	3	4
32. Did you have a bloated feeling in your abdomen / stomach?	1	2	3	4
33. Did you have problems with your clothes feeling too tight?	1	2	3	4
34. Did you experience change in bowel habit as a result of your disease or treatment?	1	2	3	4
35. Were you troubled by passing wind / gas / flatulence?	1	2	3	4
36. Have you felt full up too quickly after beginning to eat?	1	2	3	4
37. Have you had indigestion or heartburn?	1	2	3	4
38. Have you lost any hair?	1	2	3	4
39. Answer this question only if you had any hair loss: Were you upset by the loss of your hair?	1	2	3	4
40. Did food and drink taste different from usual?	1	2	3	4
41. Have you had tingling hands or feet?	1	2	3	4
42. Have you had numbness in your fingers or toes?	1	2	3	4
43. Have you felt weak in your arms or legs?	1	2	3	4
44. Did you have aches or pains in your muscles or joints?	1	2	3	4
45. Did you have problems with hearing?	1	2	3	4
46. Did you urinate frequently?	1	2	3	4
47. Have you had skin problems (e.g. itchy, dry)?	1	2	3	4
48. Did you have hot flushes?	1	2	3	4
49. Did you have night sweats?	1	2	3	4

Please go on to next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
50. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
51. Have you been dissatisfied with your body?	1	2	3	4
52. How much has your disease been a burden to you?	1	2	3	4
53. How much has your treatment been a burden to you?	1	2	3	4
54. Were you worried about your future health?	1	2	3	4

During the past 4 weeks:	Not at All	A Little	Quite a Bit	Very Much
55. To what extent were you interested in sex?	1	2	3	4
56. To what extent were you sexually active?	1	2	3	4
Answer the following two questions only if you were sexually active:				
57. To what extent was sex enjoyable for you?	1	2	3	4
58. Did you have a dry vagina during sexual activity?	1	2	3	4

Appendix I Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF)

MFSI-SF

Below is a list of statements that describe how people sometimes feel. Please read each item carefully, then circle the one number next to each item which best describes **how true each statement has been for you in the past 7 days.**

		Not at all	A little	Moderately	Quite a bit	Extremely
1.	I have trouble remembering things	0	1	2	3	4
2.	My muscles ache	0	1	2	3	4
3.	I feel upset	0	1	2	3	4
4.	My legs feel weak	0	1	2	3	4
5.	I feel cheerful	0	1	2	3	4
6.	My head feels heavy	0	1	2	3	4
7.	I feel lively	0	1	2	3	4
8.	I feel nervous	0	1	2	3	4
9.	I feel relaxed	0	1	2	3	4
10.	I feel pooped	0	1	2	3	4
11.	I am confused	0	1	2	3	4
12.	I am worn out	0	1	2	3	4
13.	I feel sad	0	1	2	3	4
14.	I feel fatigued	0	1	2	3	4
15.	I have trouble paying attention	0	1	2	3	4
16.	My arms feel weak	0	1	2	3	4
17.	I feel sluggish	0	1	2	3	4
18.	I feel run down	0	1	2	3	4
19.	I ache all over	0	1	2	3	4
20.	I am unable to concentrate	0	1	2	3	4
21.	I feel depressed	0	1	2	3	4
22.	I feel refreshed	0	1	2	3	4
23.	I feel tense	0	1	2	3	4
24.	I feel energetic	0	1	2	3	4
25.	I make more mistakes than usual	0	1	2	3	4
26.	My body feels heavy all over	0	1	2	3	4
27.	I am forgetful	0	1	2	3	4
28.	I feel tired	0	1	2	3	4
29.	I feel calm	0	1	2	3	4
30.	I am distressed	0	1	2	3	4

Appendix J Microbiome Collection Questionnaire

Patient's name:
MRN:
Date:
Cycle Number:

MICROBIOME COLLECTION QUESTIONNAIRE

1. **Have you had any fevers or infections in the past 2 weeks?**
   - o No
   - o Yes, please specify:_____
2. **Have you taken antibiotics or antifungal agents in the past 4 weeks?**
   - o No
   - o Yes, please tell us which medication(s):_____
     - o Why were you given antibiotics? _____
     - o For how long did you take it? _____
3. **How many times a week do you eat red meat?**
   - o I don't eat red meat
   - o 1-3 times a week
   - o More than 3 times a week
4. **How many times a week you eat cruciferous vegetables such as cabbage, broccoli, kale, and cauliflower?**
   - o I don't eat cruciferous vegetables
   - o 1-3 times a week
   - o More than 3 times a week
5. **Do you eat yoghurt?**
   - o I don't eat yoghurt
   - o 1-3 times a week (please specify which kind)_____
   - o More than 3 times a week (please specify which kind)_____
6. **Do you take probiotics?**
   - o No
   - o Yes, please indicate how often_____

Patient's name:
MRN:
Date:
Cycle Number:

7. Do you have any food allergies?

- o No
- o Yes, please list any food allergies:_____
- o Foods you avoid for religious, personal, or cultural reasons:_____
- o Foods your Doctor told you to avoid:_____

8. If you are CURRENTLY on a special diet, please indicate below:

- o Not on a special diet
- o Weight loss
- o Weight gain
- o Vegetarian
- o Diet for diabetes
- o Diet for heart disease
- o Diet for kidney disease
- o Other:_____

9. Are you currently taking prescribed or over-the-counter medications to lose weight or maintain your current weight?

- o No
- o Yes, I am on these weight loss medications:_____

10. Are you taking laxatives to have a bowel movement on a regular basis?

- o No
- o Yes, please describe which kind and how often:_____

Patient's name:
MRN:
Date:
Cycle Number:

11. How would you describe your bowel movements in the past 2 weeks:

- Regular bowel movements every 1-2 days with no change
- Frequent issues with diarrhea, please describe_____
- Frequent issues with constipation, please describe_____

12. Do you participate in regular physical activity?

- No
- Yes, please describe what exercise you like to do? _____
  - How long? _____
  - How many times a week? _____

13. Do you smoke?

- No
- Yes (please indicate how many cigarettes a day)_____

14. Did you swim in a chlorinated pool or use a hot tub in the past 2 weeks?

- No
- Yes, please indicate how many times a week_____

15. Did you use a tanning bed/sun lamp in the past 2 weeks?

- No
- Yes, please indicate how many times and when was the last time_____

16. Do you use vaginal douching?

- No
- Yes, please indicate how many times a week and which kind: _____

Patient's name:
MRN:
Date:
Cycle Number:

17. Have you used lubrication or any vaginal product/medication in the past 2 weeks?

- No
- Yes, please specify_____

18. Have you had any abnormal vaginal discharge or bleeding in the past 2 weeks?

- No
- Yes, please specify:_____

19. Are you currently sexually active?

- No
- Yes

Appendix K Microbiome Initial Assessment Questionnaire

- Patient's name:
- MRN:
- Date:
- Cycle Number:

MICROBIOME INITIAL ASSESSMENT QUESTIONNAIRE

1. **What is your race? (one or more categories may be selected)**

   - American Indian or Alaska Native
   - Black or African American
   - White
   - Asian, please specify: _____
   - Native Hawaiian/Other specific Islander: _____
   - Other Race, please specify:_____

2. **Are you of Hispanic or Spanish origin?**

   - No
   - Yes

3. **Were you breastfed as an infant?**

   - No
   - Yes
   - I don't know

4. **Were you delivered via vaginal delivery or C-section?**

   - Vaginal delivery
   - C-section
   - I don't know

5. **Have you had an appendectomy?**

   - No
   - Yes
   - I don't know

- Patient's name:
- MRN:
- Date:
- Cycle Number:

6. Have you ever had any bowel surgery/removal of a portion of your bowel or stomach?

- No
- Yes, please specify_____
- I don't know

7. Were you ever diagnosed with an autoimmune disease?

- No
- Yes, please specify_____

8. **How often do you use a tanning bed/sun lamp?**

- Never
- 1-3 times a year
- 4-10 times a year
- More than 10 times a year

9. Do you regularly swim or use a hot tub?

- No
- Yes, please indicate how many times a week_____

10. If your untanned skin was exposed to the summer sun for the first time for 45 minutes, would you:

- Always burn, tan minimally
- Burn moderately, tan gradually
- Burn minimally, tan well
- Rarely/never burn

Appendix L Collection Instructions: Skin

SKIN SAMPLE COLLECTION INSTRUCTIONS

During your next office visit we will collect swabs from the surface of your forearms, inguinal creases and upper back to study the bacteria on your skin. The collection will not cause any discomfort and will involve small gentle scraping on the surface of the collection sites and then using a swab to touch the area.

In order to get accurate samples, we ask you to follow the instructions below:

- Please do NOT use antibiotic-containing soaps and washes for 1 week. Most soaps/body wash products do not contain antibiotics, thus you should be fine using regular soap/body wash products.
- Please do NOT put any topical antibiotics on your skin for 7 days, unless you have a skin infection and were specifically instructed to do so by your doctor.
- Please do NOT swim in chlorinated pools, use hot tubs, or tan for 3 days prior to sample collection.
- Please abstain from using moisturizers and cosmetics or other topical products and, from washing sampled skin areas (forearms, inguinal creases, upper back) for 24 hours before your next visit.

Appendix M Collection Instructions: Vaginal

VAGINAL SAMPLE COLLECTION INSTRUCTIONS

At the time of your next visit we will collect 2 vaginal swabs for research purposes during the routine pelvic exam. The sample collection will not cause any additional discomfort and will be similar to getting a Pap smear.

In order to get accurate samples, we ask you to follow the instructions below:

- Please do NOT use any vaginal lubricants/cream/medications for 3 days prior to your visit.
- Please do NOT use vaginal douching for 3 days prior to your visit.

Appendix N Collection Instructions: Stool

STOOL SAMPLE COLLECTION INSTRUCTIONS

On the morning of your visit (or the day prior, but within 24 hours of your visit), please collect a small stool sample and fill out the Stool Sample Collection Questionnaire.

Before starting:

Please check to make sure that you have received the following:

1. A white stool collection container and toilet ring
2. Two (2) plastic tubes with a scoop attached to each lid.
3. A plastic zip-lock bag with an orange biohazard sign
4. A pair of disposable gloves

If something is missing, or if any of the following instructions are unclear, please call the research nurse.

Getting ready to collect:

- Line the white collection container with toilet paper. It is important that you do NOT use the bag with the orange biohazard sign on it, because that bag cannot be flushed down the toilet.
- Follow the instructions on the container lid for placing the container under the toilet seat. The toilet seat and collection kit should be set up like this:

- Sit on the toilet, relax, and collect a stool sample into the container.

Getting sample into the vials we provide:

1. Put on the gloves.
2. Remove the lid from the first tube provided. Using the scoop that is attached to the lid, place 2 pea-sized scoops into the tube. Please do not collect more than 2 pea-sized scoops.
3. Screw the lid onto the tube, and **make sure it is on properly and tightly**.
4. Repeat steps 2-3 with the other tube provided.

5. Place both tubes into the plastic zip-lock bag with the red biohazard sign on it, and **seal it tightly**.

Disposing of the leftover stool:

Dispose of the contents of the collection container (toilet paper, leftover stool) in the toilet.

Dispose of the white plastic collection container in the regular trash.

Remove the gloves, and throw them in the trash. **Do NOT** flush any part of the white collection container or the gloves down your toilet.

Completing the form:

When you are finished and have washed your hands, complete the "Stool Sample Collection Questionnaire" form (on the following page).

Storing before delivery:

Store the collection bag in a cool place, but not the refrigerator. Bring the tubes in the biohazard bag and the Stool Sample Collection Form with you on the morning of your visit, and give them to the research nurse.

Clinic Use Only
Study ID:
Date:
Collection:

STOOL SAMPLE COLLECTION QUESTIONNAIRE

Did you have any problems or concerns with the stool collection? (Please describe):

_____

_____

_____

1) Date stool sample collected: _____-_____-_____ **(MM/DD/YYYY)**

2) Time of collection: _____: _____ **(Hr:Min)** ☐**AM** ☐**PM**

PLEASE BRING THIS QUESTIONNAIRE AND THE SAMPLE MATERIALS TO YOUR NEXT VISIT.

APPENDIX O - CLINICAL DATA NEEDED FOR FINAL ANALYSIS

1. Did the patient have primary debulking surgery? Yes □ No □

    1a. If yes, did the patient have optimal tumor debulking (<1 cm residual disease) Yes □ No □

    1b. If no, did the patient receive neoadjuvant chemotherapy? Yes □ No □

        i. If yes, patient received neoadjuvant chemotherapy, did she have interval cytoreduction? Yes □ No □

        ii. If the patient had interval cytoreduction after neoadjuvant chemotherapy, did she have optimal tumor debulking (<1 cm residual disease) Yes □ No □

2. Did the patient have any secondary debulking at any time of tumor recurrence? Yes □ No □

    2a. If yes, did the patient have optimal tumor debulking (<1 cm residual disease) Yes □ No □

Prior Chemotherapy

3. How many prior lines of chemotherapies the patient has received? _____

4. Is the patient platinum resistant? Yes □ No □

5. Prior Avastin (Bevacizumab) exposure? Yes □ No □

    5a. If yes, for how many cycles? _____

6. Prior oral single agent Cytoxan exposure? Yes □ No □

    6a. If yes, for how many cycles? _____

7. Prior exposure to combination of oral Cytoxan and Avastin (Bevacizumab)? Yes □ No □

    7a. If yes, for how many cycles? _____

    7b. If yes, what response did she have (CR, PR, DP)?

8. Any prior exposure to immunotherapy? Yes □ No □

    8a. If yes, what kind of immunotherapy? _____

Medical history

9. Any history of high blood pressure? Yes □ No □

10. Any history of kidney disease? Yes □ No □

11. Any history of autoimmune disease? Yes □ No □

12. Any history of radiation? Yes □ No □

13. Does the patient have BRCA1 or 2 mutation? Yes □ No □

14. Any exposure of PARP-inhibitors? Yes □ No □

Follow up / Off Study

15. Any subsequent exposure to Avastin (Bevacizumab)? Yes □ No □

    15a. If yes, for how many cycles? _____

    15b. If yes, any major side effects from Avastin (Bevacizumab)? Yes □ No □ _____

16. How many additional lines of chemotherapy? _____

17. Any subsequent exposure to immunotherapy? Yes □ No □

    17a. If yes, what kind of immunotherapy? _____

18. Any new cancer diagnosis? Yes □ No □ _____

19. Any new diagnosis of autoimmune disease? Yes □ No □ _____

17 REFERENCES

1. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203-13. Epub 2003/01/17. doi: 10.1056/NEJMoa020177. PubMed PMID: 12529460.

2. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ, Odunsi K. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102(51):18538-43. doi: 10.1073/pnas.0509182102. PubMed PMID: 16344461; PMCID: 1311741.

3. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10(9):942-9. Epub 2004/08/24. doi: 10.1038/nm1093. PubMed PMID: 15322536.

4. Kryczek I, Wei S, Zhu G, Myers L, Mottram P, Cheng P, Chen L, Coukos G, Zou W. Relationship between B7-H4, regulatory T cells, and patient outcome in human ovarian carcinoma. Cancer research. 2007;67(18):8900-5. Epub 2007/09/19. doi: 10.1158/0008-5472.CAN-07-1866. PubMed PMID: 17875732.

5. Knutson KL, Krco CJ, Erskine CL, Goodman K, Kelemen LE, Wettstein PJ, Low PS, Hartmann LC, Kalli KR. T-cell immunity to the folate receptor alpha is prevalent in women with breast or ovarian cancer. J Clin Oncol. 2006;24(26):4254-61. doi: 10.1200/JCO.2006.05.9311. PubMed PMID: 16908932.

6. Kalli KR, Krco CJ, Hartmann LC, Goodman K, Maurer MJ, Yu C, Johnson EM, Erskine CL, Disis ML, Wettstein PJ, Fikes JD, Beebe M, Ishioka G, Knutson KL. An HLA-DR-degenerate epitope pool detects insulin-like growth factor binding protein 2-specific immunity in patients with cancer. Cancer Res. 2008;68(12):4893-901. doi: 10.1158/0008-5472.CAN-07-6726. PubMed PMID: 18559537; PMCID: 2744636.

7. Reuschenbach M, von Knebel Doeberitz M, Wentzensen N. A systematic review of humoral immune responses against tumor antigens. Cancer Immunol Immunother. 2009;58(10):1535-44. doi: 10.1007/s00262-009-0733-4. PubMed PMID: 19562338; PMCID: 2782676.

8. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76(2):301-14. Epub 1994/01/28. PubMed PMID: 7507411.

9. Mukai S, Kjaergaard J, Shu S, Plautz GE. Infiltration of tumors by systemically transferred tumor-reactive T lymphocytes is required for antitumor efficacy. Cancer Res. 1999;59(20):5245-9. Epub 1999/10/28. PubMed PMID: 10537304.

10. Wang DH, Guo L, Wu XH. Checkpoint inhibitors in immunotherapy of ovarian cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(1):33-9. doi: 10.1007/s13277-014-2848-2. PubMed PMID: 25409618.

11. Nair S, Boczkowski D, Moeller B, Dewhirst M, Vieweg J, Gilboa E. Synergy between tumor immunotherapy and antiangiogenic therapy. Blood. 2003;102(3):964-71. doi: 10.1182/blood-2002-12-3738. PubMed PMID: 12689940.

12. Cao Z, Shang B, Zhang G, Miele L, Sarkar FH, Wang Z, Zhou Q. Tumor cell-mediated neovascularization and lymphangiogenesis contrive tumor progression and cancer metastasis. Biochimica et biophysica acta. 2013;1836(2):273-86. doi: 10.1016/j.bbcan.2013.08.001. PubMed PMID: 23933263.

13. Kobold S, Hegewisch-Becker S, Oechsle K, Jordan K, Bokemeyer C, Atanackovic D. Intraperitoneal VEGF inhibition using bevacizumab: a potential approach for the symptomatic treatment of malignant ascites? The oncologist. 2009;14(12):1242-51. doi: 10.1634/theoncologist.2009-0109. PubMed PMID: 20008305.

14. Ferrara N. VEGF as a therapeutic target in cancer. Oncology. 2005;69 Suppl 3:11-6. doi: 10.1159/000088479. PubMed PMID: 16301831.

15. Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twiggs LB, Carson LF, Ramakrishnan S. Vascular endothelial growth factor expression in early stage ovarian carcinoma. Cancer. 1997;80(1):98-106. PubMed PMID: 9210714.

16. Bouzin C, Brouet A, De Vriese J, Dewever J, Feron O. Effects of vascular endothelial growth factor on the lymphocyte-endothelium interactions: identification of caveolin-1 and nitric oxide as control points of endothelial cell anergy. J Immunol. 2007;178(3):1505-11. PubMed PMID: 17237399.

17. Griffioen AW, Damen CA, Martinotti S, Blijham GH, Groenewegen G. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. Cancer Res. 1996;56(5):1111-17. PubMed PMID: 8640769.

18. Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res. 2010;70(15):6171-80. doi: 10.1158/0008-5472.CAN-10-0153. PubMed PMID: 20631075; PMCID: 2912959.

19. Ohm JE, Carbone DP. VEGF as a mediator of tumor-associated immunodeficiency. Immunologic research. 2001;23(2-3):263-72. doi: 10.1385/IR:23:2-3:263. PubMed PMID: 11444391.

20. Laxmanan S, Robertson SW, Wang E, Lau JS, Briscoe DM, Mukhopadhyay D. Vascular endothelial growth factor impairs the functional ability of dendritic cells through Id pathways. Biochemical and biophysical research communications. 2005;334(1):193-8. doi: 10.1016/j.bbrc.2005.06.065. PubMed PMID: 16002046.

21. Dikov MM, Ohm JE, Ray N, Tchekneva EE, Burlison J, Moghanaki D, Nadaf S, Carbone DP. Differential roles of vascular endothelial growth factor receptors 1 and 2 in dendritic cell differentiation. J Immunol. 2005;174(1):215-22. PubMed PMID: 15611243.

22. Yang L, Carbone DP. Tumor-host immune interactions and dendritic cell dysfunction. Adv Cancer Res. 2004;92:13-27. doi: 10.1016/S0065-230X(04)92002-7. PubMed PMID: 15530555.

23. Gabrilovich DI, Ishida T, Nadaf S, Ohm JE, Carbone DP. Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. Clin Cancer Res. 1999;5(10):2963-70. PubMed PMID: 10537366.

24. Yu JS, Lee PK, Ehtesham M, Samoto K, Black KL, Wheeler CJ. Intratumoral T cell subset ratios and Fas ligand expression on brain tumor endothelium. Journal of neuro-oncology. 2003;64(1-2):55-61. PubMed PMID: 12952286.

25. Bajou K, Peng H, Laug WE, Maillard C, Noel A, Foidart JM, Martial JA, DeClerck YA. Plasminogen activator inhibitor-1 protects endothelial cells from FasL-mediated apoptosis. Cancer cell. 2008;14(4):324-34. doi: 10.1016/j.ccr.2008.08.012. PubMed PMID: 18835034; PMCID: 2630529.

26. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR, Hagemann IS, Lal P, Feldman MD, Benencia F, Coukos G. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. Nat Med. 2014;20(6):607-15. doi: 10.1038/nm.3541. PubMed PMID: 24793239; PMCID: 4060245.

27. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006;368(9544):1329-38. doi: 10.1016/S0140-6736(06)69446-4. PubMed PMID: 17046465.

28. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, Li JZ, Bello CL, Theuer CP, George DJ, Rini BI. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006;24(1):16-24. doi: 10.1200/JCO.2005.02.2574. PubMed PMID: 16330672.

29. Baumann KH, du Bois A, Meier W, Rau J, Wimberger P, Sehouli J, Kurzeder C, Hilpert F, Hasenburg A, Canzler U, Hanker LC, Hillemanns P, Richter B, Wollschlaeger K, Dewitz T, Bauerschlag D, Wagner U. A phase II trial (AGO 2.11) in platinum-resistant ovarian cancer: a randomized multicenter trial with sunitinib (SU11248) to evaluate dosage, schedule, tolerability, toxicity and effectiveness of a multitargeted receptor tyrosine kinase inhibitor monotherapy. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2012;23(9):2265-71. doi: 10.1093/annonc/mds003. PubMed PMID: 22377563.

30. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM, Group TS. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med. 2007;356(2):125-34. doi: 10.1056/NEJMoa060655. PubMed PMID: 17215530.

31. Matei D, Sill MW, Lankes HA, DeGeest K, Bristow RE, Mutch D, Yamada SD, Cohn D, Calvert V, Farley J, Petricoin EF, Birrer MJ. Activity of sorafenib in recurrent ovarian cancer and primary peritoneal carcinomatosis: a gynecologic oncology group trial. J Clin Oncol. 2011;29(1):69-75. doi: 10.1200/JCO.2009.26.7856. PubMed PMID: 21098323; PMCID: 3055861.

32. du Bois A, Floquet A, Kim JW, Rau J, del Campo JM, Friedlander M, Pignata S, Fujiwara K, Vergote I, Colombo N, Mirza MR, Monk BJ, Kimmig R, Ray-Coquard I, Zang R, Diaz-Padilla I, Baumann KH, Mouret-Reynier MA, Kim JH, Kurzeder C, Lesoin A, Vasey P, Marth C, Canzler U, Scambia G, Shimada M, Calvert P,

Pujade-Lauraine E, Kim BG, Herzog TJ, Mitrica I, Schade-Brittinger C, Wang Q, Crescenzo R, Harter P. Incorporation of pazopanib in maintenance therapy of ovarian cancer. J Clin Oncol. 2014;32(30):3374-82. doi: 10.1200/JCO.2014.55.7348. PubMed PMID: 25225436.

33. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer cell. 2009;15(3):232-9. doi: 10.1016/j.ccr.2009.01.021. PubMed PMID: 19249681.

34. Loges S, Mazzone M, Hohensinner P, Carmeliet P. Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. Cancer cell. 2009;15(3):167-70. doi: 10.1016/j.ccr.2009.02.007. PubMed PMID: 19249675.

35. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer cell. 2009;15(3):220-31. doi: 10.1016/j.ccr.2009.01.027. PubMed PMID: 19249680; PMCID: 2874829.

36. Johannessen TC, Wagner M, Straume O, Bjerkvig R, Eikesdal HP. Tumor vasculature: the Achilles' heel of cancer? Expert opinion on therapeutic targets. 2013;17(1):7-20. doi: 10.1517/14728222.2013.730522. PubMed PMID: 23121690.

37. Giuliano S, Pages G. Mechanisms of resistance to anti-angiogenesis therapies. Biochimie. 2013;95(6):1110-9. doi: 10.1016/j.biochi.2013.03.002. PubMed PMID: 23507428.

38. Choi HJ, Armaiz Pena GN, Pradeep S, Cho MS, Coleman RL, Sood AK. Anti-vascular therapies in ovarian cancer: moving beyond anti-VEGF approaches. Cancer metastasis reviews. 2014. doi: 10.1007/s10555-014-9538-9. PubMed PMID: 25544368.

39. Hamanishi J, Mandai M, Abiko K, Matsumura N, Baba T, Yoshioka Y, Kosaka K, Konishi I. The comprehensive assessment of local immune status of ovarian cancer by the clustering of multiple immune factors. Clinical immunology. 2011;141(3):338-47. doi: 10.1016/j.clim.2011.08.013. PubMed PMID: 21955569.

40. Intlekofer AM, Thompson CB. At the bench: preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy. Journal of leukocyte biology. 2013;94(1):25-39. doi: 10.1189/jlb.1212621. PubMed PMID: 23625198; PMCID: 3685017.

41. Kyi C, Postow MA. Checkpoint blocking antibodies in cancer immunotherapy. FEBS letters. 2014;588(2):368-76. doi: 10.1016/j.febslet.2013.10.015. PubMed PMID: 24161671.

42. McDermott DF, Atkins MB. PD-1 as a potential target in cancer therapy. Cancer medicine. 2013;2(5):662-73. doi: 10.1002/cam4.106. PubMed PMID: 24403232; PMCID: 3892798.

43. Wang Y, Fei D, Vanderlaan M, Song A. Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro. Angiogenesis. 2004;7(4):335-45. doi: 10.1007/s10456-004-8272-2. PubMed PMID: 15886877.

44. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350(23):2335-42. Epub 2004/06/04. doi: 10.1056/NEJMoa032691
350/23/2335 [pii]. PubMed PMID: 15175435.

45. Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JI. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study. J Clin Oncol. 2007;25(33):5165-71. doi: 10.1200/JCO.2007.11.5345. PubMed PMID: 18024863.

46. Aravantinos G, Pectasides D. Bevacizumab in combination with chemotherapy for the treatment of advanced ovarian cancer: a systematic review. Journal of ovarian research. 2014;7:57. doi: 10.1186/1757-2215-7-57. PubMed PMID: 24864163; PMCID: 4033616.

47. Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Park-Simon TW, Rustin G, Joly F, Mirza MR, Plante M, Quinn M, Poveda A, Jayson GC, Stark D, Swart AM, Farrelly L, Kaplan R, Parmar MK, Perren TJ, investigators It. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. The Lancet Oncology. 2015. doi: 10.1016/S1470-2045(15)00086-8. PubMed PMID: 26115797.

48. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, Mannel RS, Homesley HD, Fowler J, Greer BE, Boente M, Birrer MJ, Liang SX, Gynecologic Oncology G. Incorporation of bevacizumab in the primary treatment of ovarian cancer. The New England journal of medicine. 2011;365(26):2473-83. doi: 10.1056/NEJMoa1104390. PubMed PMID: 22204724.

49. Eskander RN, Tewari KS. Incorporation of anti-angiogenesis therapy in the management of advanced ovarian carcinoma--mechanistics, review of phase III randomized clinical trials, and regulatory implications. Gynecologic oncology. 2014;132(2):496-505. doi: 10.1016/j.ygyno.2013.11.029. PubMed PMID: 24316305.

50. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, Sovak MA, Yi J, Nycum LR. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(17):2039-45. doi: 10.1200/JCO.2012.42.0505. PubMed PMID: 22529265; PMCID: 3646321.

51. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, Sorio R, Vergote I, Witteveen P, Bamias A, Pereira D, Wimberger P, Oaknin A, Mirza MR, Follana P, Bollag D, Ray-Coquard I. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol. 2014;32(13):1302-8. doi: 10.1200/JCO.2013.51.4489. PubMed PMID: 24637997.

52. Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, Chaput N, Puig PE, Novault S, Escudier B, Vivier E, Lecesne A, Robert C, Blay JY, Bernard J, Caillat-Zucman S, Freitas A, Tursz T, Wagner-Ballon O, Capron C, Vainchencker W, Martin F, Zitvogel L. CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. J Exp Med. 2005;202(8):1075-85. doi: 10.1084/jem.20051511. PubMed PMID: 16230475; PMCID: 2213209.

53. Gasparini G. Metronomic scheduling: the future of chemotherapy? The Lancet Oncology. 2001;2(12):733-40. doi: 10.1016/S1470-2045(01)00587-3. PubMed PMID: 11902515.

54. Shaked Y, Emmenegger U, Man S, Cervi D, Bertolini F, Ben-David Y, Kerbel RS. Optimal biologic dose of metronomic chemotherapy regimens is associated with maximum antiangiogenic activity. Blood. 2005;106(9):3058-61. doi: 10.1182/blood-2005-04-1422. PubMed PMID: 15998832; PMCID: 1895327.

55. Browder T, Butterfield CE, Kraling BM, Shi B, Marshall B, O'Reilly MS, Folkman J. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res. 2000;60(7):1878-86. PubMed PMID: 10766175.

56. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, Solary E, Le Cesne A, Zitvogel L, Chauffert B. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother. 2007;56(5):641-8. doi: 10.1007/s00262-006-0225-8. PubMed PMID: 16960692.

57. Le DT, Jaffee EM. Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective. Cancer Res. 2012;72(14):3439-44. doi: 10.1158/0008-5472.CAN-11-3912. PubMed PMID: 22761338; PMCID: 3399042.

58. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood. 2005;105(7):2862-8. doi: 10.1182/blood-2004-06-2410. PubMed PMID: 15591121.

59. Ercolini AM, Ladle BH, Manning EA, Pfannenstiel LW, Armstrong TD, Machiels JP, Bieler JG, Emens LA, Reilly RT, Jaffee EM. Recruitment of latent pools of high-avidity CD8(+) T cells to the antitumor immune response. J Exp Med. 2005;201(10):1591-602. doi: 10.1084/jem.20042167. PubMed PMID: 15883172; PMCID: 2212915.

60. Kerbel RS. Improving conventional or low dose metronomic chemotherapy with targeted antiangiogenic drugs. Cancer research and treatment : official journal of Korean Cancer Association. 2007;39(4):150-9. doi: 10.4143/crt.2007.39.4.150. PubMed PMID: 19746237; PMCID: 2739367.

61. Barber EL, Zsiros E, Lurain JR, Rademaker A, Schink JC, Neubauer NL. The combination of intravenous bevacizumab and metronomic oral cyclophosphamide is an effective regimen for platinum-resistant recurrent ovarian cancer. Journal of gynecologic oncology. 2013;24(3):258-64. doi: 10.3802/jgo.2013.24.3.258. PubMed PMID: 23875076; PMCID: 3714464.

62. Sanchez-Munoz A, Mendiola C, Perez-Ruiz E, Rodriguez-Sanchez CA, Jurado JM, Alonso-Carrion L, Ghanem I, de Velasco G, Quero-Blanco C, Alba E. Bevacizumab plus low-dose metronomic oral cyclophosphamide in heavily pretreated patients with recurrent ovarian cancer. Oncology. 2010;79(1-2):98-104. doi: 10.1159/000320602. PubMed PMID: 21079407.

63. Jurado JM, Sanchez A, Pajares B, Perez E, Alonso L, Alba E. Combined oral cyclophosphamide and bevacizumab in heavily pre-treated ovarian cancer. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2008;10(9):583-6. PubMed PMID: 18796376.

64. Garcia AA, Hirte H, Fleming G, Yang D, Tsao-Wei DD, Roman L, Groshen S, Swenson S, Markland F, Gandara D, Scudder S, Morgan R, Chen H, Lenz HJ, Oza AM. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol. 2008;26(1):76-82. doi: 10.1200/JCO.2007.12.1939. PubMed PMID: 18165643.

65. Chura JC, Van Iseghem K, Downs LS, Jr., Carson LF, Judson PL. Bevacizumab plus cyclophosphamide in heavily pretreated patients with recurrent ovarian cancer. Gynecologic oncology. 2007;107(2):326-30. doi: 10.1016/j.ygyno.2007.07.017. PubMed PMID: 17706754.

66. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine. 2012;366(26):2443-54. Epub 2012/06/05. doi: 10.1056/NEJMoa1200690. PubMed PMID: 22658127.

67. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer cell. 2015;27(4):450-61. doi: 10.1016/j.ccell.2015.03.001. PubMed PMID: 25858804; PMCID: 4400238.

68. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189-99. doi: 10.1056/NEJMoa1406498. PubMed PMID: 25409260; PMCID: 4315319.

69. Cha E, Klinger M, Hou Y, Cummings C, Ribas A, Faham M, Fong L. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. Science translational medicine. 2014;6(238):238ra70. Epub 2014/05/30. doi: 10.1126/scitranslmed.3008211. PubMed PMID: 24871131.

70. Page DB, Diab A, Yuan J, Dong Z, Emerson R, Robins H, Redmond D, Zhao C, Mu Z, Wong P, Solomon SB, Sacchini V, Morrow M, Brogi E, Patil S, Wolchok JD, Hudis CA, Norton L, Allison JP, McArthur HL. T-cell receptor (TCR) DNA deep sequencing to evaluate clonality of tumor-infiltrating lymphocytes (TILs) in early-stage breast cancer patients (pts) receiving preoperative cryoablation (cryo) and/or ipilimumab (ipi). Journal of Clinical Oncology. 2014;32(5s):(suppl; abstr 3021).

71. Althani AA, Marei HE, Hamdi WS, Nasrallah GK, El Zowalaty ME, Al Khodor S, Al-Asmakh M, Abdel-Aziz H, Cenciarelli C. Human Microbiome and its Association With Health and Diseases. Journal of cellular physiology. 2016;231(8):1688-94. doi: 10.1002/jcp.25284. PubMed PMID: 26660761.

72. Cenit MC, Matzaraki V, Tigchelaar EF, Zhernakova A. Rapidly expanding knowledge on the role of the gut microbiome in health and disease. Biochimica et biophysica acta. 2014;1842(10):1981-92. doi: 10.1016/j.bbadis.2014.05.023. PubMed PMID: 24882755.

73. Schwabe RF, Jobin C. The microbiome and cancer. Nature reviews Cancer. 2013;13(11):800-12. doi: 10.1038/nrc3610. PubMed PMID: 24132111; PMCID: 3986062.

74. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-41. doi: 10.1016/j.cell.2014.03.011. PubMed PMID: 24679531; PMCID: 4056765.

75. Morris G, Berk M, Carvalho A, Caso JR, Sanz Y, Walder K, Maes M. The Role of the Microbial Metabolites Including Tryptophan Catabolites and Short Chain Fatty Acids in the Pathophysiology of Immune-Inflammatory and Neuroimmune Disease. Molecular neurobiology. 2016. doi: 10.1007/s12035-016-0004-2. PubMed PMID: 27349436.

76. Burokas A, Moloney RD, Dinan TG, Cryan JF. Microbiota regulation of the Mammalian gut-brain axis. Advances in applied microbiology. 2015;91:1-62. doi: 10.1016/bs.aambs.2015.02.001. PubMed PMID: 25911232.

77. Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. Gut microbes and the brain: paradigm shift in neuroscience. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014;34(46):15490-6. doi: 10.1523/JNEUROSCI.3299-14.2014. PubMed PMID: 25392516; PMCID: 4228144.

78. Qiu J, Heller JJ, Guo X, Chen ZM, Fish K, Fu YX, Zhou L. The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. Immunity. 2012;36(1):92-104. doi: 10.1016/j.immuni.2011.11.011. PubMed PMID: 22177117; PMCID: 3268875.

79. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan Tg TG, Cryan JF. The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? Neurochemistry international. 2016. doi: 10.1016/j.neuint.2016.06.011. PubMed PMID: 27346602.

80. Chase D, Goulder A, Zenhausern F, Monk B, Herbst-Kralovetz M. The vaginal and gastrointestinal microbiomes in gynecologic cancers: a review of applications in etiology, symptoms and treatment. Gynecologic oncology. 2015;138(1):190-200. doi: 10.1016/j.ygyno.2015.04.036. PubMed PMID: 25957158.

81. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Berard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015;350(6264):1079-84. doi: 10.1126/science.aad1329. PubMed PMID: 26541610.

82. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, Chang EB, Gajewski TF. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084-9. Epub 2015/11/07. doi: 10.1126/science.aac4255. PubMed PMID: 26541606; PMCID: Pmc4873287.

83. Hofmann L, Forschner A, Loquai C, Goldinger SM, Zimmer L, Ugurel S, Schmidgen MI, Gutzmer R, Utikal JS, Goppner D, Hassel JC, Meier F, Tietze JK, Thomas I, Weishaupt C, Leverkus M, Wahl R, Dietrich U, Garbe C, Kirchberger MC, Eigentler T, Berking C, Gesierich A, Krackhardt AM, Schadendorf D, Schuler G, Dummer R, Heinzerling LM. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. European journal of cancer. 2016;60:190-209. doi: 10.1016/j.ejca.2016.02.025. PubMed PMID: 27085692.

84. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Nomicos E, Polley EC, Komarow HD, Program NCS, Murray PR, Turner ML, Segre JA. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome research. 2012;22(5):850-9. doi: 10.1101/gr.131029.111. PubMed PMID: 22310478; PMCID: 3337431.

85. Alekseyenko AV, Perez-Perez GI, De Souza A, Strober B, Gao Z, Bihan M, Li K, Methe BA, Blaser MJ. Community differentiation of the cutaneous microbiota in psoriasis. Microbiome. 2013;1(1):31. doi: 10.1186/2049-2618-1-31. PubMed PMID: 24451201; PMCID: 4177411.

86. Randall LM, Monk BJ. Bevacizumab toxicities and their management in ovarian cancer. Gynecologic oncology. 2010;117(3):497-504. doi: 10.1016/j.ygyno.2010.02.021. PubMed PMID: 20363017.

87. Han ES, Monk BJ. What is the risk of bowel perforation associated with bevacizumab therapy in ovarian cancer? Gynecologic oncology. 2007;105(1):3-6. doi: 10.1016/j.ygyno.2007.01.038. PubMed PMID: 17383545.

88. Zhu X, Wu S, Dahut WL, Parikh CR. Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2007;49(2):186-93. doi: 10.1053/j.ajkd.2006.11.039. PubMed PMID: 17261421.

89. Scappaticci FA, Fehrenbacher L, Cartwright T, Hainsworth JD, Heim W, Berlin J, Kabbinavar F, Novotny W, Sarkar S, Hurwitz H. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. Journal of surgical oncology. 2005;91(3):173-80. doi: 10.1002/jso.20301. PubMed PMID: 16118771.

90. Nimeiri HS, Oza AM, Morgan RJ, Friberg G, Kasza K, Faoro L, Salgia R, Stadler WM, Vokes EE, Fleming GF, Chicago Phase IIC, Consortium PMHPI, California Phase IIC. Efficacy and safety of bevacizumab plus erlotinib for patients with recurrent ovarian, primary peritoneal, and fallopian tube cancer: a trial of the Chicago, PMH, and California Phase II Consortia. Gynecologic oncology. 2008;110(1):49-55. doi: 10.1016/j.ygyno.2008.02.009. PubMed PMID: 18423560; PMCID: 2699608.

91. Kozloff M, Yood MU, Berlin J, Flynn PJ, Kabbinavar FF, Purdie DM, Ashby MA, Dong W, Sugrue MM, Grothey A, Investigators of the Bs. Clinical outcomes associated with bevacizumab-containing treatment of metastatic colorectal cancer: the BRiTE observational cohort study. The oncologist. 2009;14(9):862-70. doi: 10.1634/theoncologist.2009-0071. PubMed PMID: 19726453.

92. Verheul HM, Pinedo HM. Possible molecular mechanisms involved in the toxicity of angiogenesis inhibition. Nature reviews Cancer. 2007;7(6):475-85. doi: 10.1038/nrc2152. PubMed PMID: 17522716.

93. Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Colangelo LH, Lopa SH, Petrelli NJ, Goldberg RM, Atkins JN, Seay TE, Fehrenbacher L, O'Reilly S, Chu L, Azar CA, Wolmark N. Initial safety report of NSABP C-08: A randomized phase III study of modified FOLFOX6 with or without bevacizumab for the adjuvant treatment of patients with stage II or III colon cancer. J Clin Oncol. 2009;27(20):3385-90. doi: 10.1200/JCO.2009.21.9220. PubMed PMID: 19414665; PMCID: 2717026.

94. Chen XL, Lei YH, Liu CF, Yang QF, Zuo PY, Liu CY, Chen CZ, Liu YW. Angiogenesis inhibitor bevacizumab increases the risk of ischemic heart disease associated with chemotherapy: a meta-analysis. PloS one. 2013;8(6):e66721. doi: 10.1371/journal.pone.0066721. PubMed PMID: 23818962; PMCID: 3688569.

95. Hodi FS, Hwu WJ, Kefford R, Weber JS, Daud A, Hamid O, Patnaik A, Ribas A, Robert C, Gangadhar TC, Joshua AM, Hersey P, Dronca R, Joseph R, Hille D, Xue D, Li XN, Kang SP, Ebbinghaus S, Perrone A, Wolchok JD. Evaluation of Immune-Related Response Criteria and RECIST v1.1 in Patients With Advanced Melanoma Treated With Pembrolizumab. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34(13):1510-7. doi: 10.1200/JCO.2015.64.0391. PubMed PMID: 26951310.

96. Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements. Clin Cancer Res. 2013;19(14):3936-43. doi: 10.1158/1078-0432.CCR-13-0895. PubMed PMID: 23743568; PMCID: PMC3740724.

97. Ades F, Yamaguchi N. WHO, RECIST, and immune-related response criteria: is it time to revisit pembrolizumab results? Ecancermedicalscience. 2015;9:604. doi: 10.3332/ecancer.2015.604. PubMed PMID: 26715941; PMCID: 4679210.

98. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer. 2009;45(2):228-47. doi: http://dx.doi.org/10.1016/j.ejca.2008.10.026.

99. Oh J, Byrd AL, Deming C, Conlan S, Program NCS, Kong HH, Segre JA. Biogeography and individuality shape function in the human skin metagenome. Nature. 2014;514(7520):59-64. doi: 10.1038/nature13786. PubMed PMID: 25279917; PMCID: 4185404.

100. Bohnsack O, Hoos A, Ludajic K. 1070PADAPTATION OF THE IMMUNE RELATED RESPONSE CRITERIA: IRRECIST. Annals of Oncology. 2014;25(suppl 4):iv369. doi: 10.1093/annonc/mdu342.23.

101. Jones MP, O'Gorman TW, Lemke JH, Woolson RF. A Monte Carlo investigation of homogeneity tests of the odds ratio under various sample size configurations. Biometrics. 1989;45(1):171-81. PubMed PMID: 2720050.

102. von der Maase H, Sengelov L, Roberts JT, Ricci S, Dogliotti L, Oliver T, Moore MJ, Zimmermann A, Arning M. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(21):4602-8. doi: 10.1200/JCO.2005.07.757. PubMed PMID: 16034041.