Trial of Pembrolizumab for Advanced Penile Squamous Cell Carcinoma

Study Protocol & Statistical Analysis Plan

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CLINICAL TRIAL PROTOCOL

Phase II trial of Pembrolizumab for Advanced Penile Squamous Cell Carcinoma following previous chemotherapy

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TABLE OF CONTENTS

PSCC	Penile Squamous Cell Carcinoma
AE	Adverse Event
AJCC	American Joint Committee on Cancer
ALT	Alanine AminoTransferase
ANC	Absolute Neutrophil Count
AST	Aspartate AminoTransferase
BMP	Bleomycin-Methotrexate-Cisplatin
CAN	Copy Number Alterations
CBC	Complete Blood Counts
CDKN2A	Cyclin-Dependent Kinase inhibitor 2A
CMP	Complete Metabolic Profile
COSMIC	Catalog Of Somatic Mutations In Cancer
COX-2	Cyclo-oxygenase 2
CR	Complete Remission
CRF	Case Report Forms
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-Lymphocyte Associated protein 4
CTMC	Clinical Trials Monitoring Committee
CTNMO	Clinical Trials Network Monitoring Office
DKA	Diabetic KetoAcidosis
DLT	Dose Limiting Toxicity
DNA	Deoxyribose Nucleic Acid
DSMP	Data and Safety Monitoring Plan
ECI	Event of Clinical Interest
ECOG	Eastern Co-Operative Group
EGFR	Epidermal Growth Factor Receptor
ERCC	Excision Repair protein
FDAAA	Food and Drug Administration Amendments Act
FDAMA	Food and Drug Administration Modernization Act
FFPE	Formalix Fixed Paraffin Embedded
FISH	Fluorescent In-Situ Hybridization
HBsAg	Hepatitis B
HCV RNA	Hepatitis C virus Ribose Nucleic Acid
HER3	Human Epidermal Growth Factor Receptor 3
HER4	Human Epidermal Growth Factor Receptor 4
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
IHC	ImmunoHistoChemistry
IND	Investigational New Drug
ir	Immune Related
IRB	Institutional Review Board
irCR	Immune-Related Complete Remission

irPD	Immune-Related Progression of Disease
irPR	Immune-Related Partial Remission
irSD	Immune-Related Stable Disease
ITIM	Immunoreceptor Tyrosine based Inhibition Motif
IV	IntraVenous
KRAS	Kirsten Rat Sarcoma viral oncogene
LD	Longest Diameter
LDH	Lactate DeHydrogenase
MMP-9	Matrix Metallopeptidase-9
NCI	National Cancer Institute
PIK3CA	Phosphatidyl-4,5-Bisphosphonate 3-Kinase
OS	Overall Survival
OSP	Office of Sponsored Program
OTC	Over-The-Counter
PD-L1	Programmed Death Ligand 1
PFS	Progression Free Survival
PR	Partial Remission
PRC	Protocol review Committee
PS	Performance Status
PT	Prothrombin Time
PTEN	Phosphatase and Tensin Analog
PTT	Partial Thromboplastin Time
Q2W	Every 2 weeks
Q3W	Every 3 weeks
QAC	Quality Assurance Committee
RAS	Rat Sarcoma
RASSF	Ras-Associated Domain Family
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
T3	Thyroxine 3
T4	Thyroxine 4
TIL	Tumor Infiltrating Lymphocyte
TNM	Tumor Node Metastasis
TSP	Thrombospondin-1
ULN	Upper Limit of Normal
WBC	White Blood Cells
WHO	World Health Organization

1.0 TRIAL SUMMARY

Abbreviated Title	Pembrolizumab for Advanced Penile Squamous Cell Carcinoma
Trial Phase	II
Clinical Indication	Advanced Penile Squamous Cell Carcinoma (PSCC)
Trial Type	Interventional
Type of control	No control arm
Route of administration	Intravenous
Trial Blinding	Unblinded open label
Treatment Groups	Single arm: Pembrolizumab 200mg IV q 3 weeks.
Number of trial subjects	35 planned
Estimated enrollment period	12-18 months
Estimated duration of trial	24 months
Duration of Participation	24 months

2.0 TRIAL DESIGN

2.1 Trial Design

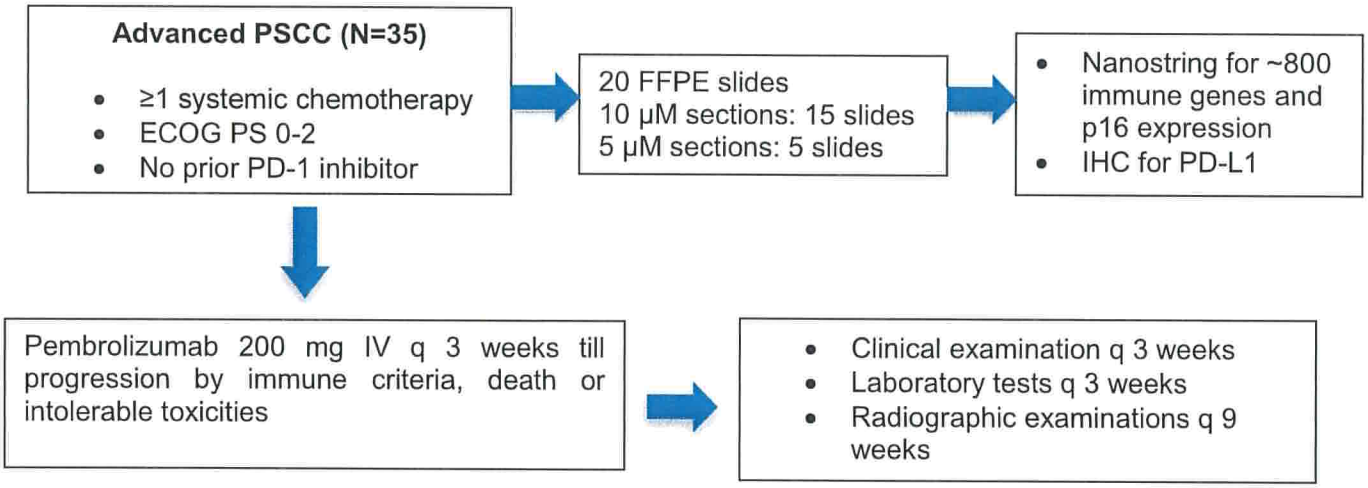
This is a non-randomized multicenter phase II trial (overall N=35, evaluable N= 32) to evaluate Pembrolizumab 200 mg IV q 3 weeks (1 cycle = 3 weeks) for patients with locally advanced unresectable or metastatic progressive PSCC following prior chemotherapy. UAB is the coordinating institution and the other participating institutions are: University of Southern California [USC] (Tanya Dorff, MD), Duke Cancer Institute (Michael Harrison, MD and Brant Inman, MD) and Emory University (Bradley Carthon, MD). Patients will undergo clinical examination every 3 weeks and radiographic work-up at baseline and then every 9 weeks (3 cycles). Patients will continue therapy until progression by immune related (ir) response criteria (RC) or intolerable toxicities [1]. Twenty archival formalin-fixed paraffin embedded (FFPE) tissue slides per patient will be acquired for future analyses including histologic sub-type, nanostring for immune signature panel (including PD-L1 and p16, see http://www.nanostring.com/products/gene_expression_panels) and IHC for PD-L1.

The eligible population of patients includes men with pathologically proven locally advanced inoperable or metastatic (lymph node or distant metastasis i.e. N+ or M1) PSCC which has received ≥1 systemic chemotherapy with ECOG PS 0-2 & optimal organ function (Hb>8.0, ANC>1500/mm3, Platelet count>100K, Total bilirubin <1.5 of ULN, creatinine clearance ≥30 ml/min, ALT and AST<2.5X ULN, for patients with liver involvement ALT and ALT<5X ULN). The disease should be measurable by RECIST 1.1 criteria and immune related response criteria are used to monitor patients [1]. Complete Blood Count (CBC) & Comprehensive Metabolic Panel (CMP) evaluations will be done on day 1 every 3 weeks. CT-scan of chest, abdomen and pelvis will be performed every 3 cycles (9 weeks). A bone scan will be performed if bone pain or high alkaline phosphatase levels are observed. Progression of disease will be defined clinically as the first occurrence of any of the following:

1) Progression in measurable disease as defined by immune related (ir) RECIST 1.1 criteria [1]: Unidimensional measurements using RECIST 1.1 will be used with with the added application of ir criteria [2]. Patients are considered to have irPR or irSD even if new lesions were present, as long as they meet the respective thresholds of response as described above. Patients are not considered to have irPD if new lesions are present and the tumor burden of all lesions does not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by RECIST 1.1 criteria as well as those patients that shift to these irRC categories.

2) Death

2.2 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

Objective: Response Rate (RR) by immune related (ir) RECIST 1.1 criteria
Hypothesis: Intravenous administration of single agent Pembrolizumab in locally advanced unresectable or metastatic PSCC will result in an anti-tumor immune response leading to a clinically meaningful RR.

3.2 Secondary Objective(s) & Hypothesis(es)

Objective: Duration of Response and Stable disease, Toxicities, Progression Free Survival (PFS) and Overall Survival (OS)
Hypothesis: Intravenous administration of single agent Pembrolizumab will be well tolerated in locally advanced or metastatic PSCC. Intravenous Pembrolizumab will produce clinically meaningful duration of response, PFS and OS.

3.3 Exploratory Objectives:

Tumor tissue immune signature panel (including PD-L1 and p16) by nanostring and IHC for PD-L1

Hypothesis: Higher PD-L1 protein expression will be associated with higher response rates. Over-expression of select immune pathway genes will be associated with higher response rate.

4.0 BACKGROUND & RATIONALE

INTRODUCTION

In developed countries, Penile Squamous Cell Carcinoma (PSCC) is relatively rare and is considered an orphan disease, but less developed countries exhibit higher incidences. In 2015, ~1700 new cases and 300 deaths from penile cancers are predicted to occur in the U.S.[3]. The median age of diagnosis is ~62 years and the majority of patients are diagnosed at a localized stage, which is managed by surgery or radiation therapy. However, PSCC is a highly aggressive malignancy characterized by early loco-regional spread with subsequent potential for distant dissemination.

Pathology

The vast majority of malignancies of the penis are Squamous Cell Cancers (SCC), but other histologic types are observed in ~5% of cases, such as melanomas, basal cell carcinomas and sarcomas [4]. The World Health Organization (WHO) classifies penile SCC, or PC, as usual, basaloid, verrucous, warty (condylomatous), papillary, sarcomatoid, adenosquamous and mixed [5]. In a surgical series of 333 patients receiving homogeneous surgery, basaloid, sarcomatoid and adenosquamous carcinomas displayed the highest histological grade and deep tissue infiltration, while verrucous, papillary and condylomatous (warty) carcinomas were associated with low grade and superficial invasion. This relationship translated into distinct clinical behavior, with a higher 10-year survival rate for verrucous, adenosquamous, mixed, papillary and warty carcinoma (100%, 100%, 97%, 92% and 90%, respectively), while patients with the usual and basaloid types had 78% and 76% 10-year survival, respectively. Of note, 75% of patients with sarcomatoid carcinoma died, usually within a year of diagnosis [6]. Interestingly, verrucous carcinomas appear to exhibit low p16 and HPV expression [7]. Grading has an established prognostic role for PC with crucial clinical implications [8, 9]. Higher grade and basaloid and warty tumors are more consistently associated with HPV, suggesting that distinct pathogenic pathways may drive tumors [10-12].

Staging of PSCC

The American Joint Committee on Cancer (AJCC) has designated staging by TNM classification to define penile cancer [13].

Primary Tumor (T)

TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor.
Tis	Carcinoma *in situ*.
Ta	Noninvasive verrucous carcinoma.
T1a	Tumor invades subepithelial connective tissue without lymph vascular invasion and is not poorly differentiated (i.e., grade 3–4).
T1b	Tumor invades subepithelial connective tissue with lymph vascular invasion or is poorly differentiated.
T2	Tumor invades corpus spongiosum or cavernosum.
T3	Tumor invades urethra.
T4	Tumor invades other adjacent structures.

Regional Lymph Nodes (N)

cNX Regional lymph nodes cannot be assessed.
cN0 No palpable or visibly enlarged inguinal lymph nodes.
cN1 Palpable mobile unilateral inguinal lymph node.
cN2 Palpable mobile multiple or bilateral inguinal lymph nodes.
cN3 Palpable fixed inguinal nodal mass or pelvic lymphadenopathy unilateral or bilateral.

Distant Metastasis (M)

M0 No distant metastasis.
M1 Distant metastasis

Table 4. Anatomic Stage/Prognostic Groups			
Stage	**T**	**N**	**M**
AJCC: Penis. In: Edge SB, Byrd DR, Compton CC, et al., eds.: AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer, 2010, pp 447-55.			
0	Tis	N0	M0
	Ta	N0	M0
I	T1a	N0	M0
II	T1b	N0	M0
	T2	N0	M0
	T3	N0	M0
IIIa	T1–3	N1	M0
IIIb	T1–3	N2	M0
IV	T4	Any N	M0
	Any T	N3	M0
	Any T	Any N	M1

Molecular biology

Epidermal growth factor receptor (EGFR) over-expression appears to be almost universal and correlated with grade but not stage [14-16]. In an American series, KRAS mutations and ERCC1 amplification appeared rare or absent, which may portend responsiveness to EGFR inhibitors and platinum chemotherapy. EGFR had the highest relative expression followed by thymidylate synthetase (TS). However, in a Spanish series (n=28), 22% of evaluable tumors had missense mutations in KRAS , suggesting there may be regional differences in biology [17]. In another study, somatic missense mutations in PIK3CA, HRAS and KRAS were found in11 of 28 PC samples (39%) [18]. PIK3CA mutations were found in all grades and stages, whereas HRAS and KRAS mutations were found in more advanced tumors. The mutations were mutually exclusive, suggesting that dysregulation of either pathway is sufficient for tumor growth. A preliminary examination of the COSMIC dataset (n=28) revealed p53 or PIK3CA mutations in 8 of 28 (29%) tumors (http://www.sanger.ac.uk/cosmic) [19]. EGFR, HER3 and HER4 protein overexpression was found in one study of 148 cases, although no *EGFR* gene amplification was detected [20]. In this study, HPV-negative tumors expressed significantly more phosphorylated EGFR than HPV-positive cancers, which correlated with phosphorylation and activation of Akt

signaling. Conversely, HER3 expression was significantly more common in HPV-positive cases, which correlated with cytoplasmic localization of Akt1. PTEN protein expression was reduced in 62% of tumors but PTEN gene loss occurred only in 4%.

The epigenetic inactivation of thrombospondin (TSP)-1 and RAS association domain family (RASSF)-1A genes by hypermethylation seemed to confer prognostic significance in one study (n=24) [21]. LN metastasis was significantly associated with negative p16 and combined LOH and promoter hypermethylation, but not with p53 alterations [22]. Similarly, another study of 148 PCs demonstrated that HPV infection may engender p16 and p21 expression and RB suppression, but no association with p53 expression was detected [23]. Nevertheless, p53 protein expression has been related to LN metastasis and poor survival in other studies [24-26]. Moreover, studies indicate the potential importance of cell-cycle regulators and pro-survival proteins, e.g. p16, p21, telomerase and the Bcl-2 family [16, 27-29].

Another study of 26 cases reported DNA sequence copy number alterations (CNAs) similar to oral and esophageal SCCs [30]. The most frequent copy number gains occurred in 8q24, 16p11-12, 20q11-13, 22q, 19q13, and 5p15, while the most common deletions occurred in 13q21-22, 4q21-32 and the X chromosome. The number of CNAs exhibited a possible correlation with clinical outcome, but the biological mechanisms remain undefined. Increased cyclo-oxygenase (COX)-2 and microsomal prostaglandin E synthase-1 were detected in penile intraepithelial neoplasia and carcinoma in one study, suggesting a pathogenic role for inflammation and a therapeutic role for COX-2 inhibitors [31]. The potential role of angiogenesis was suggested by a case series reporting the activity of sorafenib and sunitinib [32].

Prognostic factors

PSCC is classified as usual, basaloid, verrucous, warty, papillary, sarcomatoid, adenosquamous and mixed [5]. Better survival is observed for verrucous, adenosquamous, mixed, papillary and warty carcinomas, while patients with the usual and basaloid types had lower survival..Pathologic TNM staging provides prognostic stratification after surgery [34]. Furthermore, extranodal extension in inguinal LNs and pelvic LN involvement appear to be independently associated with decreased 5-year cancer specific survival (42% and 22%, respectively) [35]. Nomograms have been reported for patients following penectomy to better predict cancer-specific survival and LN metastasis [36-38]. These nomograms incorporate multiple variables in addition to stage to enhance prognostication including grade, venous or lymphatic embolization and type of surgery. Other studies have reported lymph node density, lack of koilocytosis and clear cell subtype to be prognostic [35, 38-52]. In patients with locally advanced and advanced disease undergoing systemic fist-line chemotherapy, a recent analysis identified performance status and visceral metastasis as major prognostic factors [53]. In this retrospective analysis of 140 men receiving first-line chemotherapy, the multivariate model of poor prognostic factors included visceral metastases ($p<0.001$) and ECOG-PS ≥ 1 ($p<0.001$) for both PFS and OS. A risk model showed that those with 0, 1 and both poor prognostic factors which was internally validated and demonstrated moderate discriminatory ability (c-statistic of 0.657 and 0.677 for OS and PFS). The median OS for the entire population was 9 months. Median OS was not reached, 8 and 7 months respectively for those with 0, 1 and both risk factors. Cisplatin-based regimens were associated with better OS ($p=0.017$), but not PFS ($p=0.37$), compared to non-cisplatin based regimens after adjusting for the 2 prognostic factors.

Additionally, molecular prognostic markers are suggested by some studies, e.g. p53, ki-67, E-cadherin, MMP-9, Annexins I and IV and decreased KAI1/CD82, a metastasis suppressor gene

[24, 26, 54-57]. Although HPV has been associated with high-grade tumors, the impact on outcomes is unclear with one study even demonstrating a favorable impact of HPV and another study showing a positive association with survival of p16 (CDKN2A), which is related to HPV [12, 58-60].

Current systemic chemotherapy for advanced PSCC

High-level evidence for the value of systemic therapy does not exist in this orphan disease. The efficacy of adjuvant therapy is unproven. In patients with bulky primary disease or fixed or bulky inguinal or pelvic lymph nodes (LNs), multimodality therapy including neoadjuvant chemotherapy followed by surgery and node resection is offered [61-64]. Generally, cisplatin-based combinations employed in the pre-and post-taxane era have demonstrated a similar response-rates (25-50%) and survival. One phase II trial evaluated the combination of ifosfamide, paclitaxel and cisplatin (ITP) and demonstrated responses in 50%, but only 9 patients (30.0%) remained alive and free of recurrence after a median follow-up of 34 months [64]. However, a single optimal neoadjuvant systemic regimen has not been established and the value of surgery following chemotherapy for those with LN involvement remains unproven.

Systemic chemotherapy alone is offered for men with distant metastases and clinical trials are strongly encouraged given the poor efficacy of currently available regimens. A variety of first-line cisplatin-based, systemic therapy regimens have been reported in mostly small single institution retrospective and phase II studies, as the rarity of this disease prohibits the conduct of large randomized trials [61, 65-78]. Older trials employed bleomycin and methotrexate as partners, while newer studies have investigated taxanes, 5-fluorouracil and gemcitabine combinations with median survivals ranging from 6 to 11 months. Historical data with combination BMP demonstrated a median survival of only 28 weeks [72, 79, 80]. In the largest prospective study of this regimen, there were 5 complete and 8 partial responses in 40 evaluable patients for a 32.5% response rate [80]. Unfortunately, in this study, 5 treatment related deaths occurred and 6 other patients had 1 or more life threatening toxic episodes. Hence, the toxicities of bleomycin-containing regimens have been recognized and considered to be prohibitive. However, outcomes with non-cisplatin based regimens are unclear. A recent study evaluated prognostic factors in 140 patients receiving first-line chemotherapy and identified performance status and metastatic sites as major prognostic factors [81].

Second-line therapy is also not established, and taxanes have been used with marginal activity [82]. In a prospective, multicenter phase II trial, 25 patients were enrolled and treated with paclitaxel 175 mg/m2 every 3 weeks. Partial responses were observed in 20%. The median PFS was only 11 weeks, and the median OS was 23 weeks.

Table. Reported studies of ≥ 10 patients receiving chemotherapy for advanced penile cancer

Author	Line of therapy	Regimen	Design	N	Clinical Response N (%)	Median PFS	Median OS
Gagliano [78]	First	Cisplatin	Phase II trial	26	4 (15.4)	NR	4.7 mo
Haas [80]	First	BMP	Phase II trial	40	13 (32.5)	NR	28 wk
Dexeus [72]	First	BMP	Retrospective†	14	10 (72)	NR	NR
Corral [79]	First	BMP	Phase II trial*	30	16 (55)	NR	11.5 mo

Di Lorenzo [83]	First	CF	Retrospective	25	8 (32)	20 wk	8 mo
Theodore [63]	First	CI	Phase II trial	28	8 (30.8)	NR	NR
Di Lorenzo [82]	Second	Paclitaxel$^{\pi}$	Phase II trial	25	5 (20)	11 wk	23 wk

BMP: Bleomycin-Methotrexate-Cisplatin; CF: Cisplatin-5FU; CI: Cisplatin-irinotecan; †12 of the 14 patients had penile primary site; *trial enrolled patients with squamous cell carcinoma of the penis, scrotum, bladder, renal pelvis, ureter or urethra; NR: not reported; $^{\pi}$Paclitaxel every 3 weeks.

Potential role of the Programmed Death (PD)-1 pathway in PSCC

The T-lymphocyte checkpoint receptor PD-1 and the PD-ligand (L)-1 are emerging as major therapeutic targets in multiple malignancies. Intriguingly, high expression of PD-L1 is emerging as potential predictive biomarker for the activity of PD-1 and PD-L1 inhibitors across a broad spectrum of malignancies. Preliminary data regarding PD-L1 gene expression in PSCC have been obtained at UAB by using the Nanostring platform for gene expression. Gene expressing profiling performed by the nanostring technology, which utilizes formalin-fixed paraffin embedded (FFPE) tissue using the nCounter® appears highly promising [84]. This technology provides robust and objectively obtained data from FFPE tissue using the nCounter® GX Kit and offers high levels of precision and sensitivity (>1 copy per cell) without the need for amplification of ≥100 ng of RNA. Nanostring utilizes digital counting and excellent quantitative reproducibility by employing two ~50 base probes per mRNA that hybridize in solution. Multiple successful applications utilizing the nCounter System to subtype tumors, formulate tumor prognostic signatures, or discover novel targets for therapy have been reported in other malignancies [85-88]. Notably, the 50-gene PAM50 signature (Prosigna^{TM}) derived from nanostring was approved by the US FDA in 2013 to assess the risk of distant recurrence at 10 years in postmenopausal women with hormone receptor-positive breast cancer. Indeed, the PAM50 gene score, which is enriched for proliferation-associated genes appeared more robust than the Oncotype DX® recurrence score in predicting recurrence [87]. Thus, in contrast to exome analyses, nanostring provides the level of expression of transcripts and is more objectively performed and analyzed than platforms that measure protein expression such as immunohistochemistry (IHC).

In a study performed at UAB, a total of sixteen (n=16) archival FFPE invasive chemonaive PSCC tumors were available for analysis and adjacent normal matched tissue was available for 8 of these tumors. These tumors underwent macrodissection to separate tumor tissue from normal tissue and underwent RNA was harvested from tumor and matched normal tissue by the Qiagen method and 100ng were input directly into a hybridization reaction containing color-coded molecular barcodes representing the genes. The reporter probe carries the signal and the capture probe allows the complex to be immobilized for data collection. Profiling was performed using the Nanostring platform for PD-L1 and p16 gene expression and internal control housekeeping genes utilizing a previously described method [84]. The data showed an over-expression of the PD-L1 gene in the 16 tumors compared to 8 matched normal tissue samples (Figure 1, red colors represent high expression and green represent low expression). A mean 54.6% increase of PD-L1 gene expression was observed in tumors compared to matched

normal tissue overall. PD-L1 expression by IHC is being assessed and pending at this time. Interestingly, the mean CDKN2A (p16) gene expression was 62.8% higher in tumors compared to normal tissue, suggesting the involvement of HPV in the majority of cases.

Figure: 1

In another recent study, formalin-fixed paraffin-embedded (FFPE) tumor specimens were obtained from 19 patients with PSCC [89]. PD-L1 immunohistochemical staining was done with an E1L3N rabbit monoclonal antibody with the summary of a positive score using H-score membrane staining, which considers both intensity and percentage of cells, with >5% score as a positive. HPV status was also evaluated using FISH or p16 immunohistochemical staining to evaluate for correlation with PD-L1. A total of 23 samples between primaries and lymph nodes were collected from the 19 patients with 5/23 samples (22%) positive for anti-PDL1 staining. 6 samples (26%) were positive for HPV by at least one method of testing with 2/6 (33%) HPV positive patients having positive PD-L1 membrane staining. In both of these samples, there was equivocal peritumoral inflammatory infiltration of PD-L1 cells. Thus, this analysis shows that PD-L1 is expressed in PSCC and may indicate a role for future anti-PD-L1/PD-1 targeting drugs as therapeutic options.

Programmed death (PD)-1/PD-(Ligand)-L1, are emerging as highly promising agents to induce durable remissions in multiple malignancies including melanoma, renal cell carcinoma, non-small cell lung cancer, gastric cancer and urothelial carcinoma [90-94]. Pembrolizumab was recently approved for advanced melanomas [95, 96]. Interestingly, an association of response with tumor tissue PD-L1 has been identified in melanomas and non-small cell lung cancer, although even PD-L1 negative tumors respond [92].

4.1 Pembrolizumab

4.1.1 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene PDCD-1) is an Ig superfamily

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

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (Pembrolizumab) has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

4.1.2 Preclinical and Clinical Trial Data

There are no specific preclinical or clinical data in PSCC. Refer to the Investigator's Brochure for detailed Preclinical and Clinical data.

Nonclinical Pharmacokinetics

After intravenous (IV) administration of MK-3475 to cynomolgus monkeys, systemic exposure to MK-3475 independent of sex, increased with increasing dose. Systemic exposure for the 7-day dosing interval increased after repeated dosing from 40 to 200 mg/kg. Area under the concentration-time curve (AUC) for the 7-day dosing interval (AUC[0-7 days]) after one dose appeared to be dose-proportional from 0.3 to 200 mg/kg, suggesting dose-independent pharmacokinetics (PK). Terminal half-life (t½) values from individual animals after repeated IV dosing ranged from 11.8 to 23.7 days (mean values ranged from 15.7 to 22.3 days) across the doses tested.

Safety Pharmacology/Toxicology

The potential for systemic toxicity of MK-3475 was assessed in a 1-month repeat-dose toxicity study with a 4-month recovery in cynomolgus monkeys and in a 6-month repeat dose toxicity study with a 4-month recovery period in cynomolgus monkeys. In the 1-month toxicity study, cynomolgus monkeys were administered an IV dose of 6, 40, or 200 mg/kg once weekly for a total of five doses. Four monkeys/sex/group were euthanized during Week 5. The remaining two monkeys/sex/group were euthanized during Week 23, after a four-month post-dose period. In this study, MK-3475 was well tolerated in monkeys with the systemic exposure (AUC) up to approximately 170,000 µg/day/mL over the course of the study. There was no test article-related mortality, and test article-related changes were limited to an increased incidence of inguinal swelling, and increased splenic weights in males receiving 200 mg/kg. Both of these findings were not considered adverse and there was no histopathologic correlation. Splenic weights were normal at the post-dose necropsy. Anti-MK-3475 antibodies were detected in seven (out of eight) animals in the 6 mg/kg dose group and one (out of eight) animal in the 40 mg/kg dose group, and were associated with an apparent increase in clearance of MK-3475. The presence of anti-drug antibodies (ADA) in monkeys in the low-dose group and in one monkey in the mid-dose group did not impact the pharmacodynamic response as sufficient target engagement was demonstrated for the duration of the study (with the exception of one low-dose monkey). Additionally, anti-MK-3475 antibodies were not detected in any monkeys in the high-dose group, suggesting that potential toxicity has been evaluated at the highest exposure levels in the study. Based on the lack of adverse test article-related findings in this study, the No Observable Adverse Effect Level (NOAEL) was ≥200 mg/kg.

In the 6-month toxicity study, the potential for systemic toxicity was assessed in cynomolgus monkeys administered an IV dose of 6, 40, or 200 mg/kg once every other week for approximately 6 months (a total of 12 doses) followed by a 4-month treatment free period. Three animals/sex/group were designated for interim necropsy at the end of the 6-month dosing phase (3 days after receiving the last dose in Study Week 23); and the remaining monkeys were designated for final necropsy following the 4-month treatment-free period. MK-3475 was well tolerated at all dose levels. There were no test article-related antemortem findings. There were no test article-related electrocardiographic or ophthalmic findings. There were no test article-related changes at injection sites. There were no test article-related gross observations or organ weight changes at the interim or final necropsy. Since there were no test article-related histomorphologic findings at interim necropsy, histomorphologic evaluation of tissues collected at final necropsy was not conducted. The presence of ADA was observed in five out of ten animals at 6 mg/kg/dose during the dosing phase, which correlated with an apparent increased rate of elimination of MK-3475 in these animals. No anti-MK-3475 antibodies were detected at 40 or 200 mg/kg/dose during the dosing phase, and no MK-3475 serum concentration profiles in these two groups suggested an effect of ADA on MK-3475 elimination rate. During the treatment-free period, anti-MK-3475 antibodies were detected in two animals at 6 mg/kg/dose, which already had ADA present during the dosing phase, and in two additional animals (one at 6 mg/kg/dose and one at 200 mg/kg/dose), which were ADA negative during the dosing phase. The detection of anti-MK-3475 antibodies had a minimal effect on the mean group systemic exposure to MK-3475 during the study and did not impact the evaluation of potential toxicity of MK-3475 for the duration of the 6-month study as there were no test article-related effects on any of the parameters examined and as no monkey in the mid- and high-dose groups developed ADA during the dosing phase. In conclusion, MK-3475 administered once every other week over a 6-month duration to cynomolgus monkeys was well tolerated and the no observed effect level (NOEL) was ≥200 mg/kg/dose (the highest dose tested). In addition, tissue

cross-reactivity studies using monkey and human specimens were conducted to evaluate the potential cross reactivity of MK-3475 with cryosections of cynomolgus monkey tissues and normal human tissues. Results demonstrated the expected on-target staining of the membranes of mononuclear leukocytes in both species. The off-target staining (cytoplasmic and stromal) that occurred in many tissues of both species was considered spurious binding inherent to the experimental conditions of the in vitro tissue cross reactivity studies with no *in vivo* toxicological significance.

Clinical Development

Clinical data from PN001 is presented in this report with a visit cut-off date of 26-Jul-2013. As of 26-Jul-2013, there have been 789 patients treated in PN001 with MK-3475 as a 30-minute IV infusion. Of these 789 patients, preliminary data are presented in this report from 479 patients. Data from 200 patients in Part B3 and 110 patients in Part F that were treated as of the cut-off data were not included in the analyses yet. Based upon this safety database consisting of patients treated up to 10 mg/kg once every two to three weeks, MK-3475 has been generally well-tolerated at doses up to 10 mg/kg every other week without DLTs. One (0.002%) patient assayed to date had samples confirmed positive for ADA, but no impact on safety has been observed. Five other clinical studies (PN002, PN006, PN010, PN011, and PN012) are ongoing however preliminary data analyses are not yet available. Important safety findings from these studies will be discussed in Section 5 of this report. For these 5 studies, the number of treated patients indicated in this report is based on a visit cut-off date of 18-Oct-2013 to allow for more mature enrollment data and align with the Development Safety Update Report data cutoff date.

MK-3475 PK results have been obtained from PN001 following the first dose at 1, 3 and 10 mg/kg IV of MK-3475 administered to 17 patients with solid tumors. The observed pharmacokinetic profile of MK-3475 was typical of other IgG mAbs with a half-life ($t_{1/2}$) of approximately 2 to 3 weeks. There was no indication of dose dependency of half-life in the 3 dose groups and a dose related increase in exposure was observed from 1 to 10 mg/kg. The long half-life supports a dosing interval of every 2 or 3 weeks. Exposure obtained with sparse sampling after dosing melanoma and non-small cell lung cancer (NSCLC) patients at 2 and 10 mg/kg, every 2 or 3 weeks, is consistent with this profile. As of 18-Oct-2013, PN002 has randomized 497 patients with metastatic melanoma (495 patients were treated) across 3 treatment groups as follows, MK-3475 2 mg/kg Q3W, MK-3475 10 mg/kg Q3W, and chemotherapy (investigator choice of treatment) in a 1:1:1 ratio. PN006 has randomized 68 IPI-naïve patients with unresectable or metastatic melanoma across the 3 treatment groups: 10 mg/kg Q2W, 10 mg/kg Q3W, and Ipilimumab in a 1:1:1 ratio. PN010 has randomized three patients with NSCLC across the 3 treatment groups: 10 mg/kg Q3W, 2 mg/kg Q3W, and docetaxel 75 mg/m2 Q3W in a 1:1:1 ratio. In PN011 10 patients have been treated. In PN012, 109 patients have been treated across the three cohorts as of 18-Oct-2013.

Durable objective responses have been reported in patients with melanoma and NSCLC. Adverse events have generally been manageable and infrequently require discontinuation of MK-3475 treatment.

General Investigational Approach

Six clinical trials are currently evaluating MK-3475: PN001, PN002, PN006, PN010, PN011 and PN012.

PN001 is the Phase I first in human (FIH) study of MK-3475, a dose-escalation study in patients with progressive locally advanced or metastatic carcinomas, along with subject expansion cohorts in MEL and NSCLC. PN001 is an open-label study consisting of 5 primary aspects including the initial dose escalation and subsequent patient expansions. Part A examined 3 dose levels (1, 3, and 10 mg/kg) in patients with solid tumors. With no DLTs observed and no MTD reached, additional PK cohorts were examined at various doses (Parts A-1 and A-2). Subsequent cohorts were then initiated in patients with MEL [Part B (B1, B2, and B3) and D] and NSCLC (Parts C and F) at various dose levels and dose frequencies. Preliminary data from patients enrolled into Parts B1, B2, C, and D are described in Section 5. Data from Part A (30 patients in total) are presented in Appendix 7.2 of IB. Data from Parts B3 and F are not yet available for inclusion in this IB. Each of the two disease specific cohorts (MEL and NSCLC) are enrolled to confirm tolerability and to evaluate tumor response of MK-3475.

PN002 is a Phase II study designed to evaluate 2 doses of MK-3475 versus a chemotherapy control arm in patients with IPI-refractory metastatic melanoma. Patients are randomized in a 1:1:1 ratio to receive blinded MK-3475 2 mg/kg Q3W or MK-3475 10 mg/kg Q3W, or chemotherapy (according to current clinical practice) for the treatment of MEL. Patients assigned to the control chemotherapy arm may cross-over to the experimental MK-3475 arm once progression is confirmed (approximately ≥ Week 12). PN006 is a randomized, controlled, open-label, three-arm pivotal study of two dosing regimens of MK-3475 versus IPI in patients with unresectable or metastatic MEL who have not received IPI treatment. Patients are randomized in a 1:1:1 ratio to receive 10 mg/kg Q2W, 10 mg/kg Q3W, or ipilimumab.

PN010 is multi-center, worldwide, randomized, adaptively designed Phase II/III trial of MK-3475 at two dosing schedules versus docetaxel in patients with NSCLC with PD-L1 positive tumors who have experienced disease progression after platinum-containing systemic therapy. Patients are randomized to receive 10 mg/kg Q3W, 2 mg/kg Q3W, or docetaxel 75 mg/m2 Q3W.

PN011 is an open-label, non-randomized, multi-center Phase I study of MK-3475 alone in Japanese patients with advanced solid tumors, and in combination with cisplatin/pemetrexed and carboplatin/paclitaxel in patients with advanced NSCLC. In Part A (monotherapy 3+3 design), patients with advanced solid tumors are receiving escalating doses of MK-3475 2 mg/kg (Dose level 1) or 10 mg/kg (Dose level 2) Q2W. In Part B (combination, 3+6 design), patients with advanced NSCLC will receive MK- 3475 10 mg/kg Q3W in combination with cisplatin/pemetrexed (Cohort 1), or carboplatin/paclitaxel (Cohort 2).

PN012 is a multicenter, nonrandomized, multi-cohort trial of MK-3475 in patients with PD-L1 positive advanced solid tumors. All patients will receive MK-3475 10 mg/kg Q2W. Cohort A is enrolling patients with triple negative breast cancer, Cohort B is enrolling patients with squamous cell carcinoma of the head and neck, Cohort C is enrolling patients with urothelial tract cancer of the renal pelvis, ureter, bladder, or urethra, and Cohort D is enrolling patients with adenocarcinoma of the stomach or gastroesophageal junction.

In addition, Phase I studies in combination with various standard-of-care agents may be initiated, and single-agent efficacy of MK-3475 may be evaluated in additional solid tumors.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Penile Cancer is a squamous cell carcinoma, which arises in a background of chronic inflammation (either due to lack of circumcision and/or HPV) and the PD-L1 tumor tissue gene expression and protein expression appears to be high according to data using the robust nanostring platform and IHC, respectively. Other data indicate the presence of decreased NK cell activity in PSCC, suggesting that boosting NK and T-cell activity may yield anti-tumor activity [97]. Moreover, the somatic mutation burden in PSCC appears high with approximately a third of tumors demonstrating p53 or PIK3CA mutations, and high somatic mutation burdens may select for tumors with neoantigens that may be particularly responsive to immunotherapy, e.g. melanomas, squamous cell non-small cell-lung cancer and gastroesophageal cancer [98-101].

PD-1 inhibitors have induced responses across a broad spectrum of tumors regardless of PD-L1 expression at the protein level. Therefore, a subset of patients with PSCC is likely to benefit from PD1 inhibitors regardless of tumor PD-L1 expression. This is an orphan malignancy with no approved or effective second-line agents. Hence, a strong rationale may be made to investigate the efficacy of Pembrolizumab in men with locally advanced unresectable or metastatic PSCC progressing after chemotherapy. Given the orphan disease status of PSCC and dismal outcomes with current therapy, the demonstration of response in ≥20% of patients supported by durability of responses in a nonrandomized phase II trial should constitute adequate evidence for clinically meaningful benefit. The employment of immune related response criteria will enable the optimal capture of all patterns of response, which defines progression based on a continuum and does not remove patients for progression based on conventional RECIST (e.g. new lesions) [1]

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent MK-3475 (Pembrolizumab). The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between

clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

4.2.3 Endpoints

4.2.3.1 Efficacy Endpoints

The primary endpoint of the study is Response Rate (RR) using immune related (ir) criteria. Given the poor prognosis of this patient population and data from other studies of salvage therapy, a RR ≥20% is felt to be important for this regimen, while a RR <5% is felt to be of poor interest. [1]: Unidimensional measurements using RECIST 1.1 will be used with the added application of ir criteria [2]. Patients are considered to have irPR or irSD even if new lesions were present, as long as they meet the respective thresholds of response as described above. Patients are not considered to have irPD if new lesions are present and the tumor burden of all lesions does not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by RECIST 1.1 criteria as well as those patients that shift to these irRC categories. The secondary endpoints are duration of Response and Stable disease, Toxicities, Progression Free Survival (PFS) and Overall Survival (OS).

4.2.3.2 Biomarker Research

Twenty archival formalin-fixed paraffin embedded (FFPE) tissue slides per patient may be acquired: 15 slides of 10 µM sections and 5 slides of 5 µM sections. Tumor tissue immune signature panel by nanostring (http://www.nanostring.com/products/pancancer_immune) and IHC for PD-L1 will be performed. The 5 µM section slides may be sent to Merck laboratories for immunohistochemistry for PD-L1. The tumor tissue from the 10 µM section slides may undergo evaluation of the immune signature panel by nanostring at UAB.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Men with pathologically proven PSCC

5.1.2 Subject Inclusion Criteria

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

1. Locally advanced unresectable or metastatic stage 4 (i.e. T4 or N3 or M1) PSCC
2. Radiologic evidence for progressive disease after ≥1 prior platinum containing chemotherapy regimen in the perioperative or metastatic setting.
3. Be ≥ 18 years of age on day of signing informed consent.
4. Have measurable disease based on RECIST 1.1.
5. Have a performance status of 0-2 on the ECOG Performance Scale.
6. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 days of 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** ≥30 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**

	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

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

8. Formalin-fixed paraffin embedded (FFPE) tumor tissue from previous biopsy is requested, but not mandatory.

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

5.1.3 Subject Exclusion Criteria

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

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

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

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

4. Hypersensitivity to Pembrolizumab or any of its excipients.

5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

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

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

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

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.

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

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

10. Has known history of, or any evidence of non-infectious pneumonitis that required steroids or current pneumonitis.

11. Has an active infection requiring systemic therapy.

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

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

14. Is expecting to 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.

15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

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

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

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

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5.1.4 General Guidelines and Registration Process

Eligible patients will be entered on study centrally at the Kirklin Clinic, UAB Hospitals (research nurse to be designated by Elizabeth Busby, Director of Oncology Clinical Trials, CSU, UAB) and the other participating institutions: University of Southern California [USC] (Tanya Dorff, MD), Duke Cancer Institute (Michael Harrison, MD and Brant Inman, MD) and Emory University (Bradley Carthon, MD). All sites should call the Study Monitor at UAB to verify agent availability. Following registration, patients should begin protocol treatment within 72 hours. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The Study Monitor should be notified of cancellations as soon as possible.
The Clinical Trials Network Monitoring Office (CTNMO) of the O'Neal Comprehensive Cancer Center (CCC) coordinates investigator-initiated clinical trials under Good Clinical Practice conditions at participating sites to achieve timely study subject enrolment. Once a study subject has been screened and deemed eligible for study entry by the participating site, a study-specific study subject eligibility checklist, a copy of the dated and signed consent form, and corresponding source documentation are faxed to the participating site study coordinator for eligibility verification. Subsequently, a study-specific number is assigned to the study subject and sent to the participating site. Finally, a Patient Registration Form is completed and faxed by the CTNMO site to the participating study coordinator. Queries regarding data accuracy are forwarded from the CTNMO to the participating site coordinator for clarification or correction. Once the participating site addresses queries, any corrected data forms or copies of corrected source documentation are faxed to the CTNMO.

5.2 Trial Treatments

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

Table 2 Trial Treatment

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

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

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

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

5.2.1.2 Dose Modification

Adverse events (both non-serious and serious) associated with Pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3 below. See Section 5.3, 5.4

and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.

Table 3 Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold Pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume Pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with Pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with Pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
			10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[2]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

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

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

[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

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

5.2.2 Timing of Dose Administration

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

All trial treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Every effort will be taken 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 will be 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.

5.2.3 Trial Blinding/Masking

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

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

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

5.3.1 Acceptable Concomitant Medications

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

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

5.3.2 Prohibited Concomitant Medications

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

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

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

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

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

5.4 Rescue Medications & Supportive Care

5.4.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below and in greater detail in the ECI guidance document version-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 is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document version-5). Refer to Section 5.2.1 for dose modification.

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

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

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

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and

electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:

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

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

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatitis**:

- For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
  - Treat with IV or oral corticosteroids
- For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

  4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of Pembrolizumab (MK-3475).

Table 4 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of Pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

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

5.5.2 Contraception

It is not known if Pembrolizumab has transient adverse effects on the composition of sperm. . Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom, copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2-Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 Use in Pregnancy

Not Applicable

5.5.4 Use in Nursing Women

Not Applicable

5.6 Subject Withdrawal/Discontinuation Criteria

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

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

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

  Note: For unconfirmed radiographic disease progression, please see Section 5.2.2

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 7.1.2.7.1
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment

- Investigator's decision to withdraw the subject
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with Pembrolizumab or 35 administrations of study medication, whichever is later.

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

- Administrative reasons

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

5.6.1. Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with Pembrolizumab and had at least two treatments with Pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with Pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of Pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.5.5.

5.7 Subject Replacement Strategy

Considering there may be inevaluable patients, the study design provides to enroll more patients (5-10%) than the sample size required for final analysis.

5.8 Clinical Criteria for Early Trial Termination

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

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

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

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

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

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	Screening Phase		Treatment Cycles		End of Treat-ment	Post-Treatment	
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	Day 1 of every cycle (21 days)	To be repeated every 3 cycles	Discon	Safety Follow-up	Follow Up Visits[a]
Scheduling Window (Days):	-30 days	-14 days	± 3	± 3	At time of Discon	30 days post discon	Every 8 weeks ± 7 days post discon
Administrative Procedures							
Pre-screening Consent	X						
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Medical History and physical examination	X		X				
Prior and Concomitant Medication Review	X		X				
Pembrolizumab 200 mg IV			X				
Post-study anticancer therapy status					X	X	

Survival Status			X		X	X	X
Clinical Procedures/Assessments							
Review Adverse Events		X	X		X	X	
Full Physical Examination		X	X		X	X	X
Vital Signs and Weight		X	X		X	X	
ECOG Performance Status		X	X		X	X	X
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory							
PT/INR and aPTT		X		X	X	X	
CBC with Differential		X	X		X	X	
Comprehensive Serum Chemistry Panel[b]		X	X		X	X	
Urinalysis		X	X		X	X	
T3, FT4 and TSH		X		X			
Efficacy Measurements							
Tumor Imaging (CT scan or MRI chest, abdomen and pelvis; bone scan if bone pain or raised alkaline phosphatase)	X			X	X		X
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood							
Archival or Newly Obtained Formalin-Fixed Paraffin Embedded (FFPE) tumor Tissue Collection: 15 slides of 10 µM sections, 5 of 5 µM sections	X						

[a] Follow up visit for those patients who have stopped treatment for reasons other than disease progression, Follow up with radiologic scans will be done every 8 weeks (+ 7 days) in first year after discontinuation of treatment for reasons other than progression, then after year 1 every 9 weeks (+ 7 days) thereafter till death, disease progression, start of new anti-neoplastic treatment whichever occurs first.

[b] Comprehensive Serum Chemistry Pane will include alkaline phosphatase, ALT, AST, LDH, and magnesium

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

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

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

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

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

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

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

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

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

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

7.1.1.4.2 Concomitant Medications

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

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

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

7.1.1.6 Assignment of Patient Study Number

Every patient will be assigned a unique patient study number that will have initials of the site and the sequence number for registration once the patient eligibility is confirmed (eg. First patient at UAB will be numbered UA01)

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

Patients will be advised to avoid any foods known to aggravate diarrhea, nausea or vomiting.

Since the treatment is outpatient on day 1 of each cycle, compliance will be correlated by patients visit to clinic. Patients who do not attend a minimum of 75% of scheduled study visits, unless due to exceptional circumstances, should be discussed with the sponsor and be evaluated for compliance.

The investigator and/or the sponsor can withdraw a patient from the study in the event of serious and persistent non-compliance which jeopardizes the patient's safety or render study results for this patient unacceptable.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with Pembrolizumab all AEs of unknown etiology associated with Pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document in Appendix 4 regarding the identification, evaluation and management of potential irAEs.

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

7.1.2.2 Full Physical Exam

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

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7.1.2.3 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 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

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

7.1.2.5 Tumor Imaging and Assessment of Disease by the adaptation of immune related (ir) response criteria to RECIST 1.1

Antitumor effect

Patients continue therapy with tumor CT or MRI (preferably CT) imaging performed every 9 weeks (3 cycles) until progression by immune related (ir) progression. Immune related response criteria are employed to define progression of disease [1]. Unidimensional measurements (changes in only the largest diameter) using RECIST 1.1 will be used with the added application of ir criteria [2, 102]. Baseline-selected target lesions and new measurable lesions should NOT be assessed separately. Measurements of those lesions should be combined into the Total Measured Tumor Burden (TMTB), and one combined assessment provided.

All patients will be evaluable for toxicity from the time of their first treatment with Pembrolizumab. Only those patients 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 response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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 with conventional techniques (CT, MRI) or as ≥10 mm with spiral CT scan. Lymph nodes are considered measurable if the short axis diameter is ≥15 mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. irRECIST 1.1 criteria for unidimensional lesion measurement apply to both target and new measurable lesions, i.e. new lesions are required to have a minimum 10 mm in the longest diameter for non-nodal lesions, and a minimum 15 mm in short axis for lymph nodes. Smaller lesions contribute to the non-target or new non-measurable tumor burden, but do not get measured. Smaller lesions contribute to the non-target or new non-measurable tumor burden, but do not get measured. If new measurable lesions appear in patients with no target lesions at baseline, irPD will be assessed. That irPD timepoint will be considered a new baseline, and all subsequent timepoints will be compared to it for response assessment. irPR is possible if the TMTB of new measurable lesions decreases by ≥ 30% compared to the first irPD documentation. Progression confirmation no less than 4 weeks after the initial irPD assessment is recommended especially in case of marginal disease growth and if the first irPD assessment is within the compound-specific tumor flare window.

Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

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) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

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 nontarget lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up. A substantial and unequivocal increase of non-target lesions is indicative of progression. Baseline selected non-target lesions can never convert to measurable lesions, not even if they increase in size at subsequent timepoints and become measurable. Only true new lesions can be measured and contribute to the TMTB.

Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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 when both methods have been used to assess the antitumor effect of a treatment.

Patients are considered to have irPR or irSD even if new lesions were present, as long as they meet the respective thresholds of response as described above. Patients are not considered to have irPD if new lesions are present and the tumor burden of all lesions does not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by RECIST 1.1 criteria as well as those patients that shift to these irRC categories (see Table below).

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions, unidimensional changes* %	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR‡
↓100	Stable	Any	irPR‡
↓100	Unequivocal progression	Any	irPR‡
↓≥30	Absent/Stable	Any	irPR‡
↓≥20	Unequivocal progression	Any	irPR‡
↓<30 to <20↑	Absent/Stable	Any	irSD
↓<30 to <20↑	Unequivocal progression	Any	irSD
≥20	Any	Any	irPD‡

*Decreases assessed relative to baseline, including measurable lesions only.

†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

7.1.2.6 Tumor Tissue Collection and Correlative Studies Blood Sampling

Twenty archival formalin-fixed paraffin embedded (FFPE) tissue slides per patient will be acquired: 15 slides of 10 µM sections and 5 slides of 5 µM sections. The 5 µM section slides will be sent to Merck laboratories for immunohistochemistry for PD-L1. The tumor tissue from the 10 µM section slides will undergo evaluation of the immune signature panel by nanostring at UAB. FFPE tumor tissue is demarcated from adjacent normal tissue followed by histologic macrodissection. RNA is harvested from tumor and matched normal tissue by the Qiagen method and 100ng are input directly into a hybridization reaction containing color-coded molecular barcodes representing the genes. The reporter probe carries the signal and the capture probe allows the complex to be immobilized for data collection. Profiling is performed for tumors by nanostring utilizing the nCounter customized panel of ~800 cancer-immune profile related human genes (http://www.nanostring.com/products/pancancer_immune) with internal control housekeeping genes utilizing a previously described method [84].

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

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

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	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		PK
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.
‡ If considered standard of care in your region.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with Pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.4.2 Blinding/Unblinding

This is an unblinded study.

7.1.5 Visit Requirements

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

7.1.5.1 Screening

Patients, who have confirmed diagnosis of PSCC and availability of archived biopsy specimen, will undergo screening in 2 visits. First visit will be 30 days before day 1 of cycle 1 and second visit will be 14 days prior to day 1 of cycle 1.

On first visit, the patients will be seen in outpatient clinic and a detailed history will be taken about details pertaining to their course of disease which will include smoking history, demographic information like age, and ethnicity as well as assessment of concomitant medication and adverse events seen in the past if any. Their heights will be recorded and the patients will then undergo CT scan of chest, abdomen and pelvis and details about lesions and spread of cancer will be noted. If the patient has complaints of bone pain in the past, they will also undergo an additional bone scan to determine spread of disease to the bone. All the imaging details will be recorded as baseline records which will be compared to determine disease progression in follow up scans.

At the second visit for screening in outpatient clinic, the patients will have their blood pressure and weight recorded. They will then undergo detailed physical examination by the study doctor. About 10 ml of blood will be withdrawn in laboratory for routine complete blood count with differential count along with complete metabolic panel.

The study doctor will calculate ECOG performance status from the information collected at screening visit.

All details collected in the 2 visits will be first recorded in patient case files and then transferred to CRF provided by the sponsor.

For those patients who will be determined eligible for the study, archived tumor biopsy specimen will be obtained and reviewed by central laboratory. This specimen will be used to prepare 20 Formalin Fixed Paraffin Embedded slides. Of these, 15 slides will have sections of 10 µM thickness while 5 slides will have sections of 5 µM thickness. The slides with 10 µM thickness will be sent to Dr. Eddy Yang's laboratory situated in UAB Department of Radiation Oncology for gene expression studies using nanostring technology. Immunohistochemistry studies and HPV DNA analysis will be conducted on 5 µM thick sections.

7.1.5.2 Treatment Period

Patients who are eligible for the study, will then start treatment cycles with Pembrolizumab 200 mg IV on day 1. Each cycle corresponds to duration of 3 weeks. Thus the next dose will be scheduled after 3 weeks.

History will be taken on course of disease since screening and during the cycle of treatment along with adverse events assessment during treatment cycle; weight and blood pressure will be recorded; detailed physical examination will be conducted; blood will be withdrawn for routine complete and differential blood count as well as complete metabolic profile.

On follow-up visits every 9 weeks thereof, patients will undergo CT scan of chest, abdomen and pelvis to document response of disease to the medication. If the patient had undergone baseline Bone scan at screening visit, a repeat bone scan will be conducted along with CT scan.

ECOG performance status will be calculated at each visit to determine if patient is tolerating the treatment well.

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

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

7.1.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8 weeks (56 ± 7 days) by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 9 weeks (± 7 days). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with Pembrolizumab as detailed in Section 7.1.5.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with Pembrolizumab according to the criteria in Section 7.1.5.5 will move from the follow-up phase to the Second Course Phase when they experience disease progression. Details are provided in Section 6.2 – Trial Flow Chart for Retreatment.

7.1.5.4.1 Survival Follow-up

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

7.1.5.5 Second Course Phase (Retreatment Period)

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

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

OR

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

AND

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

- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.1.2
- Subject should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

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

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

7.2 Assessing and Recording Adverse Events

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

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

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

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1.

Data and Safety Monitoring Plan

The O'Neal Comprehensive Cancer Center Data and Safety Monitoring Plan (DSMP) instituted by the CTNMO will monitor subjects treated at UAB and all of the other institutions participating in the trial. The Clinical Trials Monitoring Committee (CTMC) on a weekly basis will closely monitor adverse reactions observed during treatment. The CTMC is responsible for data and safety monitoring of the trial and adherence to the DSMP. The office of CTNMO will also report any SAE at participating sites outside UAB to the CTMC. The independent Quality Assurance Committee (QAC) is responsible for oversight of the operation of CTMC, including adherence to the DSMP. Reports from the CTMC are reviewed monthly by the QAC.

Protocol Management and Oversight of Participating Site

Dr. Lisle Nabell functions as the sponsor of the trial at UAB and at the participating sites. The participating sites will utilize their respective IRB of record. The Lead Investigator at participating site(s) will be responsible for ensuring that all the required data will be collected and entered onto the Electronic Case Report Forms (eCRFs). A teleconference between UAB CTNMO and participating sites every 3 months will ensure discussion of AEs seen across all sites. UAB PI, CTNMO manager and participating site Lead Investigators will discuss management of AEs, and impact of AEs on trial conduct. The CTNMO office will report the outcome of these discussions at CTMC meeting every month. Periodically, monitoring visits will be conducted by CTNMO manager and the participating site Lead Investigator will provide access to his/her original records to permit verification of proper data entry. At the completion of the study, all case report forms will be reviewed by the CTNMO manager.

Table 6. AE/SAE reporting requirements

Time period	**Reporting requirements**
From signing of informed consent to ≤90 days after last trial drug administration or initiation of new anticancer drug, whichever is earlier	Report all AEs and SAEs regardless of relatedness or whether the trial drug was administered. This includes all deaths.
Post-treatment (>90 days after last trial drug administration or after initiation of new anticancer drug, whichever is earlier)	Report only SAEs which are considered related to trial treatment or trial design. Death should be reported as an SAE only when considered related to trial treatment or trial design (because death is an

	endpoint and will be followed-up separately).

All adverse events, serious and non-serious, will be collected, documented and reported to the sponsor by the Lead site investigator on the appropriate CRFs / SAE reporting forms (Merck SAE report forms).

For each adverse event, the investigator will provide the onset date, end date, CTCAE grade, treatment required, outcome, seriousness, and action taken with the investigational drug. The investigator will determine the relationship of the investigational drug to all AEs as defined in Table 6.

7.2.1 Definition of an Overdose for This Protocol 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 subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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

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

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

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

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

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

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

7.2.3.1 Serious Adverse Events

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

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

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

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. Serious Adverse Events (SAEs) are reported by the participating site Lead Investigator within 24 hours to the Clinical Trial Network Monitoring Office (CTNMO) Manager (Pam Dixon) by email (pamdixon@uab.edu) or by fax (205) 975-9875. The 24 hour paging number for the CTNMO Manager is (205) 934-3411, beeper #5904. The CTNMO Manager is then responsible for reporting SAEs to the UAB IRB, FDA and protocol P.I. in accordance with study-specific requirements. SAEs occurring at CTNMO sites are reported to the UAB IRB as "non-UAB" events

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

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

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 outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck.

It is also the responsibility of the participating site Lead Investigator to report SAEs to the local site IRB and to submit copies of that report to the CTNMO Manager. It is the CTNMO Manager's responsibility to report the SAE to the Clinical Trials Monitoring Committee (CTMC), UAB IRB, sponsor/Principal Investigator (Lisle Nabell, MD from UAB) and Merck.

This submission of IND Safety Reports will be cross referenced according to local regulations to Merck Investigation New Drug (IND) at the time of submission

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

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

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

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

1. Additional adverse events:

   A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"). This document can be found in Appendix 4 and provides guidance regarding identification, evaluation and management of ECIs and irAEs.

   ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported

within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

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

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 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.

Table 7 Evaluating Adverse Events

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 subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	

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

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the 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 Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge.

		(Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

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

8.0 STATISTICAL ANALYSIS PLAN

The justification for the proposed sample size is based on an exact test for a binomial proportion with a two-sided significance level of 5%. With 32 evaluable subjects, we will have approximately 80% power (79.6%) to reject the null hypothesis that RR < 5% in favor of RR ≥ 20%. Given that 5-10% of patients may be inevaluable, up to 35 patients may be enrolled. If ≥5 patients respond, this regimen will be considered to be of significant interest. Chi-square or Fisher's exact test will be employed to determine univariate association of biomarker status with response. Descriptive statistics including frequencies and proportions will be calculated to summarize safety and toxicity outcomes. Specifically, the number of adverse events (AE) and serious adverse events (SAE) will be tabulated and summarized. Point estimates and exact binomial (Clopper-Pearson) confidence intervals (CI) will be calculated to estimate the incidence of AEs and SAEs. The precision of these estimates, i.e., width of the confidence interval, will depend on the estimated incidence of each outcome. The table below gives the exact 95% confidence intervals for a range of incidences, assuming 32 evaluable subjects.

Number of subjects experiencing event	Estimated Incidence	95% Exact CI for Incidence		Width of CI
		Lower Limit	Upper Limit	
1	0.031	0.001	0.162	0.161
2	0.062	0.008	0.208	0.200
4	0.125	0.035	0.290	0.255
5	0.156	0.053	0.328	0.275
8	0.250	0.115	0.434	0.319
16	0.500	0.319	0.681	0.362

SAS version 9.3 was used to calculate power.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

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

Clinical Supplies will be provided by Merck as summarized in Table 8.

Table 8 Product Descriptions

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

9.2 Packaging and Labeling Information

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

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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

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

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

9.5 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted below. Patient confidentiality will be ensured by using Medical Record Number (MRN).

Treatment data can be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare. Data generated as a result of the study need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the EC and the regulatory authorities.

10.2 Compliance with Financial Disclosure Requirements

All potential conflicts of interest will be available for the Principal Investigator and all coinvestigators.

10.3 Compliance with Law, Audit and Debarment

A quality assurance audit/inspection of this study may be conducted by ECs or by regulatory authorities. The quality assurance auditor will have access to all medical records, the investigator's study-related files and correspondence, and the informed consent documentation of this clinical study

10.4 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 subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.5 Quality Management System

This study will be initiated only after all required legal documentation has been reviewed and approved by the University of Alabama at Birmingham Comprehensive Cancer Center's (UAB CCC) Protocol Review Committee (PRC), Institutional Review Board (IRB) and Office of Sponsored Programs (OSP) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.

Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains in the responsibility of the treating physician of the patient.

The investigator will inform the sponsor immediately of any urgent safety measures taken to protect the study subjects against any immediate hazard, and also of any serious breaches of the protocol/ICH GCP.

10.6 Data Management

10.6.1 Source documents

Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the (e)CRFs that are transcribed from source documents will be consistent with the source documents or the discrepancies will be explained. The investigator may need to request previous medical records or transfer records, depending on the study; also current medical records will be available.

For (e)CRFs all data will be derived from source documents.

10.6.2 Direct access to source data and documents

The investigator / institution will permit study-related monitoring, audits, EC review and regulatory inspection, providing direct access to all related source data / documents. (e)CRFs and all source documents, including progress notes and copies of laboratory and medical test results will be available at all times for review by the on-site monitor, auditor and inspection by health authorities (e.g. FDA). The Clinical Trials Network Monitoring Office (CTNMO) and auditor will review all (e)CRFs, and written informed consents. The accuracy of the data will be verified by reviewing the documents described in Section 10.6.1.

11.0 APPENDICES

11.1 ECOG Performance Status

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

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

11.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

11.3 Immune related (ir) Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

Antitumor effect

Patients continue therapy with tumor CT or MRI (preferably CT) imaging performed every 9 weeks (3 cycles) until progression by immune related (ir) progression. Immune related response criteria are employed to define progression of disease [1]. Unidimensional measurements (changes in only the largest diameter) using RECIST 1.1 will be used with the added application of ir criteria [2, 102]. Baseline-selected target lesions and new measurable lesions should NOT be assessed separately. Measurements of those lesions should be combined into the Total Measured Tumor Burden (TMTB), and one combined assessment provided.

All patients will be evaluable for toxicity from the time of their first treatment with Pembrolizumab. Only those patients 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 response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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 with conventional techniques (CT, MRI) or as ≥10 mm with spiral CT scan. Lymph nodes are considered measurable if the short axis diameter is ≥15 mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. irRECIST 1.1 criteria for unidimensional lesion measurement apply to both target and new measurable lesions, i.e. new lesions are required to have a minimum 10 mm in the longest diameter for non-nodal lesions, and a minimum 15 mm in short axis for lymph nodes. Smaller lesions contribute to the non-target or new non-measurable tumor burden, but do not get measured. Smaller lesions contribute to the non-target or new non-measurable tumor burden, but do not get measured. If new measurable lesions appear in patients with no target lesions at baseline, irPD will be assessed. That irPD timepoint will be considered a new baseline, and all subsequent timepoints will be compared to it for response assessment. irPR is possible if the TMTB of new measurable lesions decreases by ≥ 30% compared to the first irPD documentation. Progression confirmation no less than 4 weeks after the initial irPD assessment is recommended especially in case of marginal disease growth and if the first irPD assessment is within the compound-specific tumor flare window.

Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

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) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

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 nontarget lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up. A substantial and unequivocal increase of non-target lesions is indicative of progression. Baseline selected non-target lesions can never convert to measurable lesions, not even if they increase in size at subsequent timepoints and become measurable. Only true new lesions can be measured and contribute to the TMTB.

Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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 when both methods have been used to assess the antitumor effect of a treatment.

Patients are considered to have irPR or irSD even if new lesions were present, as long as they meet the respective thresholds of response as described above. Patients are not considered to have irPD if new lesions are present and the tumor burden of all lesions does not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by RECIST 1.1 criteria as well as those patients that shift to these irRC categories (see Table below).

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions, unidimensional changes* %	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR‡
↓100	Stable	Any	irPR‡
↓100	Unequivocal progression	Any	irPR‡
↓≥30	Absent/Stable	Any	irPR‡
↓≥20	Unequivocal progression	Any	irPR‡
↓<30 to <20↑	Absent/Stable	Any	irSD
↓<30 to <20↑	Unequivocal progression	Any	irSD
≥20	Any	Any	irPD‡

*Decreases assessed relative to baseline, including measurable lesions only.

†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

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11.5 Events of Clinical Interest Guidance Document

11.5. Comparision of Key Elements in Radiographic Assessment of Solid Tumors [Eisenhauer 2009, Wolchock 2009]

Comparison of Key Elements in the Radiographic Assessment of Solid Tumo

	New, measurable lesions (i.e., ≥5x5 mm)
irRC	Incorporated in tumor burden
mWHO	Always represent progressive disease
RECIST 1.1	Always represent progressive disease
	New, non- measurable lesions (i.e., ≥5x5 mm)
irRC	Do not define progression (but preclude immune-related complete response)
mWHO	Always represent progressive disease
RECIST 1.1	Always represent progressive disease
	Non-index lesions
mWHO	Changes contribute to defining best overall response of complete or partial response and stable or
irRC	Contribute to defining immune-related complete response (complete disappearance required)
RECIST 1.1	Changes contribute to defining best overall response of complete or partial response and stable or
	Complete Response (CR)
irRC	Disappearance of all lesions in two consecutive observations ≥ 4 weeks apart
mWHO	Disappearance of all lesions in two consecutive observations ≥ 4 weeks apart
RECIST 1.1	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) m Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes n
	Partial Response (PR)
irRC	≥ 50 % decrease in tumor burden vs. baseline in two observations at least 4 weeks apart
mWHO	≥ 50 % decrease in SPD* of all index lesions vs. baseline in two observations at least 4 weeks apa progression of non-index lesions
RECIST 1.1	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline
	Stable Disease (SD)
irRC	50 % decrease in tumor burden vs. baseline cannot be established nor 25 % increase vs. nadir
mWHO	50 % decrease in SPD vs. baseline cannot be established nor 25 % increase vs. nadir, in absence index lesions
RECIST 1.1	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as refer Persistence of one or more non-target le- sion(s) and/or maintenance of tumour marker level above
	Progressive Disease (PD)
irRC	At least 25 % increase in tumor burden vs. nadir (at any single time point) in two consecutive obser
mWHO	At least 25 % increase in SPD vs. nadir and/or unequivocal progression of non-index lesions and/o
RECIST 1.1	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate progression of existing non-target lesions. The appearance of one or more new lesions is also cons

*SPD = sum of products of the 2 largest perpendicular diameters

^ = Non-complete response/non-progressive disease is preferred over stable disease when assessing non-target l