HOUSTON Methodist NEAL CANCER CENTER

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TITLE: Phase II Window of Opportunity Trial of **S**tereotactic Body Radiation **T**herapy and In Situ **O**ncolytic Virus Therapy in **M**etastatic Triple Negative Breast Cancer and Metastatic Non-Small Cell Lung Cancer Followed by **P**embrolizumab (STOMP)

SHORT TITLE: SBRT and Oncolytic Virus Therapy Before Pembrolizumab for Metastatic TNBC and NSCLC (STOMP)

PRINCIPAL INVESTIGATOR:	Jenny Chang, M.D.
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1.0 TRIAL SUMMARY

Abbreviated Title	Stereotactic Body Radiation Therapy and In Situ Oncolytic Virus Therapy in Metastatic Triple Negative Breast Cancer and Metastatic Non-Small Cell Lung Cancer Followed by Pembrolizumab
Trial Phase	II
Clinical Indication	Metastatic triple negative breast cancer (TNBC) and metastatic non-small cell lung cancer (NSCLC)
Trial Type	Window of opportunity
Type of control	None
Route of administration	Pembrolizumab, intravenous infusion; oncolytic virus therapy (adenovirus-mediated expression of herpes simplex virus thymidine kinase [ADV/HSV-tk], intratumoral injection; valacyclovir, oral
Trial Blinding	None
Treatment Groups	1
Number of trial subjects	57 (TNBC, 28; NSCLC, 29)
Estimated enrollment period	18–24 months
Estimated duration of trial	36 months
Duration of Participation	Continuing until disease progression, unacceptable toxicity, or up to 24 months in subjects without disease progression
Estimated average length of treatment per patient	Continuing until disease progression, unacceptable toxicity, or up to 24 months in subjects without disease progression

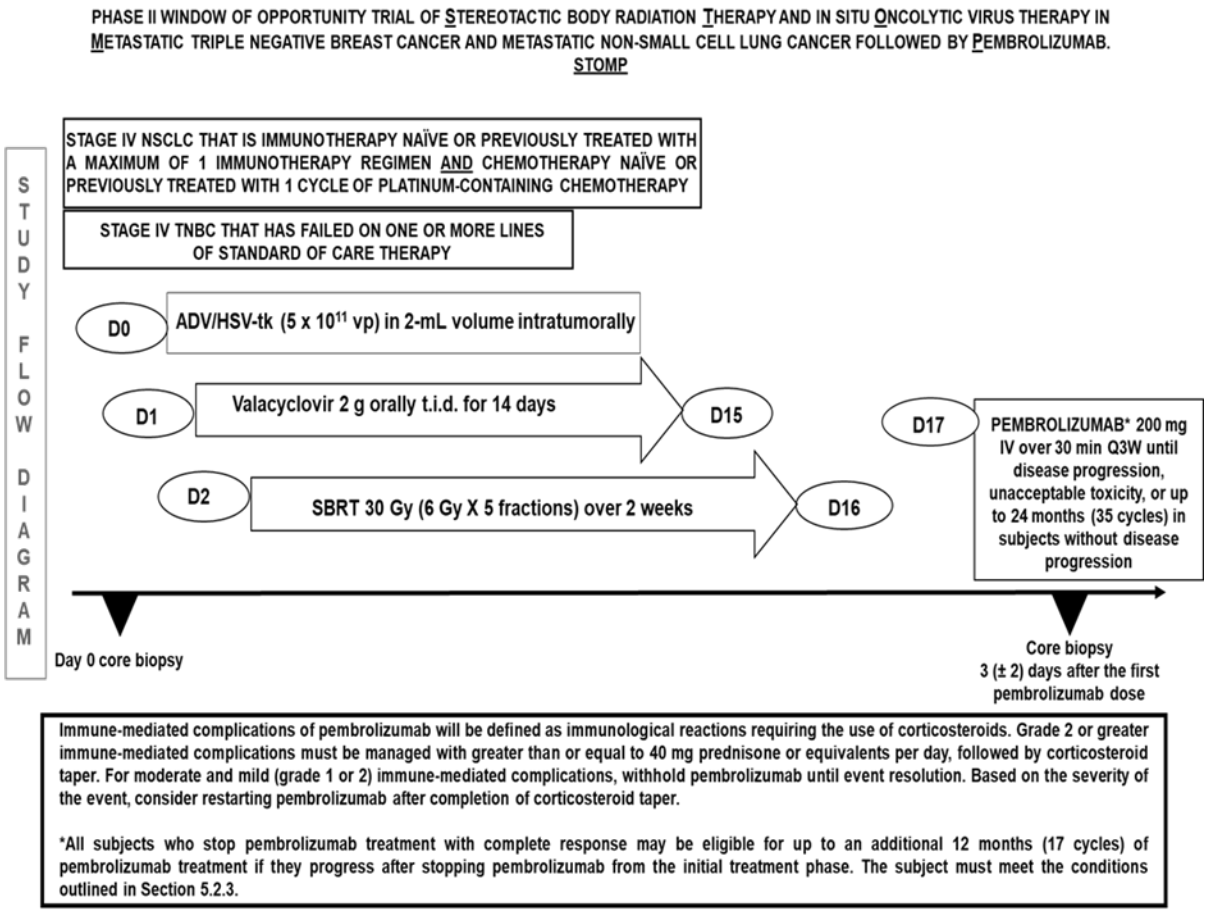
2.0 TRIAL DESIGN

2.1 Trial Design

This is a Phase II window of opportunity study evaluating the efficacy and toxicity of stereotactic body radiation (SBRT) and in situ oncolytic virus therapy (adenovirus-mediated expression of herpes simplex virus thymidine kinase [ADV/HSV-tk]) followed by pembrolizumab in patients with metastatic triple negative breast cancer (TNBC) and metastatic non-small cell lung cancer (NSCLC).

2.2 Trial Diagram

vp = viral particle; t.i.d. = three times daily; IV = intravenously; Q3W = every 3 weeks.



3.0 OBJECTIVES

3.1 Primary Objective

Objective: To determine the objective response rate (ORR) of ADV/HSV-tk plus (+) valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC. Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 will be used to assess treatment response. Modified immune-related response criteria (irRC; derived from RECIST 1.1) will also be documented.

3.2 Secondary Objectives

Objectives:
(1) To determine the duration of response (DoR) of ADV/HSV-tk + valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC.

(2) To determine the overall survival (OS) rate of ADV/HSV-tk + valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC.

(3) To determine the progression-free survival (PFS) rate of ADV/HSV-tk + valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC.

(4) To document the toxicities associated with ADV/HSV-tk + valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC. Toxicity will be defined as any treatment-related death or any ≥ Grade 3 hematological toxicity excluding alopecia and constitutional symptoms, as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03.

(5) To document the antitumor activity of ADV/HSV-tk + valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC, as assessed by the RECIST 1.1. Modified irRC will also be documented.

(6) To estimate the clinical benefit rate (CBR) of ADV/HSV-tk + valacyclovir therapy in combination with SBRT used as a window of opportunity treatment before pembrolizumab in patients with metastatic TNBC and metastatic NSCLC.

3.3 Exploratory Objectives

Objectives:

(1) To determine the abscopal effect of ADV/HSV-tk + valacyclovir therapy in combination with SBRT followed by pembrolizumab. The primary criterion for abscopal effect evaluation will be computed tomography (CT)-based response assessment (RECIST 1.1) of a non-target lesion. The secondary criterion will be immune parameters including programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) expression, immune infiltrates, and cytokine expression (interleukin [IL]-1, IL-2, IL-6, IL-12, interferon [IFN]-c, tumor necrosis factor-α [TNF-α], and granulocyte macrophage colony-stimulating factor [GM-CSF]).

(2) To measure the immune response to ADV/HSV-tk + valacyclovir therapy in combination with SBRT followed by pembrolizumab in patients with metastatic TNBC and metastatic NSCLC.

(3) To explore correlative tissue and blood-based biomarkers including but not limited to T-cell cytokine profiles (IL-1, IL-2, IL-6, IL-12, [IFN-c, TNF-α, and GM-CSF), tumor-infiltrating lymphocytes (TILs), PD-1 and PD-L1 expression, effector and suppressor immunocyte populations, and mutation load.

4.0 BACKGROUND & RATIONALE

4.1 Background

Despite the significant advances in our understanding of breast cancer biology, limited progress has been made in the treatment of advanced breast cancer. There has been little change in the OS of women with treatment-resistant metastatic breast cancer over the last several decades.[1] Notably, approximately 40,000 women with metastatic breast cancer die each year primarily due to treatment resistance and failure. TNBC is a mutationally complex breast cancer subtype with poor prognosis and no current targeted therapy options. Compared with other intrinsic breast cancer subtypes, TNBC has higher expression levels of PD-L1 and its receptor PD-1, which may hinder antitumor T cell responses. Immunotherapy has demonstrated promising efficacy in pretreated metastatic TNBC and appears to be one of future treatment choices for resistant cancers.[2]

Lung cancer is the leading cause of cancer death in the United States. Lung cancer is expected to account for an estimated 224,390 new cases (117,920 in men and 106,470 in women) and 158,080 deaths (85,920 in men and 72,160 in women) in 2016.[1] Only 16.8% of lung cancer patients are alive for 5 years or more after their diagnosis. NSCLC accounts for over 85% of all lung cancer cases. Most patients are diagnosed with advanced or metastatic (stage IIIB/IV) disease,[3] and current first-line treatment options for these patients are limited. In patients with advanced NSCLC, first-line platinum-based doublet chemotherapy yields 1-year OS rates of only 30–40% and can cause significant toxicities that may complicate treatment.[3] However, much progress has recently been made in the diagnosis and treatment of lung cancer including screening methods, minimally invasive techniques for diagnosis and treatment, and advances in radiation therapy (RT) including SBRT, targeted therapies, and immunotherapies.[4-8]

Cancer immunotherapy harnesses and boosts the innate powers of the immune system to fight cancer and represents the most promising new cancer treatment approach since the development of chemotherapeutic agents in the late 1940s. Because of the extraordinary memory and specificity of the immune system, immunotherapy has the potential to achieve complete, long-lasting remissions with few or no side effects in cancer patients, regardless of their cancer type. Immunotherapies targeting PD-1 have shown unprecedented rates of durable clinical responses in patients with various cancer types.[9-13] Upregulation of PD-L1 and its ligation to PD-1 on antigen-specific CD8+ T-cells (termed adaptive immune resistance) represents a major mechanism by which cancer tissues limit the host immune response.[14, 15] Under healthy conditions, PD-1, expressed on the cell surface of activated T-cells, functions to downmodulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to CD28 and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) that 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 its family members are type I transmembrane glycoproteins containing an Ig variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an

immunoreceptor tyrosine-based inhibition motif and immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases Src homology phosphatase (SHP)-1 and SHP-2 to the ITSM motif within its cytoplasmic tail. This leads to the dephosphorylation of effector molecules such as CD3ζ, protein kinase-θ, and ZAP70, which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 downmodulates 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, regulatory T cells (Tregs), and natural killer (NK) cells. PD-1 expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells (DCs). 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 and various tumors. Both ligands are type I transmembrane receptors containing both IgV- and Ig constant-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 and 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 have been 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 an attractive target for therapeutic intervention.

Clinical trials have demonstrated the therapeutic potential of PD-1 blockade therapy. Brahmer et al. demonstrated that, among patients with previously treated advanced squamous NSCLC, OS, response, and PFS rates were significantly better with the PD-1 inhibitor nivolumab than with docetaxel regardless of PD-L1 expression level. The confirmed ORR was significantly higher with nivolumab than with docetaxel (20% [95% confidence interval (CI): 14–28] vs. 9% [95% CI: 5–15]; P = 0.008).[16] Herbst et al. found that treatment with MPDL3280A, a PD-L1-specific monoclonal antibody (mAb), induces therapeutic responses in patients with advanced NSCLC, renal cell cancer, and melanoma.[17] Powles et al. showed that the same antibody can be used to treat urothelial bladder cancer. Among patients with a minimum of 6 weeks of follow-up, ORRs were 43% (13/30; 95% CI: 26–63) for those with tumor PD-L1 immunohistochemical (IHC) expression scores of 2 or 3 (2/3) and 11% (4/35; 95% CI: 4–26) for those with tumor PD-L1 IHC expression scores of 0 or 1 (0/1). In the tumor PD-L1 IHC expression 2/3 score group, the ORR included a 7% complete response (CR) rate (2/30). Among patients with a tumor PD-L1 IHC expression score of 2/3 and a minimum of 12 weeks of follow-up, an ORR of 52% (13/25; 95% CI: 32–70) was achieved. Sixteen of the 17 responders had ongoing responses, and all 17 responders continued on MPDL3280A treatment until the data cutoff. One patient who initially responded at the first response assessment later presented with new lesions, including a bladder mass thought to be consistent with pseudo-progression.[18] Anti-PD-L1 immunotherapy has also shown promising results in TNBC.[2] The

latest data analysis of a Phase I clinical trial of MPDL3280A in TNBC revealed a 24-week PFS rate of 27% and ORR rate of 19%, with three of four responses ongoing. This data is encouraging, because longer responses do not typically occur in metastatic TNBC patients treated with chemotherapy, the standard of care for this population.[2] Together, these studies demonstrate the durable responses and low toxicity rates of anti-PD-1 immunotherapy. This is particularly important as high-grade adverse effects have limited the use of immunotherapy for cancer treatment in the past. PD-L1/PD-1 expression in cancer cells is an obvious candidate biomarker for immunotherapy response, as PD-L1/PD-1 can directly turn off the immune response by inhibiting the activity of tumor-infiltrating cytotoxic T-lymphocytes (CTLs). However, PD-L1 and PD-1 expression in tumor cells has been shown to have little predictive power. On the other hand, Herbst et al. reported that PD-L1 expression in immune cells is a good biomarker of response to immunotherapy.[17] The finding that the complexity of the T-cell population in the tumor infiltrate can predict good response to checkpoint blockade therapy highlights the importance of identifying tumor antigens that can elicit an effective antitumor immune response. Previous studies have suggested that tumors with a high load of somatic mutations are more likely to respond to immunotherapy, as in theory these tumors would have a higher diversity of neoantigens that can trigger an immune response when CTLA-4/PD-1 inhibition is bypassed.

Pembrolizumab (MK-3475) is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Antibody-mediated PD-1 blockade with pembrolizumab and other similar agents reinvigorates the immune system, allowing for cancer cell targeting and destruction. Pembrolizumab is one of a number of closely related therapies dubbed immune checkpoint blockade.[19] On September 4, 2014, Keytruda™ (pembrolizumab) was approved by the United States Food and Drug Administration (FDA) for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. On October 2, 2015, Keytruda™ (pembrolizumab) received accelerated FDA approval for the treatment of patients with metastatic PD-L1-positive NSCLC whose disease has progressed on or after platinum-containing chemotherapy or targeted therapy against anaplastic lymphoma kinase (ALK) or epidermal growth factor receptor (EGFR), if appropriate. Keytruda™ is approved for use with a companion diagnostic, the PD-L1 IHC 22C3 pharmDx test, the first test designed to detect PD-L1 expression in NSCLC tumors. FDA approval for both disease indications was based on the results from the open label, first-in-human Phase Ib KEYNOTE-001 study (NCT01295827) of pembrolizumab in patients with progressive locally advanced or metastatic carcinomas. The melanoma cohort included patients with advanced disease previously treated with ipilimumab or ipilimumab in combination with a BRAF inhibitor (for *BRAF* mutation carriers).[20] After mandatory biopsy, patients were treated with one of three doses of pembrolizumab for 12 weeks; responders continued on treatment until disease progression. Among 173 patients treated with the recommended 2-mg/kg pembrolizumab dose, the overall response rate was 24%, with a DoR of 1.4 to 8.5 months. The NSCLC cohort included 550 patients with metastatic disease.[21] The ORR in the efficacy population, which comprised 61 patients with PD-L1 strongly positive tumors, was 41% (95% CI: 28.6–54.3); all were partial responses (PRs). At the time of the analysis, responses were ongoing in 21 of 25 (85%) patients, with 11 (44%) patients having a

DoR of ≥ 6 months. The most commonly occurring (≥ 20%) adverse events (AEs) included fatigue, decreased appetite, dyspnea, and cough. The most frequent (≥ 2%) serious AEs (SAEs) were pleural effusion, pneumonia, dyspnea, pulmonary embolism, and pneumonitis. Immune-mediated AEs occurred in 13% of patients and included pneumonitis, colitis, hypophysitis, and thyroid disorders. Multiple ongoing clinical trials in all phases (NCT02551432, NCT02520154, NCT02530502, NCT02446457, NCT02306850, NCT02402920, NCT02298959, and NCT02560636) are evaluating pembrolizumab monotherapy and combination therapies for the treatment of all stages of liquid and solid malignancies, including TNBC. The KEYNOTE-086 study (NCT02447003) is a Phase II trial of pembrolizumab monotherapy in metastatic TNBC patients. The KEYNOTE-119 study (NCT02555657) is an open label, randomized Phase III study of single-agent pembrolizumab versus single-agent chemotherapy per physician's choice for metastatic TNBC. The KEYNOTE-173 study (NCT02622074) is a Phase Ib study to evaluate the safety and clinical activity of pembrolizumab in combination with chemotherapy as neoadjuvant treatment for TNBC. Together, these studies will pave the way for expanding the indications for pembrolizumab.

RT is proven to provide local tumor control. RT has traditionally been thought to mediate tumor regression through direct cytotoxic effects. However, it is now known that RT also alters the local tumor microenvironment to affect both local and systemic antitumor immune responses. There is growing evidence that the rational integration of RT with the expanding armamentarium of clinically approved immunotherapeutics can yield potent antitumor responses exceeding those of either therapy alone.[22] The beneficial effects of RT in cancer patients extend beyond direct tumor cell cytotoxicity. Delivery of localized radiation to tumors often leads to systemic responses at distant sites, a phenomenon known as the abscopal effect, which has been attributed to the induction and enhancement of endogenous antitumor innate and adaptive immune responses. The mechanisms surrounding the abscopal effect are diverse and include trafficking of lymphocytes into the tumor microenvironment, enhanced tumor recognition and killing via upregulation of tumor antigens and antigen-presenting machinery, and induction of positive immunomodulatory pathways.[23] Cytokines play an important role in the abscopal effect. In one case, a Japanese patient receiving RT for thoracic vertebral bone metastasis experienced spontaneous regression of an unrelated hepatocellular carcinoma. Pre- and post-analyses of serum cytokine levels revealed a marked elevation in TNF-α following RT, suggesting that abscopal-mediated regression may involve such cytokines as part of the host immune response.[24] RT-induced IFN-β has been shown to enhance T-cell-dependent tumor regression by increasing the cross-priming capacity of tumor-infiltrating DCs *in vivo*. This effect can be mimicked by exogenous IFN-β delivery to tumor tissues.[25] Immune cell mediation of the abscopal effect is supported by the observation that exogenous administration of chemokines following local RT enhances tumor cell killing at distal sites. This abscopal effect was tumor-type independent and involved the infiltration of CD8+ and CD4+ lymphocytes and NK1.1+ NK cells into the tumor sites of mice.[26] The abscopal effect remains an active area of investigation in the immunotherapy field. CTLA-4, a negative regulator of cytotoxic CD8+ T-cells, has been targeted as a means to activate antitumor immune CTLs. CTLA-4 blockade has been shown to decrease the threshold of activation of endogenous tumor-reactive T-cells in mouse xenografts.[27] Local RT and CTLA-4 blockade have recently been shown to significantly reduce the motility of TILs at tumor sites, thereby allowing TILs

to engage in stable interactions with tumor targets.[28] The NK group 2, member D (NKG2D) ligand retinoic acid early inducible-1 is upregulated in irradiated neoplastic cells; interaction with its receptor, NKG2D, on CTLs costimulates and enhances tumor cell killing. T-cell receptor, NKG2D, and CTLA-4-transduced signals contribute to the stability of the immunological synapse. Their association appears to be mediated in part by increased antibody responses to the multiple tumor antigens released after RT.[29]

Multiple factors contribute to the development of an abscopal effect. The abscopal effect involves the interplay of irradiation and induction of adaptive immune responses leading to tumor cell elimination at distant sites. Antitumor CTL responses, which represent the outcome of an abscopal effect primed by irradiated tumor cells, seem to play a significant role in RT-induced antigen-specific immunity.[30] Further evidence that antigen-specific T cells are elicited after RT is borne out by studies demonstrating a significant CD8+ T-cell-mediated reduction in systemic tumor burden after local ablative RT.[31] T-cell priming following RT-mediated tumor cell death has been postulated to occur through DC cross presentation of released tumor antigens in draining lymph nodes, leading to primary or metastatic tumor rejection.

Antigen presentation by DCs seems to be crucial to RT-induced CD8+ T cell-dependent antitumor immunity. Antigens can be endogenously or exogenously loaded onto major histocompatibility complex (MHC) class I molecules. RT seems to differentially affect these two antigen presentation pathways. Presentation of endogenous antigens is blocked in irradiated DCs, whereas presentation of exogenously pulsed peptide antigens is enhanced in irradiated DCs, leading to favorable antitumor T-cell responses.[32] RT dose-dependently facilitates cell surface expression of MHC class I molecules by three different mechanisms: (1) induction of protein unfolding and degradation to increase the intracellular peptide pool; (2) enhancement of protein synthesis to increase the intracellular peptide pool; and (3) generation of radiation-specific peptide antigens to increase the diversity of the intracellular peptide pool. By increasing the quantity and/or diversity of the peptide pool, RT leads to an overall increase in the number and density of surface peptide/MHC class I complexes expressed on murine DCs.[33] In some cases, RT upregulates cancer-testis antigens, a class of potentially immunogenic tumor rejection antigens, which can be targeted with adoptive T-cell therapy and other antigen-specific immune-based approaches.[34] One significant hurdle faced by T-cell-based immunotherapies is downregulation of MHC genes, which may represent an important mechanism by which tumor cells, especially those breaching the interface between normal and malignant tissues, evade host immune surveillance.

It is generally believed that Tregs are more radioresistant than conventional effector T-cells[35] and may be over-represented in RT-treated patients compared with RT-naïve patients.[36] Radiation has been shown to upregulate transforming growth factor-β and adenosine A2A in head and neck squamous cell carcinoma patients.[37] This can provide both a growth and survival advantage to Tregs[38], thereby suppressing the potential beneficial antitumor effects of RT. Strategies to eliminate or suppress the number and activity of Tregs such as adjusting RT dose and schedule would enhance RT-induced antitumor responses.

Abscopal effects have been mainly observed in patients with lymphoid malignancies wherein radiation or treatment of local disease led to regression in distant unirradiated sites.[39-41] However, notable cases have been observed in Merkel cell carcinoma,[42] advanced uterine cervical carcinoma,[43] and hepatocellular carcinoma. Irradiation of affected lymphoid sites may be more likely to incite systemic immunity because of the higher likelihood of immune effectors trafficking through these regions and encountering released antigen. However, irradiation of affected visceral sites including bone, skin, and parenchyma has also been shown to induce abscopal effects.[43, 44] In cancer therapies, some notions of metastasis and recurrence may be explained using oligometastases and oligo-recurrence. Oligometastases is the state capable of achieving long-term survival or cure with local therapy despite active primary legions. On the other hand, oligo-recurrence is the notion that metastatic and recurrent lesions could be treated with local therapy since the primary lesions have been controlled.[45-47] SBRT provides a treatment option for oligometastases by enabling the delivery of high-dose (HD) oligo-fractionated radiation to deep-seated tumors while minimizing damage to normal tissues.[48] This HD ablative RT can also be employed in combination strategies such as adoptive cell and anti-CTLA-4 therapies. Administration of autologous DCs, produced *ex vivo* through autologous leukapheresis-derived monocytes, can also boost immune responses presumably by facilitating the presentation of tumor antigens released during RT.[49] In clinical trials, the addition of SBRT to HD IL-2 has been shown to be highly effective in patients with metastatic melanoma and renal cell cancer and represents a clinically tenable strategy given that HD IL-2 is approved for use in these malignancies. The presence of an elevated effector memory CD4+ T-cell population in the peripheral blood was associated with a clinical response in these patients.[50]

Viral vectors have also been shown to induce antitumor immune responses. Completed and ongoing clinical trials have shown that ADV/HSV-tk followed by ganciclovir (GCV) therapy (ADV/HSV-tk + GCV) has a favorable toxicity profile and antitumor activity in prostate cancer. Furthermore, this system has been shown to direct systemic antitumor activity in several experimental cancer models, including prostate cancer, and thus may serve as the basis for in situ immunomodulatory gene therapy. In a mouse model of prostate cancer, NK cells were shown to mediate the antimetastatic activity of ADV/HSV-tk + GCV. To enhance its antitumor activity, ADV/HSV-tk + GCV has been combined with ADV/IL-12. IL-12 increases NK cell proliferation and cytotoxicity. ADV/HSV-tk + GCV + ADV/IL-12 combination therapy demonstrated superior local and systemic growth suppression compared with either therapy alone. Importantly, when the metastatic tumor burden was increased to an extent that negated the growth-suppressive activity directed by ADV/HSV-tk + GCV or ADV/IL-12 alone, the combination therapy continued to demonstrate significant growth suppression. Examination of TILs showed enhanced NK lytic activity with the combination therapy.[51] Suicide gene therapy using HSV-tk + GCV therapy is being explored for the treatment of a wide variety of cancers. HSV-tk phosphorylates GCV, converting it to a non-diffusible nucleoside analog that terminates DNA synthesis, causing cell death.[52] However, gene therapy approaches for metastatic cancer are divided between those that deliver genes hematogenously to disseminated lesions, requiring methods of tissue-restricted gene expression or specific tissue targeting by the delivery vector, and those that manifest systemic antitumor capabilities following local gene expression, such as gene-modified immunotherapy.[53] With regard to the

HSV-tk system, the generation of immunologic activity has been suggested by numerous investigators to be an important aspect of therapy that may be exploited in the treatment of disseminated tumor lesions.

Several avenues of study have demonstrated the immunologic activity of HSV-tk therapy. HSV-tk + GCV-treated tumors contain areas of necrosis highlighted by infiltration of macrophages and CD4+ and CD8+ T-cells.[54-57] Furthermore, within treated tumors are detectable levels of the cytokines IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF in a fashion consistent with a Th1 (cellular based) response profile.[57,] Functional antitumor roles for these immune effectors have been suggested to impact within a treated tumor in the form of a local bystander effect and on synchronously growing non-transduced tumors or challenge tumor cell injections in the form of a distant bystander effect.[54, 58-61] Further supportive evidence has noted a significant loss of growth suppression in immunocompromised hosts.[55, 58, 59] HSV-tk + GCV may induce immune-related activities through necrosis-mediated exposure of putative tumor antigens to the cytokine-stimulated lymphocytic infiltrate. However, for the most part, direct demonstration of effector cell induction and its relevance to *in vivo* responses is lacking. In an orthotopic model of mouse prostate cancer, injection of ADV/HSV-tk + GCV therapy was found not only to inhibit local tumor growth but also to suppress spontaneous metastatic activity.[60] Furthermore, following surgical removal of a treated subcutaneous tumor, systemic activity against a tumor cell challenge injection of parental cells via the tail vein was induced by ADV/HSV-tk + GCV treatment of the primary tumor. Direct evidence of HSV-tk + GCV-mediated induction of antitumor lymphocytes was provided by a serial assay demonstrating the lytic activity of TILs isolated from HSV-tk + GCV-treated orthotopic tumors against parental tumor cells *in vitro*.[61] These studies demonstrated the induction of NK cells by ADV/HSV-tk + GCV treatment. Gene therapy performed in the absence of NK cells, but not T-cells, resulted in a modest but significant loss of HSV-tk + GCV-directed growth suppression within the primary tumor and complete abrogation of systemic activities.[61] Therefore, HSV-tk + GCV therapy appears to induce NK cells to impact local and remote tumor growth. Enhancement of NK cell activity may improve therapeutic response to ADV/HSV-tk + GCV.

Viral vectors have been increasingly studied as potential antitumor therapeutic agents. With their ability to invade and replicate within target cells, viruses have been utilized as oncolytic agents to directly lyse tumor cells. Viruses can also deliver their genetic payload into infected cells, allowing for the repair of defective tumor suppressor genes, disruption of oncogenic pathways, and production of cytokines that activate the immune system. Furthermore, viruses encoding tumor-associated antigens can infect DCs to trigger tumor-specific immune responses. The ability to engineer viruses with high levels of tumor specificity and efficient rates of infection has enhanced the safety profile of these agents, making viral vector-mediated gene therapy, either alone or in conjunction with more conventional therapies, a viable option for cancer therapy.[62]

4.1.1 Preclinical and Clinical Trial Data

4.1.1.1 Pembrolizumab Preclinical Pharmacology[63]

Pembrolizumab binds to human and Cynomolgus monkey PD-1 with comparable affinity and blocks the binding of human and Cynomolgus monkey PD-1 to PD-L1 and PD-L2 with comparable potency. Pembrolizumab does not cross-react with dog, rat, or mouse PD-1. Pembrolizumab does not bind Ig superfamily members CD28, CTLA-4, or inducible T-cell costimulator.

Pembrolizumab strongly enhances T-lymphocyte immune responses in cultured blood cells from healthy human donors, cancer subjects, and non-human primates. In T-cell activation assays using human donor blood cells, the half-maximal effective concentration has been approximately 0.1 to 0.3 nM. Pembrolizumab was found to modulate the levels of IL-2, TNF-α, IFN-γ, and other cytokines. Pembrolizumab potentiates existing immune responses only in the presence of antigen and does not non-specifically activate T-cells. In *in vitro* peripheral blood mononuclear cell and whole blood cytokine release assays, the cytokine levels induced by pembrolizumab were low and comparable to those induced by trastuzumab. Pembrolizumab does not induce antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity.

4.1.1.2 Pembrolizumab Preclinical Pharmacokinetics[63]

The pharmacokinetics (PK) of pembrolizumab were evaluated in a non-Good Laboratory Practices (GLP) single-dose PK study and two GLP repeat-dose toxicokinetic studies (1 month and 6 month) in Cynomolgus monkeys.

After single-dose intravenous (IV) administration at 0.3, 3, or 30 mg/kg in Cynomolgus monkeys, decline of serum concentration followed multiphasic kinetics. Anti–drug antibodies (ADAs) were detected in most of the treated animals. Clearance (CL) and terminal half-life (t1/2) appeared to be dose-dependent in the dose range tested, with t1/2 varying from 4 to 10 days.

In the 1-month repeat-dose (once weekly) GLP toxicity study at 6, 40, or 200 mg/kg in Cynomolgus monkeys, ADAs were detected in most of the low-dose (6 mg/kg) treated animals. The systemic exposure (area under the curve [AUC]) over the 7-day dosing interval was sex independent and increased with increasing dose. The mean t1/2 values in individual ADA-negative animals ranged from 15.7 to 22.3 days across doses.

In the 6-month repeat-dose (every other week) GLP toxicity study at 6, 40, or 200 mg/kg in Cynomolgus monkeys, ADAs were detected in most of the low-dose (6 mg/kg) treated animals. The systemic exposure to pembrolizumab was independent of sex and was approximately dose proportional across all doses. The mean t1/2 values in individual ADA-negative animals ranged from 21 to 22 days across doses.

4.1.1.3 Pembrolizumab Preclinical Safety Pharmacology/Toxicology[63]

The potential for systemic toxicity of pembrolizumab was assessed in a 1-month repeat-dose toxicity study with a 4-month recovery and 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 5

doses. Four monkeys/sex/group were euthanized during Week 5. The remaining 2 monkeys/sex/group were euthanized during Week 23, after a 4-month post-dose period. In this study, pembrolizumab 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-pembrolizumab antibodies were detected in 7 out of 8 animals in the 6 mg/kg dose group and 1 out of 8 animals in the 40 mg/kg dose group and were associated with an apparent increase in pembrolizumab CL. The presence of ADA in monkeys in the low-dose group and in 1 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 1 low-dose monkey). Additionally, anti-pembrolizumab 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 observed 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 Week 23); the remaining monkeys were designated for final necropsy following the 4-month treatment-free period. Pembrolizumab was well tolerated at all dose levels. There were no test article-related antemortem findings, electrocardiographic or ophthalmic findings, changes at injection sites, gross observations, or organ weight changes at the interim or final necropsy. As 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 5 out of 10 animals in the 6 mg/kg/dose group during the dosing phase, which correlated with an apparent increased rate of pembrolizumab elimination in these animals. No anti–pembrolizumab antibodies were detected in the 40 or 200 mg/kg/dose groups during the dosing phase, and no pembrolizumab serum concentration profiles in these 2 groups suggested an effect of ADA on pembrolizumab elimination rate. During the treatment-free period, anti–pembrolizumab antibodies were detected in 2 animals in the 6 mg/kg/dose group, which already had ADA present during the dosing phase, and in 2 additional animals (1 in the 6 mg/kg/dose group and 1 in the 200 mg/kg/dose group), which were ADA-negative during the dosing phase. The detection of anti-pembrolizumab antibodies had a minimal effect on the mean group systemic exposure to pembrolizumab during the study and did not impact the evaluation of potential toxicity of pembrolizumab for the duration of the 6-month study, because there were no test article-related effects on any of the parameters examined and no monkey in the mid- and high-dose groups developed ADA during the dosing phase. In conclusion, pembrolizumab administered once every other week over a 6-month duration to Cynomolgus monkeys was well tolerated and the NOAEL was ≥ 200 mg/kg/dose (the highest dose tested).

4.1.1.4 Pembrolizumab Clinical Trial Data[63]

4.1.1.4.1 Ongoing Studies

KEYNOTE-001 (NCT01295827) is an open label, first-in-human Phase I study of pembrolizumab in subjects with progressive locally advanced or metastatic carcinomas, especially melanoma or NSCLC. This study is active, but not recruiting patients. Part A of the study involved dose escalation that used a traditional 3+3 design. Cohorts of 3 to 6 subjects were enrolled sequentially at escalating doses of 1, 3, or 10 mg/kg administered every 2 weeks (Q2W). Once the dose escalation was completed, additional subjects were enrolled into Parts A1 and A2 to further characterize the PK and pharmacodynamics of pembrolizumab. In Parts B and D, subjects with metastatic melanoma were enrolled to assess the safety and antitumor activity of pembrolizumab. Additionally, Part B explored 3 different dose regimens in subjects with metastatic melanoma: 10 mg/kg Q2W, 10 mg/kg every 3 weeks (Q3W), and 2 mg/kg Q3W. In Part C, subjects with NSCLC (with prior systemic therapy) were enrolled at 10 mg/kg Q3W to assess the tolerability, safety, and antitumor activity of pembrolizumab. Part D explored the low and high doses of pembrolizumab identified in Parts A and B in subjects with advanced or metastatic melanoma. In Part F, non-previously treated (Cohort F-1) and previously treated (Cohort F-2) subjects with NSCLC whose tumors expressed PD-L1 were enrolled at 10 mg/kg Q2W and 10 mg/kg Q3W to characterize the tolerability, safety, and antitumor activity of pembrolizumab. A small cohort of previously treated (at least 2 lines of systemic therapy) subjects with NSCLC whose tumors did not express PD-L1 were enrolled and treated at a dose of 10 mg/kg Q2W (Cohort F-2). In Cohort F-3, previously treated subjects with NSCLC whose tumors expressed PD-L1 were enrolled at 2 mg/kg Q3W to better characterize the efficacy, safety, and antitumor activity of pembrolizumab. Each of the 2 disease-specific cohorts (melanoma and NSCLC) were enrolled to confirm tolerability and evaluate tumor response to pembrolizumab. The overall response rate and disease control rate for 655 melanoma subjects who received pembrolizumab were 31% and 51%, respectively. The ORR was 19.4%, and the median DoR was 12.5 months for 495 NSCLC patients who received pembrolizumab at a dose of 2 mg/kg Q3W, 10 mg/kg Q3W, or 10 mg/kg Q2W.[64] Median PFS and OS were 3.7 months and 12 months, respectively.

KEYNOTE-002 (NCT01704287) is a partially blinded, randomized Phase II study designed to evaluate 2 doses of pembrolizumab versus a chemotherapy control arm in subjects with ipilimumab-refractory metastatic melanoma.[65] Subjects (n = 540) were randomized to receive pembrolizumab 2 mg/kg Q3W (n = 180), pembrolizumab 10 mg/kg Q3W (n = 181), or chemotherapy (according to current clinical practice; n = 179) for the treatment of melanoma. Subjects assigned to the control chemotherapy arm could cross over to the experimental pembrolizumab arm once progression was confirmed (approximately ≥ Week 12). Based on 410 PFS events, PFS was improved in the pembrolizumab 2 mg/kg (hazard ratio [HR]: 0.57; 95% CI: 0.45–0.73; $P < 0.0001$) and pembrolizumab 10 mg/kg groups (HR: 0.50; CI: 0.39–0.64; $P < 0.0001$) compared with the chemotherapy group. The 6-month PFS rate was 34% (95% CI: 27–41) in the pembrolizumab 2 mg/kg group, 38% (95% CI: 31–45) in the pembrolizumab 10 mg/kg group, and 16% (95% CI: 10–22) in the chemotherapy group. Grade 3–4 treatment-related AEs occurred in 20 (11%) patients in the pembrolizumab 2 mg/kg group,

25 (14%) patients in the pembrolizumab 10 mg/kg group, and 45 (26%) patients in the chemotherapy group. The most common Grade 3–4 treatment-related AE in the pembrolizumab groups was fatigue (2 [1%] of 178 patients in the 2 mg/kg group and 1 [<1%] of 179 patients in the pembrolizumab 10 mg/kg group compared with 8 [5%] of 171 patients in the chemotherapy group). Other Grade 3–4 treatment-related AEs included generalized edema and myalgia (each in 2 [1%] patients) in the pembrolizumab 2 mg/kg group; hypopituitarism, colitis, diarrhea, decreased appetite, hyponatremia, and pneumonitis (each in 2 [1%] patients) in the pembrolizumab 10 mg/kg group; and anemia (9 [5%] patients), fatigue (8 [5%] patients), neutropenia (6 [4%] patients), and leucopenia (6 [4%] patients) in the chemotherapy group.

KEYNOTE-011 (NCT01840579) is an open label, non-randomized, multicenter Phase I study of pembrolizumab monotherapy in Japanese subjects with advanced solid tumors and in combination with cisplatin/pemetrexed and carboplatin/paclitaxel in subjects with advanced NSCLC in Japan. This study is active, but not recruiting patients. In Part A (monotherapy, 3+3 design), subjects with advanced solid tumors received escalating doses of pembrolizumab (dose level 1, 2 mg/kg Q2W; dose level 2, 10 mg/kg Q2W). Three patients received pembrolizumab at 2.0 mg/kg and 7 patients received pembrolizumab at 10 mg/kg. No dose-limiting toxicities were observed during cycle 1. Eighty percent of patients experienced drug-related AEs (DRAEs; mostly Grade 1 or 2); the most common DRAEs were nausea, malaise, pyrexia, and aspartate aminotransferase (AST)/alanine transaminase (ALT) increased (n = 2 each). No Grade 4 or 5 DRAEs occurred. Immune-related AEs comprised Grade 3 ALT increased (n = 1), Grade 3 AST increased (n = 1), Grade 1 pneumonitis (n = 1), and Grade 1 thyroid-stimulating hormone increased (n = 1). A partial tumor response was observed in one patient with NSCLC and in one patient with melanoma.[66] In Part B (combination, 3+6 design), subjects with advanced NSCLC receive pembrolizumab 10 mg/kg Q3W in combination with either cisplatin/pemetrexed (Cohort 1) or carboplatin/paclitaxel (Cohort 2).

KEYNOTE-012 (NCT01848834) is a multicenter, non-randomized, multicohort Phase Ib trial of pembrolizumab in subjects with PD-L1-positive advanced solid tumors. This study is active, but not recruiting patients. All subjects received pembrolizumab 10 mg/kg Q2W. Cohort A enrolled subjects with TNBC; Cohorts B and B2 enrolled subjects with squamous cell carcinoma of the head and neck; Cohort C enrolled subjects with urothelial tract cancer of the renal pelvis, ureter, bladder, or urethra; and Cohort D enrolled subjects with adenocarcinoma of the stomach or gastroesophageal junction.

KEYNOTE-013 (NCT01953692) is an open label, multicenter Phase Ib trial of pembrolizumab in subjects with hematologic malignancies. This study is recruiting patients. All subjects receive pembrolizumab at 10 mg/kg Q2W. Cohort 1 is enrolling subjects with intermediate-1, intermediate-2, or high-risk myelodysplastic syndrome who have failed at least 4 cycles of hypomethylating agent treatment. Cohort 2 is enrolling subjects with relapsed/refractory multiple myeloma. Cohort 3 is enrolling subjects with relapsed/refractory Hodgkin lymphoma who are ineligible for or refused a stem cell transplant and whose disease has relapsed after treatment with or failed to respond to brentuximab vedotin. Cohort 4a is enrolling subjects with relapsed/refractory mediastinal large B cell lymphoma who are ineligible for or refused a stem

cell transplant, and Cohort 4b is enrolling subjects with any other positive PD-L1-positive relapsed/refractory non-Hodgkin lymphoma who are ineligible for or refused a stem cell transplant.

KEYNOTE-021 (NCT02039674) is an open label, multicenter Phase I/II study of pembrolizumab at 2 dosing schedules in combination with chemotherapy or immunotherapy in subjects with locally advanced or metastatic NSCLC. This study is recruiting patients. The study is composed of 2 parts. The objective of Part 1 is to determine the recommended Phase II dose (RP2D) for pembrolizumab in combination with different chemotherapy and/or immunotherapy regimens:

- Cohort A – 1:1 randomization to carboplatin and paclitaxel plus either pembrolizumab 2 mg/kg or pembrolizumab 10 mg/kg
- Cohort B – 1:1 randomization to carboplatin, paclitaxel, and bevacizumab plus either pembrolizumab 2 mg/kg or pembrolizumab 10 mg/kg
- Cohort C – 1:1 randomization to carboplatin and pemetrexed plus either pembrolizumab 2 mg/kg or pembrolizumab 10 mg/kg
- Cohort D – ipilimumab plus pembrolizumab
- Cohort E – erlotinib plus pembrolizumab
- Cohort F – gefitinib plus pembrolizumab

The objective of Part 2 is to evaluate the antitumor activity of pembrolizumab in combination with chemotherapy or immunotherapy. Part 2 includes a randomized comparison of chemotherapy ± pembrolizumab based on the doses defined in Part 1, as well as a cohort expanding the ipilimumab cohort from Part 1:

- Cohort G – 1:1 randomization to carboplatin and pemetrexed with or without pembrolizumab 200 mg
- Cohort H – ipilimumab (RP2D from Part 1 Cohort D) plus pembrolizumab followed by pembrolizumab monotherapy

KEYNOTE-023 (NCT02036502) is an open label, multicenter Phase I trial of pembrolizumab in combination with lenalidomide (Len) and dexamethasone (Dex) or pembrolizumab and Len in subjects with relapsed/refractory multiple myeloma who have failed at least 2 lines of prior therapy, including a proteasome inhibitor (e.g., bortezomib or carfilzomib) and an immunomodulary derivative (thalidomide, pomalidomide, lenalidomide). This study is recruiting patients. The trial uses a modified 3+3 design for dose determination, followed by dose confirmation and expansion, a further evaluation of safety, and a preliminary assessment of efficacy. During dose determination, cohorts of approximately 3 to 6 subjects are enrolled and receive pembrolizumab 2 mg/kg or 1 mg/kg IV Q2W in each 28-day cycle in combination with Dex 40 mg QW and/or Len 25 mg or 10 mg on Days 1 to 21. After a preliminary maximum tolerated dose (MTD)/maximum administered dose (MAD) is identified, additional subjects are enrolled at a fixed dose of pembrolizumab 200 mg or 100 mg in combination with Len/Dex to confirm the MTD/MAD.

KEYNOTE-28 (NCT02054806) is an open label, non-randomized, multicenter, multicohort Phase Ib trial of pembrolizumab in subjects with PD-L1-positive advanced solid tumors. This study is active, but not recruiting patients. Subjects were enrolled into 1 of the following 20

solid tumor cohorts: A1, colon or rectal adenocarcinoma; A2, anal canal squamous cell carcinoma; A3, pancreas adenocarcinoma; A4, esophageal squamous cell carcinoma or adenocarcinoma (including gastroesophageal junction); A5, biliary tract adenocarcinoma (gallbladder and biliary tree but excluding ampulla of Vater cancers); A6, carcinoid tumors; A7, neuroendocrine carcinomas (well or moderately differentiated pancreatic neuroendocrine tumor); B1, estrogen receptor-positive human epidermal growth factor receptor 2-negative breast cancer; B2, ovarian epithelial, fallopian tube, or primary peritoneal carcinoma; B3 endometrial carcinoma; B4, cervical squamous cell cancer; B5, vulvar squamous cell carcinoma; C1, small cell lung cancer; C2, mesothelioma (malignant pleural mesothelioma); D1, thyroid cancer (papillary or follicular subtype); D2, salivary gland carcinoma; D3, nasopharyngeal carcinoma; E1, glioblastoma multiforme; E2, leiomyosarcoma; and E3, prostate adenocarcinoma. All subjects received pembrolizumab 10 mg/kg Q2W.

4.1.1.4.2 Summary of Pharmacokinetics Data[63]

PK samples have been obtained in the ongoing studies KEYNOTE-001, KEYNOTE-002, KEYNOTE-012, KEYNOTE-013, and KEYNOTE-023 from subjects with solid tumors (KEYNOTE-001 Part A); subjects with advanced melanoma (KEYNOTE-001 Parts B and D, KEYNOTE-002); subjects with NSCLC (KEYNOTE-001 Parts C and F); subjects with advanced solid tumors which include breast cancer (TNBC) and head and neck cancer, urothelial tract cancer, and gastric cancer (KEYNOTE-012); and subjects with hematological cancers (KEYNOTE-013 and KEYNOTE-023). Pre-dose and post-dose samples have been obtained in various cycles: cycles 1, 2, 5, and 6 for KEYNOTE-001 melanoma; cycles 1 and 6 for KEYNOTE-001 NSCLC; and cycles 1 and 2 for KEYNOTE-002, KEYNOTE-012, KEYNOTE-013, and KEYNOTE-023. Pre-dose samples only were drawn every 3 to 8 cycles thereafter. In KEYNOTE-001 Part A, extensive blood sampling was applied during cycles 1 and 2 for detailed PK analysis. Pre-dose minimum serum concentration (C_{trough}) and post-dose serum concentration (C_{max}) levels were consistent and in good agreement across the different studies. There is no meaningful difference between the pre-dose or post-dose concentration values per time point for the different indications.

The PK profile of pembrolizumab was investigated in Part A of the ongoing study KEYNOTE-001. Results have been obtained following a single dose at 1, 3, and 10 mg/kg pembrolizumab to 17 subjects with solid tumors in cycle 1 (Part A and A-1). The observed PK profile of pembrolizumab was typical of those observed for other IgG mAbs, with a t1/2 of approximately 2 to 3 weeks. There was no indication of dose dependency of t1/2 in the 3 dose groups. A dose-related increase in exposure was observed from 1 to 10 mg/kg. The long t1/2 supports a dosing interval of Q2W or Q3W.

Consistent with the 5-fold higher dose, pre-dose or post-dose concentrations of the 10 mg/kg Q3W regimens are approximately 5 times higher than those of the 2 mg/kg Q3W regimen, confirming dose proportionality of pembrolizumab exposure in this dose range. The overall geometric mean 24-week pre-dose serum concentration values from subjects treated with 10 mg/kg Q2W (N = 128 [N = 89 melanoma subjects and N = 39 subjects other cancer types]) across all studies and indications is 231 μg/mL (range, 191–296 μg/mL), which is on average 30% higher than the week 24 pre-dose concentration (155 μg/mL) from subjects treated with

10 mg/kg Q3W (range, 154–156 μg/mL; N = 168 [N = 161 melanoma subjects and N = 7 NSCLC subjects]).

In summary, the PK profile of pembrolizumab, with low CL and limited volume of distribution, is typical for therapeutic antibodies. Exposure to pembrolizumab is approximately linear in the dose range of clinical relevance (1 to 10 mg/kg).

4.1.1.4.3 Summary of Immunogenicity Data[63]

Data for immunogenicity assessment are available from subjects with advanced melanoma (KEYNOTE-001, Parts B and D, and KEYNOTE-002) and from subjects with NSCLC (KEYNOTE-001, Parts C and F). For the subjects with advanced melanoma, the observed incidence of treatment-emergent ADAs in evaluable subjects was 0.4% (1 out of 268 subjects), based on 1 subject with a confirmed treatment-emergent positive status compared with all evaluable subjects including 3 with a non-treatment-emergent positive status and 264 with a negative immunogenicity status. A subset analysis of subjects treated with pembrolizumab 2 mg/kg Q3W was performed. In this analysis, none of the 220 evaluable subjects treated with 2 mg/kg Q3W tested positive for treatment-emergent anti-pembrolizumab antibodies. None of the subjects with NSCLC were classified as treatment-emergent or non-treatment-emergent positive. To this point, only 6 of the 290 assessable subjects were treated with 2 mg/kg. Due to the majority of subjects receiving the high dose, only 16 subjects had a drug concentration with the last sample below the drug tolerance level (DTL) and could be classified as conclusively negative. The other 274 subjects were classified as inconclusive because the last sample had a drug concentration above the DTL. Thus, to date, the occurrence of treatment-emergent ADA has been observed in 1 subject or less than 1% of the subjects evaluable for immunogenicity assessment, indicating a low potential of pembrolizumab to elicit the formation of ADA. No impact of ADA on pembrolizumab exposure, efficacy, or safety has been observed.

4.1.1.4.4 Summary of Efficacy Data[63]

The overall response rate obtained in KEYNOTE-001 demonstrated the antitumor activity of pembrolizumab in subjects with melanoma (ipilimumab-naïve and previously treated with ipilimumab). The KEYNOTE-002 study demonstrated superior PFS for both pembrolizumab treatment arms compared with the chemotherapy control arm. In KEYNOTE-002, treatment with pembrolizumab lead to an overall response rate that was > 4 fold higher than the response rate of the chemotherapy control arm. This difference was highly statistically significant. The overall response rates for pembrolizumab treatment in KEYNOTE-001 and KEYNOTE-002 compared favorably to historical response rates for available treatments for melanoma, particularly in subjects who have progressed after multiple prior therapies.

4.1.1.4.5 Summary of Adverse Event Data[63]

In the pembrolizumab monotherapy trials (KEYNOTE-001, KEYNOTE-002, KEYNOTE-012, KEYNOTE-013, KEYNOTE-28, and KEYNOTE-011 [monotherapy arm]), the overall incidence of AEs ranged from 83.0% (73 of 88 subjects in KEYNOTE-012) to 100% (10 of 10 subjects in KEYNOTE-011). The most commonly reported AEs included fatigue, diarrhea, decreased appetite, nausea, and anemia. The incidence of DRAEs ranged from 39.8% (35 of

88 subjects in KEYNOTE-013) to 80.0% (8 of 10 subjects in KEYNOTE-011). The most commonly reported DRAEs across all studies were nausea, fatigue, and diarrhea. The incidence of Grade 3–5 DRAEs across studies ranged from 6.8% (6 of 88 in KEYNOTE-013) to 12.0% (187 of 1562 subjects in KEYNOTE-001/002). The most commonly reported Grade 3–5 DRAEs were anemia, ALT increased, and AST increased. Most subjects who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 subjects in KEYNOTE-28) to 12.3% (192 of 1562 subjects in KEYNOTE-001/002). The majority of AEs leading to discontinuation were not considered drug related. Discontinuations due to a DRAE were infrequent and ranged from 0% (no subjects in KEYNOTE-011) to 4.5% (4 of 88 subjects in KEYNOTE-013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, ALT increased, and AST increased. The overall pattern of AEs observed in melanoma subjects enrolled in KEYNOTE-002 demonstrates the favorable safety profile of pembrolizumab compared with chemotherapy. Consistent with prior observations from randomized comparisons of the 2 mg/kg and 10 mg/kg dose levels when given Q3W, there are no important differences in the safety profile of pembrolizumab at these 2 dose levels, and both doses appear to have a favorable safety profile compared with chemotherapy.

In the combination therapy trials KEYNOTE-021 and KEYNOTE-023, the overall incidence of AEs was 95.4% (62 of 65 subjects) and 80% (8 of 10 subjects), respectively. In KEYNOTE-021, the most commonly reported AEs across the dose regimens were fatigue (49.2%), constipation and nausea (26.2% each), decreased appetite (23.1%), diarrhea (18.5%), and anemia and alopecia (15.4% each). In KEYNOTE-023, the most commonly reported AEs across the dose regimens were neutropenia and thrombocytopenia (50.0% each), followed by anemia, respiratory tract infection, and back pain (30.0% each). The incidence of DRAEs was 86.2% (56 of 65 subjects) in KEYNOTE-021 and 60.0% (6 of 10 subjects) in KEYNOTE-023. In KEYNOTE-021, the most commonly reported DRAEs across the dose regimens were fatigue (35.4%); nausea, decreased appetite, and alopecia (13.8% each); diarrhea (12.3%); and constipation and AST increased (10.8% each). In KEYNOTE-023, the most commonly reported DRAEs across the dose regimens were neutropenia and thrombocytopenia (50.0% each); anemia (30.0%); and dysphonia, hiccups, and pruritis (20.0% each).

Grade 3–5 DRAEs were reported in 23.1% (15 of 65 subjects) in KEYNOTE-021 and 50.0% (5 of 10 subjects) in KEYNOTE-023. In KEYNOTE-021, the most common Grade 3–5 DRAEs across dose regimens were AST increased (6.2%) and anemia and ALT increased (4.6% each). In KEYNOTE-023, the only Grade 3–5 DRAEs that occurred in more than 1 subject across dose regimens were neutropenia (40.0%) and anemia (20.0%).

In KEYNOTE-021, most subjects continued treatment despite AEs, and only 4.6% of subjects discontinued study treatment due to an AE. Only 3.1% of subjects discontinued study treatment due to an AE that was considered related to the study treatment by investigators. AEs resulting in discontinuation were reported in 3.1% of subjects (2 of 65 subjects). Interstitial lung disease, dermatitis allergic, and drug eruption were the only AEs resulting in discontinuation and were reported in 1 subject each (1.5%). In KEYNOTE-023, no subjects discontinued study treatment due to an AE.

In general, the incidence of drug-related SAEs (DRSAEs) was low. Many of the events occurred in 1 subject each and/or < 1.0% each. In the pembrolizumab monotherapy trials, the most commonly reported DRSAEs (those that occurred in 3 or more subjects overall in at least 1 study) were pneumonitis (range, 0.7%–1.3% of subjects); colitis (range, 0.3%–0.9% of subjects), pyrexia (range, 0.3%–0.5% of subjects); diarrhea (range, 0.2%–0.4% of subjects); hepatitis (0.7% of subjects); nausea, adrenal insufficiency, hyponatremia, hyperthyroidism, hypophysitis, vomiting, and dyspnea (0.3% of subjects each); and dehydration, generalized edema, hypothyroidism, renal failure acute, and pericardial effusion (0.2% of subjects each). The remaining DRSAEs occurred in 1 or 2 subjects each per study. In the combination therapy trials, all DRSAEs were reported in 1 subject each. In KEYNOTE-021, 8 subjects experienced DRSAEs; the DRSAEs were as follows: anemia, febrile neutropenia, atrial fibrillation, colitis, pyrexia, hypersensitivity, ALT increased, AST increased, drug eruption, rash, and urticaria. In KEYNOTE-023, 2 subjects experienced DRSAEs; 1 subject had an event of pneumonia and the other had an event of tumor lysis syndrome. Due to the fact that KEYNOTE-021 and KEYNOTE-023 were combination studies and also were of small sample size, comparative evaluation of the AE profile of pembrolizumab combination therapy from those protocols to pembrolizumab monotherapy or to chemotherapy monotherapy in other studies cannot be made.

Among KEYNOTE-001 and KEYNOTE-002 studies, the incidence of AEs of special interest (AEOSI) was 16.1%. Overall, the most commonly reported AEOSI included hypothyroidism (7.2%), pneumonitis (2.9%), infusion reaction (2.5%), and hyperthyroidism (2.2%). The incidences of the remaining AEOSI were low (range, 0.1%–1.3%). The overall incidence of drug-related AEOSI was 14.3%. Overall, 2.6% of subjects discontinued treatment due to an AEOSI, and the most commonly reported drug-related AEOSI leading to discontinuation was pneumonitis (1.3%). Only one subject died of AEOSI (pneumonitis, 0.1% of subjects). Corticosteroids were not used to manage myositis, pericarditis, thyroiditis, type 1 diabetes mellitus (T1DM), uveitis, or vasculitis.

4.1.1.5 HSV-tk Preclinical and Clinical Trial Data

Many laboratories have shown the *in vitro* susceptibility of mouse and human prostate cancer cells to HSV-tk/prodrug treatment. Our group has evaluated the effects of ADV-tk + GCV on *in vivo* tumor growth and survival in mouse prostate cancer models.[67] An adenoviral vector carrying the HSV-tk gene under the control of the Rous sarcoma virus promoter was administered by direct intratumoral injection.[67-69] Tumor growth was significantly delayed in the ADV-tk + GCV group compared with the control groups (ADV-tk + saline and ADV-β-galactosidase [control vector] + saline or GCV).[67] Tumor volume in the ADV-tk + GCV-treated group was 16% of that in the combined control groups. However, with longer follow-up, ADV-tk + GCV-treated tumors began to grow at a rate similar to control tumors. Mean survival was 14.2 ± 0.6 days for the combined control groups and 21.2 ± 1.3 days for the treatment group ($P < 0.001$ by Mantel-Cox log-rank analysis). Animal studies have also shown that ADV-tk treatment can protect against metastases. In an orthotopic model, the spontaneous metastasis rate was significantly lower for the ADV-tk + GCV treatment group compared with the pooled control groups (12.5% [2/16] vs. 71.4% [10/14]; $P = 0.0032$ by Fisher's Exact test).[70]

Similar results were obtained in experimental metastasis models (i.e., tail vein injection of tumor cells). HSV-tk/GCV gene therapy in combination with radiation significantly prolonged survival compared with either therapy alone and sham treatment (22.9 ± 1.34 days vs. 16.9 ± 0.84 days and 11.3 ± 0.77 days, respectively; $P < 0.001$ by log-rank and Wilcoxon tests).[70]

Based on extensive efficacy and toxicity studies in mice, cotton rats, and non-human primates,[68, 71] our group designed a dose escalation Phase I clinical study to evaluate the potential toxicity of ADV/HSV-tk in humans (Regulatory Affairs Certification [RAC] protocol # 9601-144). The study was evaluated and approved by the local institutional review board [IRB] and institutional biosafety committee and national (RAC and FDA) regulatory bodies. Subjects included men with locally recurrent prostate cancer after RT. Vector particles (2×10^9 to 2×10^{12}) were administered in a single intratumoral injection guided by ultrasound imaging. Three patients achieved PRs, and only occasional transient toxicities were observed (Phase I Study of Adenoviral Vector Delivery of the HSV-tk Gene and the Intravenous Administration of Ganciclovir in Men with Local Recurrence of Prostate Cancer after Radiation Therapy, National Institutes of Health-Office of Recombinant DNA Activities # 9601-144).[71]

Based on our favorable preclinical and clinical trial data, a Phase I/II study was designed to analyze the efficacy and toxicity of HSV-tk gene therapy in combination with RT in prostate cancer patients.[72] In this study, intravenous GCV was replaced with valacyclovir (Valtrex, Glaxo Wellcome), an orally administered medication that is rapidly and almost completely metabolized in the liver to acyclovir. The trial consisted of 3 treatment arms: Arm A, low-risk patients treated with HSV-tk/valacyclovir + RT; Arm B, high-risk patients treated with the same regimen as Arm A with the addition of hormonal therapy; and Arm C, patients with stage D1 (positive pelvic lymph nodes) who received the same regimen as Arm B with the addition of 45 Gy to the pelvic lymphatics. Fifty-nine patients (29 in Arm A, 26 in Arm B, and 4 in Arm C) completed the trial. The median age was 68 years (range, 39–85 years). The median follow-up for the entire group was 13.5 months (range, 1.4–27.8 months). Only Arm A patients were observed to have an increase in prostate specific antigen (PSA) on day 14, which then declined appropriately. All patients in Arm A (median follow-up, 13.4 months) and Arm B (median follow-up, 13.9 months) had biochemical control at last follow-up. Three patients in Arm C (with pretreatment PSA of 335, 19.6, and 2.5 ng/mL and a combined Gleason score of 8, 9, and 9 involving all biopsy cores) had biochemical failure at 3, 3, and 7.7 months. Two patients had distant failure in bone and 1 patient in the para-aortic lymph nodes outside the radiation portal. Six to twelve prostate biopsies performed in these 3 patients revealed no evidence of residual carcinoma. In Arm A, biopsy showed no evidence of carcinoma in 66.7% (18/27), 92.3% (24/26), 91.7% (11/12), 100% (8/8), and 100% (6/6) of patients at 6 weeks, 4 months, 12 months, 18 months, and 24 months after treatment, respectively. In Arm B, biopsy showed no evidence of carcinoma in 96% (24/25), 90.5% (19/21), 100% (14/14), 100% (7/7), and 100% (2/2) of patients at 6 weeks, 4 months, 12 months, 18 months, and 24 months after treatment, respectively. This was the first reported trial of its kind in the field of prostate cancer that aimed to expand the therapeutic index of RT by combining it with in situ gene therapy.[73]

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

The PD-1 pathway represents a major tumoral immune resistance mechanism. Viral vector-based gene therapy such as ADV/HSV-tk + GCV has also been shown to induce antitumor immune activity by several mechanisms including enhancing NK proliferation and cytotoxicity, boosting cytokine stimulatory activity, and increasing lymphocytic infiltrate. The therapeutic potential of HSV-tk gene therapy is greatly enhanced by a "bystander effect" in which cytotoxicity is conferred to non-transduced neighboring cells. RT has been shown to augment endogenous antitumor innate and adaptive immune responses. The beneficial effects of RT in cancer patients extend beyond direct tumor cell cytotoxicity. Delivery of localized radiation to tumors often leads to systemic responses at distant sites, a phenomenon known as the abscopal effect, which has been attributed to the induction and enhancement of endogenous antitumor innate and adaptive immune responses. The mechanisms surrounding the abscopal effect are diverse and include trafficking of lymphocytes into the tumor microenvironment, enhanced tumor recognition and killing via upregulation of tumor antigens and antigen-presenting machinery, and induction of positive immunomodulatory pathways. Thus, RT in combination with immunomodulators can produce significant local control and induce antitumor responses at distant sites by triggering and amplifying endogenous cellular immune responses. Here, we propose a rational combination of ADV/HSV-tk in situ gene therapy plus SBRT followed by immunomodulatory therapy with pembrolizumab for the treatment of metastatic TNBC and metastatic NSCLC.

TNBC is an aggressive, difficult-to-treat disease with poor prognosis and no current targeted therapy options. Compared with other intrinsic breast cancer subtypes, TNBC has higher expression levels of PD-L1 and its receptor PD-1, which may hinder antitumor T cell responses. Lung cancer remains the leading cause of cancer-related deaths worldwide. NSCLC accounts for over 85% of all lung cancer cases. Most patients are diagnosed with advanced or metastatic (stage IIIB/IV) disease, and current first-line treatment options for these patients are limited. In patients with advanced NSCLC, first-line platinum-based doublet chemotherapy yields 1-year OS rates of only 30–40% and can cause significant toxicities that may complicate treatment.[3] Pembrolizumab has demonstrated promising efficacy in TNBC and NSCLC.[64, 74] In a Phase Ib study, 18.5% of 27 evaluable patients with heavily pretreated recurrent or metastatic TNBC responded to pembrolizumab (10 mg/kg Q2W).[74] One patient had a CR, 4 patients had a PR, 7 patients had stable disease (SD), and 12 patients had progressive disease (PD). Results of an ongoing Phase Ib study have demonstrated the antitumor activity and acceptable side effect profile of pembrolizumab in patients with advanced NSCLC. These study results have led to FDA accelerated approval of Keytruda™ (pembrolizumab) for the treatment of patients with metastatic PD-L1-positive NSCLC whose disease has progressed on or after platinum-containing chemotherapy or targeted therapy against ALK or EGFR, if appropriate. Use of ADV/HSV-tk in situ gene therapy plus SBRT before pembrolizumab may represent a window of opportunity to enhance pembrolizumab efficacy in patients with metastatic TNBC and metastatic NSCLC.

4.2.2 Rationale for Dose Selection

An open label Phase I trial (KEYNOTE-001) is being conducted to evaluate the safety and clinical activity of single-agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered Q2W in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first-in-human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg, and 10 mg/kg Q2W). No MTD has been identified to date. Ten mg/kg Q2W, the highest dose tested in KEYNOTE-001, 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 pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life.[63] Pharmacodynamic data 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 PK analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, CL and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between CL and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed-dose regimen relative to a 2 mg/kg Q3W body weight-based regimen are anticipated to remain well within the established exposure margins of 0.5–5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e., 5-fold higher dose and exposure). The population PK evaluation revealed a lack of 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 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 Q3W will provide exposures that 1) are optimally consistent with those obtained with 2 mg/kg dose Q3W; 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 reduce potential dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

ADV/HSV-tk will be administered intratumorally (single-site injection) at 5 x 10^{11} virus particles (vp) on Day 0. This dose is based on the toxicity results from the Phase I/II study of ADV-tk oncolytic virus therapy in combination with chemoradiation in patients with pancreatic adenocarcinoma.[75]

The dose of valacyclovir to be used in this study is 2 g orally three times daily (t.i.d.) for 14 days (Day 1 to Day 15). This dose has been calculated to give a similar AUC as 10 mg/kg of IV acyclovir administered every 8 hours. This is the same dose regimen used in a previous Phase I clinical trial of ADV/HSV-tk + acyclovir and topotecan in patients with recurrent ovarian cancer.[76]

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints

The primary efficacy endpoint will be the ORR of ADV/HSV-tk + valacyclovir therapy and SBRT followed by pembrolizumab in patients with metastatic TNBC and metastatic NSCLC. Secondary efficacy endpoints are antitumor activity (RECIST 1.1 in addition to modified irRC), DoR, OS rate, PFS rate, and CBR.

4.2.3.2 Biomarker Research

Blood and tissue-based markers associated with response to the study treatment will be evaluated, including but not limited to T-cell cytokine profiles (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF), TILs, PD-1 and PD-L1 expression, effector and suppressor immunocyte populations, and mutation load.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

The TNBC cohort will be comprised of patients with histologically confirmed locally advanced or metastatic TNBC that has relapsed on or is refractory to 1 or more lines of standard of care therapy. Patients must have received no more than one prior line of cytotoxic therapy for metastatic disease. Taxane and anthracycline-based chemotherapy must have been received, unless contraindicated. The NSCLC cohort will be comprised of male and female patients aged ≥ 18 years with histologically or cytologically confirmed metastatic NSCLC that is

immunotherapy naïve or previously treated with a maximum of 1 immunotherapy regimen AND chemotherapy naïve or previously treated with 1 cycle of platinum-containing chemotherapy. Patients will be screened for *EGFR* and *ALK* mutation. *EGFR*/*ALK* mutation-negative patients and patients with *EGFR* or *ALK* genomic tumor aberrations that have failed FDA-approved targeted therapy for these aberrations will be eligible for enrollment in the study. Written informed consent is required before performing any study-specific tests or procedures. Signing of the Informed Consent Form can occur outside the 28-day screening period. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before study entry. Results of standard-of-care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study entry (except where otherwise specified) may be used for screening assessments rather than repeating such tests. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

5.1.2 Subject Inclusion Criteria

Subjects must meet all the following inclusion criteria in order to be eligible for participation in this trial:

1. Willing and able to provide written informed consent/assent for the trial.
2. Male or female ≥ 18 years of age on the day of informed consent signing.
3. Histologically confirmed locally advanced or metastatic TNBC that has relapsed on or is refractory to standard of care therapy. TNBC is defined as estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2) negative. ER and progesterone receptor negativity are defined as <10% IHC staining. HER2 test result is negative if a single test (or both tests) performed show:
    - IHC 1+ or 0
    - In situ hybridization negative based on
        - Single-probe average HER2 copy number < 4.0 signals/cell
        - Dual-probe HER2/CEP17 ratio < 2 with an average HER2 copy number < 4.0 signals/cell

    OR histologically or cytologically confirmed metastatic NSCLC that is immunotherapy naïve or previously treated with a maximum of 1 immunotherapy regimen AND chemotherapy naïve or previously treated with 1 cycle of platinum-containing chemotherapy. *EGFR*/*ALK* mutation-negative NSCLC patients and NSCLC patients with *EGFR* or *ALK* genomic tumor aberrations that have failed FDA-approved targeted therapy for these aberrations will be eligible for enrollment in the study.
4. Measurable disease based on RECIST 1.1, a target lesion of suitable diameter (at least 1 cm) for SBRT with the exception of a primary breast tumor, and a non-target lesion (visceral metastatic lesion) at least 1 cm in diameter for abscopal effect evaluation.
5. Willing to provide biopsy tissues as required by the study.
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
7. Adequate organ function as defined in Table 1. All screening labs should be performed within 28 days of trial treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	

Absolute neutrophil count (ANC)	≥1,500/μL (without granulocyte colony stimulating factor support within 14 days of assessment)
Platelets	≥100,000/μL
Hemoglobin	≥8 g/dL without transfusion or erythropoietin dependency (within 7 days of assessment)
White blood cell count (WBC)	>2,500/μL and <15,000/μL
Lymphocyte count	≥500/μL
Renal	
Serum creatinine	<2 X upper limit of normal (ULN)
Hepatic	
Serum total bilirubin	≤1.0 X ULN (Subjects with known Gilbert's disease who have serum bilirubin level ≤3 X ULN may be enrolled)
Aspartate transaminase (AST) and Alanine transaminase (ALT)	≤2.5 X ULN with normal alkaline phosphatase (≤5 X ULN for subjects with liver metastases) **OR** ≤1.5 X ULN in conjunction with alkaline phosphatase >2.5 X ULN
Albumin	≥2.5 mg/dL
Coagulation	
International normalized ratio (INR) or prothrombin time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
Activated partial thromboplastin time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
[a]CrCl should be calculated per institutional standard.	

8. Life expectancy ≥ 6 months.
9. ≥ 4 weeks since any major surgery.
10. A 2-week washout period post any prior systemic anticancer therapy, RT, targeted anticancer therapy or mAb, and/or investigational therapy is required prior to trial entry. Subject should be recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to a previously administered therapy.
    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
11. Female subjects of childbearing potential should have a negative serum pregnancy (β-human chorionic gonadotropin [β-HCG]) within 7 days prior to receiving the first dose of the trial treatment and should not be lactating.
12. Female subjects of childbearing potential (Section 5.5.2) must be willing to use an adequate method of contraception as outlined in Section 5.5.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

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

13. Male subjects of childbearing potential (Section 5.5.2) must agree to use an adequate method of contraception as outlined in Section 5.5.2 – Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

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

5.1.3 Subject Exclusion Criteria

Subjects meeting any of the following exclusion criteria must be excluded from participating in the trial:

1. Unwilling or unable to comply with the study protocol.
2. Subjects for who bone metastases are the only available non-target lesions for abscopal effect evaluation.
3. Subjects with tumors for which SBRT is not considered appropriate standard therapy with the exception of subjects with a primary breast tumor. This includes subjects with target lesions less than 1 cm in diameter and those with large central lung lesions.
4. 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.
5. Known history of active tuberculosis (Bacillus Tuberculosis).
6. Known or suspected hypersensitivity to pembrolizumab or any of its excipients or any component of the proposed regimen (gene vector/valacyclovir).
7. Known gallbladder or bile duct disease (i.e., infection or cholecystitis) or acute or chronic pancreatitis.
8. ECOG performance status of ≥2 or oxygen dependence (e.g., advanced chronic obstructive pulmonary disease).
9. Inability to swallow food or any condition of the upper gastrointestinal tract that precludes administration of oral medications (valacyclovir).
10. Congestive heart failure: New York Association class III or IV heart failure or unstable angina (see Section 12.4).
11. Sustained or clinically significant cardiac arrhythmias including sustained ventricular tachycardia, ventricular fibrillation, clinically significant bradycardia, advanced heart block (Mobitz II or higher atrioventricular nodal block), prolonged corrected QT interval (longer than 470 milliseconds), or history of acute myocardial infarction.
12. Concomitant disease(s) that could prolong QT such as autonomic neuropathy (caused by diabetes or Parkinson's disease), human immunodeficiency virus (HIV), cirrhosis, uncontrolled hypothyroidism, or cardiac failure.
13. History of syncope or family history of idiopathic sudden death.
14. 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.
15. Known active central nervous system 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 4 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.
16. 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.

17. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.
18. Active infection requiring systemic therapy.
19. 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.
20. Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
21. Pregnant or breastfeeding, expecting to conceive or father children within the projected duration of the trial, starting with the prescreening or screening visit through 120 days after the last dose of trial treatment, or is unwilling to practice an effective method of birth control. Women of childbearing potential must have a negative serum pregnancy test within 7 days prior to administration of trial treatment.
22. Prior treatment with gene vector therapy.
23. Prior treatment with immunomodulatory therapy or immunotherapy (TNBC cohort only)
24. Prior treatment with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent (TNBC cohort only).
25. Known history of HIV (HIV 1/2 antibodies).
26. History of liver disease such as cirrhosis or known active hepatitis B (e.g., hepatitis B surface antigen reactive) or hepatitis C (e.g., hepatitis C virus RNA [qualitative] is detected).
27. History of or current alcohol misuse/abuse within the past 12 months.
28. Major surgery within 4 weeks prior to study enrollment.
29. Received a live vaccine within 30 days of planned start of trial therapy.
    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and are not allowed.

5.2 Trial Treatments

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

Table 2 Trial Treatments

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
ADV/HSV-tk	5 x 10^{11} vp in 2-mL volume	Once	Intratumorally	Day 0	Experimental
Valacyclovir	2 g	t.i.d. for 14 days	Orally	Day 1 - Day 15	Experimental
SBRT*	30 Gy	6 Gy X 5 fractions over 14 days	-	Day 2 - Day 16	Experimental

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

vp = viral particles; t.i.d. = three times daily; Q3W = every 3 weeks; IV = intravenous.

*Primary breast tumors will be treated with three-dimensional RT (short course of 5−8 fractions).

**Pembrolizumab will continue until disease progression, unacceptable toxicity, or up to 24 months (35 cycles) in subjects without disease progression. All subjects who stop pembrolizumab treatment with complete response may be eligible for up to an additional 12 months (17 cycles) of pembrolizumab treatment if they progress after stopping pembrolizumab from the initial treatment phase. The subject must meet the conditions outlined in Section 5.2.3.

ADV/HSV-tk

The tumor will be identified by positron emission tomography (PET)/CT by the radiation oncologist. ADV/HSV-tk will be administered intratumorally as a single injection using a 21-gauge needle. For nodal, lung, and liver lesions, ADV/HSV-tk will be administered after fiducial placement for SBRT. For primary lung tumors, ADV/HSV-tk will be administered under endobronchial ultrasound (EBUS) guidance. In cases where the tumor cannot be accessed by EBUS, a CT-guided injection will be performed. For primary breast tumors, ADV/HSV-tk will be administered under ultrasound guidance. For liver and lung metastatic lesions, ultrasound or CT-guided injection of ADV/HSV-tk will be performed. EBUS will be performed with the patient under sedation. Patients will be monitored until the sedation has worn off. For CT-guided injections, local anesthesia will be used and patients will be monitored for several hours after the procedure.

SBRT

Primary lung tumors and metastatic lesions (breast and lung) will be treated with SBRT (6 Gy X 5 fractions over 2 weeks). Primary breast tumors will be treated with three-dimensional (3D) RT (short course of 5−8 fractions over 2 weeks). Tumor target will be decided by PET/CT scan. For the planned dosing regimen, SBRT simulation will be done before treatment. For subjects with multiple tumors, the largest tumor will be selected. Sedation is not necessary for SBRT.

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.

5.2.1.2 Dose Modification

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

Table 3 Dose Modification Guidelines for Pembrolizumab-Related AEs

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

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event. [a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued from treatment. [b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. Refer to Table 4 – Infusion Treatment Guidelines for further management details. [c] 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.			

ADV/HSV-tk oncolytic virus therapy: Significant toxicity has not been observed with the administration of ADV/HSV-tk oncolytic virus therapy alone or in combination with RT or chemotherapy in previous clinical trials.[71, 73, 75, 76] Fever may occur due to the local inflammatory and immune response to ADV-tk, which will be treated with acetaminophen as necessary. If local inflammation is extensive or infection develops, valacyclovir will be stopped and antibiotics and/or anti-inflammatory therapy will be given as appropriate.

In both the TNBC and NSCLC cohorts, ADV/HSV-tk dose will be de-escalated to 5×10^9 vp if unacceptable toxicity occurs with the planned dose of ADV/HSV-tk. If toxicity occurs with this dose modification, the dose will be further de-escalated to 5×10^6 vp.

Dosing interruptions for pembrolizumab and SBRT/3D RT 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. Patients whose treatment is interrupted or permanently discontinued due to an AE including abnormal laboratory value must be followed at least once a week for 4 weeks and subsequently at 4-week intervals until resolution or stabilization of the event, whichever comes first. Dose interruptions should be reported on the appropriate Dosage Administration case report form (CRF). The maximum time allowed for toxicity-related treatment interruption is 21 days (3 weeks) from the intended dosing day. If interruption is > 3 weeks, the patient must be discontinued from the study treatment. However, the patient will continue to be followed for toxicity.

Valacyclovir: For patients with serum creatinine level of 1.6−2.0 × ULN, valacyclovir dose will be reduced 50% (i.e., 1 g t.i.d.).

5.2.2 Timing of Dose Administration

All trial treatments will be administered on an outpatient basis.

ADV/HSV-tk (5×10^{11} vp) in a 2-mL total volume will be injected intratumorally on Day 0 of the study. For nodal, lung, and liver lesions, ADV/HSV-tk will be administered after fiducial placement for SBRT.

Valacyclovir will be orally administered at a dose of 2 g t.i.d. for 14 days from Day 1 to Day 15 of the study.

Primary lung tumors and metastatic lesions (breast and lung) will be treated with SBRT (30 Gy; 6 Gy X 5 fractions). Primary breast tumors will be treated with a short course (5−8 fractions) of 3D RT. SBRT and 3D RT will be administered over 2 weeks from Day 2 to Day 16 of the study.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion Q3W (± 1 day). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

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

5.2.3 Second Course

All subjects who stop pembrolizumab treatment with CR may be eligible for up to an additional 12 months (17 cycles) of pembrolizumab treatment if they progress after stopping pembrolizumab from the initial treatment phase. This retreatment is termed the Second Course Phase of this trial and is only available if the trial remains open and the subject meets the following conditions:

Either

- Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR based on the RECIST 1.1, and
    - Was treated with at least 8 cycles of pembrolizumab before discontinuing treatment, and
    - Received at least 2 cycles of pembrolizumab beyond the date when the initial CR was declared

OR

- Had CR and stopped pembrolizumab treatment after completion of 24 months (35 cycles) of pembrolizumab for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined radiographic disease progression by the RECIST 1.1 after stopping initial pembrolizumab treatment, and
    - No new anticancer treatment was administered after the last dose of pembrolizumab, and

- The subject meets all of the safety parameters listed in the inclusion criteria and none of the safety parameters listed in the exclusion criteria, and
- The study is ongoing

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

5.2.4 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 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 CRF including all prescription, over-the-counter, 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 30 days after the last dose of trial treatment should be recorded for SAEs and events of clinical interest (ECIs) as defined in Section 7.2.

5.3.2 Prohibited Concomitant Medications/Therapies

Subjects are prohibited from receiving the following until disease progression is documented per modified irRC and RECIST 1.1:

- Antineoplastic systemic chemotherapy, hormone therapy, or targeted/biological therapy.
- Immunotherapy not specified in this protocol.
- RT not specified in this protocol.

NOTE: Patients are allowed to have palliative radiotherapy to a painful site if they are otherwise benefitting from the trial treatment per the treating physician's judgment and and it is not the target index lesion for tumor response assessment.

- Investigational agents other than pembrolizumab and ADV/HSV-tk.

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, bacillus Calmette-Guérin, and typhoid vaccine.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an ECI 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 that 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 AEs with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1.2 for dose modification.

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

- **Pneumonitis**:

- For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- For **Grade 3–4 events**, immediately treat with IV 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 gastrointestinal consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis**, administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis**, treat with IV steroids followed by HD oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **T1DM if new onset (including diabetic ketoacidosis) or ≥ Grade 3 hyperglycemia if associated with ketosis (ketonuria) or metabolic acidosis**
  - For **T1DM** or **Grade 3–4** Hyperglycemia
    - Insulin replacement therapy is recommended for T1DM and for Grade 3–4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis**:
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3–4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

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

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

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3–4** events, treat with IV 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 infusion completion.

Table 4 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 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5 hr (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg p.o. (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg p.o. (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

IV = intravenous; NSAID = nonsteroidal anti-inflammatory drug; p.o. = orally.

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

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

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

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

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

OR

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

OR

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

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

(1) practice abstinence[†] from heterosexual activity;

OR

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

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

- intrauterine device
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)

- male condom or female condom (cannot be used together)
- hormonal contraceptive: contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and Ethic Review Committees (ERCs)/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

Subjects should be informed that the trial therapy may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of trial therapy initiation throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 Use in Pregnancy

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

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.5.4 Use in Nursing Women

It is unknown whether pembrolizumab and valacyclovir are excreted in human milk. It is known, however, that among women taking valacyclovir, concentrations of valacyclovir in breast milk are about four times higher than in the mother's blood. The safety of valacyclovir in breastfeeding infants has not been established. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breastfeeding are not eligible for enrollment.

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 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
- Dose interruption that exceeds 21 days
- Unacceptable adverse experiences
- Any Grade 4 or greater AE
- Severe (Grade 3) or life-threatening (Grade 4) immune-mediated complication
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Death
- Administrative reasons
- Discontinuation of pembrolizumab treatment may be considered for subjects who have attained a confirmed CR and have been treated for at least 8 cycles (at least 24 weeks) of pembrolizumab and have received at least 2 cycles of pembrolizumab beyond the date when the initial CR was declared. These subjects may be eligible for second course treatment as described in Section 5.2.3.
- Completion of 24 months (35 cycles) of pembrolizumab

NOTE: Subjects who stop pembrolizumab after completion of 24 months (35 cycles) may be eligible for retreatment if they progress after stopping pembrolizumab provided they meet the requirements detailed in Section 5.2.3. Subjects may be retreated in the Second Course Phase (Retreatment) for up to an additional 12 months (17 cycles [calculated starting with the first dose]).

The End of Treatment and Follow-up visit procedures are listed in Section 7.1.5 (Visit Requirements). At the end of treatment (EOT), each subject will be followed for 30 days for AE monitoring (SAEs will be collected for 90 days after EOT as described in Section 7.2.3.1). Subjects who discontinue for reasons other than PD 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 OS until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.7 Clinical Criteria for Early Trial Termination

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

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to 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 Trial Flow Chart

Visit	Screening[a]	Baseline[b]	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10*	EOT	
Day	**-28 to -1**	**-7 to -1**	**0±5**	**2±5**	**4±5**	**7±5**	**9±5**	**11±5**	**17±5**	**38±5**	**59±5**	**80*±5**		
Informed Consent	X													
Inclusion/ Exclusion	X													
Demographics	X													
Medical History	X													
Physical Exam[c]	X		X						X	X	X	X	X	
Height	X													
Weight	X		X						X	X	X	X	X	
ECOG Performance Status	X		X						X	X	X	X	X	
12-Lead ECG and MUGA Scan or Echocardiogram[d]	X												X	
Concomitant Therapies	**Continuous from screening period**													
Hematology[e]	X		X						X	X	X	X	X	
Clinical Chemistry[e]	X		X						X	X	X	X	X	
Urinalysis[f]	X													
Serum Pregnancy (β-hCG)[g]	X													
Tumor Assessments (modified irRC and RECIST 1:1)[h]	X												X	
Brain MRI (NSCLC patients only)[i]	X													
Biopsy[j]			X						X					
Correlative Studies Blood Collection[k]	X								X		X			
AEs and SAEs			**From informed consent signing up to 30 days after the last treatment dose[m]**											
ADV/HSV-tk Administration[l]			X											
Valacyclovir Administration[l]			X											
SBRT Administration[l]				X	X	X	X	X						
Pembrolizumab Administration[l]									X	X	X	X		

Abbreviations: AE = adverse event; ALT = alanine transaminase; ANC = absolute neutrophil count; aPTT = activated partial thromboplastin time; AST = aspartate transaminase; β-hCG, beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CBC = complete blood count; CT = computed tomography; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end of treatment; GM-CSF = granulocyte macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; INR = international normalized ratio; LDH = lactate dehydrogenase; MRI = magnetic resonance imaging; MUGA = multigated acquisition; Q3W = every 3 weeks; PD-1 = programmed death-1; PD-L1 = programmed death-ligand 1; PET = positron emission tomography; p.o. = orally; PT = prothrombin; RT = radiation therapy; SAE = serious adverse event; T3 = triiodothyronine; T4 = thyroxine; Th1 = T-helper; t.i.d. = three times daily; TIL = tumor-infiltrating lymphocyte; TNF = tumor necrosis factor; TSH = thyroid-stimulating hormone; vp = viral particles; WBC = white blood cell. A window of ± 5 days is allowed for visits and study assessments (except as otherwise specified).

a. Within 28 days prior to Day 0 of ADV/HSV-tk.

b. Within 7 days prior to Day 0 of ADV/HSV-tk. Only screening procedures not performed within 7 days of ADV/HSV-tk dosing are required at baseline.

c. The baseline symptom-medical history and physical examination are not required if the screening medical history and physical examination were conducted within 7 to 28 days prior to Day 0 of ADV/HSV-tk. Vital sign (blood pressure, respiratory rate, pulse, and oral temperature) measurements will be performed at each physical exam.

d. A 12-lead ECG will be performed at screening, at EOT, and when clinically indicated. MUGA scan or echocardiogram will be performed only if clinically indicated.

e. A blood sample for CBC with platelet count and differential WBC count will be obtained at screening, on Days 0 and 17, every 3 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated.

If a patient is found to have an ANC <1.5 x 10^9/L, platelet count < 100 x 10^9/L, or both, the CBC with differential should be repeated at least every other day until the ANC and platelet count have exceeded these values.

A blood sample for clinical chemistry panel (albumin, glucose, sodium, potassium, carbon dioxide, chloride, calcium, BUN, creatinine, total bilirubin, total protein, alkaline phosphatase, AST, and ALT) and evaluation of phosphorus, magnesium, and LDH will be obtained at screening, on Days 0 and 17, every 3 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. PT/INR and aPTT will be tested at screening and on Day 17. TSH, free T4, and total T3 will be tested at screening and when clinically indicated.

f. Urinalysis (blood, glucose, protein, specific gravity) will be performed at screening and when clinically indicated.

g. For women of childbearing potential, the results of a serum β-hCG pregnancy test must be negative within 7 days before the first treatment dose is administered. If the screening serum β-hCG pregnancy test is performed more than 7 days before ADV/HSV-tk dosing, it must be repeated at baseline, with results known to be negative prior to the first dose of the study treatment. Testing is to be repeated as clinically indicated.

h. For tumor assessments, CT scan of the chest, abdomen, and pelvis will be performed at screening, every 8 weeks thereafter and/or at the discretion of the treating physician, when clinically indicated, and 30 days after the last dose of pembrolizumab. For patients with equivocal CT scan results, PET scan will be performed. For abscopal effect evaluation, CT-based response assessment of non-target lesions will be performed at screening, every 8 weeks thereafter, and 30 days after the last dose of pembrolizumab.

i. Brain MRI will be performed on NSCLC patients only at screening.

j. In patients with accessible tumor, biopsies will be conducted at Day 0 and 3 (± 2) days after the first dose of pembrolizumab. Patients whose disease progresses while on treatment will also undergo biopsy before they start their new treatment. Banked tumor tissue obtained as part of the patient's standard care and additional biopsy tissues will be evaluated for correlative biomarkers including cytokine expression (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF), TILs, PD-1 and PD-L1 expression, and mutation load. Cytokine expression, TILs, and PD-1 and PD-L1 expression will also be analyzed for abscopal effect evaluation.

k. Blood samples for correlative studies will be collected at screening, 3 (± 2) days after the first dose of pembrolizumab, and end of Cycle 2 of pembrolizumab. An additional sample will be collected from patients whose disease progresses while on treatment. Blood will be collected into standard vacutainer tubes (3 green top tubes).

l. ADV/HSV-tk (5 x 10^{11} vp) in a 2-mL total volume will be injected intratumorally (single-site injection) on Day 0 of the study. The study coordinator will dispense the 14-day quantity of valacyclovir to the patient on Day 0. The patient will be instructed to take valacyclovir (2 g) p.o. t.i.d. for 14 days (from Day 1 to Day 15 of the study). Primary lung tumors and metastatic lesions (breast and lung) will be treated with SBRT of 30 Gy (6 Gy X 5 fractions). Primary breast

tumors will be treated with a short course (5-8 fractions) of three-dimensional RT. SBRT and three-dimensional RT will be administered over 2 weeks from Day 2 to Day 16 of the study. Pembrolizumab at a dose of 200 mg will be intravenously infused over 30 min Q3W (± 1 day) starting on Day 17 (± 1 day) of the study and continuing until disease progression, unacceptable toxicity, or up to 24 months (35 cycles) in subjects without disease progression. All subjects who stop pembrolizumab treatment with complete response may be eligible for up to an additional 12 months (17 cycles) of pembrolizumab treatment if they progress after stopping pembrolizumab from the initial treatment phase. The subject must meet the conditions outlined in Section 5.2.3.
m. AEs and SAEs will be captured from the time of informed consent signing up to 30 days after the final dose of pembrolizumab. Study treatment-related SAEs and any study patient death occurring beyond 30 days after the last dose of pembrolizumab should also be reported.
*Patients will be treated until disease progression, unacceptable toxicity, or up to 24 months in subjects without disease progression.

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.

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 Anticancer Therapy Status

The investigator or qualified designee will review all new antineoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anticancer 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 anticancer therapy has been initiated, the subject will move into survival follow-up.

7.1.2 Clinical Procedures/Assessments

A visit window of ±5 days is allowed for study visits and assessments (except as otherwise specified).

7.1.2.1 Adverse Event 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 the NCI CTCAE v4.03 (see Section 12.2). Toxicities will be characterized in terms of seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For both the TNBC and NSCLC cohorts, a safety run-in period will be implemented for the initial subjects enrolled onto the study. An initial cohort of 3 patients will be followed for toxicity through the first cycle of pembrolizumab. **The PI will submit a status update for the first 3 patients after each individual is enrolled onto this trial via a Reportable Event application.**

Please refer to Section 7.2 for detailed information regarding the assessment and recording of AEs and Section 8.0 for detailed information regarding the run-in cohorts.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam at baseline, on Days 0 and 17, every 3 weeks thereafter until completion of the protocol-specified treatment, and at EOT. Vital signs including temperature, pulse, respiratory rate, and blood pressure will be measured at each physical exam. Height will be measured at screening only. Weight will be measured at screening, on Days 0 and 17, every 3 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated.

7.1.2.3 Directed Physical Exam

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

7.1.2.4 ECOG Performance Scale

The investigator or qualified designee will assess ECOG performance status (see Section 12.1) at screening, on Days 0 and 17, every 3 weeks thereafter until completion of the protocol-specified treatment, and at EOT.

7.1.2.5 Electrocardiogram/Multigated Acquisition Scan/Echocardiogram

A 12-lead electrocardiogram ECG will be performed at screening, at EOT, and when clinically indicated. Multigated acquisition scan or echocardiogram will only be performed when clinically indicated.

7.1.2.6 Brain Magnetic Resonance Imaging

Brain magnetic resonance imaging will be performed on NSCLC patients only at screening.

7.1.2.7 Tumor Imaging and Assessment of Disease and Abscopal Effect Evaluation

Tumor assessments will be performed at screening, every 8 weeks thereafter until completion of the protocol-specified study treatment and/or at the discretion of the treating physician, when clinically indicated, and 30 days after the last dose of pembrolizumab. Tumor imaging will consist of CT scan of the chest, abdomen, and pelvis. Disease status will be assessed using RECIST 1.1 in addition to modified irRC (see Section 12). For patients with equivocal CT scan results, PET scan will be performed. For abscopal effect evaluation, CT-based response assessment (RECIST 1.1) of a non-target lesion will be performed at screening, every 8 weeks thereafter until completion of the protocol-specified study treatment, and 30 days after the last dose of pembrolizumab.

Modified irRC is based on recent cancer immunotherapy clinical studies showing that immunotherapy-mediated tumor regressions or stabilizations may only become evident or prominent over time. For this trial, the concepts of the irRC[77] are combined with RECIST 1.1 to come up with the modified irRC, which uses unidimensional measurements. For modified irRC, only target and measurable lesions are taken into account. In contrast to the RECIST 1.1, the modified irRC (a) require confirmation of both progression and response by imaging at 8 weeks after initial imaging and (b) do not necessarily score the appearance of new lesions as PD if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%. All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are defined as follows:

- New measurable lesions: incorporated into tumor burden.
- New non-measurable lesions: do not define progression but precludes immune-related CR (irCR).
- Overall irCR: complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes must also have a reduction in short axis to 10 mm or less.
- Overall immune-related PR (irPR): ≥ 30% decrease in the sum of the longest diameters of target and new measurable lesions.
- Overall immune-related SD (irSD): Sum of the longest diameters of target and new measurable lesions neither irCR, irPR, (compared to baseline), or immune-related PD (irPD) (compared to nadir).

- Overall irPD: ≥ 20% increase in the sum of the longest diameters of target and new measurable lesions (compared to nadir), confirmed by repeat, consecutive observations at least 4 weeks (normally it should be done at 6 weeks) from the date first documented.

Documentation of irPD (based on the modified irRC) does not mandate discontinuation of the study treatment even after irPD is confirmed with CT scan 8 weeks after the initial observation of irPD.

RECIST 1.1 are defined as follows:

Target Lesions (Main Tumor)

- CR: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
- PR: At least a 30% decrease in the sum of the diameters of target lesions, using the baseline sum diameters as the reference.
- PD: At least a 20% increase in the sum of the diameters of target lesions, using the smallest sum on study (includes the baseline sum) as the reference. In addition to the relative 20% increase, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression)
- SD: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, using the smallest sum diameters while on study as the reference.

Non-Target Lesions

- CR: Disappearance of all non-target lesions and normalization of tumor marker levels. All lymph nodes must be non-pathological in size (< 10 mm short axis).
  Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker levels above the normal limits.
- PD: Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.
- *Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or investigator).*

7.1.2.7.1 Second Course (Retreatment) Tumor Imaging

Tumor imaging must be performed within 4 weeks prior to restarting treatment with pembrolizumab. The first on-study imaging assessment should be performed at 8 weeks (± 7 days) after the restart of treatment. Subsequent tumor imaging should be performed every 8 weeks (± 7 days) or more frequently, if clinically indicated.

7.1.2.8 Tumor Tissue and Blood Collection and Correlative Studies

In patients with accessible tumor, biopsies will be conducted at Day 0 and 3 (± 2) days after the first dose of pembrolizumab. Subjects whose disease progresses while on treatment will undergo an additional biopsy before starting their new treatment. Banked tumor tissue obtained as part of the subject's standard of care and collected biopsy tissues will be evaluated for Th1 response by cytokine expression (IL-1, IL-2, IL-6, IL-12, IFN-c, TNF-α, and GM-CSF); TILs; PD-1 and PD-L1 expression; and mutation load. PD-1 and PD-L1 expression, immune infiltrates, and cytokine expression will also be analyzed for abscopal effect evaluation.

Blood samples for correlative studies will be collected at screening, 3 (± 2) days after the first dose of pembrolizumab, and end of Cycle 2 of pembrolizumab. An additional sample will be collected from subjects whose disease progresses while on treatment. Blood samples will be collected into standard vacutainer tubes (2 green top tubes). Samples will be evaluated for profile of circulating suppressor and effector immunocytes and cytokines.

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)

A blood sample for clinical chemistry panel (sodium, potassium, carbon dioxide, chloride, calcium, glucose, albumin, blood urea nitrogen, creatinine, total bilirubin, total protein, alkaline phosphatase, AST, and ALT) and evaluation of phosphorus, magnesium, and lactate dehydrogenase will be obtained at screening, on Days 0 and 17, and every 3 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. PT/INR and aPTT will be tested at screening and on Day 17. Thyroid-stimulating hormone, free thyroxine, and total thriiodothyronine will be tested at screening and when clinically indicated. A blood sample for complete blood count (CBC) with platelet count and differential WBC count will be obtained at screening, on Days 0 and 17, every 3 weeks thereafter until completion of the protocol-specified treatment, at EOT, and when clinically indicated. If a patient is found to have an ANC < 1.5 x 10^^{9}/L, platelet count < 100 x 10^^{9}/L, or both, the CBC with differential should be repeated at least every other day until the ANC and platelet count have exceeded these values. For women of childbearing potential, the results of a serum β-hCG pregnancy test must be negative within 7 days before the administration of the first dose of trial treatment. If the screening serum β-hCG pregnancy test is performed more than 7 days before ADV/HSV-tk dosing, it must be repeated at baseline. Pregnancy testing is to be repeated as clinically indicated. Urinalysis (blood, glucose, protein, specific gravity) will be performed at screening and when clinically indicated. Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hemoglobin Platelet count WBC count (total and differential) ANC	Albumin Alkaline phosphatase ALT AST Lactate dehydrogenase Carbon dioxide Creatinine Calcium Chloride Glucose Phosphorus Potassium Sodium Magnesium Total bilirubin Total protein Blood urea nitrogen	Blood Glucose Protein Specific gravity Microscopic exam *(If abnormal)* results are noted	Serum β- hCG† PT (INR) aPTT Total thriiodothyronine Free thyroxine Thyroid-stimulating hormone
†Perform on women of childbearing potential only.			

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 AEs 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.

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 Post-Treatment Visits

7.1.5.1.1 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 anticancer 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 the beginning of a new antineoplastic therapy, whichever occurs first. SAEs that occur

within 90 days of EOT or before initiation of a new anticancer treatment should also be followed and recorded.

7.1.5.1.2 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 6 weeks (42 ± 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 antineoplastic therapy, disease progression, death, or end of the study.

7.1.5.1.3 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anticancer 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.

7.2 Assessing and Recording Adverse Events

An AE 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 AE can, therefore, be any unfavorable and unintended sign (e.g., abnormal laboratory finding), 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 study treatment is also an AE. Progression of the cancer under study is not considered an AE.

All AEs that occur after informed consent form signing must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment, or a procedure.

From the time of informed consent signing through 30 days following cessation of treatment, all AEs must be reported by the investigator. AE reporting will be performed according to 21 CFR 312.32. AEs will be recorded at each examination on the Adverse Event CRFs/worksheets. AEs (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the subject's CRF. The reporting timeframe for AEs meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-SAEs for outcome.

AEs will not be collected for subjects during the prescreening period as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

The occurrence of AEs should be sought by non-directive questioning of the patient at each study visit. AEs may also be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each AE should be evaluated to determine:

1. Severity grade (CTCAE Grade 1–4)
2. Duration (Start and end dates or if continuing at the Safety Follow-up Visit)
3. Relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown)
7. Whether it is serious, where a SAE is defined as in Section 7.2.3.1.

All AEs should be treated appropriately. Such treatment may include changes in study treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an AE is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

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 pembrolizumab overdose. 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 AE(s) is associated with ("results from") pembrolizumab overdose, the AE(s) is reported as a SAE, even if no other seriousness criteria are met.

If a dose of pembrolizumab meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious ECI, using the terminology "accidental or intentional overdose without adverse effect."

For valacyclovir overdose, activated charcoal may be administered to aid in the removal of unabsorbed drug. General supportive measures are recommended. Acute renal failure and neurological symptoms, including confusion, hallucinations, agitation, decreased consciousness and coma, and nausea and vomiting may occur. Caution is required to prevent inadvertent overdose. Many of the reported cases involved renally impaired and geriatric patients receiving repeated overdoses due to lack of appropriate dosage reduction.

All reports of overdose with and without an AE must be reported within 24 hours to the Sponsor (hmccsaereports@houstonmethodist.org; FAX 713-790-5106) 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 AEs, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

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

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

Such events must be reported within 24 hours to the Sponsor (hmccsaereports@houstonmethodist.org; FAX 713-790-5106) 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 SAE is any untoward medical occurrence that at any dose:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication and not associated with any deterioration in condition

  - elective or preplanned treatment for a preexisting condition that is unrelated to the indication under study and has not worsened since the start of study drug
  - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
  - social reasons and respite care in the absence of any deterioration in the subject's general condition;
- Is a congenital anomaly/birth defect;
- Is another important medical event. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent.
- **Note:** In addition to the above criteria, AEs meeting either of the below criteria, although not serious per International Conference on Harmonisation (ICH) definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
  - Is a new cancer (that is not a condition of the study);
  - Is associated with an overdose.

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

From the time of informed consent form signing, any SAE or follow up to a SAE, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.1 for additional details), that occurs to any subject must be reported within 24 hours to the Sponsor (hmccsaereports@houstonmethodist.org; FAX 713-790-5106) and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment, or a procedure.

From the time of informed consent form signing through 90 days following cessation of treatment or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any SAE or follow up to a SAE, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.1 for additional details), whether or not related to the study treatment, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

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

All subjects with SAEs must be followed up for outcome.

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

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by the FDA, European Union, Pharmaceutical and Medical Devices agency, and 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 the FDA.

7.2.3.2 Events of Clinical Interest

Selected non-serious and serious AEs are also known as ECI and must be reported within 24 hours to the Sponsor (hmccsaereports@houstonmethodist.org; FAX 713-790-5106) and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220).

From the time of informed consent form signing, any ECI or follow up to an ECI that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment, or a procedure.
From the time of informed consent form signing through 90 days following cessation of treatment or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI or follow up to an ECI, whether or not related to the study treatment, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.
ECIs for this trial include:
1. an overdose of pembrolizumab, 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 3 X ULN and an elevated total bilirubin lab value that is greater than or equal to 2 X ULN and, at the same time, an alkaline phosphatase lab value that is less than 2 X ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

7.2.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 7.2.3- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the study treatment and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

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

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study.

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

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all AEs according to the NCI CTCAE v4.03. Any AE which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the Adverse Event CRFs/worksheets.

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

Table 6 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all AEs as to:

V4.03 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (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 SAE is any untoward medical occurrence that at any dose:	
	†**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 AE that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a SAE. A pre-existing condition is a clinical condition that is diagnosed prior to the use of the study treatment and is documented in the subject's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis); or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); or	
	Is an overdose (whether accidental or intentional). Any AE associated with an overdose is considered a SAE for collection purposes. An overdose that is not associated with an AE is considered a non-serious ECI and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	

	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a SAE 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 AE. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the AE cause the study treatment to be discontinued?	
Relationship to the study treatment	Did the study treatment cause the AE? The determination of the likelihood that the study treatment caused the AE 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 study treatment and the AE based upon the available information. **The following components are to be used to assess the relationship between the study treatment and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the study treatment caused the AE:	
	Exposure	Is there evidence that the subject was actually exposed to the study treatment such as: reliable history, acceptable compliance assessment, 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 study treatment? Is the time of onset of the AE compatible with a drug-induced effect?
	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 Merck product 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 Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the 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) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN AE 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 SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the CRFs/worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to 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 of 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 the AE is more likely explained by another cause than the Merck product. (Also entered for a subject with overdose without an associated AE.)

7.2.5 Sponsor Responsibility for Reporting Adverse Events

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

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analysis Plan Summary

Sample Size: For the TNBC cohort, the null and alternate hypotheses are 19% ORR[78] and 39% ORR, respectively. For the NSCLC cohort, the null and alternate hypotheses are 20% ORR[79] and 40% ORR, respectively. For the TNBC cohort, the target response rate is 39%. With a sample size of 28 patients, if the observed number of responders is 11 (39.3%); the 95% CI estimate will have a margin of error of 18.1 percentage points (i.e., 21.2% to 57.4%) based on the normal approximation. For the NSCLC cohort, the target response rate is 40%. With a sample size of 29 patients, if the observed number of responders is 12 (41.4%); the 95% CI estimate will have a margin of error of 17.9 percentage points (i.e., 23.5% to 59.3%) based on the normal approximation. For both the TNBC and NSCLC cohorts, we will implement a safety run-in period for the initial subjects enrolled onto the study. An initial cohort of 3 patients will be followed for toxicity through the first cycle of pembrolizumab. **The PI will submit a status update for the first 3 patients after each individual is enrolled onto this trial via a Reportable Event application.** If only 1 of 3 patients experience toxicity, then 3 more patients will be enrolled. If no more than 1 of 6 patients experience toxicity, then the trial will be fully opened. The staggering interval will be 5 weeks for the run-in cohort.

Statistical Analysis: DoR, OS rate, PFS rate, and CBR will be analyzed. Safety profiles will also be assessed through summaries of AEs, SAEs, AEs leading to treatment discontinuation, and treatment-related death. The safety analysis will report the frequency of all AEs and laboratory abnormalities as well as the frequency of dose interruptions and toxicity-related treatment discontinuation. Toxicity rates will be presented using the worst NCI CTCAE grade per patient.

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 products in accordance with the protocol and any applicable laws and regulations.

Pembrolizumab will be provided by Merck as summarized in Table 7.

Table 7 Product Descriptions

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

9.2 Packaging and Labeling Information

Investigational products will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Investigational Product Disclosure

This trial is open label; therefore, the subject, trial site personnel, 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

Investigational products 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.

Investigational products 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 investigational product 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 products 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 that appropriate records of disposal are kept.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the FDA Modernization Act and FDA Amendments Act, 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.

10.2 Data Management

CRFs will be designed and utilized to capture all patient data. An electronic database will be designed to store patient CRFs. Data quality control will be performed regularly by the research coordinator/research nurse to ensure timely, accurate, and complete patient data collection. Queries will be generated and resolved prior to the generation of interim and final summary reports.

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12.0 APPENDICES

12.1 ECOG Performance Status Scale

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.
Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.	

12.2 Common Terminology Criteria for Adverse Events V4.03

The descriptions and grading scales found in the revised NCI CTCAE version 4.03 will be utilized for AE reporting. (http://evs.nci.nih.gov/ftp1/CTCAE/About.html)

12.3 Modified irRC (derived from RECIST 1.1)

Overall responses derived from changes in index, non-index, and new lesions

Measurable Response	Non-Measurable Response		Overall Response Using Modified irRC
Index and New, Measurable Lesions (Tumor Burden)	**Non-Index Lesions**	**New, Non-Measurable Lesions**	
Decrease 100%	Absent	Absent	irCR*
Decrease 100%	Stable	Any	irPR*
Decrease 100%	Unequivocal progression	Any	irPR*
Decrease ≥ 30%	Absent / Stable	Any	irPR*
Decrease ≥ 30%	Unequivocal progression	Any	irPR*
Decrease < 30% to increase < 20%	Absent / Stable	Any	irSD
Decrease < 30% to increase < 20%	Unequivocal progression	Any	irSD
Increase ≥ 20%	Any	Any	irPD

*Assuming that the response (irCR and irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart.

irCR, immune-related complete response; irPR, immune-related partial response; irSD, immune-related stable disease; irPD, immune-related progressive disease.

12.4 New York Heart Association Functional Classifications

Class	Description
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on physical activity without discomfort. Symptoms of cardiac insufficiency or of angina syndrome may be present at rest. If any physical activity is undertaken, discomfort is increased.