Title: Phase Ib study to test the safety and potential synergy of pembrolizumab (anti-PD-1) and AMG386 (angiopoietin-2 (Ang-2)) in patients with advanced solid tumor

NCT Number: NCT03239145
IRB Approval Date: Version 5.0 Protocol Version Date 12Aug2021

Phase Ib study to test the safety and potential synergy of pembrolizumab (anti-PD-1) and AMG386 (angiopoietin-2 (Ang-2)) in patients with advanced solid tumor

Protocol Version Date: August 12, 2021

Protocol Version No.: 5.0

DF/HCC Protocol No.: 17-217

[redacted]

Study Disease(s):

1. Solid Tumors
2. Melanoma
3. Ovarian Cancer
4. Kidney cancer
5. Colorectal Adenocarcinoma

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[redacted]

[redacted]

Agent(s): **Pembrolizumab (KEYTRUDA®)**: Merck & Co., Inc.
Trebananib (AMG386): Amgen, Inc.

IND #: 133954

[redacted]

STUDY SUMMARY

Abbreviated Title	Pembrolizumab and Trebananib in advanced solid tumors
Trial Phase	Ib
Clinical Indication	Phase Ib: all solid tumors Expansion cohorts: melanoma, ovarian, renal, and colorectal cancer
Trial Type	Interventional
Type of control	No control
Route of administration	Intravenous
Trial Blinding	Unblinded Open Label
Treatment Groups	Phase Ib: one cohort for all solid tumors Expansion cohorts: melanoma, ovarian, renal, and colorectal cancer
Number of trial participants	60 patients
Estimated enrollment period	1.5 years
Estimated duration of trial	3-4 years
Duration of Participation	2 years
Estimated average length of treatment per patient	1-2 years

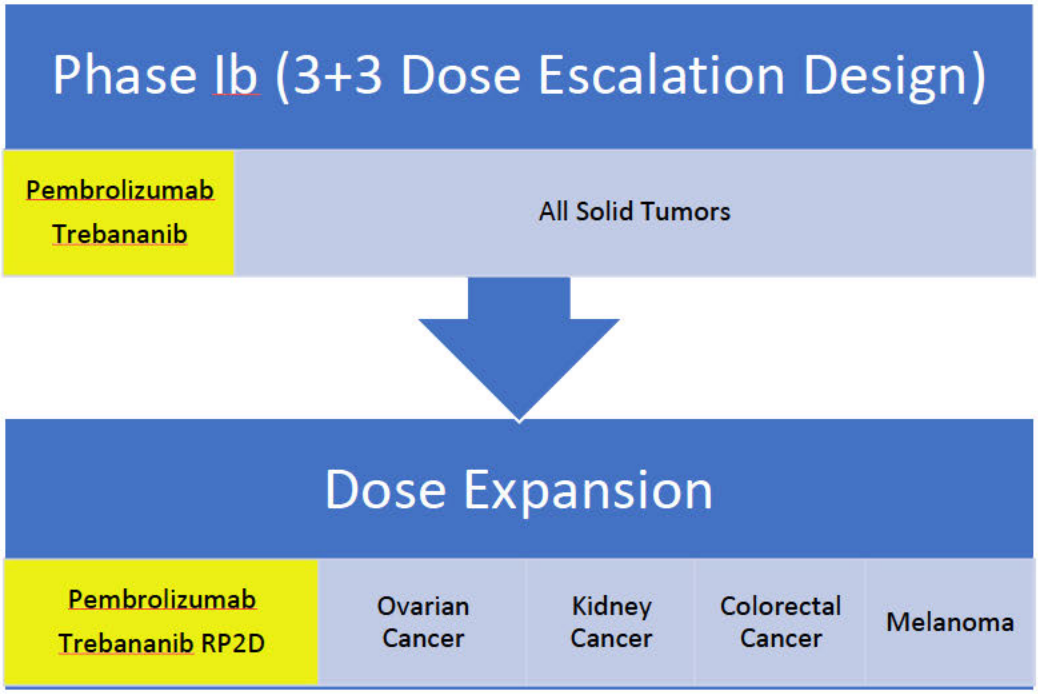
PROTOCOL AMENDEMENT VERSION 4.0 RATIONALE:

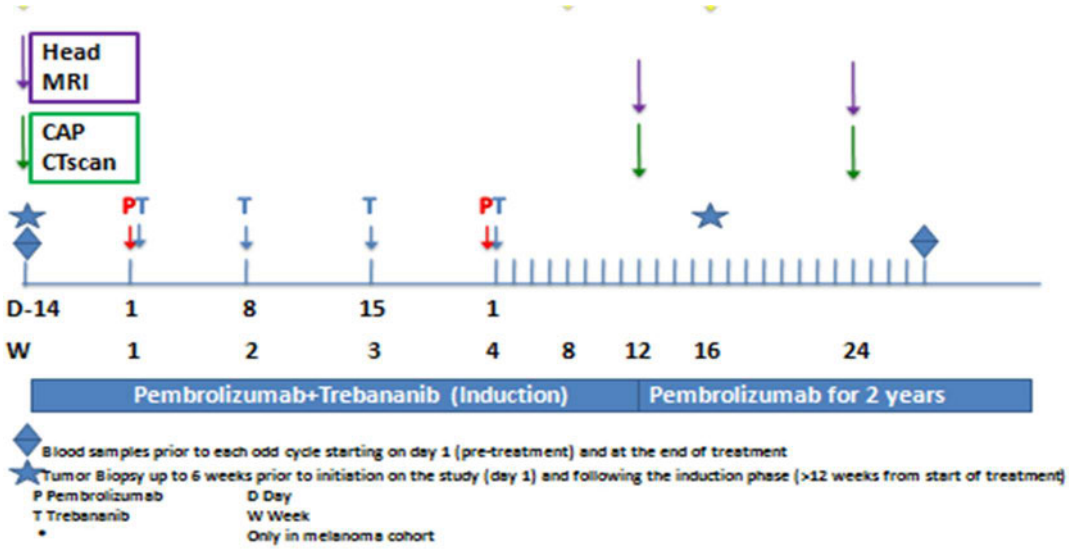
At the time of this protocol amendment Version 4.0, the study is in the dose expansion phase, with the RP2D determined to be 200 mg of pembrolizumab every 3 weeks + 30 mg/kg trebananib every week. The main reason for amending the protocol is to shift the focus of which disease indications are being studied in expansion:

- The melanoma and RCC cohorts will be closed to accrual. Due to competing studies and changes in standard of care options, the accrual rate to these disease cohorts has been slow, with 4 patients being enrolled to the RCC cohort and no patients being enrolled in the melanoma cohort. The ovarian cohort will remain open to accrual with the originally planned sample size of 12 patients.
- Due to data suggesting clinical activity, the colorectal cancer cohort sample size will be expanded by an additional 25 participants, increasing the colorectal cancer cohort to a total of 37 patients.

Minor administrative changes and clarifications have been made throughout the protocol to reflect these changes.

STUDY SCHEMA





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1. OBJECTIVES

1.1 Study Design

This is a prospective trial which will accrue participants with solid tumors to evaluate the safety, clinical, and immunological effect of the combination of pembrolizumab (MK-3475) and trebananib (AMG386). The treatment will include an induction phase of pembrolizumab and trebananib for 4 cycles (12 weeks) followed by pembrolizumab alone for 2 years. The study will accrue up to 60 participants. This trial will be conducted in 2 parts. Part I will use a standard 3+3 dose escalation design in all solid tumors. The goal of Part I will be to identify the recommended part 2 (expansion cohort) doses (RP2D) for the combination of pembrolizumab plus trebananib . Dose escalation will begin in dose cohort +1. If two or more patients in dose cohort +1 experience a DLT, the next cohort of patients will be enrolled into dose cohort -1. Should dose cohort -1 prove too toxic, enrollment to the study will stop. If the toxicity profile of dose cohort +1 is acceptable, the next cohort will be enrolled into dose cohort +2. Should dose cohort +2 have acceptable toxicity, that will be the RP2D; otherwise, dose cohort +1 will be the RP2D.

Part II has 4 dose expansion cohorts to be treated at the RP2D, which was determined to be 200 mg pembrolizumab every 3 weeks + 30 mg/kg trebananib every week:

- melanoma
- renal cell carcinoma (RCC)
- ovarian cancer
- colorectal cancer (CRC)

Initially, 12 patients were to be treated in each cohort. However, as of protocol amendment Version 4.0, no patients were enrolled to the melanoma cohort and only 4 patients were enrolled to the RCC cohort. Due to slow accrual in these specific cohorts, the melanoma and RCC cohorts will no longer be open to accrual. Accrual will continue in the ovarian cohort, and the CRC cohort will be expanded to allow enrollment of 37 patients total.

Safety assessments will include all patients receiving one or more doses of the study drug combinations. Secondary and correlative endpoints will be based on the cohorts of patients enrolled in Part II of the trial. We will summarize secondary and correlative endpoints according to disease indication and, in an exploratory fashion, with all patients combined. Pre-and on-treatment biopsies will be obtained for all patients enrolled in the dose expansion cohorts and archival tissue will be collected for selected dose escalation patients when available.

1.2 Primary Objectives

- Part 1: To determine the safety, tolerability, and RP2D for trebananib when given with pembrolizumab in patients with metastatic solid tumor.

- Part 2 (expansion cohort): To determine the safety and tolerability of the RP2D of trebananib, determined in Part 1, when given with pembrolizumab in patients

withunresectable stage III or stage IV melanoma, metastatic renal cell, ovarian, or colorectal cancer.

1.3 Secondary Objectives

- To obtain preliminary estimates of progression free survival (PFS) at 6 months.
- To obtain preliminary estimates of the rate of 1-year overall (OS).
- To obtain preliminary estimates of the response rate (RR).
- To obtain preliminary estimates of time to progression.
-

1.4 Exploratory Objectives

- To determine the effect of the combination on vasculopathy, immune infiltration, and tumor necrosis by staining pathologic specimens for VEGF/VEGFR expression, phosphoTie-2 and other targets.
- To investigate immune responses in the periphery to VEGFR, Tie-2, and other angiogenic molecules and tumor-specific antigens as a function of treatment.

2. BACKGROUND

2.1 Background: Pembrolizumab

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

2.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades (Disis. 2010). 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 (Thompson, Dong et al. 2007) (Galon, Pagès et al, 2012).

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) (Sharpe, Freeman et al. 2002). 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 (Okazaki, Chikuma et al. 2013). 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 (Sharpe, Freeman et al. 2002). 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 participants with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda® (pembrolizumab) has been approved in the United Stated for the treatment of unresectable or metastatic melanoma, metastatic non-small cell lung cancer (NSCLC) whose tumors express programmed death ligand 1 (PD-L1), and recurrent metastatic squamous cell carcinoma of the head and neck,

2.1.2 Preclinical and Clinical Trial Data

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

2.1.3 Previous experience with immune checkpoint blockade (CTLA-4) and anti-angiogenesis

Human immune responses against cancer can be suppressed through various mechanisms during disease progression such that cancers evade immune recognition and anti-tumor effector functions. The expression of immune regulatory molecules such as cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) and programmed death 1 (PD-1), etc. inhibits the proliferation and function of conventional T cells. Immune checkpoint blockade with ipilimumab (CTLA-4 blockade) has revealed improved survival in patients with metastatic melanoma (Robert, Thomas et al., Hodi, O'Day et al. 2010). Blockade of PD-1 and PD-L1 interactions has also revealed durable clinical benefits in patients with a variety of cancers including melanoma, non-small cell lung cancer, and renal cell carcinoma (Topalian, Hodi et al. 2012, Hamid, Robert et al. 2013). Currently, efforts are needed to better understand treatment modality combinations that could improve efficacy of immune checkpoint blockade. This would include clinical benefits in cancers that exhibit limited efficacy to checkpoint blockade alone. There is increasing evidence of the role that angiogenic factors play in affecting immune regulation as well as immune effector cell trafficking into tumors. We have recently demonstrated that soluble VEGF (sVEGF) predicts clinical benefit to ipilimumab therapy (Yuan, Zhou et al. 2014).

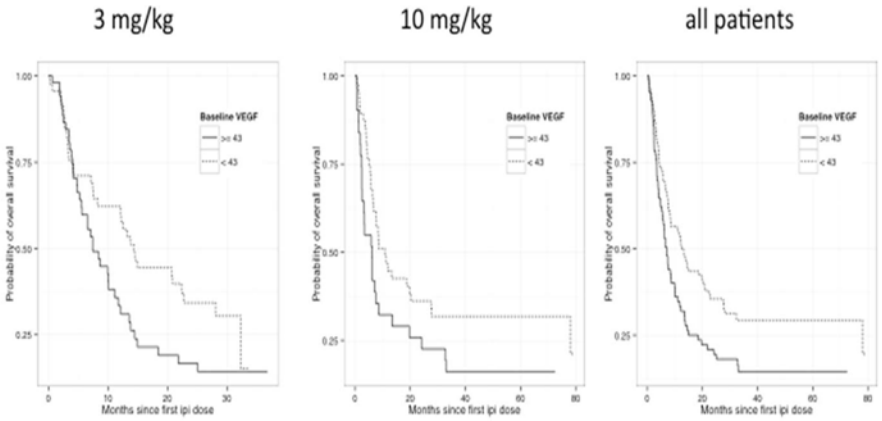


Figure 1: Baseline VEGF value correlated with patient overall survival. Kaplan-Meier curves demonstrating the difference in OS for patients with $VEGF^{low}$ and $VEGF^{hi}$ are statistically significant: 3 mg/kg dose of ipilimumab (median OS 14.33 vs 7.44 months, p=0.0367); 10 mg/kg dose of ipilimumab (median OS 10.85 vs 6.16 months, p=0.0477); all patients (median OS 12.87 vs 6.56 months p=0.006).

Anti-CTLA-4 blockade

Ipilimumab is a fully human monoclonal antibody that blocks the costimulatory checkpoint molecule CTLA-4. The anti-tumor mechanism of action involves amplification of T cells by blocking endogenous CTLA-4 with resultant T cell proliferation and tumor cell killing. Activity has been observed when administered as a single agent or in combination with other immunotherapies such as vaccines or interleukin-2 (IL-2) as we well as when combined with chemotherapy, and in multiple indications including melanoma.

CTLA-4 blockade with ipilimumab leads to improved overall survival in patients with advanced melanoma as documented in two phase III studies, emphasizing the antitumor activity of immune checkpoint blockade. The published response rate is approximately 18%, with a substantial number of responses being durable and/or complete. Recent long-term analyses of follow up for patients treated with ipilimumab reveal a durable 22% survival rate with an inflection and flattening of the survival curves at approximately 3 years (ECCO 2013), demonstrating long-term benefits for patients.

The question remains what treatment modalities exist to combine with checkpoint blockade to improve efficacy. Anti-angiogenesis has been one modality we have pursued.

Combination of anti-CTLA-4 and anti-VEGF therapy in patients with advanced melanoma
Our own experience with ipilimumab includes its administration to patients following a therapeutic vaccine. In patients in whom pre-existing sites of disease were biopsied following treatment, we have observed the consistent presence of an immune mediated vasculopathy around the vasculature feeding the tumor deposit associated with extensive tumor necrosis (Figure 1) (Hodi, Mihm et al. 2003).

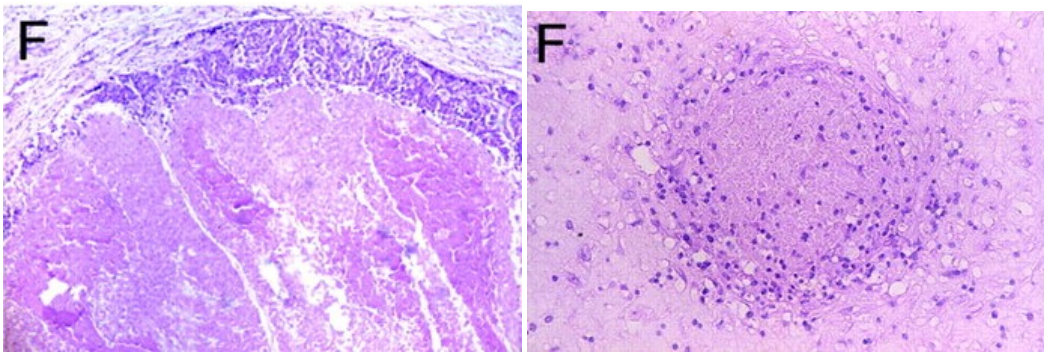


Figure 2: Left: Melanoma tumor deposit post-ipilimumab demonstrating extensive hemorrhagic tumor necrosis with rim of viable tumor heavily infiltrated with granulocytes and lymphocytes.
Right: Melanoma tumor deposit post-ipilimumab with severe tumor vasculopathy accompanied by perivascular and intramural lymphoid infiltrates associated with luminal thrombosis. (Magnification: ×125).

In addition to this evidence, VEGF is known to be a potent inhibitor of dendritic cell maturation. VEGF inhibition has also been demonstrated to facilitate T cell trafficking across endothelia. As a result of these observations, we initiated and completed a phase 1 trial of the combination of bevacizumab and ipilimumab in patients with unresectable stage III or IV melanoma. The results of the trial provide the first experience of combining anti-angiogenesis with immune checkpoint blockade.

The primary endpoints of the trial were the safety and preliminary activity of the combination of the two treatments for patients with advanced melanoma. Patients received ipilimumab every 3 weeks for four doses then every 12 weeks, and bevacizumab every 3 weeks. Patients could

continue treatment with good performance status (PS), ≤40% increase in sum of the longest diameter, and ≤2 new target lesions. Cohort 1 comprised 10 mg/kg ipilimumab plus 7.5 mg/kg bevacizumab. Following the induction dosing every three weeks for four cycles, bevacizumab was continued every 3 weeks as tolerated, and ipilimumab was administered every 12 weeks as tolerated. With ≥3/5 patients not experiencing DLT, Cohort 2 enrolled at 10 mg/kg ipilimumab plus 15 mg/kg bevacizumab. Twelve additional patients were treated at MTD. With the FDA approval of ipilimumab at 3 mg/kg additional Cohorts (12 patients each) were added and received ipilimumab 3 mg/kg with 7.5 or 15 mg/kg of bevacizumab (Cohorts 3 and 4, respectively). A total of forty-six patients were treated. The combination showed promising evidence of activity, including a 32% overall response rate (ORR) (6 PR, 1 CR) and an additional 32% rate of durable (>6 months) stable disease. Inflammatory events included giant cell arteritis (1), hepatitis (2), and uveitis (2). Median follow-up at the time of latest analysis was 17.3 months (95% CI: 11.1 to 30.2 months).

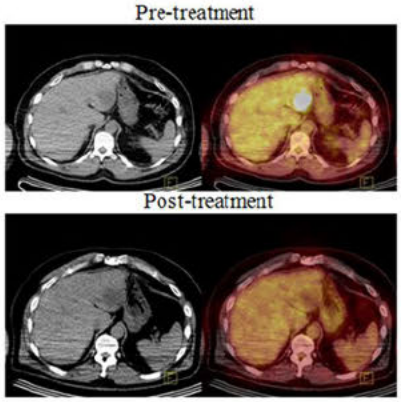


Figure 3: Clinical activity of ipilimumab plus bevacizumab by PET-CT (CT images on left, PET images on right). Pretreatment demonstrates an FDG avid liver metastasis. With treatment the metastasis is no longer metabolically avid but anatomically still present. With continued treatment and follow up, this lesion regressed approximately four months later without evidence of additional disease.

Radiographic examples of pseudo-progression and delayed best response were also observed. Thirty-one patients reported a best response of CR, PR, or SD, resulting in a disease-control percentage of 67.4% (95% exact CI: 52% to 81%).

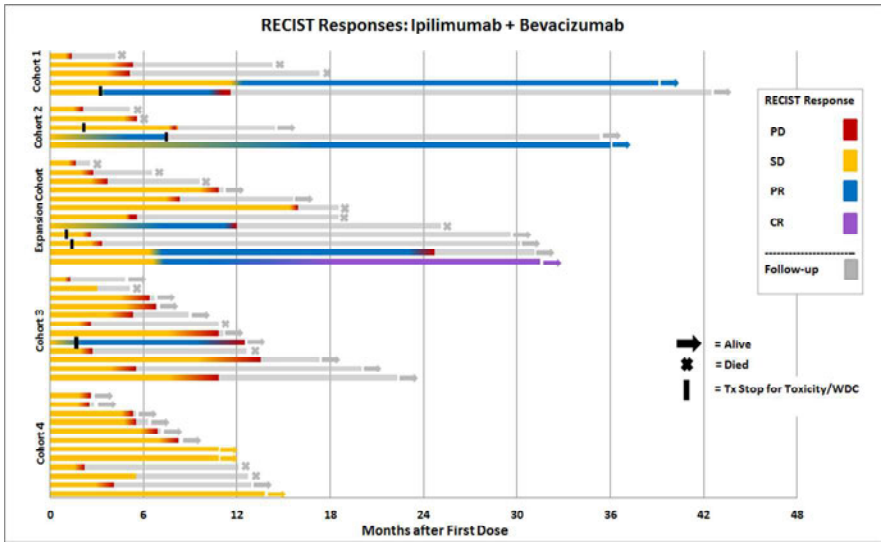


Figure 4: Activity in treated patients by cohort according to RECIST criteria. Arrows indicate alive at time of analysis. Crosses indicate death. Black bars indicate discontinuation of treatment other than due to progressive disease. Five patients came off trial due to toxicity requiring systemic steroids. One patient withdrew consent after week 12 without dose-limiting toxicity. PD= progressive disease. SD = stable disease. PR = partial response. CR = complete response.

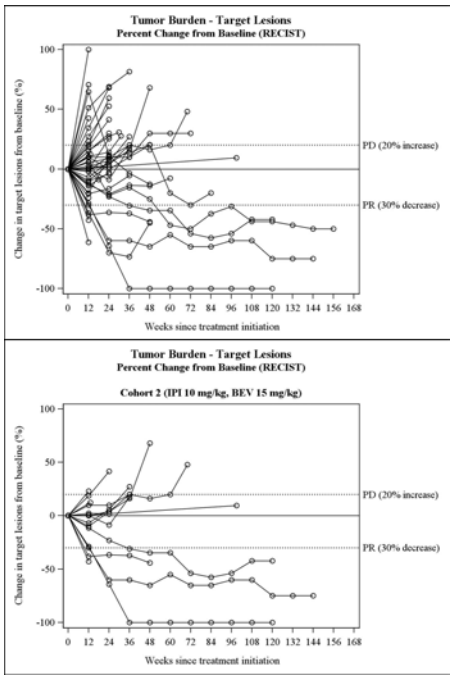


Figure 5: Response kinetics in treated patients. Baseline tumor measurements are standardized to zero. A. Entire treatment population (cohorts 1-4). B. Cohort 2 patients (MTD). Horizontal line PD = progressive disease representing 20% increase. Horizontal line PR represents 30% decrease from baseline.

The highest percentage with disease control was 76.5%, which was reported in Cohort 2 (95% exact CI: 50% to 93%). A number of patients had durable responses, with several achieving best response after months of therapy. A patient in Cohort 2 (MTD) experienced approximately 7 months of stable disease before a partial response and subsequently had a complete response beginning approximately 17 months following the initiation of therapy. Eight patients in Cohorts 1 and 2 remain alive for months after discontinuation of therapy. There is significantly shorter

follow-up time for Cohorts 3 and 4. The median time to progression (based on mWHO) was 9.0 months, 95% CI (5.5 to 14.5 months). Median overall survival was 25.1 months (95% CI: 12.7 to ∞).

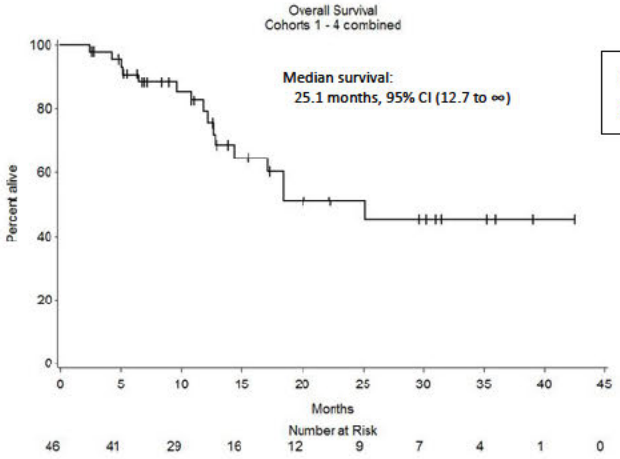


Figure 6: Kaplan-Meier estimates of overall survival.

For this trial, the Kaplan-Meier estimate of 1-year OS was 79% (95% CI 62% to 89%) and the Kaplan-Meier estimate of 6-month progression-free survival (PFS) was 63% (95% CI 47% to 75%). This compares favorably with the expected 1-year survival rate of 25% in 2nd line, and 35% in 1st line patients. Both 6 month PFS and 1-year OS values are in excess of the 95% confidence limit upper boundary to be expected for trials of similar size as described in the Korn meta-analysis of phase II trials for metastatic melanoma (Korn, Liu et al. 2008). This is also superior to the overall survival for the phase II and III trials previously reported. These data reveal that anti-angiogenic therapy with bevacizumab VEGF-A blockade and immune checkpoint blockade with ipilimumab can be safely administered and resulted in a significant proportion of patients receiving clinical benefit.

Correlatives Revealing Mechanisms of Action for Combination Therapy of Bevacizumab and Ipilimumab

A number of notable observations were made in correlative laboratory and pathological investigations in this trial. Marked infiltration with $CD3^+$, $CD4^+$, and $CD8^+$ T cells as well as $CD163^+$ cells (monocyte/macrophage lineage) were observed after treatment with ipilimumab plus bevacizumab. In contrast, patients treated only with ipilimumab demonstrated a lesser degree of immune cell infiltration while on therapy.

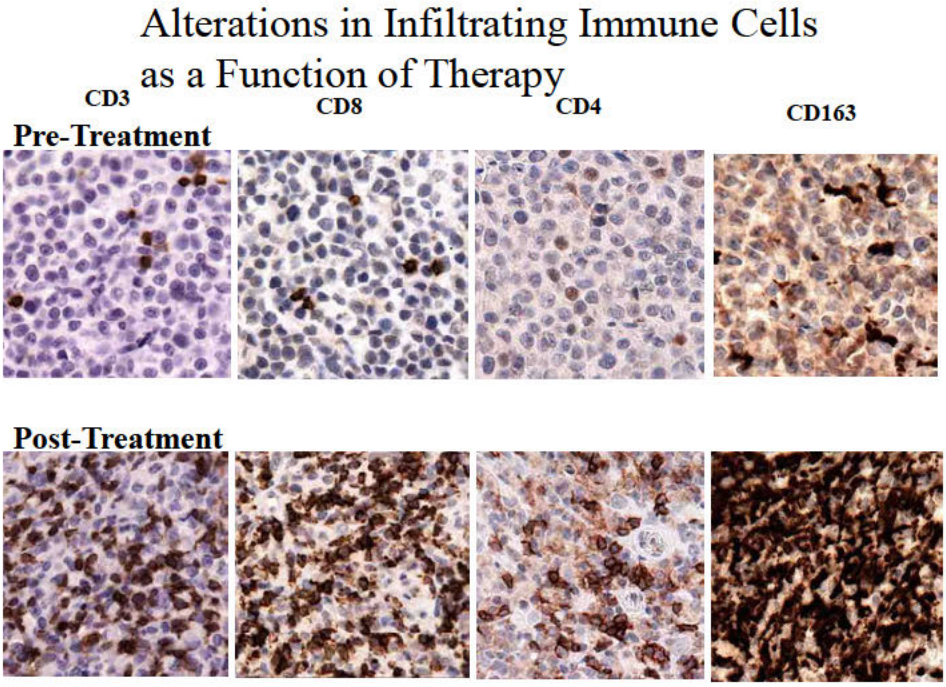


Figure7: Changes in tumor deposits resulting from treatment with bevacizumab plus ipilimumab. Phenotypic characterization of immune cell infiltrates in biopsies from responders before and after initiation of therapy. Significant infiltration by $CD3^{+}CD8^{+}$ T cells and $CD163^{+}$ dendritic macrophages with minimal change in $Foxp3^{+}$ component were observed.

The immune infiltrate appeared to form tertiary lymphoid aggregates, which was associated with dendritic cell infiltration and evidence of local endothelial activation similar to that seen in high endothelial venules in lymph nodes.

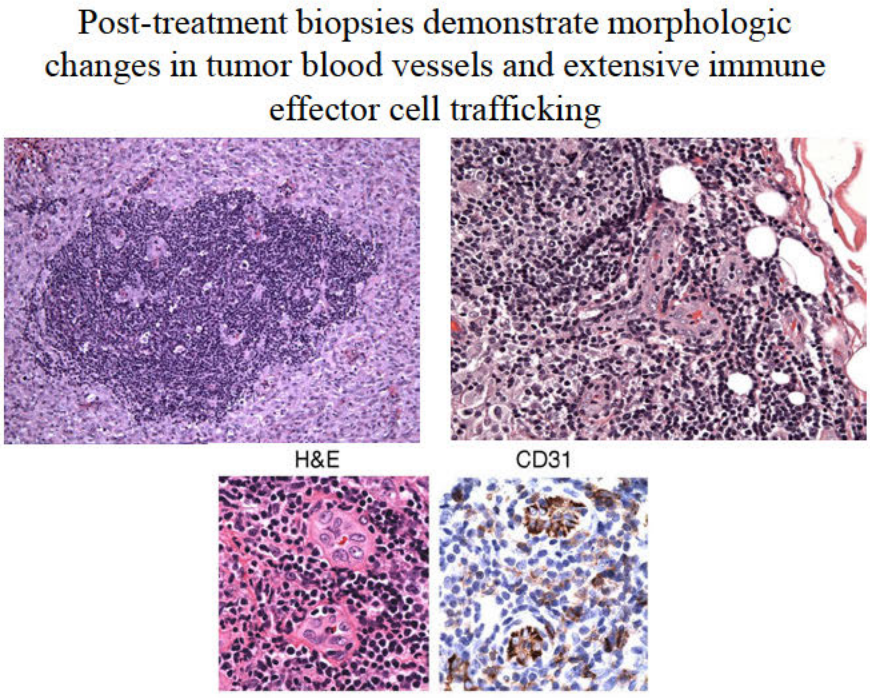


Figure8: Lymphoid aggregates and morphologic changes in endothelial cells

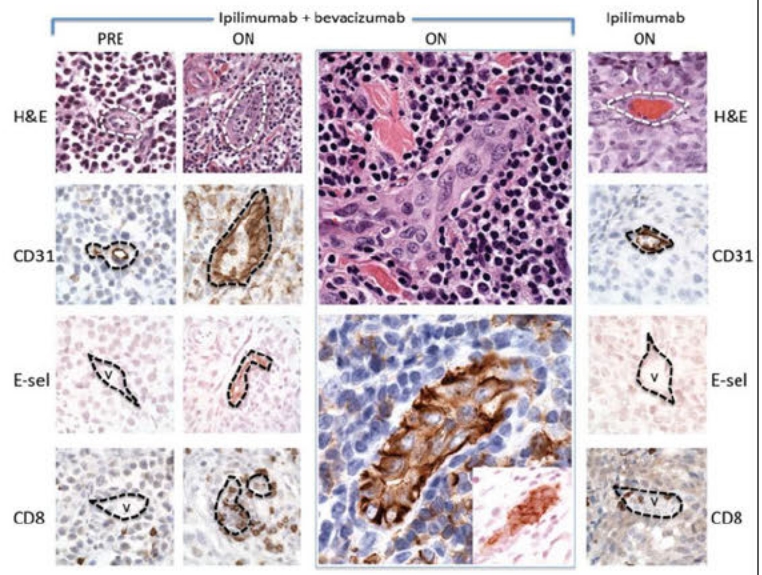
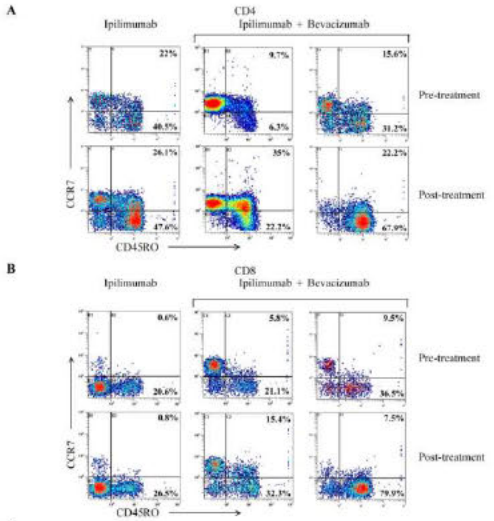


Figure 9: Blood vessels before and after initiation of therapy. Endothelial cells lining small vessels within melanomas of ipilimumab plus bevacizumab treated patients were plump as assessed by H&E and CD31 staining, in contrast to pre-treatment samples and on treatment samples from patients receiving ipilimumab alone (column to extreme right; v = vessel). Endothelial cells in tumor deposits of patients receiving ipilimumab plus bevacizumab were also associated with increased expression of E-selectin, and adhesion and diapedesis of $CD8^+$ T cells. Enlarged central panels highlight the focally occlusive appearance of this endothelial activation (top H&E, bottom CD31, [inset, E-selectin]). Base membrane of vessels approximated by dotted line.

In addition, flow cytometry analysis on peripheral PBMC indicated a marked increase in the number of patients exhibiting a ≥50% increase in levels of circulating $CD4^+$ and $CD8^+$ memory cells (CD45Ro), a phenomenon that has not been previously observed in studies of ipilimumab alone.



C

	Ipilimumab (3 mg/kg) (N=18)	Ipilimumab (3 mg/kg) + Bevacizumab (N=24)	Ipilimumab (10 mg/kg) + Bevacizumab (N=22)
CD4			
$CCR7^+CD45RO^+$	0 (0%)	7 (29%) *	8 (36%) **
$CCR7^-CD45RO^+$	2 (11%)	10 (42%) *	11 (50%) *
CD8			
$CCR7^+CD45RO^+$	1 (6%)	10 (42%) *	8 (36%) *
$CCR7^-CD45RO^+$	1 (6%)	9 (38%) *	9 (41%) *

Figure 10: T cell responses of melanoma patients after treatment with ipilimumab alone or ipilimumab plus bevacizumab. A. Example of changes as a function of treatment in $CD4^+CCR7^+CD45RO^+$ and $CD4^+CCR7^-CD45RO^+$ T cell populations to ipilimumab plus bevacizumab treatment, compared to changes with ipilimumab treatment alone. B. Example of changes as a function of treatment to $CD8^+CCR7^+CD45RO^+$ and $CD8^+CCR7^-CD45RO^+$ T cell populations to ipilimumab plus bevacizumab treatment, compared to the responses to ipilimumab treatment alone. C. Numbers of melanoma patients that have at least 50% enhancement in $CD4^+/CD8^+$ $CCR7^+CD45RO^+$ and $CD4^+/CD8^+$ $CCR7^-CD45RO^+$ T cell populations following treatment with ipilimumab (3mg/kg), or ipilimumab (3mg/kg) plus bevacizumab, or ipilimumab (10mg/kg) plus bevacizumab. * indicates $P<0.05$ between ipilimumab and ipilimumab plus bevacizumab. ** indicates $P<0.01$.

Angiopoietin-2 and its relevance to immune checkpoint blockade

Angiopoietin-2

Angiopoietin-1 is constitutively expressed in many adult tissues and is required for normal vascular homeostasis, whereas Ang-2 is predominantly expressed in tissues undergoing vascular remodeling and in hypoxic tumor microenvironments (Nasarre, Thomas et al. 2009). Ang-2 is a critical regulator of blood vessel maturation (Augustin, Koh et al. 2009). The molecule, which is in normal tissue almost exclusively produced by endothelial cells (EC), functions as a vessel-destabilizing molecule that facilitates the activities of other endothelial-acting cytokines by controlling the Ang-2/Tie-2 signaling pathway (Wong, Haroon et al. 1997; Augustin, Koh et al. 2009). Several studies have demonstrated that elevated levels of Ang-2 and higher Ang-2/Ang1 ratios compared to levels in normal tissues are associated with a worse prognosis in a number of different tumor types. The expression patterns of Ang-2 in normal tissues versus tumor suggest that Ang-2 may be a promising target for cancer therapy. Circulating Ang-2 was identified as a biomarker for progression and metastasis in melanoma (Helfrich, Edler et al. 2009) Furthermore, Ang-2 was found to be expressed by tumor-associated endothelial cells and melanoma cells; siRNA silencing of Ang-2 lead to strongly reduced invasive and migratory capacity of melanoma cells.

Angiopoietin-2 in ipilimumab studies

In our previous experience in early ipilimumab studies, serologic screening of cDNA expression libraries identified angiopoietin-2 (Ang-2) as a target of high titer antibodies in treated patients. Furthermore, a number of patients who experienced favorable clinical outcomes from these studies have developed high titer antibodies to Angiopoietin-2 (Ang-2) as a function of treatment.

Humoral responses to angiopoietins are associated with clinical benefits

Patient	Anti-ang-1 antibodies	Anti-ang-2 antibodies
K008	5	5
L19	25	25
M34	16	16
OV65	4	4
K027	32	32
M22	3	8
L18	16	16
M28	16	16
M35	16	16
M30	3	3
M9	2	2

8/11 patients showed long-term survival (≥ 4.5 yrs)

Figure 11: Humoral immune response to Ang-2 in treated melanoma patients with favorable clinical outcomes.

These antibodies in patients have proven to be functional in TIE-2 binding assays as well as tube formation assays (TIE-2 receptor signaling in endothelial cells), suggesting that synergy of

immune checkpoint blockade may go beyond VEGF and include the family of angiogeneic factors including angiopoietin 2.

Anti-angiopoietin Abs in sera block Tie-2 mediated signaling in endothelial cells

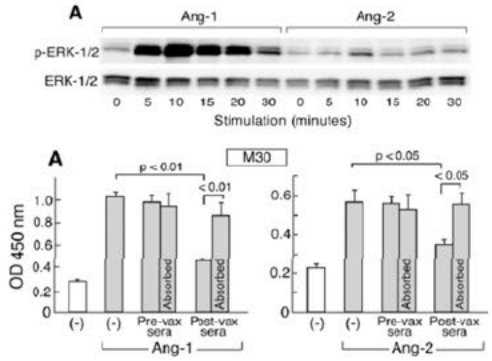


Figure 12: Patient humoral responses to Ang-2 are functional in TIE-2 binding assays

Anti-angiopoietin Abs in sera block tube formation by endothelial cells

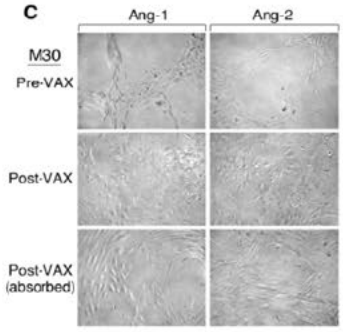


Figure 13: Patient humoral responses to Ang-2 are functional in tube forming assays

To investigate the potential influence of Ang-2 relative to immune checkpoint activity, we first assessed pre-treatment and post-treatment Ang-2 levels by Luminex relative to clinical outcomes of patients receiving ipilimumab therapy. There was a correlation with increased post-treatment levels of Ang-2 and clinical responses in a cohort of ipilimumab treated patients, suggesting that Ang-2 may play a role in disease progression in these patients.

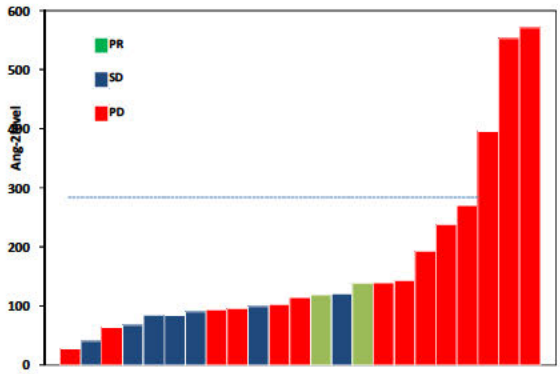


Figure 14: Ratios of post/pre-treatment Ang-2 level and clinical outcomes to ipilimumab. Comparison is from pre-treatment to week 12 of treatment in melanoma patients.

Angiopoietin-2 in ipilimumab combined with bevacizumab studies

We next assessed the development of antibodies to Ang-2 as a function of ipilimumab or ipilimumab plus bevacizumab in melanoma patients. In a cohort of 48 patients, 16 patients developed high-titer antibodies to Ang-2 as a function of treatment by ELISA and confirmed by immunoblotting.

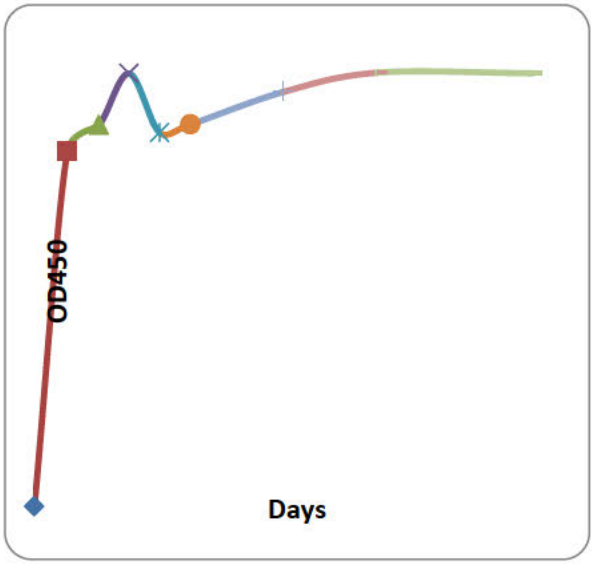


Figure 15: Example of changes in Ang-2 humoral immunity as a function of treatment with ipilimumab plus bevacizumab in a patient with metastatic melanoma

We next correlated percent changes from baseline Ang-2 antibody titers to clinical outcomes in ipilimumab treated melanoma patients demonstrating a trend in patients experiencing a response and magnitude of antibody titer changes.

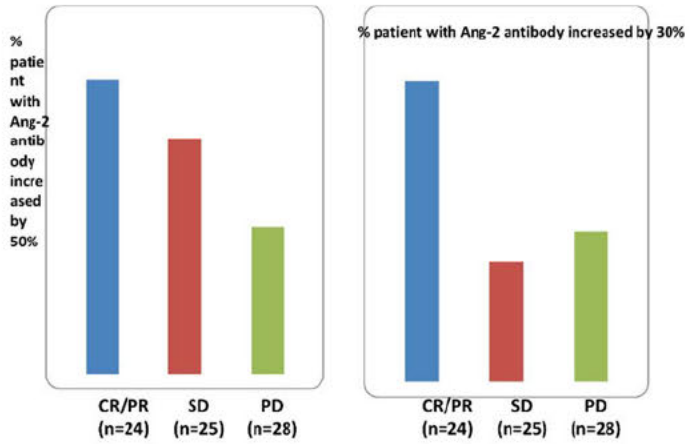


Figure 16: Changes from baseline Ang-2 antibody titers to clinical outcomes in ipilimumab treated melanoma patients.

We then assessed cytokines, circulating endothelial cells, and tumor associated endothelial cells. Assays for biologically active molecules involved in tumor related angiogenesis and the means to assess the immune responses to these molecules have been developed in the laboratory. We have established means to assay for VEGF, bFGF, as well as circulating endothelial cells (CEC) and circulating progenitor cells (CPC) (Duda, Cohen et al. 2007).

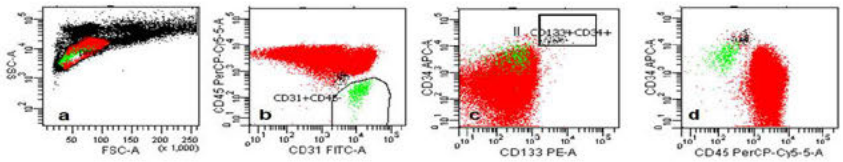


Figure 17. Identification and enumeration of CEC and CPC in whole blood samples of melanoma patients by multicolor flow cytometric analyses. Mononuclear cellular events were gated on the forward-side scatter plot (red in a). CEC in the mononuclear cellular population were identified as CD31brightCD45-CD34+CD133- (green in b-d) and CPC as CD133+CD34brightCD31+CD45dim (black in b–d). CEC and CPE were 0.6% and 0.15% of blood mononuclear cells, respectively, within the typical ranges of CEC (from 0.1% to 6.0%) and CPE (from 0.01 to 0.20%) in blood mononuclear cells from a normal donor.

We have also established a Luminex platform for circulating cytokine analyses. Analyses of samples from ipilimumab treated and ipilimumab plus bevacizumab treated patients are in process.

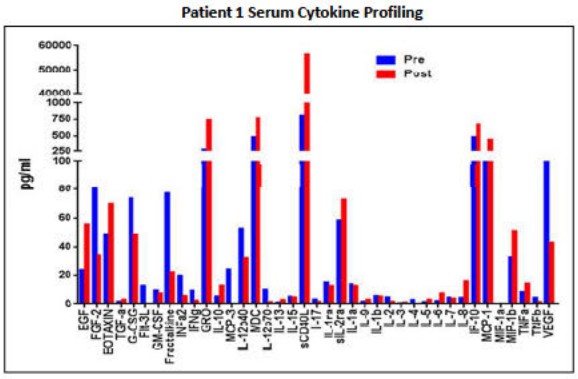


Figure 18. Example of serum cytokine profiling of ipilimumab plus bevacizumab treated patients using Luminex technology. Thirty-nine cytokines were analyzed. The levels of many cytokines altered as function of the treatment.

Effect of Ipilimumab + Bevacizumab on CEC

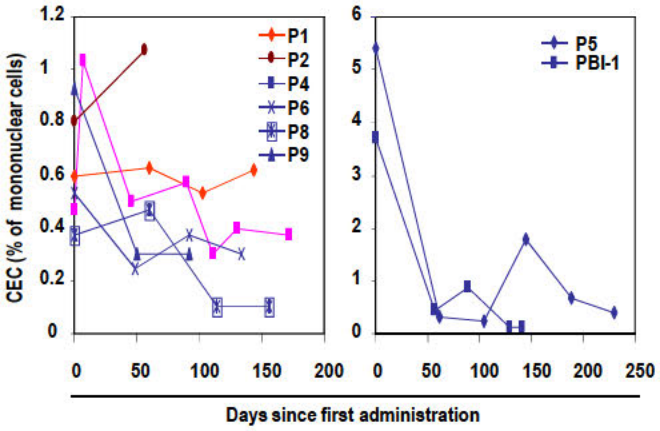


Figure 19: Treatment with ipilimumab plus bevacizumab decreased circulating CEC in the blood of melanoma patients.

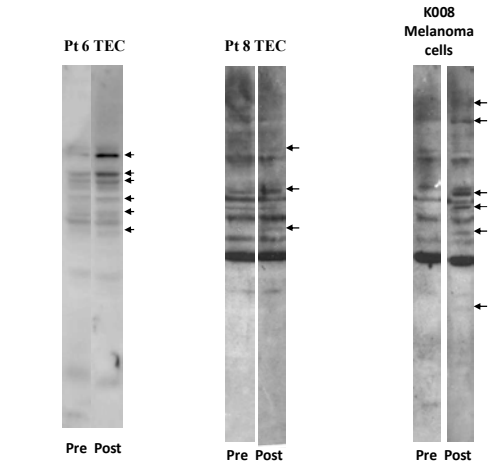


Figure 20: Treatment with ipiliumab plus bevacizumab resulted in humoral immune recognition of targets on tumor associated endothelial cells (TEC) isolated from fresh post-treatment biopsies as well as targets on melanoma cells.

2.2 Trebananib

Angiopoietin 2 (Ang2) is an autocrine cytokine, and it is expressed in endothelial cells and stored in Weibel-Palade bodies for rapid releasing upon stimulation (Reiss, Droste et al. 2007 Imhof, Aurrand-Lions et al. 2006). Tie-2, its receptor is predominately expressed on embryonic endothelium, and also found in the entire quiescent vasculature in a wide range of adult tissue (Dumont, Gradwohl et al. 1993; Wong, Haroon et al. 1997). Ang2 functions as an antagonist of Ang1/Tie-2 signaling in angiogenesis (Gale, Thurston et al. 2002; Augustin, Koh et al 2009; Maisonpierre, Suri et al 1997). In varieties of tumors, Ang2 expression by EC is up-regulated (Tsutsui, Inoue et al 2006; Sfiligoi, de Luca et al 2003; Currie, Gunningham et al 2002; Detjen, Rieke et al. 2010; Helfrich, Edler et al. 2009; Lind, Wikstrom et al 2005). In tumor microenvironment, vessel destabilization and regression by Ang2 is involved in vasculature remodeling and facilitates VEGF dependent angiogenesis (Augustin, Koh et al 2009; Holash, Maisonpierre et al 1999). Disruption of Ang2 with blocking antibody and genetic depletion indicates important roles in tumor angiogenesis and tumor growth (Brown, Cao et al. 2010; Hashizume, Falcon et al. 2010; Mazzieri, Pucci et al. 2011; Oliner, Min et al. 2004; Nasarre, Thomas et al. 2009).

Tie-2 is also found to be expressed on a proangiogenic subpopulation of myeloid cells in circulation and tumors called Tie-2-expressing monocytes/macrophages (TEM) (De Palma, Venneri et al. 2003; De Palma, Venneri et al. 2005; Coffelt, Scholz et al. 2010). Ang2 augments the ability of TEM in angiogenesis and facilitates TEM toward an M2-like macrophage phenotype (Coffelt, Scholz et al. 2010; Pucci, Venneri et al. 2009). Furthermore, Ang2 stimulates the immunno-suppressive properties of TEM via suppression of T cell activation, promotion of Treg expansion, and upregulations of IL10 and CCR17 (Coffelt, Scolz et al. 2010).

Trebananib is an angiopoietin-1/antiopoietin-2 neutralizing peptibody. Preclinical studies with trebananib showed significant inhibitions of several tumor types (Neal, Wakelee et al. 2010; Coxon, Bready et al. 2010). Recently the TRINOVA-1 phase 3 trials investigated the combination of trebananib and paclitaxel and showed significant reduction in disease progression but no improvement in overall survival in ovarian cancer patients (Monk et al. 2014; Monk et al. 2016). In metastatic colorectal cancer trebananib in combination with FOLFIRI did not improve PFS (Peeters et al. 2013), however, the ongoing VENGEANCE study in combination with bevacizumab suggests clinical activity in this setting (Mooi et al. 2015). The RCC studies suggested improved responses and PFS of trebananib in combination with sunitinib but not with sorafenib (Atkins et al. 2015; Rini et al. 2012).

These indicate important roles of angiopoietin-Tie-2 axis in tumor progression. However, mechanisms of trebananib on tumor immunity are not clearly elucidated, in particular, its roles in T cell and $CD14^{+}Tie\text{-}2^{+}$ monocyte immunity. Current studies suggest that interactions of Ang2 with $CD14^{+}Tie\text{-}2^{+}$ monocytes exert inhibitory effects on T cell activation, and trebananib shows partially restoration of suppression of T cell activation. In addition, initial studies showed that Tie-2 pathway increases PD-L1 expression and decrease ICOSL in $CD14^{+}Tie\text{-}2^{+}$ monocytes. These strongly suggest complicated suppression mechanism of T cell activation by Tie-2 signaling.

Generation of $CD14^{+}Tie\text{-}2^{+}$ monocytes

$CD14^{+}Tie\text{-}2^{+}$ monocytes play critical roles in T cell via Ang2/Tie-2 axis {Coffelt, 2011 #2961}. As shown in Figure 21, approximately 20% $CD14^{+}Tie\text{-}2^{+}$ monocytes were generated for studies of T cell suppression and trebananib. Tie-2 expressing Lenti virus was also generated for investigations of signals of Tie-2 and Ang2. Monocytes were further transduced with the virus. Approximately 36% $CD14^{+}Tie\text{-}2^{+}$ monocytes were generated after the infection.

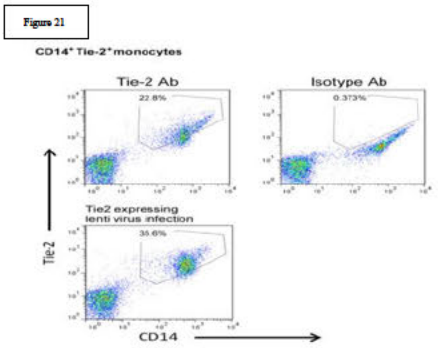


Figure 21. Expression of CD14 and Tie-2 on monocytes. Monocytes were isolated by adherent cell selections. Expressions of CD14 and Tie-2 on the monocytes were analysed by cytometry.

Effects of Ang2 and trebananib on T cell proliferation via enriched and Tie-2-infected $CD14^{+}Tie\text{-}2^{+}$ monocytes

Based on $CD14^{+}Tie\text{-}2^{+}$ monocytes and Tie-2-infected $CD14^{+}Tie\text{-}2^{+}$ monocytes, effects of Ang2 and trebananib on T cell proliferation were further examined. As shown in Figure 22, Ang2 exerts inhibitory effects on T cell proliferation, and the inhibitions were Tie-2 level dependent of $CD14^{+}$ monocytes. Trebananib partially restored Ang2 induced suppression. These suggest neutralizing effects of trebananib on Ang2 in T cell suppression.

Figure 22

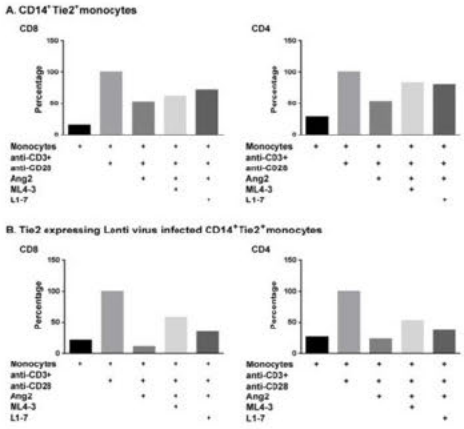


Figure 22. Effects of Ang1 and AMG386 on T cell proliferation. Pan T cells were stained with CSFE and further cocultured with $CD14^{+}Tie\text{-}2^{+}$ monocytes in the presence or absence of anti-CD3+CD28 beads and/or AMG386 as indicated. A. $CD14^{+}Tie\text{-}2^{+}$ monocytes. B. $CD14^{+}Tie\text{-}2^{+}$ monocytes transduced with Tie-2 expressing Lenti virus. Data represent precentage of proliferated T cells. The percentage of anti-CD3+CD28 bead group are presented as 100%.

<u>Tie-2 signaling of $CD14^{+}Tie\text{-}2^{+}$ monocytes involved in T cell suppression</u>

To further investigate the inhibitory mechanism of $CD14^{+}Tie\text{-}2^{+}$ monocytes on T cell activation, PD-L1 and ICOSL are examined. As shown in Figure 23, expression of PD-L1 was remarkably increased in Tie-2 infected $CD14^{+}Tie\text{-}2^{+}$ monocytes, in comparison with parental cells. Interestingly, expression of ICOSL was decreased over 50% in Tie-2 infected $CD14^{+}Tie\text{-}2^{+}$ monocytes, in comparison with parental cells (Figure 24).

It is shown that Ang2 stimulates Tie-2-expressing monocytes to suppress T cell activation (Coffelt, Muthana et al. 2011). Current studies are in consistent with this notion, in spite that the experiment system need to be further confirmed. Trebananib partially reverse Ang2 induced suppression. These support trebananib functions as neutralizing peptibodies.

Figure 23

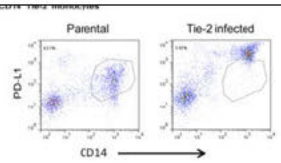


Figure 23. Expression of PD-L1 on $CD14^{+}Tie\text{-}2^{+}$ monocytes. Expression of PD-L1 on $CD14^{+}Tie\text{-}2^{+}$ monocytes and $CD14^{+}Tie\text{-}2^{+}$ monocytes infected with Tie-2 expressing Lenti virus were analyzed by cytometry.

Figure 24

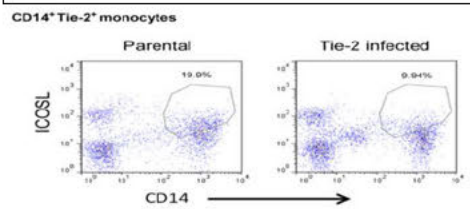


Figure 24. Expression of ICOSL on $CD14^{+}Tie\text{-}2^{+}$ monocytes. Expression of ICOSL on $CD14^{+}Tie\text{-}2^{+}$ monocytes and $CD14^{+}Tie\text{-}2^{+}$ monocytes infected with Tie-2 expressing Lenti virus were analyzed by cytometry.

Furthermore, the inhibitory effects are dependent on Tie-2 levels of $CD14^{+}$ monocytes. It suggests critical roles of Tie-2 signaling in TEM induced T cell suppression. Analyses with gene signature, phenotype, and superior proangiogenic abilities indicate that TEM and MHC-II^{low} TAM are analogous (Pucci, Venneri et al. 2009; Movahedi, Laoui et al. 2010). Functionally, both MHC-II^{high} and MHC-II^{low} cells are capable of suppressing T cell activation. MHC-II^{high} cell-mediated suppression is iNOS dependent, whereas, TEM derived IL10, which is induced by Ang2, suppresses T cell activation in both *in vitro* and mouse tumor *in vivo* models (Coffelt, Muthana et al. 2011). Whether trebananib reverse the suppression via IL10 pathway needs to be investigated.

PD-L1 is a membrane bound protein, primarily expressed on dendritic cells and monocytes {Keir, Buttie et al. 2008). The recepter for the ligand is PD1, which is expressed on activated T cells and B cells, DC, and monocytes (Keir, Buttie et al. 2008). During the engagement of T cells with antigen/MHC complex, interaction of PDL1 with PD1 exerts inhibitory effects on T cell activation, leading to immune suppression (Sharpe, Freeman et al. 2002; Keir, Buttie et al. 2008; Sharpe, Freeman et al. 2002). Our preliminary data show that Tie-2 siganling is associated with PD-L1 expression on $CD14^{+}Tie\text{-}2^{+}$ monocytes. It is of interest whether Ang2 and trebananib affects PD-L1 expression on $CD14^{+}Tie\text{-}2^{+}$ monocytes.

ICOS is a member of CD28 immunoglubulin superfamily, expressed upon T cell activation (Sharpe, Freeman et al. 2002). Its ligand is ICOSL, which expressed on B cells, dendritic cells, monocytes/macrophages, and T cells (Sharpe, Freeman et al. 2002; Coyle, Gutierrez-Ramos et al. 2001; Chambers, 2001). ICOS pathway is involved in functions of T helper cells, formation of germinal centers, and collaboration of T/B cells (Sperling, Bluestone et al. 2001; Mak, Shahinian et al. 2003). Disruption of ICOS/ICOSL pathway by genetic depletion showed important roles in anti-tumor therapy by CTLA4 blockade (Fu, He et al. 2011). Our preliminary data showed that Tie-2 signaling is associated with down-regulation of ICOSL expression on $CD14^{+}Tie\text{-}2^{+}$ monocytes. It is of interest whether Ang2 and trebananib affects ICOSL expression on $CD14^{+}Tie\text{-}2^{+}$ monocytes.

sPD-L1 is recently identified and characterized in our Lab (manuscript preparation). It is also secreted from mature DC, melanoma and renal tumor cells (Frigola, Inman et al. 2012; Frigola, Inman et al. 2011) (our unpublished data). It showed suppression of T cell activation (Frigola, Inman et al. 2011) (our unpublished data). Elevated sPD-L1 is associated with tumor progression in patients with renal cell carcinoma (Frigola, Inman et al. 2011). Our unpublished data also

indicated that higher levels of sPD-L1 are associated with progressive diseases in advanced melanoma patients, and expression of PD-L1 is associated with secretion of sPD-L1. Whether sPD-L1 secretion occurs and how Ang2 and trebananib affects sPD-L1 secretion in $CD14^{+}Tie\text{-}2^{+}$ monocytes are worthy of further investigation.

In summary, Tie-2 pathway plays roles in the regulation of expression of PD-L1, ICOSL, and IL10, which are involved in the modulations of T cell and tumor immunity. Impacts of Ang2 and its neutralizing trebananib on Tie-2 pathway are worthy of further investigation.

2.3 Rationale

2.3.1 Rationale for the Trial and Selected Participant Population

The combination of CTLA-4 and VEGF blockade appears to be well tolerated in patients with advanced melanoma. The clinical efficacy data and correlative studies of the immune response suggest that the combination has enhanced antitumor immunostimulatory effects beyond those observed with CTLA-4 blockade alone. One potential mechanism is that VEGF blockade promotes normalization of tumor blood vessels and permits enhanced egress of tumor specific lymphocytes and other immune effectors. Another, non-exclusive mechanism is that VEGF may inhibit some aspects of an effective adaptive immune response itself, including endothelial cell activation and dendritic cell maturation, and that blockade of VEGF further augments the anti-tumor immune response. The emergence of antibodies to Ang-2 after vaccination or CTLA-4 blockade observed in our studies suggests that synergy of immune checkpoint blockade may go beyond VEGF and include the family of angiogenic factors including Ang-2. There also remains the potential role for Ang2 blockade at influencing immune suppressor cells such as myeloid and M2 macrophages. These data in conjunction with the increasing evidence for Ang-2 as an important target for cancer therapy provide the rationale for the proposed trial of combined blockade.

Given the currently available data and clinical experiences, there are several cancer types that are reasonable to target based on this biology and clinical activity when considering combination therapies of immune checkpoint blockade and anti-angiogenesis. Pembrolizumab has demonstrated significant clinical activity in melanoma patients who have previously been treated with ipilimumab or are ipilimumab-naïve (Hamid, Robert et al. 2013, Robert, Ribas et al. 2014). Overall response rate is approximately 26-38% with evidence for durable benefit. The anti-PD-1 antibody nivolumab has also demonstrated significant clinical activity with a response rate of approximately 31%, median overall survival of 16.8 months, and median response duration of two years. Furthermore with this clinical activity, improved outcomes and understanding of combination approaches are needed. In addition, single agent activity of ziv-aflibercept as anti-angiogenesis in metastatic cutaneous or uveal melanoma included a 7.5% response rate and a median overall survival of 16.3 months (Tarhini Frankel, and Margolin, 2011). With the ipilimumab and bevacizumab combination clinical experience (Hodi, Lawrence et al. 2014), the single agent activity of zivaflibercept in melanoma, as well as known clinical activity for pembrolizumab in melanoma, melanoma is one disease to include investigations with this novel combination. Clinical activity with immune checkpoint blockade with ipilimumab and PD-1 agents has also been seen in renal cell carcinoma, and anti-VEGF agents are a mainstay of

treatment for this disease (Choueiri 2013, Escudier, Albiges et al. 2013, McDermott and Atkins 2013, Mooney, Paluri et al. 2014). Currently available are a number of VEGF TKI agents that have demonstrated clinical activity but with the development of resistance. Therefore, treatment of renal cell carcinoma patients with such combinations should also be considered as improvement of patient outcomes is still warranted. Anti-angiogenesis with bevacizumab and Ziv-aflibercept has become a mainstay treatment for colorectal cancer in combination with chemotherapy (Damin and Lazzaron 2014) (Patel and Sun 2014) (Dietvorst and Eskens 2013). There remains a need for improving patient outcomes from these combinatorial approaches. Improved understanding of checkpoint blockade as well as combinations is warranted in this disease with unmet need. Finally, ipilimumab has demonstrated activity in ovarian cancer (Hodi, Mihm et al. 2003). Bevacizumab is an active agent used in combination therapy for ovarian cancer. As part of the mainstay in this disease, there remains an unmet need in platinum-resistant ovarian cancer (Jayson, Kohn et al. 2014, Syrios, Banerjee et al. 2014).

Accumulating clinical experience with trebananib has demonstrated both activity and a favorable safety profile. VEGF and PD-1 blockade has also shown successful combination in pre-clinical animal models. Ang-2 and VEGF blockade together has revealed synergy in pre-clinical animal models and have successfully been combined in clinical trials. Ang-2 plays an important role in the proangiogenic and immune inhibitory effects of TIE-2 positive monocytes. The addition of Ang-2 inhibition to PD-1 blockade with pembrolizumab may further complement the reversal of angiogenic immune suppression and improve immune cell trafficking. We hypothesize to test whether the combination of Ang-2 inhibition with PD-1 blockade is tolerable and safe. We next seek to determine if the combination augments anti-tumor activity through evidence of clinical responses and biomarker responses.

In order to gain greater safety as well as preliminary efficacy data and correlatives to determine mechanisms of potential synergy in these patient populations, expanded cohorts will be treated at the recommended part 2 (expansion cohort) dose for pembrolizumab plus trebananib.

2.3.2 Rationale for Dose Selection/Regimen/Modification

2.3.2.1 Rationale Pembrolizumab Dose Selection

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

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg Q2W, representing an approximate 5- to 7.5 – fold exposure range (refer to IB, Section 5.2.2)
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distribution of exposures, supporting suitability of 200 mg Q3W

- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK]

2.3.2.2 Rationale Trebananib Dose Selection

The safety, pharmacokinetics, and antitumor activity of Trebananib were evaluated in Phase I study in patients with advanced solid tumors. Thirty-two patients received weekly intravenous trebananib doses of 0.3, 1, 3, 10, or 30 mg/kg in sequential cohorts. One DLT was reported in the 30 mg/kg cohort (respiratory arrest), which likely was caused by tumor burden that was possibly related to trebananib. The most common toxicities were fatigue and peripheral edema. Proteinuria (n = 11) was observed without clinical sequelae. Only four patients (12%) experienced treatment-related toxicities greater than grade 1. A maximum-tolerated dose was not reached. PK was dose-linear and the mean terminal-phase elimination half-life values ranged from 3.1 to 6.3 days. Serum trebananib levels appeared to reach steady-state after four weekly doses, and there was minimal accumulation. No anti-trebananib neutralizing antibodies were detected (Herbst, Hong et al. 2009). The safety of trebananib was also evaluated in a Phase I study in the Japanese population using dose escalation of 3, 10, or 30 mg/kg. Trebananib was well tolerated at all dose levels and no DLT was observed (Doi, Ohtsu et al. 2012). Trebananib has been evaluated as monotherapy and in combination with chemotherapy or other biologic agents across tumor types, including mixed solid tumors, ovarian, breast, renal, gastric, hepatic, and colorectal cancers. As of the study-specific data cutoff dates, 3611 participants have been enrolled into 20 studies in the trebananib clinical program, of whom 3561 participants have received ≥ 1 dose of trebananib or trebananib placebo, at doses ranging from 0.3 mg/kg to 30 mg/kg intravenously (IV) once weekly (QW). No maximum tolerated dose for trebananib monotherapy has been identified at doses up to 30 mg/kg IV QW. Most of early phase 1b/2 studies (in combination with other agents) were conducted with 3 and 10 mg/kg every week (QW) doses. Later, based on exposure response modeling the 15 mg/kg QW dose was elected in all 3 of the large Phase 3 trials and this dose was explored further in a few Phase 1b/2. Few small studies using 30 mg/kg QW in combination with chemotherapy or other agents are currently ongoing and there is no controlled studies comparing 15 to 30 mg/kg.

A summary of anticipated overlapping toxicities with pembrolizumab includes:

- Diarrhea
- Fatigue
- Nausea and vomiting
- Shortness of breath
- Skin itching
- Rash
- Cough
- Joint pain
- Fever
- Swelling

- Weakness
- Back pain
- Abdominal pain
- Anemia
- Inflammation of the bowel

2.3.3 Rationale for Endpoints

2.3.3.1 Efficacy Endpoints

The primary endpoint of this study is to determine the safety, tolerability and recommended dosing for the combination of pembrolizumab plus trebananib. Our secondary objective are to obtain in preliminary fashion the efficacy of the combination including PFS at 6 months, the rate of 1-year overall (OS), the response rate (RR) and time to progression in diseases where anti-angiogenesis had shown to be effective. Chest, abdomen, and pelvic CT scan will be obtained every twelve weeks for determination of therapeutic efficacy. Standard solid tumor response criteria (RECIST) will be obtained. To further explore the clinical observations in some patients receiving ipilimumab of delayed responses and disease burden increasing before stable disease or response attained, we will incorporate proposed immune response criteria to assess for clinical activity. These immune response criteria (irRC) (Hodi, Hoos et al. 2008) (Wolchok et al., 2009) will be captured and compared to standard response criteria for solid tumors.

2.4 Correlative Studies Background

Antigen-specific T cell responses are controlled by co-stimulatory and co-inhibitory molecules positively and negatively. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1, CD279) are among the key co-inhibitory molecules, broadly categorized as “checkpoint molecules” (Pardoll DM, 2012). CD279 is up-regulated on activated T lymphocytes and mediate immunosuppression when binding to its ligands B7-H1 (CD274) and B7-DC (CD273). Blockade of CD279 or CD274 induced durable objective response in patients with advanced melanoma, renal cell carcinoma and non-small cell lung cancers in clinical trials (Topalian S, 2012; Brahmer J, 2012; Hamid O, 2013). Moreover, immunohistochemical staining performed on pretreatment tumor tissues from patients with anti-CD279 treatment showed that none of 17 patients with CD274 negative tumors had an objective response whereas 36% (9/25) patients with CD274 positive tumors had an objective response (P=0.006). This striking difference suggests that CD274 expression on tumor specimen might be a useful biomarker to predict response to anti- CD279 treatment.

Anti-vascular endothelial growth factor pathway therapies preferentially target immature tumor blood vessels and leave behind normalized and resistant blood vessels. Angiopoietin-2 (ANGPT2)/TIE pathway is largely confined to vasculature. It has two receptors TIE1 and TIE2 as well as three ligands, angiopoietin-1, angiopoietin-2 (ANGPT2) and angiopoietin-4. ANGPT2 plays an important role in vascular remodeling and angiogenesis. It acts as context-specific antagonist of angiopoietin-1/TIE2 signaling, destabilizes the quiescent blood vessels as a prerequisite to sprouting angiogenesis in the presence of proangiogenic stimulation or vascular

regression in the absence of such stimuli. Therapeutics targeting the ANGPT2/TIE pathways including selective anti-ANGPT2 antibodies is in development (Gerald D, 2013; Karlan BY, 2012; Hashizume H, 2010). Serum ANGPT2 is found to be a biomarker for tumor progression and survival in various malignancies (Helfrich I, 2009).

We further hypothesize that not only CD274 but also CD279 and CD273 protein expression in tumor tissues might be associated with favorable clinical response, and might be served as biomarkers for patient selection for CD279 blockade in clinical treatment.

We also hypothesize that ANGPT2 protein expression in tumor tissue might be a biomarker to identify a group of patients who might have objective responses on anti-ANGPT2 or/with anti-vascular endothelial growth factor therapies.

3. PARTICIPANT SELECTION

Participants must meet all eligibility criteria below prior to registration. See Section 4 for further information regarding registration procedures and Section 9 for study calendar and further details.

All assessments are to occur within 28 days of registration except where noted otherwise. The participant must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the participant prior to enrollment.

Following registration, any additional laboratory assessments obtained prior to start of treatment will not be used to re-confirm eligibility.

3.1 Eligibility Criteria

In order to be eligible for participation in this trial, the participant must meet the following criteria on screening examination:

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

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

3.1.3 Have measurable disease based on RECIST 1.1.

3.1.4 In dose escalation (Phase I), patients must have histologically or cytologically confirmed metastatic disease from any solid tumor that is incurable and fulfills one of the following criteria:

a) Has demonstrated progression of disease following at least one line of effective systemic therapy. Prior treatment with anti-CTLA-4 antibody (including ipilimumab) is allowable

OR

b) For which effective therapy does not exist

3.1.5 In dose expansion (part 2), patients must have histologically or cytologically confirmed unresectable or metastatic melanoma, renal cell carcinoma, ovarian cancer, or colorectal cancer.

3.1.6 Renal cell patients must have had at least one prior VEGF TKI.

3.1.7 Ovarian cancer patients must be resistant to platinum therapy (i.e. within 6 months of last platinum therapy).

3.1.8 Patients with colorectal cancer should have progressed on at least one fluorouracil plus irinotecan or oxaliplatin containing regimen.

3.1.9 Patients with melanoma should have unresectable or metastatic disease. Melanoma patients with BRAF V600E or V600K mutation-positive melanoma who have previously received a BRAF inhibitor with or without a MEK inhibitor) are eligible.

3.1.10 In the dose expansion cohort patients should be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion (pre-treatment) and an on-treatment biopsy. Newly-obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Patients for whom newly-obtained samples cannot be provided (e.g., inaccessible, participant safety concern, or unwilling to undergo biopsy) may submit an archived specimen only upon agreement from the Sponsor. An on-treatment biopsy will be collected approximately halfway through the induction period, about 6 weeks from the start of study treatment (sometime between Cycle 2 Day 8– Cycle 3 Day 1).

3.1.11 Have a performance status of 0 or 1 on the ECOG Performance Scale (see Appendix A) up to 28 days before treatment initiation

3.1.12 Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed up to 28 days before treatment initiation.

Table 1. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for participant with creatinine levels > 1.5 X institutional ULN
Hepatic	
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
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) 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 ≤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
[a]Creatinine clearance should be calculated per institutional standard.	

3.1.13 Negative protein on screening urinalysis

3.1.14 Female participant of childbearing potential should have a negative serum pregnancy test performed during the screening period.

Female participants of childbearing potential (Section 5.11.2) must be willing to use an adequate method of contraception as outlined in Section 5.11.2 – Contraception, for the course of the study through 120 days after the last dose of study medication. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

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

3.1.15 Male participants of reproductive potential (Section 5.11.2) must agree to use an adequate method of contraception as outlined in Section 5.11.2- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

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

3.2 Exclusion Criteria

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

3.2.1 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.

3.2.2 Has a diagnosis of immunodeficiency including participants infected with Human Immunodeficiency Virus (HIV).

3.2.3 Is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

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

3.2.5 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.

3.2.6 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.

3.2.7 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 in situ cervical cancer.

3.2.8 Lesions suspected to be at higher-risk for bleeding such as bowel involvement with tumor that invades into the bowel wall or involves the intraluminal component of bowel by imaging or direct visualization or central pulmonary lesions.

3.2.9 Ulcerated skin lesions

3.2.10 Poorly-controlled hypertension as defined BP > 150/100 mmHg, or SBP > 180 mmHg when DBP < 90 mmHg, on at least 2 repeated determinations on separate days within 3 months prior to study enrollment.

3.2.11 History within 6 months prior to treatment of myocardial infarction, severe/unstable angina pectoris, CABG, NYHA class III or IV CHF, stroke or TIA.

3.2.12 History within 3 months prior to treatment of Grade 3-4 GI bleeding/hemorrhage, treatment resistant peptic ulcer disease, erosive esophagitis or gastritis, infectious or inflammatory bowel disease, diverticulitis, pulmonary embolus, or other uncontrolled thromboembolic event.

3.2.13 Patients who are less than 4 weeks post-op after major surgery.

3.2.14 History of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab and trebananib including history of allergic reactions to bacterially produced proteins.

3.2.15 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.

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

3.2.17 Treatment within 30 days prior to enrollment/randomization with strong immune modulators including but not limited to systemic cyclosporine, tacrolimus, sirolimus, mycophenolate mofetil, methotrexate, azathioprine, rapamycin, thalidomide, and lenalidomide.

3.2.18 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, interstitial lung disease or active, non-infectious pneumonitis, nephritis, pancreatitis, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.19 Has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or current pneumonitis/interstitial lung disease..

3.2.20 Has an active infection requiring systemic therapy.

3.2.21 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 participant to participate, in the opinion of the treating investigator.

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

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

3.2.24 Patient with ovarian cancer and colorectal cancer who have received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 antibody. Melanoma and renal cell carcinoma patients who received prior anti-PD-1 or anti-PD-L1 or CTLA-4 antibodies are allowed to participate.

3.2.25 Has received trebananib or another angiopoietin-2 directed therapy (prior treatment with bevacizumab is not an exclusion criteria)

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

3.2.27 Has received a live vaccine or live-attenuated vaccine within 30 days prior to the first dose of study drug. Administration of killedvaccines is allowed.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Participant Research Titled Centralized Participant Registration (SOP #: REGIST-101A) or Decentralized Participant Registration (SOP #: REGIST-101B) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

This is a prospective trial which will accrue participants with solid tumors to evaluate the safety, clinical, and immunological effect of the combination of pembrolizumab and trebananib. The treatment will include an induction phase of pembrolizumab and trebananib for 4 cycles (12 wks) followed by pembrolizumab alone for up to 2 years. This trial will be conducted in 2 parts:

Part I will use a standard 3+3 dose escalation design in all solid tumors (refer to Table 2. Dose escalation will begin in dose cohort +1 (refer to Table 3). If two or more patients in dose cohort +1 experience a DLT, the next cohort of patients will be enrolled into dose cohort -1. Should dose cohort -1 prove too toxic (i.e. two or more patients experience a DLT), enrollment to the study will stop. If the toxicity profile of dose cohort +1 is acceptable per the escalation decision rules in Table 2, the next cohort will be enrolled into dose cohort +2. Should dose cohort +2 have acceptable toxicity per the escalation decision rules in Table 2, that will be the RP2D; otherwise, dose cohort +1 will be the RP2D.

Table 2. Dose Escalation Schema

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

Table 3. Part I Dose Escalation Schedule

Dose Cohort	Pembrolizumab every 3 weeks IV	Trebananib weekly IV	N
- 1	200mg	3 mg/kg	3+3
+1	200mg	15 mg/kg	3+3
+2	200mg	30 mg/kg	3+3

Part II has four dose expansion cohorts to be treated at the RP2D of pembrolizumab and trebananib: melanoma (closed to accrual), renal cell carcinoma (closed to accrual), ovarian cancer, and colorectal cancer. For ovarian cancer, 12 patients will be enrolled and treated. For CRC patients, a total of 37 patients will be enrolled on the expansion cohort. For the RCC cohort, 4 patients were enrolled and treated in the expansion cohort.

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

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 4. Each treatment cycle will be 3 weeks (21 days) long.

The treatment will include an induction phase of pembrolizumab and trebananib for 4 cycles (12 wks) followed by pembrolizumab alone for 2 years.

Table 4. Regimen Description for Parts I and II

Regimen Description					
Agent	**Pre-medications**	**Dose**	**Route**	**Schedule *****	**Cycle Length**
Pembrolizumab	Not routinely necessary unless prior infusion reaction	200mg at a final concentration of 1 mg/mL to 10 mg/mL in NS or D5	IV over approximately 30 minutes (range: 25-40 minutes)	Day 1	21 days (3 weeks)
Trebananib	Not routinely necessary unless prior infusion reaction	** mg/kg at a final concentration of 30mg/mL in NS	****IV over approximately 30 minutes	Day 1,8,15	
Doses as appropriate for assigned dose level. * The treatment will include an induction phase of pembrolizumab and trebananib for 4 cycles (12 wks) followed by pembrolizumab alone for 2 years.					

**** First dose of trebananib should be administered over 60 minutes.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 2.3.2

Details on the preparation and administration of pembrolizumab and trebananib are provided in the pharmacy manual.

5.3 Agent Administration

Treatment will be administered on an outpatient basis. Dose for weight-based drugs should be flagged for recalculation at the start of each cycle should the weight of a participant change by more than 5% from the previous cycle's Day 1 weight. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

5.3.1 Timing of Dose Administration: Pembrolizumab

Pembrolizumab should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 9). Pembrolizumab may be administered with trebananib up to 2 days before or after the scheduled Day 1 of each cycle due to administrative reasons. Pembrolizumab will be administered before trebananib.

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

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

5.3.2 Timing of Dose Administration: Trebananib

Trebananib should be administered on Day 1, 8, and 15 of each 21-day cycle after all procedures/assessments have been completed. Trebananib may be administered with pembrolizumab up to 2 days before or after the scheduled Day of each cycle due to administrative reasons (±2 days).

The first dose of trebananib will be administered by IV infusion over a 60-minute period. If the initial dose administration is well tolerated, future administrations may be given over approximately 30 minutes (treatment cycle intervals may be increased due to toxicity a described in section 5). A window of -5 to +10 minutes is permitted (i.e. infusion time is 30 minutes: -5 min/+10 min). Trebananib will be administered within 2 hours after Pembrolizumab.

The Investigational Product Instruction Manual (IPIM) contains specific instructions for the preparation of the trebananib infusion fluid and administration of infusion solution.

5.3.3 Dose Modifications

5.3.3.1 Pembrolizumab Dose Modification and Toxicity Management for Immune-Related AEs Associated with Pembrolizumab and Combination Therapy

AEs associated with pembrolizumab exposure, including coadministration with additional compounds, may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab/combination treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab/combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided in Table 5.. See Section 5.9 for supportive care guidelines, including use of corticosteroids.

Attribution of Toxicity:

When study interventions are administered in combination, attribution of an adverse event to a single component is liely to be difficult. Therefore, while the investigator may attribute a toxicity event [to the combination, to [trebananib alone] or to pembrolizumab alone], for adverse events listed in Table 5, both interventions must be held according to the criteria in Table 5 Dose Modification and Toxicity Management Guidelines for Immune-Related Adverse Events Associated with Pembrolizumab.

Holding Study Interventions:

When study interventions are administered in combination, if the AE is considered immune-related, both interventions should be held according to the recommended dose modifications.

Restarting Study Interventions:

Participants may not have any dose modifications (no change in dose or schedule) of pembrolizumab in this study, as described in Table 5.

- If the toxicity does not resolve or the criteria for resuming treatment are not met, the participant must be discontinued from all study interventions
- If the toxicities do not resolve and conditions are aligned with what is defined in Table 5, the combination of trebananib and pembrolizumab may be restarted at the discretion of the

investigator. [In these cases where the toxicity is attributed to the combination of trebananib and pembrolizumab or trebananib alone, re-initiation of pembrolizumab as a monotherapy may be considered at the principal investigator's discretion.]

Table 5 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab monotherapy and IO combinations

General instructions:

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

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

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

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
	Grade 3 or 4	Withhold or permanently discontinue [d]	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

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

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

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up

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

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

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).

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

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 6.

Table 6. Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion .** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 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 participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug intervention.**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
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 participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. ** In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov.		

Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical/surgical events and/or unforeseen circumstances not related to study intervention. However, intervention is to be restarted within 3 weeks of the originally scheduled dose and within 42 days of the previously administered dose, unless otherwise discussed with the Sponsor. The reason for study intervention interruption should be documented in the patient's study record.

If pembrolizumab is held or treatment delayed, trebananib should also be delayed such that the two drugs are administered on the same day. If pembrolizumab is to be permanently discontinued, the patients may continue trebananib treatment alone.

5.3.3.2 Trebananib Dose Modification and Toxicity Management

Criteria for dose modification as well as specific toxicity management are described below:

For infusion reaction and delayed infusion related reactions: Any potential infusion reaction should be classified based upon severity and time of onset relative to the infusion, and recorded in the case history (and AE eCRF) as an infusion reaction in addition to the underlying symptom.

For infusion reactions occurring within 24 hours of infusion of trebananib and deemed by the investigator to be related to trebananib infusion:

- Mild (grade 1) or moderate (grade 2) – treat per appropriate medical guidelines. Trebananib dosing may resume; however all subsequent doses of trebananib should be administered no faster than over 60 minutes.
- Severe (grade 3) or life-threatening (grade 4) –permanently discontinue trebananib

For infusion – related reactions occurring more than 24 hours after the infusion of trebananib, regardless of whether the reaction is deemed related or not –related to trebananib:

- All grades – treat per appropriate medical guidelines. Contact the Priniciple Investigator prior to any additional administration of trebananib.

Hemorrhage: Participants who develop any new grade of hemorrhage in the central nervous system will permanently discontinue trebananib.

Participants who develop grade 3 or 4 hemorrhage in any other organ system (pulmonary, GI, etc.) will permanently discontinue trebananib.

Thromboembolic Events: For grade 1 or 2 venous thromboembolic event, no dose modification is required. For grade 3 venous thromboembolic event, or asymptomatic grade 4 venous thromboembolic event (asymptomatic pulmonary embolus), hold trebananib. If the planned duration of the full-dose anticoagulant is ≤ 2 weeks, trebananib should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is > 2 weeks, trebananib may be resumed during the period of full dose anticoagulation if the following criterion is met:

- The participant must be therapeutically anti-coagulated with an approved anticoagulant agent according to standard prescribing guidelines.

Participants, while on anticoagulation, who develop a second thromboembolic event of grade 2 or higher, should permanently discontinue trebananib.

For symptomatic grade 4 venous thrombosis, permanently discontinue trebananib.

For any grade of arterial thromboembolic event, trebananib will be permanently discontinued.

Edema/lymphedema: A higher incidence of peripheral edema was observed in the trebananib arms vs. the placebo arm in the 20060342 study. To provide a common framework for reporting edema (including lymphedema) investigators should report new edema or edema that significantly worsens from pre-study baseline, and manage their participants as defined in this section.

All adverse events of peripheral edema must be reported and graded as found in this section and NOT per CTCAE v4.0.

Edema of a visceral organ or body cavity, such as pulmonary congestion, ascites, or pleural effusion, should NOT be reported as per this section but per CTCAE v4.0.

Investigators should attempt to ascertain the etiology of edema, which may include, but is not limited to, thromboembolism, chronic corticosteroid dosing, tumor obstruction of lymphatic or blood vessels, congestive heart failure, iatrogenic fluid overload, renal insufficiency, nephrotic syndrome or other significant hypoalbuminemic states.

Edema/lymphedema should be classified as:

- LOCALIZED (confined to a single body area, e.g. lower extremities only) or
- GENERALIZED (contiguous extension to more than a single body area).

Additionally, both localized and generalized edema/lymphedema should be graded as follows:

- Grade 1 (MILD): defined as trace thickening or faint discoloration of the affected area.
- Grade 2 (MODERATE): defined as moderate thickening or marked discoloration; leathery skin texture; papillary formation.
- Grade 3 (SEVERE): defined as severe symptoms that may involve skin blistering or skin breakdown; limitations of activities of daily living (ADL).

The management of edema / lymphedema should be as follows based upon the above grading:

- Grades 1 or 2 – Continue trebananib dosing per protocol and treat edema / lymphedema per appropriate medical and institutional guidelines
- Grade 3 or 4 – Discontinue trebananib permanently. Treat edema /lymphedema per appropriate medical and institutional guidelines

Hypertension: Hypertension (HTN) should be managed per institutional standards and appropriate medical guidelines. trebananib should be held for systolic BP > 200 mmHg or diastolic BP > 110 mmHg, or symptomatic hypertension. trebananib may be resumed at the pre-hold dose upon resolution of symptomatic HTN, systolic BP ≤ 140 mmHg and diastolic BP ≤ 90 mmHg, or returned to participant's baseline blood pressure. If the participant's BP is elevated upon first reading, measurements should be confirmed with a repeat measurement.

For any grade of hypertensive crisis or hypertensive encephalopathy, permanently discontinue trebananib.

Hypokalemia: Participants should have their serum potassium checked as outlined in the Section 9 and managed per institutional guidelines. If hypokalemia is present, replacement should be managed with either oral and/or parenteral replacement, according to institutional practice and to the degree of hypokalemia present. It is recommended that the participant's serum potassium level should be maintained within the normal range, as much as possible, during study treatment. Isolated hypokalemia without clinical sequelae should not be reported as an adverse event.

Pleural Effusion and Ascites: Pleural effusion and ascites should be graded according to CTCAE v4.0. However, since trebananib is known to cause or worsen pre-existing pleural effusions and ascites, these adverse events should be managed as noted below:
Life-threatening pleural effusions or ascites:

- Institute emergency measures per institutional guidelines
- Permanently discontinue trebananib

For all non-life-threatening pleural effusion or ascites:

- Treat per institutional guidelines which may include,

o non-investigational diuretics,
o thoracentesis, chest tube drainage or paracentesis
o pleurodesis
- Investigators should document each paracentesis and /or thoracentesis that occurs while a participant is on study.

Additional toxicities to permanently discontinue trebananib for:
- Gastrointestinal perforation
- Compromised wound healing
- Fistula formation
- Nephrotic syndrome or thrombotic microangiopathy (TMA)
- Reversible posterior leukoencephalopathy syndrome (RPLS)
- Ascites or pleural effusion requiring therapeutic invention

Trebananib should be temporarily suspended at least 4 weeks prior to an elective surgery. Trebananib should not be resumed until 4 weeks after major surgery, or 2 weeks after minor surgery, and until wounds are healed and drainage tubes removed.

Any grade 1 or 2 toxicities other than those listed above considered to be related to trebananib or the combination of trebananib and pembrolizumab should be managed according to standard medical practice.

When a participant experiences a grade-3 or 4 toxicity other than those listed above considered to be related to trebananib or the combination of trebananib and pembrolizumab, trebananib should be held until the toxicity resolves to ≤ grade 1 or the patient's baseline. When resumed trebananib will be dose reduced by one dose level, however, patients at dose level -1 requiring a reduction will discontinue trebananib.

If more than three doses of trebananib need to be held then trebananib will be discontinued.

If a toxicity is assessed as related to trebananib and not pembrolizumab, pembrolizumab may be continued when the toxicity is improved to grade 1 or resolved. If trebananib is to be permanently discontinued, the patient may continue treatment with pembrolizumab alone. If the patient continues pembrolizumab alone due to a toxicity related to trebananib, then they are permitted to skip any Day 8 and Day 15 visits and assessments that coincide with the administration of trebananib.

5.4 Criteria to Resume Treatment

For non-autoimmune or inflammatory events, patients may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:
- Patients may resume treatment in the presence of Grade 2 fatigue.
- Patients with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in

the presence of Grade 2 AST/ALT OR total bilirubin.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies (not including drug-related adrenal insufficiency or hypophysitis) adequately controlled with only physiologic hormone replacement may resume treatment after replacement correction and clinically stable regimen.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the treatment should resume at the earliest convenient point that is within the 12 week delay period.

If treatment is delayed >12 weeks, the patient must be permanently discontinued from study therapy, except as specified in Section 5.12 (Duration of Therapy).

5.5 Definition of Dose-Limiting Toxicity (DLT)

Dose –limiting toxicities (DLTs) will be assessed during the first 3 weeks of treatment so that labs and other evaluations taken at the end of the first cycle (start of the second cycle) are included in the assessment period. A DLT is defined as an adverse event that is (a) related to pembrolizumab and/or trebananib with an attribution of possible, probable, or definite, and (b) occurs during and/or begins during the first 3 weeks of the study treatment and (c) meets any of the following criteria:

<u>Hematological toxicities</u>
- A Grade 4 hematological toxicity unrelated to an underlying medical condition
- Arterial thromboembolic event

<u>Non-hematological toxicities:</u>
- A non-hematological toxicity of grade 3 unrelated to an underlying medical condition
- Eye pain of grade 2 or higher.
- Urine protein: creatinine > 3.5 or >2g protein on 24hour urine collection
- Two delays of treatment (not due to scheduling non-compliance) each lasting more than 10 days within 4 cycles of drug.

Exclusions to Dose limiting toxicities:

- Grade 3 electrolyte toxicities that can be corrected to Grade 1 or less within 24 hours will not be considered dose limiting.
- Grade 3 hypertension that can be controlled with oral medications and does not require treatment delay for > 7 days will not be considered a DLT.
- Grade 3 diarrhea that can be corrected to Grade 1 or less within 48 hours after anti-diarrhea medication will not be considered dose limiting.
- Grade 3 nausea and vomiting that can be corrected to Grade 1 or less within 48 hours after anti-emetic therapy will not be considered a DLT.

- Grade 3 rise in creatinine that can be corrected to Grade 2 or less after 2 liters of intravenous fluids within 24 hours will not be considered dose limiting.
- Grade 3 elevation in AST/ALT if AST/ALT levels were <1.5 times the baseline level will not be considered a DLT.
- Grade 3 elevation on ALP (Alkaline phosphatase) will not be considered a DLT.
- Grade 3 lymphopenia will not be considered a DLT.
- Grade 3 rash that returns to ≤ grade 2 after 1 week of symptomatic treatment will not be considered a dose limiting toxicity.

Management and dose modifications associated with the above adverse events are outlined in Section 5.3.3.

Dose escalation will proceed within each cohort according to the following schema. Dose-limiting toxicity (DLT) is defined above.

5.6 Definition of Maximum Tolerated Dose

The MTD will be based on the assessment of DLTs as defined in section 5.5 and will not exceed the 30mg/kg trebananib weekly dose. The MTD will be defined as the dose at which fewer than one-third of participants experience a DLT to pembrolizumab and trebananib.

5.7 Trial Blinding/Masking

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

5.8 Stratification

No stratification based on age, gender or other characteristic will be used in this trial.

5.9 General Concomitant Medication and Supportive Care Guidelines

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 DFCI Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician.

5.9.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and

fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 9.2.13.

5.9.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy

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

Participants should not receive strong immune modulators including but not limited to systemic cyclosporine, tacrolimus, sirolimus, mycophenolate mofetil, methotrexate, azathioprine, rapamycin, thalidomide, lenalidomide, and targeted immune modulators such as abatacept (CTLA4Ig), adalimumab, alefacept, anakinra, belatacept (LEA29Y), efalizumab, etanercept, infliximab, or rituximab.

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 describes other medications which are prohibited in this trial. There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.10 Pembrolizumab Rescue Medications & Supportive Care

5.10.1 Supportive Care Guidelines

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outline along with the dose modification guidelines in Section 5.3.3 [Table 5]. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Table 5 in Section 5.3.3 for dose modification.

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

5.11 Diet/Activity/Other Considerations

5.11.1 Diet

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

5.11.2 Contraception

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

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

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

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

amenorrhea, a single FSH measurement is insufficient.);
OR

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

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

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

(1) practice abstinence† from heterosexual activity;

OR

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

Acceptable methods of contraception are‡:
Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female participant's male partner
- contraceptive rod implanted into the skin
- Combination method (requires use of two of the following):
  - diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
  - cervical cap with spermicide (nulliparous women only)
  - contraceptive sponge (nulliparous women only)
  - male condom or female condom (cannot be used together)
  - hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

  †Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the participant's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.
  ‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for participants participating at sites in this country/region.

Participants 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 participants of childbearing potential must adhere to the contraception requirement

(described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a participant of childbearing potential will not reliably comply with the requirements for contraception, that participant should not be entered into the study.

5.11.3 Use in Pregnancy

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab and trebananib, the participant will immediately be removed from the study. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor, Merck, and Amgen. This should be reported without delay within 24 hours to the Sponsor and within 2 working days to Merck and Amgen if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).
The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male participant impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor, Merck, and Amgen and followed as described above and in Section 6.2.

5.11.4 Use in Nursing Women

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

5.12 Duration of Therapy and Criteria for Taking a Participant Off Protocol Therapy/Participant Withdrawal

Discontinuation of study intervention does not represent withdrawal from the study.

As certain data on clinical events beyond study intervention discontinuation may be important to the study, they must be collected through the participant's last scheduled follow-up, even if the participant has discontinued study intervention. Therefore, all participants who discontinue study intervention prior to completion of the protocol-specified treatment period will still continue to be monitored in this study and participant in the study visits and procedures unless the participant has withdrawn from the study.

Participants may discontinue study intervention at any time for any reason or be discontinued from the study intervention at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study intervention by the investigator or the Sponsor if the study intervention is inappropriate, a study plan is violated, or for administrative and/or other reasons.

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for two years with pembrolizumab and three months with trebananib or until one of the following criteria applies:

- Disease progression
  Note: For unconfirmed radiographic disease progression, please see Section 11.1.6.
  Note: A participant may be granted an exception to continue on treatment despite radiographic disease progression if clinically stable, please see Section 11.1.6.
- Intercurrent illness that prevents further administration of treatment.
- Participant or their legal representative (such as a parent or legal guardian) decides to withdraw from the study.
- Investigator decides to withdraw the participant
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- The participant has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The participant is lost to follow up.
- The participant completes 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study pembrolizumab, whichever is later
- Administrative reasons
- Unacceptable adverse event(s), including:
  - Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
  - Grade 3 drug-related autoimmune or inflammatory event including uveitis, pneumonitis, diarrhea, colitis, neurologic adverse events, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
  - Any Grade 3 or 4 drug-related laboratory imbalance or electrolyte abnormality, not associated with underlying organ pathology and that do not require treatment except for electrolyte replacements do not require treatment discontinuation, with the following exceptions with approval of the Principal Investigator:
  - Hypophysitis or pan-hypopituitarism any grade should discontinue treatment.
  - Grade 4 amylase or lipase abnormalities that are not associated with DM, associated liver or gall bladder inflammation clinical manifestations of pancreatitis may stay on study.
  - Any drug-related liver function test (LFT) abnormality that meets the following criteria requires discontinuation: Grade 4 AST or ALT.

- Grade 3 drug-related thrombocytopenia >7 days or associated with bleeding requires discontinuation.
- For patients with skin-only toxicity, when symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Discontinue pembrolizumab if unable to reduce corticosteroid dose for irAEs to ≤10 mg. Pembrolizumab treatment may be restarted and the dose modified as specified in the protocol.
- Patients with endocrinopathy (e.g. thyroiditis, hypophysitis) and no other autoimmune/inflammatory event may be restarted after a short course of steroids and/or on a stable replacement regimen.

- Any dosing interruption lasting >12 weeks with the following exceptions:
  - Dosing interruptions >12 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a participant with a dosing interruption lasting >12 weeks, the Principal Investigator must be consulted.
  - Tumor assessments should continue as per protocol even if dosing is interrupted.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

An ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the DF/HCC website at http://www.dfhcc.harvard.edu/research/clinical-research-support/document-library-forms-sops-etc/.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, *F. Stephen Hodi, MD* by phone (617-632-5053) or by pager (44131).

5.13 End of Treatment Evaluation and Follow-Up

The End of Treatment and Follow-up visit procedures are listed in Section 9 (Protocol Flow Chart) and Section 11 (Visit Requirements). After the end of treatment, each participant will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 6).

Participants who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up.

5.14 Long-Term Follow-Up and Study Completion

Patients will be followed every 12 weeks after discontinuing study treatment.

Patients removed from study for unacceptable adverse event(s) will be followed until progression.

All patients will be followed for at least a year from the time of initiation of treatment or until death, whichever occurs first.

After documented disease progression each participant will be followed by telephone or medical record review for overall survival until death, withdrawal of consent, or the end of the study as detailed below.

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

A ODQ Off Study Form will be filled out when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

5.15 Discontinuation of Study Therapy after Stable Disease, PR, or CR

Participants who discontinue treatment may be eligible for up to one year of additional treatment with pembrolizumab when they experience radiographic recurrence of disease via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the participant meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Participants will resume treatment at the same dose and schedule of time of initial discontinuation.

5.16 Clinical Criteria for Early Trial Termination

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

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

2. Plans to modify the development of the study drug

3. If for any reason Merck or Amgen makes a decision to no longer supply study drug.

5.17 Participant Replacement Strategy

Additional participants may be enrolled to ensure that the required number of evaluable participants in each cohort is achieved. A participant that discontinues the trial for progressive disease or a drug-related AE will not be replaced and will be counted in the evaluable population of participants for the respective cohort.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

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

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

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

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

All adverse events that occur after the consent form is signed but before presenting for cycle 1 day 1 must be reported by the investigator ONLY if they cause the participant to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of cycle 1 day 1 through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 6.3 The investigator will make every attempt to follow all participants with non-serious adverse events for outcome.

Adverse events will not be collected for participants during the pre-screening period (for determination of archival tissue status) as long as that participant has not undergone any protocol-specified procedure or intervention.

6.1 Definition of an Overdose for Pembrolizumab and Reporting of Overdose to the Sponsor and to Merck

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

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

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

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

6.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck and Amgen

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) that occurs during the trial.

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

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

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220) and Amgen Global Safety (Attn: Amgen Global Safety - FAX is #:1-888-814-8653).

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

6.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck or Amgen products that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another important medical event

- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor, Merck and Amgen in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck and Amgen for collection purposes.
    - Is a new cancer (that is not a condition of the study);
    - Is associated with an overdose.

For the time period beginning when the consent form is signed until the participant presents for cycle 1 day 1, any serious adverse event that occurs to any participant must be reported within 24 hours to the IND holder and within 2 working days to Merck Global Safety and Amgen ONLY if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any participant from the time the participant presents for Cycle 1 Day 1 through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck or Amgen product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety and Amgen. Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the IND holder and to Merck Global Safety.

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

[redacted]

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

6.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

6.3.3 Expedited Reporting to the Food and Drug Administration (FDA)

The IND holder will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

6.3.4 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

6.3.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

6.3.6 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck or Amgen as described in Section 6.3.1- Immediate Reporting of Adverse Events to the IND holder and to Merck and Amgen, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the IND holder within 24 hours and to Merck Global Safety and Amgen Global Safety within 2 working days either by electronic or paper media.

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

The IND holder will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the participants in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety and Amgen

Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

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

6.3.7 Events of Clinical Interest for Pembrolizumab

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the IND holder and within 2 working days to Merck Global Safety. [redacted]

For the time period beginning when the consent form is signed until the participant presents for cycle 1 day 1, any ECI, or follow up to an ECI, that occurs to any participant must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety ONLY if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning when the participant presents for cycle 1 day 1 through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product., as defined in Section 6.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

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

6.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade

recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.
as defined in Section 6.3.1. In addition, attribution of AEs must be classified based on the following definitions:

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

Table 15. Adverse Events Evaluation

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

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 participant, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of participant taking the product regardless of time to diagnosis); or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the IND holder within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the participant was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

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

Safety assessments will be based on medical review of AE reports and the results of vital sign measurements, physical examinations and clinical laboratory tests. The incidence of AEs will be tabulated and reviewed for potential significance and clinical importance. The reporting period for safety data will be from the date of first on-study dose to 70 days after the last dose is received. Additionally, from the time of consent forward, any occurrence of a serious adverse event (SAE) must be reported to the IND holder. The DSMC at DFCI will have oversight of this study.

6.5 Sponsor Responsibility for Reporting Adverse Events

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

7. PHARMACEUTICAL INFORMATION

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

7.1 Product Descriptions

7.1.1 Description

7.1.1.1 Pembrolizumab Description

Pembrolizumab is a humanized anti-PD-1 mAb of the IgG4/kappa isotype with a stabilizing S228P sequence alteration in the fragment crystallizable (Fc) region. Pembrolizumab binds to human PD-1 and blocks the interaction between PD-1 and its ligands. The theoretical molecular weight of the polypeptide is 146,288 Da and its theoretical pI is 7.5. Additional information on pembrolizumab nomenclature is detailed in the following table:

Code Name	MK-3475 (Anti–PD-1)
Other Code Name	MK3, 02P106, ORG 307448-0, SCH 900475 (Anti–PD-1)
Chemical Name	Humanized X PD-1-mAb (H409A11) IgG4
CAS Number	1374853-91-4
CAS Name	Anti-(human protein PDCD1 (programmed cell death 1)) immunoglobulin G4 (human-Mus musculus monoclonal heavy chain) disulfide with human-Mus musculus monoclonal light chain, dimer
Trade Name	KEYTRUDA®

7.1.1.2 Trebananib Description

Trebananib is a novel Fc fusion protein directed against Ang1 and Ang2, expressed recombinantly in *Escherichia coli (E coli)*. The molecule is a non-glycosylated homodimer engineered by fusing an immunoglobulin G1 (IgG1) Fc domain to 4 copies of an anti-Ang1 / anti-Ang2 peptide. Each monomeric unit contains 10 cysteine residues that are involved in 4 intrachain disulfide bonds and 2 interchain disulfide bonds. Trebananib contains 287 amino acids. The molecular weight is approximately 63.5 kDa.

7.1.2 Form

7.1.2.1 Pembrolizumab Form

Two drug product (DP) dosage forms are available for pembrolizumab: a white to off-white lyophilized powder, 50 mg/vial, and a liquid, 100 mg/vial, both in Type I glass vials intended for single use only. The drug products are manufactured using facilities and practices under Good Manufacturing Practice (GMP) requirements.

Pembrolizumab Powder for Solution for Infusion, 50 mg/vial is a lyophilized powder that is reconstituted with sterile water for injection prior to use. It is manufactured using either the fully formulated DS or the partially formulated DS. The fully formulated DS uses L-histidine as a buffering agent, polysorbate 80 as surfactant, and sucrose as stabilizer/tonicity modifier. Pembrolizumab DP using the partially formulated DS is formulated with L-histidine as a buffering agent, polysorbate 80 as a surfactant, and sucrose as a stabilizer/tonicity modifier, and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (if necessary).

Pembrolizumab Solution for Infusion 100 mg/vial is a liquid DP (manufactured using the fully formulated DS with L-histidine as a buffering agent, polysorbate 80 as a surfactant, and sucrose as a stabilizer/tonicity modifier).

Pembrolizumab will be provided by Merck as summarized in the following table:

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

7.1.2.2 Trebananib Form

Trebananib is provided as a sterile, preservative-free, lyophilized powder for reconstitution with sterile water for injection (sWFI) and dilution in normal saline (0.9% sodium chloride) for IV administration. Each sterile vial contains specified amount of deliverable drug product, that when reconstituted with a specified volume of sWFI contains an isotonic formulation of 30 mg/mL trebananib formulated with 10 mM histidine, 4% (weight/volume [w/v]) mannitol, 2% (w/v) sucrose, 10 mM arginine hydrochloride, and 0.01% (w/v) polysorbate 20 to a pH of 7.1. Each vial is for single use only. Lyophilized vials are manufactured in 4 presentations based on the deliverable drug product. The vial presentations, vial sizes, deliverable amount, and reconstitution volume are provided in the table below.

Vial Presentation	Vial size	Deliverable Amount	Reconstitution Volume (mL sWFI)
54 mg	5 cc	54 mg	2.0
150 mg	20 cc	150 mg	5.0
240 mg	20 cc	240 mg	8.0
600 mg	50 cc	600 mg	20.0

cc = cubic centimeters; mg = milligram; sWFI = sterile water for injection

7.1.3 Packaging and Labeling Information

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

7.1.4 Clinical Supplies Disclosure

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

7.1.5 Storage, Handling, and Preparation Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than

that stated in the protocol.

7.1.6 Administration

7.1.6.1 Pembrolizumab Administration

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

7.1.6.2 Trebananib

Trebananib should be administered on Day 1, 8, and 15 of each 21-day cycle after all procedures/assessments have been completed. Trebananib may be administered up to 2 days before or after the scheduled Day of each cycle due to administrative reasons (±2 days).

The first dose of trebananib will be administered by IV infusion over a 60-minute period. If the initial dose administration is well tolerated, future administrations may be given over approximately 30 minutes (treatment cycle intervals may be increased due to toxicity a described in section 5). Trebananib will be administered immediately after Pembrolizumab.

7.1.7 Ordering

Investigative sites will order and acquire Pembrolizumab directly from Merck. Pembrolizumab will be supplied from an investigational supply.

Investigative sites will order and acquire trebananib directly from Amgen, Inc. Trebananib will be supplied from an investigational supply.

7.1.8 Accountability

The investigator, or a responsible party designated by the investigator, is responsible for keeping accurate records of the clinical supplies received from Merck and Amgen, the amount dispensed to and returned by the participants and the amount remaining at the conclusion of the trial agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form.

7.1.9 Destruction and Return

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

8. ADMINISTRATIVE AND REGULATORY DETAILS

8.1 Confidentiality

8.1.1 Confidentiality of Data

The investigator affirms to the Sponsor that information furnished to the investigator by the Sponsor will be maintained in confidence, and such information will be divulged to the institutional review board, ethics review committee (IRB/ERC) or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this trial will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

8.1.2 Confidentiality of Participant Records

By signing this protocol, the investigator agrees that the Sponsor (or Sponsor representative), IRB/ERC, or regulatory authority representatives may consult and/or copy trial documents in order to verify worksheet/case report form data. By signing the consent form, the participant agrees to this process. If trial documents will be photocopied during the process of verifying worksheet/case report form information, the participant will be identified by unique code only; full names/initials will be masked prior to transmission to the Sponsor. By signing this protocol, the investigator agrees to treat all participant data used and disclosed in connection with this trial in accordance with all applicable privacy laws, rules and regulations.

8.1.3 Confidentiality of Investigator Information

Information with respect to the investigator, and all sub-investigators and trial site personnel, may be used and disclosed for trial management purposes, as part of a regulatory submissions, and as required by law. This information may include:

- name, address, telephone number and e-mail address;
- hospital or clinic address and telephone number;
- curriculum vitae or other summary of qualifications and credentials; and
- other professional documentation.

Consistent with the purposes described above, this information may be transmitted to the Sponsor, and subsidiaries, affiliates and agents of the Sponsor, in your country and other countries, including countries that do not have laws protecting such information. Additionally, the investigator's name and business contact information may be included when reporting certain serious adverse events to regulatory authorities or to other investigators. By signing this protocol, the investigator expressly consents to these uses and disclosures. In order to facilitate contact between investigators, the Sponsor may share an investigator's name and contact information with other participating investigators upon request.

8.1.4 Confidentiality of IRB/IEC Information

The Sponsor is required to record the name and address of each IRB/IEC member that reviews and approves this trial. The Sponsor is also required to document that each IRB/IEC meets regulatory and ICH GCP requirements by requesting and maintaining records of the names and qualifications of the IRB/IEC members and to make these records available for regulatory agency review upon request by those agencies.

8.2 Compliance

8.2.1 Compliance with Financial Disclosure Requirements

Regulations, Financial Disclosure by Clinical Investigators (21 CFR Pa rt 54). It is the Sponsor's responsibility to determine, based on these regulations, whether a request for Financial Disclosure information is required. It is the investigator's/sub-investigator's responsibility to comply with any such request. The investigator/sub-investigator(s) agree, if requested by the Sponsor in accordance with 21 CFR Part 54, to provide his/her financial interests in and/or arrangements with the Sponsor to allow for the submission of complete and accurate certification and disclosure statements.

The investigator/sub-investigator(s) further agree to provide this information on a Certification/Disclosure Form, commonly known as a financial disclosure form, provided by the Sponsor or through a secure password-protected electronic portal provided by the Sponsor. The investigator/sub-investigator(s) also consent to the transmission of this information to the Sponsor in the United States for these purposes. This may involve the transmission of information to countries that do not have laws protecting personal data.

8.2.2 Compliance with Law, Audit, and Debarment

The investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of Good Clinical Practice (e.g., International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial. The investigator also agrees to allow monitoring, audits, IRB/ERC review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents. The investigator agrees not to seek reimbursement from participants, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the investigator by the Sponsor. The investigator shall prepare and maintain complete and accurate trial documentation in compliance with Good Clinical Practice standards and applicable federal, state and local laws, rules and regulations; and, for each participant participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Trial documentation will be promptly and fully disclosed to the Sponsor by the investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to cure deficiencies in the trial documentation and worksheets/case report forms. The investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for participant participation, adverse event reports, participant source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the investigator agrees that documentation shall be retained until at least 2 years after the last approval of a marketing application in an ICH region or until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. The Sponsor will determine the minimum retention period and notify the investigator when documents may be destroyed. The Sponsor will determine the minimum retention period and upon request, will provide guidance to the investigator when documents no longer need to be retained. The sponsor also recognizes that documents may need to be retained for a longer period if required by local regulatory requirements. All trial documents shall be made available if required by relevant regulatory authorities. The investigator must consult with and obtain written approval by the Sponsor prior to destroying trial and/or participant files. ICH Good Clinical Practice guidelines recommend that the investigator inform the participant's primary physician about the participant's participation in the trial if the participant has a primary physician and if the participant agrees to the primary physician being informed. The investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this trial. Persons debarred from conducting or working on clinical trials by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's trials. The investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the trial is debarred or if any proceeding for debarment is pending or, to the best of the investigator's knowledge, threatened. In the event the Sponsor prematurely terminates a particular trial site, the Sponsor will promptly notify that trial site's IRB/IEC.

8.2.3 Compliance with Trial Registration and Results Posting Requirements

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

8.3 Quality Management System

By signing this protocol, the Sponsor agrees to be responsible for implementing and maintaining a quality management system with written development procedures and functional area standard operating procedures (SOPs) to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of Good Clinical Practice, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical trial.

8.4 Data Management

The investigator or qualified designee is responsible for recording and verifying the accuracy of participant data. By signing this protocol, the investigator acknowledges that his/her electronic signature is the legally binding equivalent of a written signature. By entering his/her electronic signature, the investigator confirms that all recorded data have been verified as accurate. Detailed information regarding Data Management procedures for this protocol will be provided separately.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 28 days prior to start of registration except where noted otherwise. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 2 days of the protocol-specified date, unless otherwise noted.

Screening and Induction

Trial Period:	**Induction**						
Treatment Cycle/Title:	**Screening Visit**	**Cycle 1** [J]			**Cycles 2, 3, and 4** [J]		
Scheduling Window (Days):	-28 to -1	1± 2	8± 2	15± 2	1± 2	8± 2	15± 2
Informed Consent	x						
Inclusion/Exclusion Criteria	x						
Demographics and Medical History	x						
Prior and Concomitant Medication Review	x	x	x	x	x	x	x
Pembrolizumab Administration		x			x		
Trebananib Administration (induction phase only for 12 weeks)[I]		x	x	x	x	x	x

Review Adverse Events		x	x	x	x	x	x
Physical Examination	x	x	x	x	x	x	x
Vital Signs and Weight	x	x	x	x	x	x	x
ECOG Performance Status	x	x	x	x	x	x	x
Single EKG	x						
HIV, Hepatitis C Antibody, Hepatitis B Surface Antigen	x						
Pregnancy Test – Serum β-HCG[A]	x	x	x	x	x	x	x
PT/INR and aPTT	x	x	x	x	x	x	x
CBC with Differential	x	x	x	x	x	x	x
Comprehensive Serum Chemistry Panel, lipase and amylase	x	x	x	x	x	x	x
Tumor markers: CEA and CA125[H]		x			x		
Urinalysis [B]	x	x			x		
T3, FT4 and TSH	x	x	x	x	x	x	x
Chest, Abdomen and Pelvic CT scan[C]	X[E]				x		
Brain MRI[D]	X[E]				x		
Archival or Newly Obtained Tissue Collection[F]	x	X					
Correlative Studies Blood Collection[G]		x	x	x			

Post-Induction

Trial Period:	**Post Induction**
Treatment Cycle/Title:	Cycles 5 and beyond for up to 2 years total
Scheduling Window (Days):	Day 1 (± 2)
Prior and Concomitant Medication Review	x
Pembrolizumab Administration	x
Review Adverse Events	x
Physical Examination	x
Vital Signs and Weight	x

ECOG Performance Status	x
Pregnancy Test – Urine[A]	x
PT/INR and aPTT	x
CBC with Differential	x
Comprehensive Serum Chemistry Panel, lipase and amylase	x
Tumor markers: CEA, CA125H	x
Urinalysis [B]	x
T3, FT4 and TSH	x
Chest, Abdomen and Pelvic CT scan[C]	x
Brain MRI[D]	x
Correlative Studies Blood Collection[G]	x

Post Treatment

	Post-Treatment			
Treatment Cycle/Title:	**End of Treatment Visit**	**Safety Follow-up**	**Follow Up Visits**	**Survival Follow-Up**
Scheduling Window (Days):	At time of Discontinuation	30 days post discontinuation (± 3)	Every 12 weeks post discontinuation (± 7) for at least a year	Every 12 weeks (± 7)
Prior and Concomitant Medication Review	x	x		
Post-study anticancer therapy status			x	X
Survival Status				x
Review Adverse Events	x	x	x	
Physical Examination	x	x	x	
Vital Signs and Weight	x	x	x	
ECOG Performance Status	x	x	x	
CBC with Differential	x	x		

Comprehensive Serum Chemistry Panel, lipase and amylase	x	x		
Urinalysis [B]		x		
T3, FT4 and TSH		x		
Chest, Abdomen and Pelvic CT scan[C]	x		x	
Brain MRI[D]	x		x	
Archival or Newly Obtained Tissue Collection[G]	x			
Correlative Studies Blood Collection[G]	x			

[A] For women of child-bearing potential, a negative serum β-HCG pregnancy test must be performed during screening. A urine pregnancy test should be performed within 24 hours of starting treatment. A urine pregnancy test may be performed at all other timepoints. If a urine pregnancy test result is positive, a serum pregnancy test must be completed and resulted as negative for a participant to receive treatment.

[B] Proteinuria will be monitored by urine protein: creatinine ratio at least every six weeks. If appropriate, 24-hour urine collection for protein may be obtained.

[C] CT scan of the chest, abdomen, and pelvis will be performed every 12 weeks (± 7 days)

[D] Brain MRI to be performed only on melanoma and RCC patients. Brain MRI should be performed during screening. Repeat brain MRI should be performed if there are any neurological symptoms or findings. Brain MRI with and without contrast is preferred. If a patient is not able to obtain an MRI, CT imaging with contrast is acceptable. If a patient is not able to receive IV contrast, CT head without contrast is acceptable.

[E] Imaging for baseline tumor assessment is to be performed within 28 days prior to the start of study registration.

[F]Biopsy of site(s) of pre-existing disease will be performed up to 6 weeks (42 days) prior to initiation of treatment on Day 1. On-treatment biopsies should be performed approximately midway through the Induction period, approximately 6 weeks from the start of study treatment (sometime between Cycle 2 Day 8 – Cycle 3 Day 1). If for any reason removal of tissue requires a greater procedure, the biopsy must be done >28 days from the last planned dose trebananib

[G] Serial blood/serum samples to be collected prior to the administration of pembrolizumab at the following time points: Cycle 1 Days 1, 8, and 15; Prior to Day 1 of every odd cycle (Cycle 3, Cycle 5, Cycle 7, etc.); End of treatment visit.

[H]Tumor markers: CEA to be done on participants with colorectal cancer on day 1 of every cycle. CA125 to be done on participants with ovarian cancer on day 1 of every cycle.

[I]If trebananib is discontinued due to an AE, participants may omit any Day 8 and Day 15 visits/assessments going forward.

10. TRIAL PROCEDURES AND ASSESSMENTS

The Study Calendar (Section 9) summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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

10.1 Administrative Procedures

10.1.1 Informed Consent

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

10.1.2 General Informed Consent

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

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

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

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

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

10.1.3 Inclusion/Exclusion Criteria

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

10.1.4 Medical History

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

10.2 Prior and Concomitant Medications Review

10.2.1 Prior Medications

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

10.2.2 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 6.

10.3 Disease Details and Treatments

10.3.1 Disease Details

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

10.3.1.1 Prior Treatment Details

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

10.3.1.2 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a participant initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the participant will move into survival follow-up.

10.3.1.3 Assignment of Screening Number

All consented participants will be given a unique screening number that will be used to identify the participant for all procedures that occur prior to randomization or treatment allocation. Screening numbers will be assigned and maintained by the clinical research coordinator at the lead site. Each participant will be assigned only one screening number. Screening numbers must not be re-used for different participants. Any participant who is screened multiple times will retain the original screening number assigned at the initial screening visit.

10.3.1.4 Trial Compliance (Medications)

Interruptions from the protocol specified treatment plan for 12 weeks between doses due to toxicity require consultation between the investigator and the Sponsor and written documentation of the collaborative decision on participant management. Administration of trial medications will be witnessed by the investigator and/or trial staff. The total volume of trial treatments infused will be compared to the total volume prepared to determine compliance with each dose administered. The instructions for preparing and administering pembrolizumab and trebananib are provided in the Investigational Product Instruction Manual.

10.3.2 Clinical Procedures/Assessments

10.3.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each participant to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 6 for detailed information regarding the assessment and recording of AEs.

10.3.2.2 Physical Exam

The investigator or qualified designee will perform a physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A physical exam should be performed during screening, prior to the administration of each dose of trial treatment and as specified in the Trial Flow Chart (Section 9).

10.3.2.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 9). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

10.3.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

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

10.3.2.5 Laboratory Tests for Hematology, Chemistry, Urinalysis, and other laboratory tests

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 14

Pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing with the exception of the urine pregnancy test for women of child bearing potential (which must be performed within 24 hours prior to dosing). Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

Table 14. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test	Thyroid stimulating hormone (TSH)
	Uric Acid	Creatinine	
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		CEA for colorectal participants
	Potassium		CA125 for ovarian participants
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		
	Lipase		
	Amylase		

10.4 Other Procedures

10.4.1 Screening

The informed consent form must be signed prior to completing any protocol-specified procedure. Within 28 days prior to registration (except where noted below), potential participants will be evaluated to determine if they fulfill the entry requirements as set forth in Section 5.1. Screening procedures may be repeated after consultation with the Sponsor. After providing consent, participants will be assigned a screening number. Results of a test performed prior to the participant signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame. Exceptions to the 28-day screening window include the following assessments/tests:

- For women of reproductive potential, a urine pregnancy test will be performed within 24 hours prior to the first dose of trial treatment. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required (performed by the local study site laboratory).

Participants may be rescreened after initially failing to meet the inclusion/exclusion criteria. Results from assessments performed during the initial screening period are acceptable in lieu of a repeat screening test if performed within the specified time frame and the inclusion/exclusion criteria is met.

10.4.2 Withdrawal/Discontinuation

When a participant discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 6 – Adverse Events: List and Reporting Requirements. Participants who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 10.5.4. After discontinuing treatment following assessment of CR, these participants should return to the site for a Safety Follow-up Visit (described in Section 10.5.1) and then proceed to the Follow-Up Period of the study (described in Section 10.5.2).

10.5 Post Treatment Visits

10.5.1 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. Participants will be followed until the beginning of a new anti-neoplastic therapy. 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. Participants who are eligible for retreatment with pembrolizumab (as described in Section 10.5.4) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

10.5.2 Follow-Up Visits

Participants who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 12 weeks (84± 7 days) by radiologic imaging to monitor disease status for at least 1 year. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the participant begins retreatment with pembrolizumab as detailed in Section 10.5.4. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Participants who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 11.3.4 will move from the follow-up phase to the Second Course Phase when they experience disease progression. Details are provided in Section 9 – Trial Flow Chart for Retreatment.

10.5.3 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 (84± 7 days) to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

10.5.4 Second Course Phase (Retreatment Period)

Participants who stop pembrolizumab and trebananib with SD or better may be eligible for up to an additional 17 cycles (approximately 1 year) of additional pembrolizumab treatment if they progress after stopping study treatment from the initial treatment phase. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the participant meets the following conditions:

Either

- Stopped initial treatment with pembrolizumab and trebananib after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
    - Was treated with at least 8 cycles of study treatment before discontinuing therapy and
    - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

- Had SD, PR or CR and stopped pembrolizumab and trebananib treatment after completion of 35 administrations (approximately 2 years) of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression by RECIST 1.1 after stopping their initial treatment with pembrolizumab and trebananib and
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab and trebananib
- Meets all of the safety parameters listed in the inclusion criteria and none of the safety parameters listed in the exclusion criteria
- The study is ongoing

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

An objective response or disease progression that occurs during the Second Course Phase for a participant will not be counted as an event for the primary analysis of either endpoint in this study.

Participants who have experienced an initial disease progression by RECIST 1.1 and have an iSD, iPR, or iCR per iRECIST after completion of 35 administrations of study intervention for reasons other than disease progression or intolerability may be considered for Second Course Phase after consultation with the Sponsor.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

Although the clinical benefit of these drugs has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 12 weeks (± 7 days). In addition to a baseline scan, confirmatory scans will also be obtained 4-6 weeks following initial documentation of an objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)

[*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes. 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.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic 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.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable

lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. 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.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in 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. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice

thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

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 RECIST 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 data 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 from 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. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign

this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Treatment Beyond Progression

Immunotherapeutic agents such as Pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

If radiologic imaging shows progressive disease (PD), tumor assessment may be repeated by the site approximately 4 weeks later in order to confirm continued PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction or stabilization in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms continued PD, patients will be discontinued from study therapy. If reimaging is no worse than the prior scan 4 weeks prior, patients may continue therapy and be reimaged in another 8 weeks. If evidence for continued, increasing progression with subsequent imaging, the patient will be discontinued. In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions. The decision to continue study treatment after the first evidence of disease progression determined by radiologic imaging is at the Investigator's discretion based on the clinical status of the patient as described in the table below.
Patients may receive study treatment while waiting for confirmation of continued PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

Table 7. Treatment Beyond Progression

	Clinically Stable		**Clinically Unstable**	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of PD	Repeat imaging at approximately 4 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at approximately 4 weeks to confirm PD if possible	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment	No additional imaging required	N/A
Repeat scan shows SD, PR, or CR	Continue regularly scheduled imaging assessments every 12 weeks	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 12 weeks	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion
Tumor imaging will be assessed every 12 weeks (84 ±7 days).				

11.1.7 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.2 Antitumor Effect

11.2.1 Anti-Tumor Effect Using irRECIST

Definition of Tumor Response Using Immune-Related Response Criteria (irRC)

The sum of the longest diameter of lesions (SPD) at tumor assessment using the immune-related response criteria (irRC) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Target Lesions Response Using irRC
irComplete Response (irCR): Complete disappearance of all target lesions. This category encompasses exactly the same participants as "CR" by the mWHO criteria.
irPartial Response (irPR): Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all target and all new measurable lesions (i.e., Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by $\geq$ 25% when compared to SPD at nadir.
irStable Disease (irSD): Does not meet criteria for irCR or irPR, in the absence of progressive disease.
irProgressive Disease (irPD): At least 25% increase Percentage Change in Tumor Burden (i.e., taking SPD of all target lesions and any new lesions) when compared to SPD at nadir.
Definition of Non-Target Lesions Response Using irRC
irComplete Response (irCR): Complete disappearance of all non-target lesions. This category encompasses exactly the same participants as "CR" by the mWHO criteria.
irPartial Response (irPR) or irStable Disease (irSD): non-target lesion(s) are not considered in the definition of PR; these terms do not apply.
irProgressive Disease (irPD): Increases in number or size of non-target lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the target lesions increases by the required amount).

Impact of New Lesions on irRC
New lesions in and by themselves do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any participant from the study.

Definition of Overall Response Using irRC
Overall response using irRC will be based on these criteria:
Immune-Related Complete Response (irCR): Complete disappearance of all tumor lesions (target and non-target together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.
Immune-Related Partial Response (irPR): The sum of the products of the two largest perpendicular diameters of all target lesions is measured and captured as the SPD baseline. At

each subsequent tumor assessment, the SPD of the two largest perpendicular diameters of all target lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).
Immune-Related Stable Disease (irSD): irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
Immune-Related Progressive Disease (irPD): It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
At least 25% increase in the SPD of all target lesions over baseline SPD calculated for the target lesions.
At least a 25% increase in the SPD of all target lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the target lesions. (Hodi, Hoos et al. 2008) (Wolchok et al., 2009)

Table 10. Immune-Related Response Criteria Definitions

Target Lesion Definition	Non-Target Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Percent change in tumor burden (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	–100%	irCR
Partial Response	Any	Any	Any	≥ –50%	irPR
				<–50% to <+25%	irSD
				>+25%	irPD
Stable Disease	Any	Any	Any	<–50% to <+25%	irSD
				>+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

Immune-Related Best Overall Response Using irRC (irBOR)
irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual participant in the study. For the assessment of irBOR, all available assessments per participant are considered.
irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

12. TUMOR TISSUE COLLECTION AND CORRELATIVE STUDIES BLOOD SAMPLING

Correlative sciences will include fresh biopsies of pre-existing sites of disease and following treatment to assess histologically for vasculopathy, immune infiltration, and tumor necrosis; stain pathologic specimens for VEGF/VEGFR expression, phosphoTie2; monitor circulating levels of and development of anti-trebananib antibodies as a function of treatment. Baseline and on-treatment values of a number of inflammatory and angiogenic cytokines will be monitored. Pilot studies will include the investigation of immune responses to other angiogenic molecules as a function of treatment. Flow cytometry of PBMC will be monitored for changes in levels of naïve and memory CD4, CD8 and other lymphocyte populations. Cellular and humoral immune responses to established antigens as a function of treatment will be performed. These will include melanosomal differentiation antigens as well as novel tumor associated antigen targets, Muc-1, CEA, CA-125, and NY-Eso-1 as examples.

12.1 Fresh tumor biopsies:

Biopsies of fresh tumor will be obtained prior to treatment initiation on day 1 and approximately halfway through the induction period (between cycle 2 day 8 and cycle 3 day 1). Dedicated funds are currently available at our institution for obtaining post-treatment biopsies in patients receiving immune based therapies.

Formalin fixed-paraffin embedded (FFPE) tumor slices will be prepared and H&E stained for assessment of TIL in pre- and post-treatment tumor samples. To identify different immune cell populations (effector/memory/ CD8 cells, T regulatory cells, dendritic cells, tumor associated macrophages, NK cells, TEM) immunohistochemical staining will be performed on FFPE tumor slices using the following antibodies:

> CD3, CD4, CD8, CD25, FoxP3, Indoleamine 2,3 deoxygenase-1 (IDO), CD11c, CD83, CD86, CD56, CD14, CD16, PD-L1, and Tie-2.

We have recently developed immunohistochemical staining on paraffin embedded tissues for PD-L1, PD-L2, TIM-3 and LAG-3 through our Center for Immuno-oncology Pathology Core [redacted] PD-L1 IHC has recently been established in a CLIA approved laboratory and we are working to finalize the remaining assays for CLIA laboratory conduct. We may also send tissue out to an outside laboratory for PD-L1 testing.

[redacted]

1. Chen BJ et al., Clin Cancer Res. 2013 Jul 1;19(13):3462-73, PMID:23674495

2. Shi M et al., Am J Surg Pathol. 2014, Jul 14, PMID: 25025450.

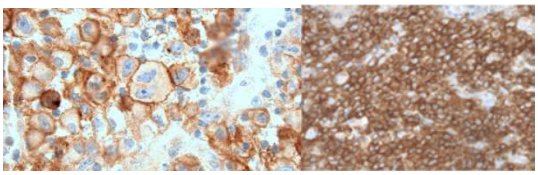


Figure 25. Control Hodgkin lymphoma stained for PDL1 (left) and mediastinal large B-cell lymphoma stained for PDL2 (right).

As part of the validation of the assays in a CLIA-certified laboratory, identical cases were stained multiple times and under a variety of staining conditions and the results reviewed by two certified pathologists. A positive control sample (classical Hodgkin lymphoma for PD-L1 expression; primary mediastinal large B-cell lymphoma for PD-L2 expression) and negative control sample (benign lymph node) is stained with each experimental tissue biopsy sample. The controls are reviewed by a certified pathologist at the time of review of the experimental sample.

An IHC assay for PD-1 (CD279, clone NAT105, Cell Marque Inc.) expression has been in standard surgical pathology diagnostic practice for several years and used to confirm the diagnosis of angioimmunoblastic T-cell lymphoma (AITL).

3. Yu H et al., Am J Clin Pathol. 2009, Jan;131(1):33-41. PMID:19095563.

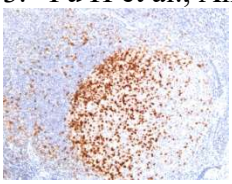


Figure 26. Control tonsil tissue stained for PD1.

PD-1 IHC is performed routinely in the CLIA-certified laboratory and interpreted by a certified pathologist with an appropriate control (reactive lymph node, intra-follicular T-cells are positive for PD-1) as described above.
Manuscript #1 above describes a semi-quantitative scoring method, which is in accordance with typical biomarker scoring in anatomic and surgical pathology. Briefly, staining is scored according to intensity (0= no staining, 1= weak staining, 2= moderate staining, 3= strong staining), staining pattern (M= predominantly cell membrane; C= predominantly cell cytoplasm), and the percentage of cells showing positive staining (0-100%). The semi-quantitative scoring is performed for: 1) the neoplastic tumor cells and 2) the non-neoplastic infiltrating immune cells. In the research setting, all cases are reviewed by two pathologists and any discordant results resolved by consensus review. Significantly discordant scoring results have been rare during case evaluations (Chen BJ et al., 2013).

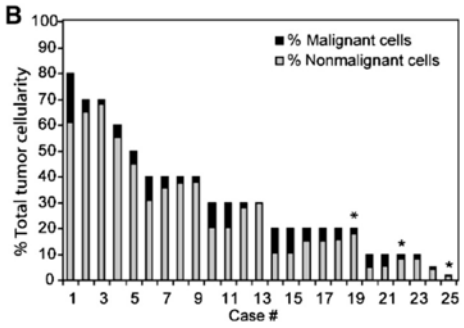


Figure 27. Semi-quantitative assessment of malignant and non-malignant cell expression of PDL1 in Hodgkin lymphoma, from Chen BJ et al.

Digital, quantitative scoring of stained tissue is performed using the Aperio slide scanning and analysis platform. Quantitative assessment of positive staining uses the commercially provided algorithm for cell identification and positive pixels counting within a pre-defined DAB (brown, chromogenic) channel. We have shown that this method of analysis shows good correlation with pathologists' scoring:

4. Mino-Kenudson M et al., Clin Cancer Res. 2010 Mar 1;16(5):1561-71. PMID:20179225.

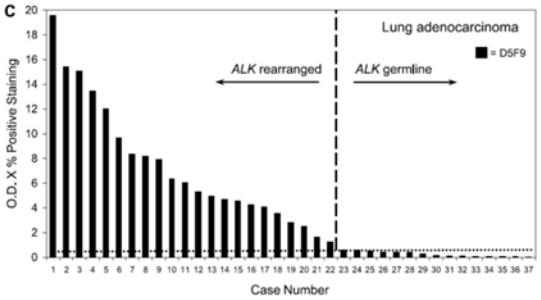


Figure 28. Aperio-base quantitative assessment of ALK protein expression in lung cancers, from Mino-Kenudson et al.

We have also used this method to score PD-L1 expression in tumor cells:

5. Green MR et al., Blood. 2010 Oct 28;116(17):3268-77. PMID: 20628145.

The correlation between quantitative IHC as determined by Aperio analysis, and semi-quantitative scoring, as determined by visual interpretation, will be determined as part of this study.

The scoring for markers (such as the PD-Ligands) that stain macrophages, dendritic cells, and other cells of heterogeneous morphology will be semi-quantitative and performed by a pathologist using a modified H-score to capture 1) the percentage of neoplastic cells positive for biomarker expression, intensity of expression, and membrane or cytoplasmic expression, and 2) the percentage of non-neoplastic cells (macrophages, dendritic cells, endothelial cells) positive for biomarker expression, intensity of expression, and membrane or cytoplasmic expression.

Scoring for PD-1 and other markers that stain lymphoid cells (CD3, CD4, CD8, CD25, FOXP3,

IDO, CD16, CD56, LAG-3, TIM-3) will primarily be performed by automated analysis using the Aperio system.

Aperio scoring for PD-1+ (and other lymphoid markers) lymphocytes will be accomplished using a standard Aperio algorithm, developed for quantifying nuclear stains, but that we have found is applicable to quantifying membrane staining of cells with a very high N:C ratio- such as lymphocytes (Nuclear algorithm). The output is number of positive-staining cells per unit area (microns2).

The data derived from the analyses above will be used as individual data points compared to other clinical (response to treatment) and pathological (histomorphological) data in the study. A goal will be to determine whether individual data points (i.e. number of PD-1+ T-cells/ unit area) are of prognostic value, or if combined data using two or more data (an "immuno-score") provides prognostic data. These investigations are exploratory and will be performed in conjunction with the biostatisticians associated with this study.

For image analysis:

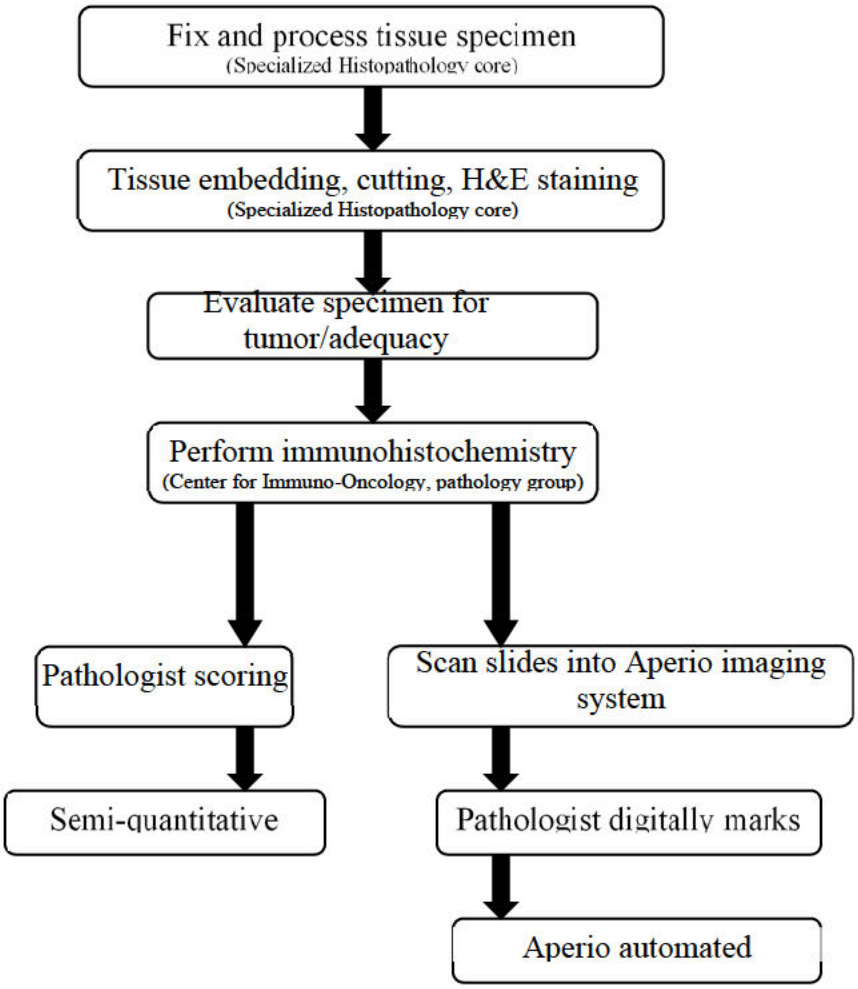
1. IHC stained slides will be digitally scanned using the Aperio ScanScope XT ([redacted]

[redacted]

3. Quantitative analysis is performed using analytical software associated with ImageScope, specifically Aperio Color Deconvolution V.9 (for PD-Ligands) and nuclear algorithm (for PD-1+ lymphocytes) and the results given as the percentage of positive pixels per unit area (for PD-Ligands) or number of positive cells per unit area (for PD-1+ lymphocytes). Intensity of staining is also captured automatically using the above algorithms and assigned a score (0, 1, 2, or 3) based upon the average optical density of the region or cells. All results are exported into an excel spreadsheet.

4. Individual scoring data will be compared to clinical parameters to determine if there is an association with outcome. Scores using a combination of biomarker data will also be considered.

Below, we provide a schematic of the workflow for the tissue-based biomarker analysis:



The list of antibodies to be used for correlative studies is included in table 11.

Table 11. Included is the prioritization of the markers (1= highest, 2= intermediate, 3= lowest) and the antibody clones, source of antibody, and status of validation.

IHC Biomarkers	Priority	Clone/ Cat #	Source	Host species	Dilution	Optimized?
PD-L1	1	9A11	G. Freeman	Mouse	1/125	Yes
PD-L2	1	9E 6	G. Freeman	Mouse	1/10000	Yes
PD-1	1	EH33	G. Freeman	Mouse	1/600	Yes
CD3	1	IS503	Dako	Rabbit	1/250	Yes
CD4	1	4B12	Vector Labs	Mouse	1/200	Yes
CD8	1	144B	Dako	Mouse	1/100	Yes
FOXP3	1	206D	BioLegend	Mouse	1:50	Yes
TIM3	1	AF2365	R&D Systems	Goat	1:50	Yes
LAG3	1	17B4	LifeSpan BioSc	Mouse	1/200	Yes
Tie2	2	AF313	R&D Systems	Goat	1/500	Yes
ANGPT2	2	sc-74403	Santa Cruz Bio	Mouse	1/200	Yes
IDO1	2	ab55305	Abcam	Mouse	1/100	No
CD38	3	SPC32	Abcam	Mouse	1/300	Yes
CD56	3	123C3	Dako	Mouse	1/100	Yes

CD14	3	ab49755	Abcam	Mouse	1/100	Yes
CD16	3	ab183354	Abcam	Rabbit	1/100	No
CD11c	3	EP1347Y	Abcam	Rabbit	1/500	Yes

The cut-off of 5% for PD-L1 tumor positivity is in accordance the criteria used in a prior study examining the use of this biomarker to predict clinical response in patients treated with a PD-1 antibody (Topalian SL et al., NEJM, 2012, PMID: 22658127).

The semi-quantitative scoring for this study is in accordance with those published previously (Topalian et al., 2012, Chen BJ et al., 2013) and, as described above, will include scores for both the neoplastic and non-neoplastic cells within the tumor microenvironment. Data derived from pathologist visual scoring (semi-quantitative, but with increased specificity for delineating neoplastic and non-neoplastic cells) and pathologist-assisted, automated scoring (quantitative, but without accurately delineating neoplastic and non-neoplastic cells) for each marker of interest will be assessed for its clinical value. As necessary, the data from combinations of makers will also be considered (i.e. combined scores from PD-L1 and PD-L2 expression). All data will be analyzed in conjunction with the biostatistics group.

12.2 Peripheral blood:

Serial blood/serum samples will be collected prior to each odd cycle (Cycle 1, Cycle 3, Cycle 5, etc.) prior to pembrolizumab administration starting on day 1 (pre-treatment) and at the end of the treatment. A panel of cytokines and chemokines will be tested in serum using Luminex cytokine assay. Changes in cytokine production in immune cell subsets as a function of treatment will be determined by ELISA and intracellular cytokine staining. Absolute lymphocyte count (ALC) will be monitored.

Peripheral blood mononuclear cells (PBMCs) will be collected from whole blood to assess immune cell populations. Surface staining with a panel of antibodies (CD3, CD4, CD8, CD19, CD25, FoxP3, CD11c, CD83, CD86, CD56) and intracytoplasmatic cytokine staining, followed by flow cytometry will be performed in order to identify different T cell populations, their activation status, myeloid-derived suppressor cells (CD11b, CD14, CD19, CD33, HLA-DR) and the production of different cytokines as well as other immune cell population as described in table 12.

Table 12. Cytokines and Immune Cells Subsets

		FITC	PE	ECD	PE-Cy5	PE-Cy7
1	Treg (1); intracellular	FoxP3	CTLA-4	CD3	CD25	CD4
2	T, B, monocyte, NK,NKT	CD19	CD56	CD14	CD45	CD3
3	T cell subset	CD8	TCRγδ	CD4	TCRαβ	CD3
4	CD4 T cell naïve memory	CCR7	CD57	CD45RO	CD28	CD4
5	CD8 T cell naïve memory	CCR7	CD57	CD45RO	CD28	CD8
6	Myeloid DC	Lineage (CD3,CD14, CD16,CD19,CD56)	CD86	HLA-DR	CD11c	CD45
7	Plasmacytoid DC	Lineage (CD3,CD14, CD16,CD19,CD56)	CD86	HLA-DR	CD45	CD123
8	PD1-ICOS	ICOS	PD-1	CD3	CD8	CD4
9	41BB-OX40	CD3	4-1BB	CD8	OX-40	CD4
10	CD127 Treg	CD3	CD127	CD25	CD27	CD4
11	NK,NKT	CD16	NKG2D	CD3	CD56	CD8
12	BDCA.DC	BDCA-2	BDCA-1	CD14,CD19	BDCA-3	CD45

Given the results from our study of ipilimumab plus bevacizumab, we will specifically analyze changes as a function of treatments for $CCR7^{+/-}/CD45RO^{+}$ cell populations for both the $CD4^{+}$ and $CD8^{+}$ compartments.

Serologic changes to antibody responses will be assessed for tumor antigens NY-ESO-1, MART-1, MUC-1, and MAGE-3 when appropriate.

Table 13. Biomarkers

Biomarker name	Assay	Tissue/Body Fluid Tested
CD274 (PD-L1)	Immunohistochemistry	Tissue
CD273 (PD-1)	Immunohistochemistry	Tissue
CD279 (PD-1)	Immunohistochemistry	Tissue
ANGPT2	Immunohistochemistry	Tissue
Circulating endothelial cells (CEC) and progenitor cells (CPC)	Surface staining and FACS	Blood
Circulating cytokines/Chemokines (as indicated in figure 17)	Luminex assay, ELISA	Blood
Circulating angiogenic factors (VEGF, Ang-2, PLGF, PDGF, HGF, FGF, BMP-9, endothelin, endoglin, leptin)	Luminex assay	Blood
Humoral response to angiogenic factors	ELISA and Western blot	Blood
Humoral response to	ELISA and Western blot	Blood

immunosuppressive molecules		
Expression of angiogenic factors and immunosupresive molecules	IHC	Tumor tissues
Circulating Tie-2 expressing Monocytes (TEM)	Surface staining and FACS	Blood
Tumor infiltrating TEM	IHC and/or Surface staining and FACS	Tumor tissues

Antigen-specific T cell responses are controlled by co-stimulatory and co-inhibitory molecules positively and negatively. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1, CD279) are among the key co-inhibitory molecules, broadly categorized as "checkpoint molecules" (Pardoll DM, 2012). CD279 is up-regulated on activated T lymphocytes and mediate immunosuppression when binding to its ligands B7-H1 (CD274) and B7-DC (CD273). Blockade of CD279 or CD274 induced durable objective response in patients with advanced melanoma, renal cell carcinoma and non-small cell lung cancers in clinical trials (Topalian S, 2012; Brahmer J, 2012; Hamid O, 2013). Moreover, immunohistochemical staining performed on pretreatment tumor tissues from patients with anti-CD279 treatment showed that none of 17 patients with CD274 negative tumors had an objective response whereas 36% (9/25) patients with CD274 positive tumors had an objective response (P=0.006). This striking difference suggests that CD274 expression on tumor specimen might be a useful biomarker to predict response to anti- CD279 treatment.

Anti-vascular endothelial growth factor pathway therapies preferentially target immature tumor blood vessels and leave behind normalized and resistant blood vessels. Angiopoietin-2 (ANGPT2)/TIE pathway is largely confined to vasculature. It has two receptors TIE1 and TIE2 as well as three ligands, angiopoietin-1, angiopoietin-2 (ANGPT2) and angiopoietin-4. ANGPT2 plays an important role in vascular remodeling and angiogenesis. It acts as context-specific antagonist of angiopoietin-1/TIE2 signaling, destabilizes the quiescent blood vessels as a prerequisite to sprouting angiogenesis in the presence of proangiogenic stimulation or vascular regression in the absence of such stimuli. Therapeutics targeting the ANGPT2/TIE pathways including selective anti-ANGPT2 antibodies is in development (Gerald D, 2013; Karlan BY, 2012; Hashizume H, 2010). Serum ANGPT2 is found to be a biomarker for tumor progression and survival in various malignancies (Helfrich I, 2009).

<u>Peripheral Blood Mononuclear Cell isolation and staining procedure for Flow Cytometry</u>
Peripheral Blood Mononuclear Cells (PBMCs) will be isolated from 25 ml of blood by Ficoll centrifugation. Aliquots of 5-10 million cells will be immediately frozen with 500ul of Fetal Bovine Serum (FBS) containing 15% Dimethyl Sulfoxide (DMSO). The cryovials containing cells will be placed in special freezing containers at -80C overnight. After which, cryovials will be transferred to liquid nitrogen. After a minimum of 48 hours in liquid nitrogen storage, cells will be thawed in a 37C water bath. A single vial will be resuspended in approximately 10ml of warm RPMI with 10% FBS. Cells will be centrifuged at 1800 RPM for 5 minutes, and supernatant will be aspirated. The cell pellet will then be resuspended in 2ml of ice cold Phosphate Buffered Saline (PBS) containing 2.5% FBS (staining solution) and human anti-CD16/CD32 blocking antibodies and incubated on ice. After 15 minutes, cells will be aliquoted

into a V-bottom 96 well plate (approx. 500,000 cells/100ul/well) and incubated with specific antibodies at manufacturer's recommended concentrations for 45 minutes in the dark. In the case of intracellular markers (e.g. FoxP3) plates will be incubated with antibodies targeting membrane markers, fixed with 1% Formaldehyde and treated with a cellular permeabilization reagent (e.g., saponin) prior to the addition of the intracellular protein targeting antibodies. Cells will be spun down at 1800 RPM at 4C for 5 minutes, and washed twice with cold staining solution. After washing, plates will be incubated on ice in the dark with 3uM of DAPI for 10 minutes. Cells will be washed once more with staining solution and then finally resuspended in 150ul of staining solution.

Antibody panels for Flow Cytometry
The following immune cell populations will be detected in PBMCs using specific marker antibodies and flow cytometry gating strategies: Regulatory T cells (CD4+/CD25+/FoxP3+), Effector T cells (CD4+/CD69+), Naïve T cells (CD4+/CD69-), Memory T cells (CCR7+/CD45RO+), CD8 Cytotoxic cells (CD8+/CD3+), Plasmocytoid Dendritic cells (CD123+/CD303+), Myeloid Dendritic cells (CD11c+/CD141+), Natural Killer cells (CD3-/CD56+), Natural Killer T cells (a/bTCR+/NKG2D+), Classic Monocytes (CD14+), and Monocytic Myeloid-Derived Suppressor cells (CD14+/HLADR-).

Detection of soluble biomarkers
Plasma from heparin treated blood will be collected, aliquoted and stored at -80C. Via Cytokine/Chemokine Multiplex assays, concentration levels will be assessed for up to 50 biomarkers including (but not limited to) the following inflammatory mediators: IL-6, IFNg, TNFa, IL-10, IP-10, IL-1b, CXCL16, VEGF, and Ang1. The assays will be performed following manufacturer Standard Operating Procedures for each biomarker group panel.

12.3 Integrated Correlative Studies

Immunohistochemical Staining for CD274, CD273, CD279, and ANGP2

We hypothesize that not only CD274 but also CD279 and CD273 protein expression in tumor tissues might be associated with favorable clinical response, and might be served as biomarkers for patient selection for CD279 blockade in clinical treatment. We also hypothesize that ANGPT2 protein expression in tumor tissue might be a biomarker to identify a group of patients who might have objective responses with anti-vascular endothelial growth factor therapies.

Assay, patient and specimen information

Immunohistochemical (IHC) staining of CD274, CD273, CD279 and ANGPT2 will be used as integrated markers in the clinical trial, which might be used in the future phase II trials to identify a group of patients who would have a good response to the treatment as a stratification variable. Tumor specimens will be collected from metastatic deposits ovarian cancer, colorectal cancer and renal cell carcinoma. Pre-treatment archived specimens will be retrieved if no fresher tumor can be obtained prior to treatment initiation on day 1. Post-treatment tissues will be collected and fixed by 10% neutral buffered formalin overnight, dehydrated and paraffin

embedded. Four-micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. [redacted]

Primary antibody characteristics

Mouse monoclonal anti-CD279, anti-CD274 and anti-CD273 antibodies were generated in the [redacted] The antibodies are human gene product and can recognize all isoforms. The specificity of these antibodies was confirmed by western blotting in human cancer cell lines and bands were at expected mass. The IHC staining was abolished in knock-down cancer cells. CD279 is expressed on the surface of activated T cells. CD274 is present in macrophages, dendritic cells, T cells, B cells and in multiple cancers (Sznol M, 2013). CD273 is expressed on dendritic cells, macrophages and bone marrow-derived mast cells whereas its expression on cancers is under exploration (Rozali EN, 2012). No cross-reactive proteins that may confound interpretation of IHC staining were identified. The antigens are stable when the period between tissue sectioning and staining is more than 30 days.

ANGPT2 antibody will be purchased from Santa Cruz biotechnology company [Ang-2 (F-1): sc-74403]. It is a mouse monoclonal antibody raised against amino acids of human origin. The antibody specificity has been confirmed by western blotting. ANGPT2 expression is observed on endothelial cells and some cancers (Buehler D, 2013; Helfrich I, 2009). The antigen is stable when the period between tissue sectioning and staining is more than 30 days.

Design of immunohistochemical assay

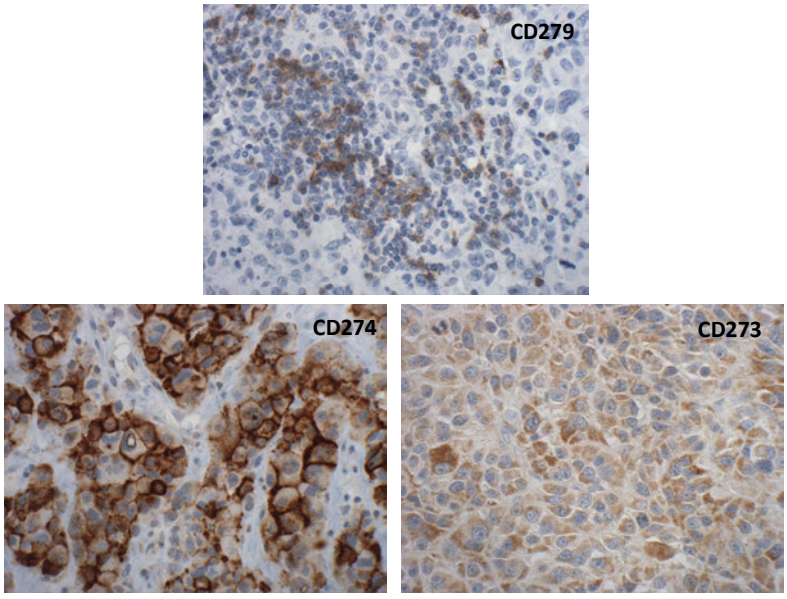
The IHC assay for CD274, CD273 and ANGPT2 is semi-quantitative while CD279 stained slides will be scanned by an automated scanning microscope and quantitatively analyzed [redacted] after they are evaluated and positive cells are manually counted by a pathologist.

Standard EnVision two-step (indirect) staining method will be utilized. Four-micrometer-thick sections will be cut, deparaffinized, rehydrated and participanted to heat mediated antigen retrieval in citrate buffer (pH 6) (Invitrogen) by steaming for 30 minutes. After cooling, tissue sections will be incubated with peroxidase block (DAKO, Carpinteria, CA) for five minutes, then serum free protein block (DAKO) for 20 minutes. Slides will be incubated at room temperature for one hour with a primary antibody. Antibodies will be diluted in Da Vinci Green Diluent (Biocare Medical, Concord, CA). Envision™ anti-mouse HRP-labeled polymer (DAKO) will be applied to the sections for 30 minutes, followed by visualization by using the chromogen 3, 3-diaminobenzidine (DAKO). All the sections will then be counterstained with haematoxylin, dehydrated, mounted and coverslipped. Positive and negative controls shall be included in each staining. Known positive stained Hodgkin lymphoma (CD274), tonsil (CD279 and ANGPT2) and melanoma (CD273) slides will be used as external control (separate slides). Stained slides

will be stored at room temperature.

Figure 29 shows the immunohistochemical staining of CD279, CD274 and CD273 in advanced melanoma.

In our pilot study, immunoreactivity for CD274 was detected in the cytoplasm and cell membrane while CD273 and CD279 expression was observed in the cytoplasm. ANGPT2 expression is present in the cytoplasm (Buehler D, 2013). Scoring for CD274, CD273 and ANGPT2 will be semi-quantitative/ordered categorical. The percentage of the tumor cells staining positive for CD274, CD273 or ANGPT2 and the intensity of the tumor cells will be recorded as 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (intense staining). Absolute CD279 positive cells will be counted under microscope lens x 20 power field. Representative 5 areas will be chosen to count. The average number from 5 areas will be recorded and be compared with data from image analysis.



Assay performance

The results will be obtained on retrospective data sets. Protocols of these three antibodies have been optimized, standardized to minimize staining variance. Positive control and negative controls were used and stained separately with each batch of slides. The IHC staining of three markers (CD274, CD273 and CD279) has been performed in two different labs by three different technicians on whole tissue sections of Hodgkin lymphomas, melanomas, lung cancers and renal cell carcinomas. Three readers were involved, confirming the good reproducibility of the assay.

Tumor will be considered positive if >5% (CD274) (Topalian S, 2012) or 10% (CD273 and ANGPT2) of the tumor cell population demonstrates unequivocally staining, respectively. CD279 positivity was defined as >3% positive cells/HPF (Bachireddy P, 2013).

[redacted]

interpretation.

For CD279 staining, slides will be scanned by an automated scanning microscope and analyzed by Aperio image analysis system (Leica Biosystems). Tumor areas will be marked by a pathologist to exclude non-neoplastic areas, such as stroma, normal epithelial and necrotic regions. The software will be used to count the number of positive cells in each tissue. The percentage of CD279 positive cells will be calculated. Data will be compared with that of manual counting by a pathologist to exclude tissue artifacts that cannot be recognized by computer image software.

[redacted]

12.4 Exploratory/Ancillary Correlative Studies

Monitoring Peripheral Blood for Changes in Immune Function and Angiogeneic Factors

Correlative sciences will be expanded from the previous determined biology and analyses developed through our experience thus far with ipilimumab and ipilimumab-bevacizumab treated patients. Subpopulations of PBMCs will be isolated, including but not limited to dendritic cells, T cells, and B cells. Phenotype changes in these cell populations by flow cytometry will be determined as a function of treatment. These include regulatory and effector immune panels, naïve and memory CD4, CD8 and NK lymphocyte populations. Given its importance in immune regulation and association, we will evaluate Tie-2 expressing monocytes (TEM). Changes in antigen specific responses to known tumor associated antigen epitopes (MART-1, NY-ESO-1) will be determined utilizing HLA-A20201 peptide system for APC (including DC maturation and presentation) and targets.

Both humoral and cellular immune responses will be investigated by ELISAs, ELISPOTs, and cytotoxic T cell chromium release assays. We will investigate the effects on tumor vasculature and vascular active molecules as a function of treatment with the two-drug combination. We have recently reported that pre-treatment serum VEGF levels predict clinical benefit to ipilimumab(Yuan, Zhou et al. 2014). We will therefore investigate angiogenic cytokines pretreatment levels as well as changes in levels as a function of treatment. Studies will include monitoring VEGF, bFGF, and HGF levels. Circulating endothelial cells and progenitors will be studied as a function of treatment.

For the analysis of cytokines, chemokines, and immune cell populations from serum or blood, data will be combined from all four diseases, resulting in longitudinal measurements for 48 patients. Serum marker levels will be summarized descriptively and graphically. The time course of expression levels will also be summarized graphically by patient, noting disease group and times of disease progression.

12.5 Sites Performing Correlative Study

[redacted]

13. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

13.1 Data Reporting

13.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

13.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

13.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date

participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

14. STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

This protocol is a phase Ib trial to assess the safety and tolerability of the combination of pembrolizumab with trebananib, determine the recommended dosing, and to obtain preliminary estimates of efficacy. Dose-finding will be based on a standard 3+3, dose escalation design conducted in patients with solid tumors (Section 2.1). After a recommended dose has been determined from the dose escalation, a dose expansion study will be conducted in three cohorts of patients: ovarian, renal, or colorectal cancer. For ovarian cancer 12 patients will be enrolled and treated. For CRC patients total of 37 patients will be enrolled on the expansion cohort. For the RCC cohort only 4 patients will be enrolled on the expansioncohort .

Safety assessments will include all patients receiving one or more doses of either of the study drugs. Secondary and correlative endpoints will be based on the cohorts of patients enrolled in the dose expansion portion of the trial, and may include those patients in the dose escalation who received the recommended dose and had the same tumor type. We will summarize secondary and correlative endpoints according to disease indication and, in an exploratory fashion, with all patients combined.

14.2 Sample Size, Accrual Rate and Study Duration

Dose Escalation: The dose escalation portion of the trial will enroll between 6 and 12 patients. The minimum would occur if dose cohorts 1 and -1 stop due to excess toxicity after the first three patients in each are assessed for DLT. The maximum is based on two cohorts of 6 patients each. Patients in any dose escalation cohort who cease treatment during the DLT assessment window due to rapid progression of disease will not be counted as having a DLT, and may be replaced.

Dose Expansion: Total sample size will be 53 evaluable patients. The same criteria used to define evaluable patients for objective response in section 14.5 should be used here.

Patients with melanoma, ovarian cancer, colorectal cancer or renal cancer who receive the recommended dose in the dose escalation will be included in the dose expansion analyses.

We will close the melanoma cohort and stop enrolling on the current RCC cohort after 4 patients have been enrolled due to low accrual rate. We will continue enrolling ovarian cancer patients on the ovarian expansion cohort as planned up to 12 patients.

We performed an interim analysis of the 15 CRC patients who received the RP2D (3 in the dose escalation and 12 in the dose expansion) and observed a response rate of 7% (1 PR), 4 patients (26%) with best RECIST response of stable disease for a median of 10 months, and median OS of 9 months. The disease control rate (DCR), defined as best RECIST response of complete or partial response or stable disease of at least 6 months duration, was 33%. Since this activity exceeds the historical control in patients who were heavily pre-treated with chemotherapy (DCR 15%, median OS 5 months) (Grothey, Lancet. 2013 Jan 26;381(9863):303-12) we decided to expand the CRC cohort by adding 25 patients. We will expand the CRC cohort with a target sample size of 40 patients (15 already enrolled plus 25 additional) to assess the DCR in these patients. This will be a single-stage design that will have 85% power to detect a difference in DCR of 15% versus 30% with a one-sided alpha=0.1 using a chi-squared test. The null DCR of 15% will be rejected if there are 9 or more patients with disease control out of 40 CRC patients. For analysis, we will present the DCR with a one-sided 90% exact confidence interval.

14.3 Patient Characteristics

Patient characteristics will be summarized by cohort. Patient demographics and disease characteristics will be summarized using descriptive methods such as number and percent for factors measured categorically, and median and range for continuous characteristics.

A detailed summary of patient disposition will be provided, which will include the numbers and proportions of patients enrolled in the study, treated under the protocol, as well as completing the study or discontinuing.

14.4 Treatment Compliance

The numbers of dose reductions or delays, numbers of cycles of treatment received, and dose intensity will be summarized for all treated patients for both study drugs.

14.5 Endpoint Definitions

Rate of DLT: The proportion of patients with DLT in each dose escalation cohort.

Objective response rate (ORR): Objective response will be determined by the best overall response designation (per RECIST 1.1) recorded between the date of first dose of trial therapy and the date of objectively documented disease progression or cessation of trial therapy, whichever occurs first. The objective response rate will be the proportion of patients achieving complete or partial response as their best response to therapy.

Progression-free survival (PFS): Time from start of trial treatment until objective disease progression (per RECIST 1.1) or death, whichever occurs first. Deaths that occur after 12 weeks following the date last known progression-free will not be counted as PFS events. For patients without progression or death within 12 weeks following the date last known progression-free, follow-up will be censored at the date of last disease assessment. Overall survival (OS): Time from start of trial treatment to death from any cause. For patients who are lost to follow-up or who have no documentation of death at the time of final analysis, follow-up will be censored at

the date of last assessment of vital status.

Time-to-progression (TTP): Time interval between the dates of the start of trial treatment and first documentation of progressive disease. In the absence of documented progressive disease, follow-up will be censored at date of last disease assessment. Deaths without prior progression will be censored events.

14.6 Efficacy Analysis

Secondary endpoints will be used to gain preliminary estimates of efficacy. The analyses of secondary endpoints will be based on patients enrolled in the expansion cohorts and will be summarized within disease type. ORR will be estimated for each disease cohort and will be summarized with 90% confidence intervals estimated using exact binomial methods. For samples of size 12, the confidence intervals will be no wider than 0.5. Time-to-event endpoints (i.e., PFS, OS, TTP) will be summarized using the product-limit method of Kaplan-Meier; confidence intervals will be based on log(-log(survival) methodology. Six-month PFS, 12-month OS, and median TTP will be presented, by disease, with 90% confidence intervals. With few patients in each disease, statistical testing will be of low power. Therefore, the analyses within disease will be primarily descriptive and will not rely on p-values.

14.7 Safety Analysis

Safety data will be presented for all patients who receive at least one dose of either of the study drugs. All adverse events recorded during the trial will be summarized and presented by disease cohort or dose level. The incidence of events that are new or worsening from the time of first dose of treatment will be summarized by dose level or disease cohort, system organ class and/or preferred term, severity (based on CTCAE V4 grade), type of adverse event, and relation to study treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, and type of adverse event.

Safety data from the expansion cohorts will be used to further characterize the safety profile of the drug combination and evaluate the toxicity of the MTD from the dose escalation. We assume that toxicities will be similar for each of the expansion cohorts; however, we will summarize safety data in both the aggregate and by cohort to allow for different adverse event types. Late toxicities observed and assessed as treatment related in the expansion cohort will be monitored. If two or more are observed, enrollment in the expansion cohort will be halted for further review and changing the RP2D will be considered. Given that trebananib is limited in use during only the first 12 weeks (4 cycles), we anticipate there is less chance that the combination of trebananib plus pembrolizumab will exhibit late toxicity in comparison to pembrolizumab given alone.

The following table summarizes the probability of observing at least one patient with a treatment-related toxicity for varying true, but unknown, incidence rates. Estimates in the table are based on individual expansion cohorts of size 12, and also for all 48 patients treated at the MTD.

Underlying incidence rate of treatment-related toxicity	Probability of 1 or more of 12 with treatment-related toxicity	Probability of 1 or more of 48 with treatment-related toxicity
0.01	0.11	0.38
0.05	0.46	0.91
0.10	0.72	0.99
0.15	0.86	>0.99
0.20	0.93	>0.99
0.25	0.97	>0.99

For each expansion cohort of size 12, the probability of observing at least one patient with a dose-related toxicity is greater than 0.70 if the incidence rate of that toxicity is 0.10 or higher. For the aggregated safety data, there is greater than 0.90 probability of observing a toxicity if the incidence rate is 0.05 or higher.

14.8 Proposed analyses of correlative endpoints

For the analysis of cytokines, chemokines, and immune cell populations from serum or blood, data will be combined from all four disease groups according to treatment, resulting in longitudinal measurements for approximately 48 patients. Serum marker levels will be summarized descriptively and graphically. The time course of expression levels will also be summarized graphically by patient, noting disease group and times of disease progression. Since patients may have rapid disease progression and terminate treatment early, the use of linear mixed models includes partial data in the analysis allowing characterization of outcome. Transformations will be applied to the outcome measures to stabilize variability and normalize the distributions, when appropriate.

In an exploratory analysis, we will summarize changes in biomarker levels in the subsets of patients who complete 6 months of treatment, recognizing that this may be a select group of participants with less severe disease. Fold-changes will be calculated comparing 6-month and pre-treatment levels (6-month/pre) and summarized descriptively. We estimate that approximately 40% of patients will complete 24 weeks of treatment, resulting in 19-20 patients.

Biomarkers from tissue will be assessed using IHC. Analyses relating baseline expression levels with response will be based on approximately 48 patient samples from newly obtained excisional biopsies or archival tissue. Paired biopsies will be needed from 20 patients in the dose expansion cohort, ideally 5 per disease indication. An endpoint of interest in the tissue analysis would be the proportion of patients with at least a 50% decrease in CD137 M2 macrophages. We anticipate a null proportion of 0.20 with at least a 50% decrease. The combination of trebananib with pembrolizumab would show important biomarker response if the proportion with 50% decrease in CD137 M2 macrophages is at least 0.45. With 20 paired biopsies, an exact binomial test with nominal, two-sided, 0.1-significance level will have at least 80% power to detect the difference between proportions of 0.20 and 0.47.

15. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data

are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

16. REFERENCES

Arai F, Hirao A, Ohmura M, et al. Tie-2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 2004;118:149-61.

Atkins, MB, Gravis G, Drosik K, Demkow T, Tomczak P, Wong SS, Michaelson MD, Choueiri TK, Wu B, Navale L, Warner D, Ravaud A. Trebananib (AMG 386) in Combination with Sunitinib in Patients with Metastatic Renal Cell Cancer: An Open-label, Multicenter, Phase II Study. JCO (2015) 33, 3431-3438.

Augustin HG, Koh GY, et al. (2009). "Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system." Nat Rev Mol Cell Biol 10(3): 165-177

Bachireddy P, Hainz U, Rooney M, Pozdnyakova O, Aldridge J, Zhang W, Liao X, Hodi FS, O'Connell K, Haining WN, Goldstein NR, Canning CM, Soiffer RJ, Ritz J, Hacohen N, Alyea EP 3rd, Kim HT, Wu CJ. Reversal of in situ T cell exhaustion during effective human anti-leukemia responses to donor lymphocyte infusion. Blood. 2013 Dec 19. [Epub ahead of print]

Buehler D, Rush P, Hasenstein JR, Rice SR, Hafez GR, Longley BJ, Kozak KR. Expression of Angiopoietin-TIE System Components in Angiosarcoma. Mod Pathol. 2013 August; 26(8): 1032–1040.

Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012 Jun 28;366(26):2455-65.

Brown, J. L., Z. A. Cao, M. Pinzon-Ortiz, J. Kendrew, C. Reimer, S. Wen, J. Q. Zhou, M. Tabrizi, S. Emery, B. McDermott, L. Pablo, P. McCoon, V. Bedian and D. C. Blakey (2010). "A human monoclonal anti-ANG2 antibody leads to broad antitumor activity in combination with VEGF inhibitors and chemotherapy agents in preclinical models." Mol Cancer Ther 9(1): 145-156.

Chambers, C. A. (2001). "The expanding world of co-stimulation: the two-signal model revisited." Trends Immunol 22(4): 217-223.

Chen BJ, Chapuy B, Ouyang J, Sun HH, Roemer MG, Xu ML, Yu H, Fletcher CD, Freeman GJ, Shipp MA, Rodig SJ. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res. 2013 Jul 1;19(13):3462-73.

Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy--inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res. 2012 Dec 15;18(24):6580-7.

Coffelt, S. B., Y. Y. Chen, M. Muthana, A. F. Welford, A. O. Tal, A. Scholz, K. H. Plate, Y. Reiss, C. Murdoch, M. De Palma and C. E. Lewis (2011). "Angiopoietin 2 stimulates TIE2-expressing monocytes to suppress T cell activation and to promote regulatory T cell expansion." J Immunol 186(7): 4183-4190.

Coffelt, S. B., A. O. Tal, A. Scholz, M. De Palma, S. Patel, C. Urbich, S. K. Biswas, C. Murdoch, K. H. Plate, Y. Reiss and C. E. Lewis (2010). "Angiopoietin-2 regulates gene expression in TIE2-expressing monocytes and augments their inherent proangiogenic functions." Cancer Res 70(13): 5270-5280.

Coxon, A., J. Bready, H. Min, S. Kaufman, J. Leal, D. Yu, T. A. Lee, J. R. Sun, J. Estrada, B. Bolon, J. McCabe, L. et al. "Context-dependent role of angiopoietin-1 inhibition in the suppression of angiogenesis and tumor growth: implications for AMG 386, an angiopoietin-1/2-neutralizing peptibody." Mol Cancer Ther 9(10): 2641-2651.

Coyle, A. J. and J. C. Gutierrez-Ramos (2001). "The expanding B7 superfamily: increasing complexity in costimulatory signals regulating T cell function." Nat Immunol 2(3): 203-209.

Currie, M. J., S. P. Gunningham, K. Turner, C. Han, P. A. Scott, B. A. Robinson, W. Chong, A. L. Harris and S. B. Fox (2002). "Expression of the angiopoietins and their receptor Tie2 in human renal clear cell carcinomas; regulation by the von Hippel-Lindau gene and hypoxia." J Pathol 198(4): 502-510.

Daly C, Eichten A, et al. (2013). "Angiopoietin-2 functions as a Tie-2 agonist in tumor models, where it limits the effects of VEGF inhibition." Cancer Res 73(1): 108-118.

Damien Gerald, Sudhakar Chintharlapalli, Hellmut G. Augustin, Laura E. Benjamin. Angiopoietin-2: An Attractive Target for Improved Antiangiogenic Tumor Therapy. Cancer Res March 15, 2013 73:6 1649-1657.

Davis S, Papadopoulos N, Aldrich TH, et al. Angiopoietins have distinct modular domains essential for receptor binding, dimerization and superclustering. Nature Structural Biology 2003;10:38-44.

De Palma, M., M. A. Venneri, R. Galli, L. Sergi Sergi, L. S. Politi, M. Sampaolesi and L. Naldini (2005). "Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors." Cancer Cell 8(3): 211-226.

De Palma, M., M. A. Venneri, C. Roca and L. Naldini (2003). "Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells." Nat Med 9(6): 789-795.

Detjen, K. M., S. Rieke, A. Deters, P. Schulz, A. Rexin, S. Vollmer, P. Hauff, B. Wiedenmann, M. Pavel and A. Scholz (2010). "Angiopoietin-2 promotes disease progression of neuroendocrine tumors." Clin Cancer Res 16(2): 420-429.
Disis, M.L., Immune regulation of cancer. J Clin Oncol, 2010. 28(29): p. 4531-8.

Doi T1, Ohtsu A, Fuse N, Yoshino T, Tahara M, Shibayama K, Takubo T, Weinreich DM. Phase 1 study of trebananib (AMG 386), an angiogenesis targeting angiopoietin-1/2 antagonist, in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol. 2013 Jan;71(1):227-35.

Duda DG, Cohen KS, et al. (2007). "A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood." Nat Protoc 2(4): 805-810.

Dumont, D. J., G. J. Gradwohl, G. H. Fong, R. Auerbach and M. L. Breitman (1993). "The endothelial-specific receptor tyrosine kinase, tek, is a member of a new subfamily of receptors." Oncogene 8(5): 1293-1301.

Erdag G, Schaefer JT, Smolkin ME, Deacon DH, Shea SM, Dengel LT, Patterson JW, Slingluff CL Jr. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. Cancer Res. 2012 Mar 1;72(5):1070-80.

Fiedler, U., Y. Reiss, M. Scharpfenecker, V. Grunow, S. Koidl, G. Thurston, N. W. Gale, M. Witzenrath, S. Rosseau, N. Suttorp, A. Sobke, M. Herrmann, K. T. Preissner, P. Vajkoczy and H. G. Augustin (2006). "Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation." Nat Med 12(2): 235-239.

Frigola, X., B. A. Inman, C. J. Krco, X. Liu, S. M. Harrington, P. A. Bulur, A. B. Dietz, H. Dong and E. D. Kwon (2012). "Soluble B7-H1: differences in production between dendritic cells and T cells." Immunol Lett 142(1-2): 78-82.

Frigola, X., B. A. Inman, C. M. Lohse, C. J. Krco, J. C. Cheville, R. H. Thompson, B. Leibovich, M. L. Blute, H. Dong and E. D. Kwon (2011). "Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma." Clin Cancer Res 17(7): 1915-1923.

Fu, T., Q. He and P. Sharma (2011). "The ICOS/ICOSL pathway is required for optimal antitumor responses mediated by anti-CTLA-4 therapy." Cancer Res 71(16): 5445-5454.

Gale, N. W., G. Thurston, S. F. Hackett, R. Renard, Q. Wang, J. McClain, C. Martin, C. Witte, M. H. Witte, D. Jackson, C. Suri, P. A. Campochiaro, S. J. Wiegand and G. D. Yancopoulos (2002).

"Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1." Dev Cell 3(3): 411-423.

Galon J, Pagès F, Marincola FM, Thurin M, Trinchieri G, Fox BA, Gajewski TF, Ascierto PA. The immune score as a new possible approach for the classification of cancer. J Transl Med. 2012 Jan 3;10:1

Gerald D, Chintharlapalli S, et al. (2013). "Angiopoietin-2: an attractive target for improved antiangiogenic tumor therapy." Cancer Res 73(6): 1649-1657.

Hamid O, Robert C, et al. (2013). "Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma." N Engl J Med 369(2): 134-144.

Hansen TM, Moss AJ, Brindle NP. Vascular endothelial growth factor and angiopoietins in neurovascular regeneration and protection following stroke. Current Neurovascular Research 2008;5:236-45.

Hashizume H, Falcón BL, Kuroda T, Baluk P, Coxon A, Yu D, Bready JV, Oliner JD, McDonald DM. Complementary Actions of Inhibitors of Angiopoietin-2 and VEGF on Tumor Angiogenesis and Growth. Cancer Res March 15, 2010 70:6 2213-2223;

Helfrich I, Edler L, Sucker A, Thomas M, Christian S, Schadendorf D, Augustin HG. Angiopoietin-2 levels are associated with disease progression in metastatic malignant melanoma. Clin Cancer Res. 2009 Feb 15;15(4):1384-92. doi: 10.1158/1078-0432.CCR-08-1615

Helfrich I, Edler L, et al. (2009). "Angiopoietin-2 levels are associated with disease progression in metastatic malignant melanoma." Clin Cancer Res 15(4): 1384-1392.

Herbst RS1, Hong D, Chap L, Kurzrock R, Jackson E, Silverman JM, Rasmussen E, Sun YN, Zhong D, Hwang YC, Evelhoch JL, Oliner JD, Le N, Rosen LS. Safety, pharmacokinetics, and antitumor activity of AMG 386, a selective angiopoietin inhibitor, in adult patients with advanced solid tumors. J Clin Oncol. 2009 Jul 20;27(21):3557-65.

Hodi F, Hoos A, et al. (2008). "Novel efficacy criteria for antitumor activity to immunotherapy using the example of ipilimumab, an anti-CTLA-4 monoclonal antibody." J Clin Oncol 26(May 20 suppl): abstr 3008.

Hodi FS, Mihm MC, et al. (2003). "Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients." Proc Natl Acad Sci U S A 100(8): 4712-4717.

Hodi FS, O'Day SJ, et al. (2010). "Improved Survival with Ipilimumab in Patients with Metastatic Melanoma." N Engl J Med 363: 711-723.

Holash, J., P. C. Maisonpierre, D. Compton, P. Boland, C. R. Alexander, D. Zagzag, G. D. Yancopoulos and S. J. Wiegand (1999). "Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF." Science 284(5422): 1994-1998.

Holash, J., S. J. Wiegand and G. D. Yancopoulos (1999). "New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF." Oncogene 18(38): 5356-5362.

Horner A, Bord S, Kelsall AW, Coleman N, Compston JE. Tie-2 ligands angiopoietin-1 and angiopoietin-2 are coexpressed with vascular endothelial cell growth factor in growing human bone. Bone 2001;28:65-71.

Huang H, Bhat A, Woodnutt G, Lappe R. Targeting the ANGPT-TIE-2 pathway in malignancy. Nat Rev Cancer 2010;10:575-85.

Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. Cancer Res. 2013 May 15;73(10):2943-8.
Imhof, B. A. and M. Aurrand-Lions (2006). "Angiogenesis and inflammation face off." Nat Med 12(2): 171-172.

Jordan KR, Amaria RN, Ramirez O, Callihan EB, Gao D, Borakove M, Manthey E, Borges VF, McCarter MD. Myeloid-derived suppressor cells are associated with disease progression and decreased overall survival in advanced-stage melanoma patients. Cancer Immunol Immunother. 2013 Nov;62(11):1711-22.

Keir, M. E., M. J. Butte, G. J. Freeman and A. H. Sharpe (2008). "PD-1 and its ligands in tolerance and immunity." Annu Rev Immunol 26: 677-704.

Karlan BY, Oza AM, Richardson GE, Provencher DM, Hansen VL, Buck M, Chambers SK, Ghatage P, Pippitt CH Jr, Brown JV 3rd, Covens A, Nagarkar RV, Davy M, Leath CA 3rd, Nguyen H, Stepan DE, Weinreich DM, Tassoudji M, Sun YN, Vergote IB. Randomized, double-blind, placebo-controlled phase II study of AMG 386 combined with weekly paclitaxel in patients with recurrent ovarian cancer. J Clin Oncol. 2012 Feb 1;30(4):362-71.

Koga K, Todaka T, Morioka M, et al. Expression of angiopoietin-2 in human glioma cells and its role for angiogenesis. Cancer Res 2001;61:6248-54.

Korn EL, Liu PY, et al. (2008). "Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials." J Clin Oncol 26(4): 527-534.

Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med. 2012 Oct 25;367(17):1596-606.

Lind, A. J., P. Wikstrom, T. Granfors, L. Egevad, P. Stattin and A. Bergh (2005). "Angiopoietin 2 expression is related to histological grade, vascular density, metastases, and outcome in prostate cancer." Prostate 62(4): 394-399.

Maine CJ, Aziz NH, Chatterjee J, Hayford C, Brewig N, Whilding L, George AJ, Ghaem-Maghami S. Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. Cancer Immunol Immunother. 2013 Dec 3.

Monk, BJ, Poveda A, Vergote I, Raspagliesi F, Fujiwara K, Bae DS, Oaknin A, Ray-Coquard I, Provencher DM, Karlan BY, Lhommé C, Richardson G, Rincón DG, Coleman RL, Marth C, Brize A, Fabbro M, Redondo A, Bamias A, Ma H, Vogl FD, Bach BA, Oza AM. Final results of a phase 3 study of trebananib plus weekly paclitaxel in recurrent ovarian cancer (TRINOVA-1): Long-term survival, impact of ascites and progression-free survival-2. Gyn Onc (2016) 143, 27-34

Monk, BJ. Poveda A, Vergote I, Raspagliesi F, Fujiwara K, Bae DS, Oaknin A, Ray-Coquard I, Provencher DM, Karlan BY, Lhommé C, Richardson G, Rincón DG, Coleman RL, Herzog TJ, Marth C, Brize A, Fabbro M, Redondo A, Bamias A, Tassoudji M, Navale L, Warner DJ, Oza AM. Anti –angiopoietin therapy with trebananib for recurrent ovarian cancer (TRINOVA-1): a randomized, multicenter, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. (2014) 15, 799-808.

Mooi, J, Chong G, Brown S, Wong R, Price TJ, Skrinos E, Tebbutt NC. Dual targeting of VEGF-A and Angiopoietins without Chemotherapy in mCRC: Results of the VENGEANCE Study. (2015) ASCO abstract # 3533

Fiedler, U., Y. Reiss, M. Scharpfenecker, V. Grunow, S. Koidl, G. Thurston, N. W. Gale, M. Witzenrath, S. Rosseau, N. Suttorp, A. Sobke, M. Herrmann, K. T. Preissner, P. Vajkoczy and H. G. Augustin (2006). "Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation." Nat Med 12(2): 235-239.

Frigola, X., B. A. Inman, C. J. Krco, X. Liu, S. M. Harrington, P. A. Bulur, A. B. Dietz, H. Dong and E. D. Kwon (2012). "Soluble B7-H1: differences in production between dendritic cells and T cells." Immunol Lett 142(1-2): 78-82.

Frigola, X., B. A. Inman, C. M. Lohse, C. J. Krco, J. C. Cheville, R. H. Thompson, B. Leibovich, M. L. Blute, H. Dong and E. D. Kwon (2011). "Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma." Clin Cancer Res 17(7): 1915-1923.

Fu, T., Q. He and P. Sharma (2011). "The ICOS/ICOSL pathway is required for optimal antitumor responses mediated by anti-CTLA-4 therapy." Cancer Res 71(16): 5445-5454.

Gale, N. W., G. Thurston, S. F. Hackett, R. Renard, Q. Wang, J. McClain, C. Martin, C. Witte, M. H. Witte, D. Jackson, C. Suri, P. A. Campochiaro, S. J. Wiegand and G. D. Yancopoulos (2002). "Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1." Dev Cell 3(3): 411-423.

Hashizume, H., B. L. Falcon, T. Kuroda, P. Baluk, A. Coxon, D. Yu, J. V. Bready, J. D. Oliner and D. M. McDonald (2010). "Complementary actions of inhibitors of angiopoietin-2 and VEGF on tumor angiogenesis and growth." Cancer Res 70(6): 2213-2223.

Helfrich, I., L. Edler, A. Sucker, M. Thomas, S. Christian, D. Schadendorf and H. G. Augustin (2009). "Angiopoietin-2 levels are associated with disease progression in metastatic malignant melanoma." Clin Cancer Res 15(4): 1384-1392.

Holash, J., P. C. Maisonpierre, D. Compton, P. Boland, C. R. Alexander, D. Zagzag, G. D. Yancopoulos and S. J. Wiegand (1999). "Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF." Science 284(5422): 1994-1998.

Holash, J., S. J. Wiegand and G. D. Yancopoulos (1999). "New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF." Oncogene 18(38): 5356-5362.

Imhof, B. A. and M. Aurrand-Lions (2006). "Angiogenesis and inflammation face off." Nat Med 12(2): 171-172.

Keir, M. E., M. J. Butte, G. J. Freeman and A. H. Sharpe (2008). "PD-1 and its ligands in tolerance and immunity." Annu Rev Immunol 26: 677-704.

Lind, A. J., P. Wikstrom, T. Granfors, L. Egevad, P. Stattin and A. Bergh (2005). "Angiopoietin 2 expression is related to histological grade, vascular density, metastases, and outcome in prostate cancer." Prostate 62(4): 394-399.

Maisonpierre, P. C., C. Suri, P. F. Jones, S. Bartunkova, S. J. Wiegand, C. Radziejewski, D. Compton, J. McClain, T. H. Aldrich, N. Papadopoulos, T. J. Daly, S. Davis, T. N. Sato and G. D. Yancopoulos (1997). "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis." Science 277(5322): 55-60.

Mak, T. W., A. Shahinian, S. K. Yoshinaga, A. Wakeham, L. M. Boucher, M. Pintilie, G. Duncan, B. U. Gajewska, M. Gronski, U. Eriksson, B. Odermatt, A. Ho, D. Bouchard, J. S. Whorisky, M. Jordana, P. S. Ohashi, T. Pawson, F. Bladt and A. Tafuri (2003). "Costimulation through the inducible costimulator ligand is essential for both T helper and B cell functions in T cell-dependent B cell responses." Nat Immunol 4(8): 765-772.

Mazzieri, R., F. Pucci, D. Moi, E. Zonari, A. Ranghetti, A. Berti, L. S. Politi, B. Gentner, J. L. Brown, L. Naldini and M. De Palma (2011). "Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells." Cancer Cell 19(4): 512-526.

Movahedi, K., D. Laoui, C. Gysemans, M. Baeten, G. Stange, J. Van den Bossche, M. Mack, D. Pipeleers, P. In't Veld, P. De Baetselier and J. A. Van Ginderachter (2010). "Different tumor

microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes." Cancer Res 70(14): 5728-5739.

Nasarre, P., M. Thomas, K. Kruse, I. Helfrich, V. Wolter, C. Deppermann, D. Schadendorf, G. Thurston, U. Fiedler and H. G. Augustin (2009). "Host-derived angiopoietin-2 affects early stages of tumor development and vessel maturation but is dispensable for later stages of tumor growth." Cancer Res 69(4): 1324-1333.

Neal, J. and H. Wakelee (2010). "AMG-386, a selective angiopoietin-1/-2-neutralizing peptibody for the potential treatment of cancer." Curr Opin Mol Ther 12(4): 487-495.

Oliner, J., H. Min, J. Leal, D. Yu, S. Rao, E. You, X. Tang, H. Kim, S. Meyer, S. J. Han, N. et al. (2004). "Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2." Cancer Cell 6(5): 507-516.

Okazaki T1, Chikuma S, Iwai Y, Fagarasan S, Honjo T. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. Nat Immunol. 2013 Dec;14(12):1212-8.

Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.

Peeters, A, Strickland H, Lichinitser M, Suresh AVS, Manikhas G, Shapiro J, Rogowski W, Huang X, Wu B, Warner D, Jain R, Tebbutt NC. A Randomized, Double-blind, Placebo-controlled Ph2 study of Trebananib (AMG 386) in Combination with FOLFIRI in Patients with Previously Treated Metastatic Colorectal Cancer. M BJC (2013) 108, 503-511Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011 Sep 16;146(6):873-87.

Pucci, F., M. A. Venneri, D. Biziato, A. Nonis, D. Moi, A. Sica, C. Di Serio, L. Naldini and M. De Palma (2009). "A distinguishing gene signature shared by tumor-infiltrating Tie2-expressing monocytes, blood "resident" monocytes, and embryonic macrophages suggests common functions and developmental relationships." Blood 114(4): 901-914.

Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010 Apr 2;141(1):39-51.

Rini, B, Szczylik C, Tannir NM, Koralewski P, Tomczak P, Deptala A, Dirix LY, Fishman M, Ramlau R, Ravaud A, Rogowski W, Kracht K, Sun YN, Bass MB, Puhlmann M, Escudier B. AMG 386 in Combination with Sorafanib in Patients with Metastatic Clear Cell Cancer of the Kidney. Cancer (2012) 118, 6152-616.

Rozali EN, Hato SV, Robinson BW, Lake RA, Lesterhuis WJ. Programmed death ligand 2 in cancer-induced immune suppression. Clin Dev Immunol. 2012;2012:656340.

Robert C, Thomas L, et al. "Ipilimumab plus dacarbazine for previously untreated metastatic melanoma." N Engl J Med 364(26): 2517-2526.Ruiz de Almodovar C, Lambrechts D, Mazzone

M, Carmeliet P. Role and therapeutic potential of VEGF in the nervous system. Physiological reviews 2009;89:607-48.

Reiss, Y., J. Droste, M. Heil, S. Tribulova, M. H. Schmidt, W. Schaper, D. J. Dumont and K. H. Plate (2007). "Angiopoietin-2 impairs revascularization after limb ischemia." Circ Res 101(1): 88-96.

Shankar LK, Hoffman JM, Bacharach S, et al. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2006;47:1059-66.

Sharpe, A.H. and G.J. Freeman, The B7-CD28 superfamily. Nat Rev Immunol, 2002. 2(2): p. 116-26.

Sfiligoi, C., A. de Luca, I. Cascone, V. Sorbello, L. Fuso, R. Ponzone, N. Biglia, E. Audero, R. Arisio, F. Bussolino, P. Sismondi and M. De Bortoli (2003). "Angiopoietin-2 expression in breast cancer correlates with lymph node invasion and short survival." Int J Cancer 103(4): 466-474.

Shim WS, Ho IA, Wong PE. Angiopoietin: a TIE(d) balance in tumor angiogenesis. Molecular cancer research : MCR 2007;5:655-65

Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res March 1, 2013 19:5 1021-1034.

Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013 Oct;13(10):739-52.

Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, Dubreuil O, Carpentier AF, Tartour E, Taieb J. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res. 2013 Jan 15;73(2):539-49.

Thompson, R.H., Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, Kwon ED. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res, 2007. 13(6): p. 1757-61.

Topalian SL, Hodi FS, et al. (2012). "Safety, activity, and immune correlates of anti-PD-1 antibody in cancer." N Engl J Med 366(26): 2443-2454.

Tsutsui, S., H. Inoue, K. Yasuda, K. Suzuki, H. Takeuchi, T. Nishizaki, H. Higashi, S. Era and M. Mori (2006). "Angiopoietin 2 expression in invasive ductal carcinoma of the breast: its relationship to the VEGF expression and microvessel density." Breast Cancer Res Treat 98(3): 261-266.

Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412-20.

Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369:122-33.

Wong, A. L., Z. A. Haroon, S. Werner, M. W. Dewhirst, C. S. Greenberg and K. G. Peters (1997). "Tie2 expression and phosphorylation in angiogenic and quiescent adult tissues." Circ Res 81(4): 567-574.

Yuan J, Zhou J, et al. (2014). "Pretreatment Serum VEGF is Associated with Clinical Response and Overall Survival in Advanced Melanoma Patients Treated with Ipilimumab." Cancer Immunology Research 2(127-32).

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.