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Title: *Targeting PD-1 Therapy Resistance with Focused High or High and Low Dose Radiation in Squamous Cell Carcinoma of the Head and Neck (SCCHN)*

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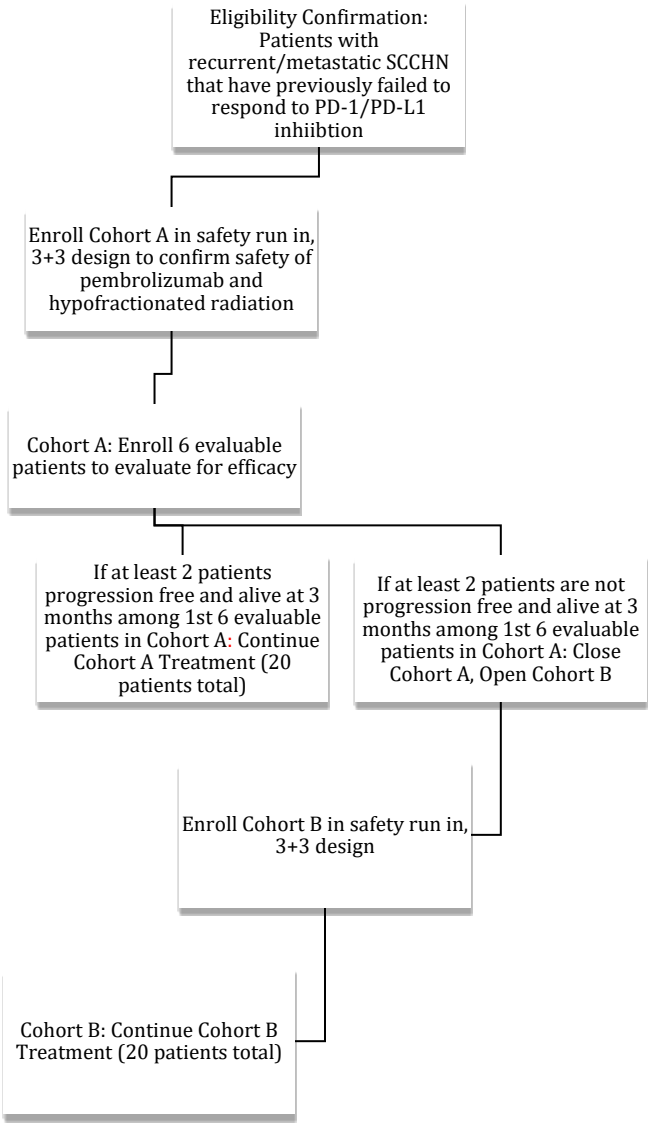
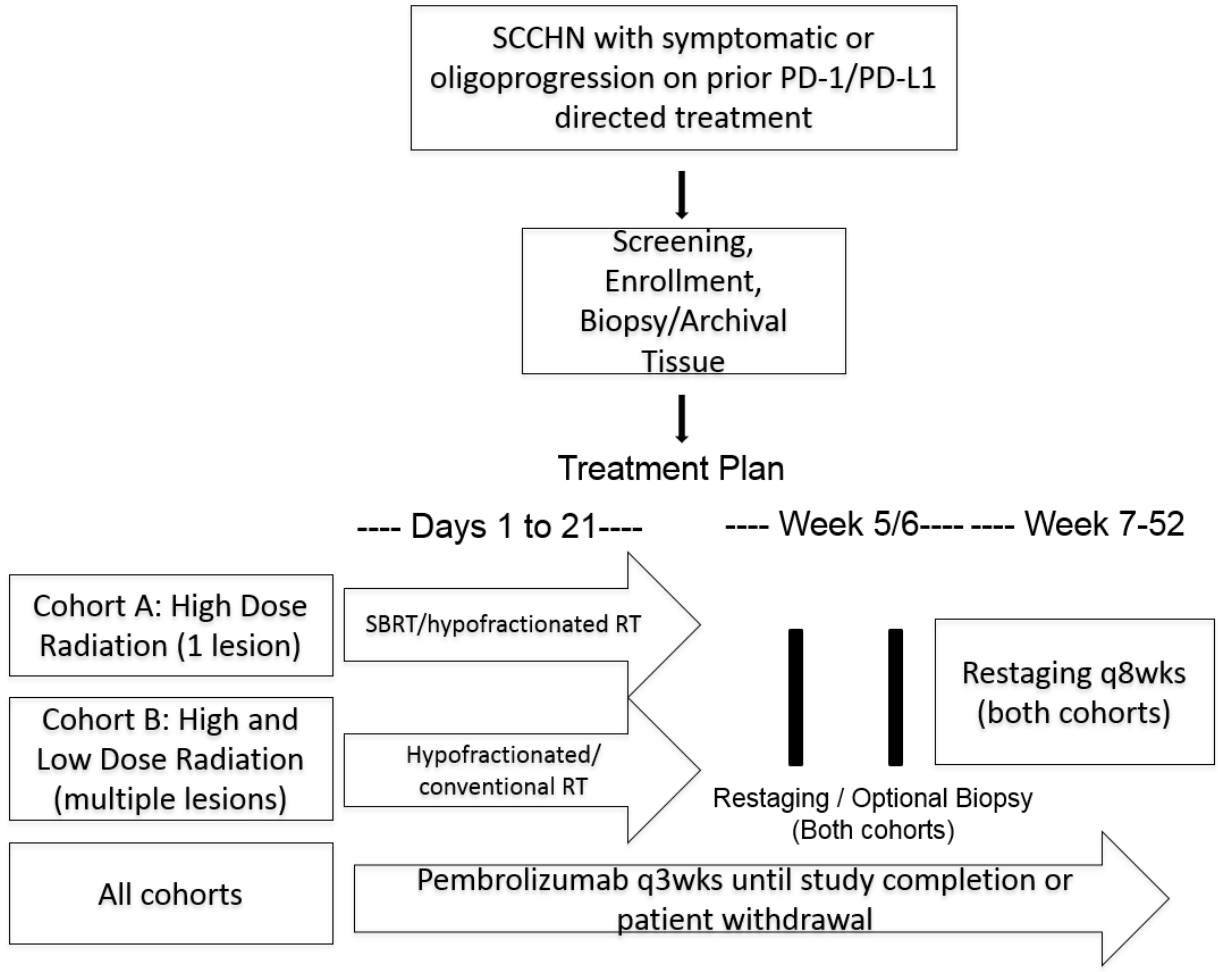
Trial Enrollment – Study Schema



TREATMENT SCHEMA (21 Day Cycles)



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1. OBJECTIVES

1.1 Study Design

This is an open label phase 2 study with two cohorts testing the hypothesis that the use of targeted radiation will invigorate the immune system and improve outcomes in patients with metastatic squamous cell carcinoma of the head and neck (SCCHN) who have previously progressed on or shortly after prior therapy targeting either the PD-1 receptor or the PD-L1 ligand. Thus radiation will be tested for its ability to delay progression in patients with limited systemic options. All patients will have previously demonstrated radiologic disease progression, symptomatic disease progression, or lack of clinical benefit/systemic response while receiving or shortly following prior agents targeting the PD-1 pathway. These patients will have lesions amenable for radiation therapy that will be irradiated in conjunction with pembrolizumab. In cohort A, only a single lesion will be targeted with 8 Gy of radiation for each of 3-5 fractions, in an attempt to provide palliative benefit and delay further progression when given in combination with pembrolizumab. Pembrolizumab will be administered intravenously every 21 days until disease progression. If there is no evidence of delayed progression among the first 6 patients treated in Cohort A, this cohort will close. Cohort B will begin enrolling after Cohort A has been closed. In this cohort, patients will receive irradiation of between 1 and 4 lesions in combination with pembrolizumab. These lesions may receive a combination of high (8 Gy x 3-5 fractions) with/without low dose radiation (20 Gy / 5 fractions) with the goal of maximally decreasing tumor burden to delay progression. Pembrolizumab will be administered intravenously every 21 days until disease progression. Correlative questions will also be incorporated in this study.

1.2 Primary Objective

- To determine progression free survival using RECIST 1.1 following treatment with pembrolizumab in combination with radiation in patients with metastatic SCCHN who have previously progressed on PD-1/PD-L1 inhibitors.

1.3 Secondary Objectives

- To confirm the safety and tolerability of radiation with pembrolizumab in patients with SCCHN.

- To determine local response rates within the irradiated fields.

- To determine overall response rates (RECIST 1.1. and irRC) and abscopal response rates (when applicable) following radiation therapy given in combination with pembrolizumab (defined as at least a 30% decrease in the longest diameter of the best responding abscopal lesion).

- To estimate progression-free and overall survival at 6 and 12 months post treatment

- When possible, to investigate mechanisms of resistance to PD-1 directed therapy in SCCHN by evaluating PD-L1 expression, and T cell infiltration in initial biopsies taken from patients who

have previously progressed on PD-1 directed therapy, and subsequently, to investigate changes in these parameters engendered by radiation therapy

- To explore the effect of radiation in combination with pembrolizumab on circulating T-cell populations, and T-cell receptor diversity.

- To evaluate the feasibility of using hypothesis guided radiation to identify lesions to be irradiated that have the greatest likelihood of producing an abscopal immune response

2. BACKGROUND

2.1 Study Agents: Pembrolizumab

2.1.1 Pembrolizumab

2.1.1.1 Mechanism of action

Cancer immunotherapy is based on the premise that the body's immune system can recognize a tumor as foreign and mount an effective antitumor response capable of eliminating that tumor. This likely requires immune recognition of specific tumor antigens, but also effective functioning of activated T-cells capable of eliminating tumor cells as they arise and causing tumor shrinkage where existing tumor deposits are present. Conversely, tumor progression is likely intimately intertwined with mechanisms by which tumors evade immune recognition and attack.

One mechanism by which tumors may evade immune attack is by coopting inherent immune checkpoints that function under normal circumstances to maintain immune homeostasis and prevent harmful autoimmunity. Thus, one strategy that exists for cancer immunotherapy is to modulate these regulatory immune checkpoints that largely exist on the surface of T-cells. This can ideally overcome tumor mediated immune suppression, and potentiate nascent antitumor immune responses that might otherwise have been unable to lead to meaningful tumor regression.

Programmed death receptor-1 (PD-1, CD279), is a 55 kD type I transmembrane protein is a member of the CD28 family of T-cell constimulatory molecules that also includes CD28, CTLA-4, ICOS, and BTLA[1]. PD-1 contains an intracellular membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal immunoreceptor tyrosine-based switch motif (ITSM). Two ligands specific for PD-1 have been identified: PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273) [2]. PD-L1 and PD-L2 have been shown to down-regulate T-cell activation upon binding to PD-1 in both murine and human systems[2, 3]. PD-1 delivers a negative signal by the recruitment of SHP-2 to the phosphorylated tyrosine residue in the ITSM in its cytoplasmic region[4, 5]. PD-1 is primarily expressed on activated T cells, B cells, and myeloid cells[5].

Further evidence for a negative regulatory role of PD-1 comes from studies of PD-1-deficient mice. PD-1-deficient mice develop various autoimmune phenotypes, including dilated cardiomyopathy, a lupus-like syndrome with arthritis and nephritis, and accelerated diabetes mellitus[6-8]. The emergence of these autoimmune phenotypes is dependent upon the genetic background of the mouse

strain and many of these phenotypes emerge at different times and show variable penetrance. In addition to the phenotypes of null mutations, PD-1 inhibition by antibody-mediated blockade in several murine models has been found to play a role in the development of autoimmune diseases such as encephalomyelitis, graft-versus-host disease, and type I diabetes[9, 10]. Taken together, these results suggest that PD-1 blockade has the potential to activate anti-self T-cell responses, but these responses are variable and dependent upon various host genetic factors. Thus, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self-antigens.

Preclinical animal models of tumors have shown that blockade by PD-1 by monoclonal antibodies (mAbs) can enhance the anti-tumor immune response and result in tumor rejection. Antitumor activity by PD-1 blockade functions in PD-L1-positive tumors as well as in tumors that are negative for the expression of PD-L1[11-16]. This suggests that host mechanisms (ie, expression of PD-L1 in antigen-presenting cells) limit the antitumor response. Consequently, both PD-L1 positive and negative tumors may be targeted using this approach. In humans, constitutive PD-L1 expression is normally limited to macrophage-lineage cells, although expression of PD-L1 can be induced on other hematologic cells as well, including activated T cells. However, aberrant expression of PD-L1 by tumor cells has been reported in a number of human malignancies [17-22]. PD-L1 expressed by tumor cells has been shown to enhance apoptosis of activated tumor-specific T cells in vitro [5]. Moreover, the expression of PD-L1 may protect the tumor cells from the induction of apoptosis by effector T cells[23]. Retrospective analyses of several human tumor types suggest that tumor over-expression (as measured by IHC) of PD-L1 may permit immune evasion by tumors. In renal cell carcinoma, high surface expression levels of PD-L1 on tumor cells are related to tumor aggressiveness [20, 21]. Subjects with high tumor and/or lymphocyte PD-L1 levels are 4.5 times more likely to die from their cancer than subjects exhibiting low levels of PD-L1 expression. In addition, in multivariate analysis, high expression of PD-L1 is correlated to have a worse overall survival rate compared to low expression levels of PD-L1[24].

2.1.1.2 Clinical pharmacology and safety

Pembrolizumab (MK-3475) (previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

Clinical data are derived from an ongoing, first-in-human phase I study (PN001, NCT01295827) to evaluate the safety and clinical activity of Pembrolizumab as a monotherapy, sponsored by Merck Sharp & Dohme. There are five parts to this study (Parts A-D and F) (Investigator's Brochure, 2014).

Part A was a 3+3 dose escalation study in subjects with solid tumors to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics, and to determine a maximum tolerated dose (MTD) or preliminary recommended phase 2 doses (RP2Ds). Doses were 1, 3, and 10 mg/kg every 2 weeks (Q2W); doses of either 2 mg/kg or 10 mg/kg were also administered every 3 weeks (Q3W). All 3 dose levels were well tolerated and no dose-limiting toxicities (DLTs) were

observed; therefore, the MTD was not determined. The RP2D was determined by the sponsor based on safety, PK, and pharmacodynamic measurements, along with the strength of antitumor activity signals observed.

Pharmacokinetics
The half-life ($t_{1/2}$) of pembrolizumab is approximately 4 weeks and there is no indication of dose dependency or half-life in the three dose groups (1,3, and 10 mg/kg) (Investigator's Brochure, 2014). The long $t_{1/2}$ supports a dosing interval of every 2 or 3 weeks.

There was a dose-related increase in exposure from 1 to 10 mg/kg. Serum concentrations of pembrolizumab were lower by a factor of approximately 5 in patients receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W. Steady-state trough concentrations were 20% greater in the patients receiving 10 mg/kg Q2W than in those receiving the same dose Q3W.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2mg/kg and comparable doses of MK-3475 in solid tumors is based on: 1) similar efficacy and safety of MK-3475 when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of MK-3475 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 MK -3475 (as assessed by the population PK model) and 4) the assumption that the dynamics of MK -3475 target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of MK-3475 showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe. A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

Anti-Drug Antibodies (ADA) Data

The occurrence of ADA has been observed in less than 1% of the patients screened, indicating a low potential of pembrolizumab to elicit the formation of ADA. No impact of ADA on pembrolizumab exposure has been observed.

Pembrolizumab has also been well tolerated specifically in metastatic and recurrent head and neck cancer patients. KEYNOTE 012 is a Phase Ib study of pembrolizumab in patients with human papillomavirus (HPV)-negative and HPV-positive head and neck cancer. This trial enrolled an initial 60 patient cohort with recurrent and/or metastatic squamous cell carcinoma of the head and neck for treatment with single agent pembrolizumab. Preliminary results of this cohort were reported at Annual Meeting of the American Society of Clinical Oncology (ASCO) in 2014 [25]. In general, pembrolizumab was well tolerated with 58.3% reporting a DR AE and 16.7% reporting a Grade 3-5 DR AE. DR AEs with an incidence ≥ 5% were fatigue (10, 16.7%), pruritis (6, 10%), rash (5, 8.3%), nausea (4, 6.7%), decreased appetite (3, 5.0%), and myalgia (3, 5.0%). Of these DR AEs, Grade 3-5 was seen in rash (2, 3.3%). The reported pre- specified AEs were adrenal insufficiency (1, 1.7%); diarrhea (1, 1. 7%); pruritis (1, 1.7%); rash (2, 3.3%); rash, macular (1, 1.7%); pneumonitis (0); alanine aminotransferase (ALT) increase (2, 3.3%); and aspartate aminotransferase (AST) increase (2, 3.3%).

2.1.1.3 Clinical Efficacy

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and leads ultimately to tumor rejection, either as a mono-therapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a mono-therapy in models of squamous cell carcinoma, pancreatic carcinoma, MEL and colorectal carcinoma. Blockade of the PD-1 pathway effectively promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-α, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo [26-30]. Experiments have confirmed the in vivo efficacy of PD-1 blockade as a mono-therapy (see the Investigator's Brochure).

When treated with pembrolizumab monotherapy, the overall response rate (ORR) for ipilimumab (IPI)-treated patients with melanoma was 25%/27% according to the Response Evaluation Criteria in Solid Tumors (RECIST)/investigator-assessed immune-related response criteria (irRC), respectively (Investigator's Brochure, 2014). The ORR for IPI-naïve patients with melanoma was 39%/43% by RECIST/investigator-assessed irRC, respectively. The majority of responses were seen in patients with melanoma by 16 weeks of therapy; however, some responses have been reported after 24 weeks or more of therapy with pembrolizumab. Responses can be delayed, and in some patients, a RECIST-defined progression followed by response has been observed. The preliminary ORR for 38 patients with non-small cell lung cancer was 21%/24% by RECIST/investigator-assessed irRC, respectively (Investigator's Brochure, 2014).

Ongoing clinical trials of pembrolizumab are being conducted in advanced melanoma, non-small cell lung cancer, a number of advanced solid tumor indications and hematologic malignancies. For study details please refer to the IB.

Trials evaluating pembrolizumab in head and neck cancer have demonstrated clinical activity in patients with recurrent and/or metastatic disease. KEYNOTE 012 is a Phase Ib study of pembrolizumab in patients with human papillomavirus (HPV)-negative and HPV-positive head and neck cancer. This trial enrolled an initial 60 patient cohort with recurrent and/or metastatic squamous cell carcinoma of the head and neck for treatment with single agent pembrolizumab. Preliminary results of this cohort were reported at Annual Meeting of the American Society of Clinical Oncology (ASCO) in 2014 [25], showing an overall response rate (confirmed and unconfirmed) of 19.6% (10 partial responses [PRs], 1 complete response [CR] out of 56 patients evaluable for response). An additional 16/56 patients (28.6%) experienced stable disease (SD), with 51% of patients experiencing some numerical decrease in tumor burden from baseline. Seventeen total patients with CR, PR, or SD remain on therapy at the time of the reporting for > 6 months. There were no new or unexpected toxicity signals in this patient cohort, with infrequent grade 3-4 drug-related (DR) adverse events (AEs).

Preliminary PD-L1 biomarker data from KEYNOTE 012 suggests that the response rate may be enhanced for patients with high PD-L1 IHC expression. Using a Youden-Index derived PD-L1 IHC cutpoint, the response rate (RR) in patients with high PD-L1 expression was 45.5% (5/11), compared to 11.4% (5/44) in low PD-L1 expression patients.

More recently, PD-1 inhibition with pembrolizumab has become standard treatment for patients with recurrent / metastatic SCCHN who are platinum refractory regardless of PD-L1 expression or in the first line setting as monotherapy for patients with PD-L1 CPS>1% based on the results of the landmark Keynote 48 data.[31] Pembrolizumab / chemotherapy is also a treatment option in the first line setting for patients with recurrent / metastatic SCCHN irrespective of PD-L1 expression based on results from the Keynote 48 study.[31] Unfortunately, however, the majority of patients either fail to respond to PD-1 inhibition or demonstrate progression after initial response.

2.2 Study Disease

SCCHN is the 5th most common type of cancer with an incidence of approximately 550,000 new cases worldwide each year. There are over 50,000 cases diagnosed each year in the United States, with over 10,000 deaths annually. HNCs describe malignancies of the upper aerodigestive tract that include squamous cell carcinomas (SCCHN) of the oral cavity, nasopharynx, pharynx and larynx. While tobacco and alcohol use are the most common risk factors for HNCs, Epstein-Barr virus and human papilloma virus (HPV) may also play a role in the development of carcinomas of the nasopharynx and squamous cell carcinomas of the oropharynx, respectively.

A TNM-based classification is used to clinically stage all SCCHN for treatment and outcome measures (American Joint Committee on Cancer [AJCC] Cancer Staging Manual 7th edition, 2010). At initial diagnosis, SCCHN can be of early stage (ES - 33%; Stage I/II), locally advanced (LA - 52 - 60%; stage III/IV-A/IV-B) or metastatic (met - ~10%; Stage IV-C). With standard of care treatment, the 5-year survival for ES is 80%, for LA it is 50% and for metastatic disease it is 25%. Approximately 50% of the treated population returns with recurrent or refractory disease. For recurrent or refractory disease, the 1-year survival rate is 5% - 33% by various estimates with a median OS of 6 to 9 months [32, 33]. Metastatic and recurrent HNC that is no longer amenable

to local surgical/radiation therapy causes substantial morbidity and high mortality, with a median progression free survival (PFS) of <= 6 months due to the lack of effective therapeutic options.

Treatment of recurrent or metastatic disease consists of cytotoxic chemotherapies such as methotrexate, organoplatinum compounds, fluorouracil (5-FU), or taxanes - either alone or in combination - and the biologic agent cetuximab. However, median survival for patients who develop metastatic disease is typically less than 1 year, even with aggressive treatment [34]. Patients whose disease progresses after platinum based therapy have a poor prognosis. In a retrospective analysis of 151 subjects with platinum-refractory recurrent metastatic SCCHN, 68 patients (45%) receiving best supportive care had a median OS of 56 days (95% CI, 46 - 67 days) whereas in 43 subjects (28%), second line chemotherapy (platinum based or methotrexate) had no ORR and median OS was 107 days (95% CI, 83 - 135 days) [35]. Even worse are patients who progress after initial treatment for metastatic disease (refractory or platinum-resistant disease) who have the worst prognosis with median OS of 3 - 4 months and 1 year survival rate of < 5%. Thus, metastatic or recurrent HNC remains an area of high unmet medical need.

Head and neck cancers represent an attractive target for immune based therapy given a predominance of viral and tobacco induced etiologies, both of which may render tumors particularly sensitive to immune checkpoint blockade [36]. Additionally, a significant number of SCCHN tumors may express PD-L1 on the surface of tumor cells [37], which has been suggested as a potential predictive biomarker of response to PD-1 inhibiting therapy in SCCHN as well as other malignancies. In addition to those patients whose tumors express PD-L1 on their surface, SCCHN tumors that do not express PD-L1 may also respond to PD-1 blockade [36].

2.3 Radiation therapy

Radiation is commonly used throughout the body for patients with metastatic disease for palliative purposes. Head and neck cancer patients, specifically, may require palliative radiation for bone pain or metastatic bony sites that could progress and cause fracture or functional compromise, enlarging lung lesions, liver lesions, or head and neck disease that may cause local symptoms or threaten critical structures. Palliative radiation regimens used vary based on institutional practices and patient and disease factors, but are generally in the range of 8 to 37.5 Gy delivered in 1 to 15 fractions. There is some evidence that a higher bioequivalent dose will result in more durable disease control [38, 39] and preclinical evidence suggests that higher radiation doses may stimulate increased immunologic effects [40]. In the head and neck specifically, a retrospective study conducted at Prince Margaret Hospital that compared multiple fractionation regimens demonstrated that radiation dose was an independent predictor of both overall survival and treatment response on multivariate analysis [41]. The scheme of 24 Gy delivered in three 8 Gy fractions was prospectively investigated in a radiation dose escalation trial in patients with oligometastatic disease and found to be well tolerated [42]. This fractionation was also evaluated in the preclinical setting in combination with immune checkpoint blockade with CTLA-4 inhibition and found potentiate abscopal, out-of-field effects better as compared to various other fractionation regimens[43]. In a prospective trial given in combination with the CTLA-4 inhibitor ipilimumab, 8 Gy delivered in each of 2 or 3 fractions was also well tolerated, although efficacy was likely limited by tumors that demonstrated high levels of PD-L1 [44]. In this study, radiation was only administered to 1 metastatic deposit with the hope of stimulating out of field responses.

While the dose of 24 Gy in 3 fractions is appropriate for palliative radiation therapy throughout the body, higher doses are indicated in clinical scenarios relevant to patients with head and neck cancers, specifically patients with locally recurrent disease within the head and neck region. Vargo et al. [45]describe a promising locoregional PFS rate of 60% at 1 year in a prospective phase 2 study with the combination of radiation to the head and neck delivered with stereotactic body radiotherapy (SBRT) to a dose of 40-44 Gy over 5 fractions in combination with cetuximab; however, median overall survival was only 10 months, highlighting the need for more effective accompanying systemic therapy. Other prospective and retrospective studies, reviewed in Baliga et al., [46]also demonstrate promising local control rates with treatment doses up to, or in some cases exceeding, 40 Gy; however, distant failure is common and survival is poor.

Lower dose palliative radiation is extremely well tolerated and effective for more radiosensitive tumors [47, 48]. Lower dose radiation has been demonstrated to result in significant T-cell infiltration into the tumor microenvironment[49], and immunotherapy has consistently demonstrated the ability to potentiate the local effects of radiation in various animal models [50]; therefore it is our intention to take advantage of the potential synergy of pembrolizumab with radiotherapy to increase the local control and palliative benefit of 20Gy administered to lesions that may benefit from palliative radiation. Given the favorable side effect profile of lower dose palliative radiation, it is feasible to deliver to multiple sites in the body concurrently to decrease the overall disease burden and impact anti-tumor immune parameters.

2.4 Rationale

PD-1 directed therapy has demonstrated activity in squamous cell carcinomas of the head and neck (SCCHN), with response rates of 14-18% and a 31-32% disease control rate in studies testing PD-1 and PD-L1 inhibitors [25, 51]. However, the majority of head and neck patients treated with PD-1 directed therapy will likely experience disease progression. Although mechanisms of primary and secondary resistance to PD-1 therapy in head and neck cancer remain undefined, studies in non-small cell lung cancer patients and melanoma patients treated with PD-1 therapy suggest that resistance may be mediated in part by: 1) Failure to recognize tumor antigens and generate an initial immune response [52]; and/or 2) Limited access of effector T-cells to the tumor microenvironment to mediate an anti-tumor immune response potentiated by PD-1 blockade [53]. There is also evidence that large volume / bulky metastatic disease may be more immune suppressive and may respond less well to immune checkpoint blockade therapy.[54, 55]

Radiation therapy has known immunologic activity, and has demonstrated synergy with PD-1 blockade in multiple preclinical models [44, 56]. Specifically, radiation may overcome two hypothesized mechanisms of resistance to PD-1 therapy. Preclinical studies and translational investigations in human patients have demonstrated that radiation may lead to increased antigen recognition and epitope spreading [44, 57]. Preclinical and human studies have also demonstrated that radiation may impact the tumor microenvironment, leading to increased infiltration of effector T-cells, and decreased levels of inhibitory regulatory T-cells and myeloid derived suppressor cells [58]. In certain circumstances, these effects may lead to responses in patients who had previously progressed on checkpoint blockade, as has been demonstrated in case reports [57] and retrospective studies [59], including our experience that found improved systemic response following palliative radiotherapy in melanoma patients treated with ipilimumab [60]. Multiple

radiation fractions / courses could potentially overcome resistance to PD-1 directed therapy and prime a systemic response in certain patients compared to a single fraction of radiation.

Preclinical studies demonstrate that fractionated high dose radiation may be optimal to promote targeted immunologic death of irradiated tumor cells leading to recognition of tumor antigens and a systemic anti-tumor immune response [40]. However, this single irradiated lesion may or may not lead to T-cell infiltration of remote, unirradiated lesions, and it is generally not feasible to irradiate multiple lesions to high doses because of toxicity concerns. In contrast, low dose radiation may be best suited to lead to T-cell infiltration and preferential expansion of effector as opposed to inhibitory T-cells within the tumor microenvironment [49]. Mathematical modeling also suggests that the site of the lesion irradiated to high dose may affect T-cell trafficking of activated T-cells to unirradiated lesions [61].

This study is informed by the preclinical and clinical data referenced above. In order to explore mechanisms of resistance to PD-1 blockade and optimize the use of targeted radiation to overcome this resistance, this phase 2 study will enroll SCCHN patients who have progressed on or shortly after prior PD-1 directed therapy. Restaging will be performed after initial course of radiation and pembrolizumab, and pembrolizumab will continue in patients that demonstrate a sustained response.

2.5 Correlative Studies Background

There is great utility to identifying mechanisms of resistance to PD-1 blockade as well as the mechanisms by which radiation may overcome this resistance. Based on prior studies conducted in patients across several disease types, expression of PD-L1 on tumor cells and tumor infiltrating immune cells could potentially serve as a predictor of response, at least in patients treated with PD-1 therapy alone. Tumor infiltrating lymphocytes at baseline may also serve as a predictive marker in patients treated with PD-1 blockade, although this has not yet been demonstrated in head and neck cancer. Additionally, it is unknown if these mechanisms of resistance may be impacted by focal radiation therapy, either delivered to a high or low total dose.

By performing biopsies on patients with disease resistant to PD-1 inhibition, and analyzing the tumor tissue for PD-L1 expression and infiltrating lymphocytes, we will investigate these potential mechanisms of resistance to PD-1 checkpoint blockade. Additionally, serial biopsies and blood collection obtained when feasible will evaluate the impact of radiation on these parameters and association with subsequent response.

In addition to radiation dose, the location of the irradiated lesions may also impact the ability of targeted radiation to stimulate an abscopal response [61] due to the likelihood of T-cells trafficking from the irradiated lesions to distant sites of disease. Algorithms incorporating T-cell homing may potentially be able to provide rational basis to target lesions most likely to generate an abscopal response.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

1. Pathologically confirmed squamous cell carcinoma of the head and neck with evidence of metastatic disease or locally recurrent disease. Patients without pathologic or cytologic evidence of metastatic disease should have unequivocal evidence of metastasis from physical examination or radiologic evaluation.
2. Patients must have evidence of radiologic or clinical disease progression during or within 6 months of previous treatment with systemic PD-1 directed therapy, or have stable disease on prior PD-1 therapy (at least 6 doses) and/or have been deemed not to derive clinical benefit from PD-1 directed treatment. PD-1 directed treatment includes treatment with antibodies targeting the PD-1 receptor such as pembrolizumab or nivolumab, as well as PD-L1 targeted antibodies such as durvalumab. These agents may have been administered as part of a clinical trial, and/or in combination with other immunologic agents such as CTLA-4 inhibitors or other investigational or standard of care agents/treatments.
3. Patients must have least 1 non-CNS based lesions. Palliative radiation must be potentially indicated for at least one lesion, and this lesion must be a reasonable candidate for radiation to a dose of 8 Gy in each of 3-5 fractions as deemed by a treating radiation oncologist in terms of the ability to meet standardly accepted radiation dose constraints. Any unirradiated lesions must not require urgent palliative local treatment.
4. Prior systemic therapy: Patients must be at least 2 weeks from prior chemotherapy, biological agents, immunotherapy or any investigational drug product, with adequate recovery of toxicity,
5. Prior radiation therapy: Patients must be at least 2 weeks from prior radiation therapy
6. Concurrent administration of other cancer specific therapy during the course of this study is not allowed.
7. Only patients 18 years and older are eligible. There is no upper age limit but the patients must be able to medically tolerate the regimen. Adverse event data are currently unavailable on the use immune checkpoint blockade for participants < 18 years of age, and thus children are excluded from this study.
8. ECOG performance status ≤1 (see Appendix A).
9. Ability to understand and the willingness to sign a written informed consent document
10. Female subjects of childbearing potential must have a negative serum pregnancy test at screening.
11. Female and male subjects of childbearing potential must agree to use an adequate method of contraception as outlined in section 5.7.1. Contraception is required prior to study entry and for the duration of study participation and 4 months after completion of pembrolizumab administration.
    - Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
12. Participants must have normal organ and marrow function as defined below:

- leukocytes ≥3,000/mcl
- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL

- hemoglobin >= 9 g/dl
- total bilirubin ≤1.5 × institutional upper limit of normal (ULN)
- AST(SGOT)/ALT(SGPT)≤2.5 × institutional ULN
- AST(SGOT)/ALT(SGPT)For patients with documented liver metastases, ≤ 5 × institutional ULN
- creatinine ≤1.5 ×within normal institutional ULN

OR

- creatinine clearance ≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.
- International normalized ratio (INR) or Prothrombin Time (PT) <1.5 times the upper limit of normal unless subject is receiving anticoagulant therapy, as long as PT or PTT is within therapeutic range of intended use of anticoagulants.
- Activated Partial Thromboplastin Time (aPTT) <1.5 times the upper limit of normal unless subject is receiving anticoagulant therapy, as long as PT or PTT is within therapeutic range of intended use of anticoagulants.

Laboratory tests required for eligibility must be completed within 14 days prior study entry. Baseline tumor measurements must be documented from tests within 28 days of study entry. Other non-laboratory tests must be performed within 28 days of study entry.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

1. Metastatic disease impinging on the spinal cord or threatening spinal cord compression.
2. Surgical fixation of bone lesion to be irradiated is required and indicated to provide mechanical stability.
3. Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Participants with previously diagnosed brain metastases are eligible if they have completed treatment at least 2 weeks prior to trial therapy initiation, are neurologically stable, and have recovered from the acute effects of radiotherapy or surgery. Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥2 weeks before the initiating protocol therapy. Treatment for brain metastases may include surgery, whole brain radiotherapy, radiosurgery, or a combination as deemed appropriate by the treating physician.
4. Participants who are receiving any other investigational agents.
5. History of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab or previous toxicity attributed to pembrolizumab or other PD-1 directed therapy that led to permanent drug discontinuation.
6. Uncontrolled intercurrent illness including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
7. Clinically significant electrocardiogram (ECG) abnormality, including a marked Baseline prolonged QT/QTc ([QT interval/corrected QT interval], e.g., a repeated demonstration of a QTc interval >500 ms).

8. Pregnant women are excluded from this study because immunotherapy has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with immunotherapy, breastfeeding should be discontinued if the mother is treated on this protocol.
9. Individuals with a history of a different malignancy are ineligible **except** for the following circumstances: if they have been disease-free for at least 2 years and are deemed by the investigator to be at low risk for recurrence of that malignancy; or if diagnosed and treated for cervical cancer in situ, low risk prostate cancer (treated or PSA being monitored without treatment) or basal cell or squamous cell carcinoma of the skin.
10. HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for interaction between immunotherapy and these medications.
11. Has history of (non-infectious) pneumonitis that required steroids, evidence of interstitial lung disease or active, non infectious pneumonitis.
12. Active, suspected or prior documented autoimmune disease that has required systemic treatment in the last 2 years with immune modifying agents (e.g. replacement therapy such as thyroxine, insulin or physiologic corticosteroids is not an exclusion criteria).
13. The subject is known to be positive for HepBsAg, or HCV RNA.
14. Lack of availability for follow up assessments.
15. The investigator's belief that the subject is medically unfit to receive pembrolizumab and or unsuitable for any other reason.
16. Has received a live vaccine within 30 days of planned start of study therapy.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Women, minorities and other underrepresented populations are all at risk to develop squamous cell carcinoma of the head and neck, with smoking and alcohol use as the critical risk factors for the disease. Socioeconomically-deprived populations tend to have higher rates of smoking and potentially higher risks of alcohol use, and therefore it is possible this study will be more likely to enroll these individuals. However, there is no reason to think that immune checkpoint blockade combined with radiation will have a differential effect on these populations. The eligibility criteria should not substantially differentially affect their enrollment in the trial, although some minority populations are more likely to have certain comorbid conditions such as heart failure, which may preclude them from the trial.

4. REGISTRATION PROCEDURES

Patients who meet all eligibility criteria (through screening) will be registered in Clinical Trials Management System (CTMS) "OnCore" by the study team. Registration must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible.

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

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator (PI). If a participant does not receive protocol therapy within 14 days following registration, the participant's registration on the study must be canceled.

The following source documents are required for registering the patient in OnCore:

- Copy of screening lab results
- Signed participant consent form
- Eligibility Checklist

4.1 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.2 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Coordinating Center by the Study Coordinator. All sites should call the Study Coordinator to verify dose level availabilities. Following registration, participants should begin protocol therapy within 10 days. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.3 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and faxed (617) 582-8911 or e-mailed to the Study Coordinator:

- Copy of hematology panel (CBC with platelets), chemistry panel, coagulation panel (PT/PTT), pregnancy test (if applicable), EKG, TSH, and HIV test results along with any provider's notes that document disease, lesions, or other details relevant to protocol treatment
- Signed participant consent form
- HIPAA authorization form
- Eligibility Checklist

The participating site will then call or e-mail the Study Coordinator to verify eligibility. The Study Coordinator will follow DF/HCC policy (REGIST-101) and register the participant on the protocol. The Study Coordinator will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site. The Study Coordinator will also contact the participating site and verbally confirm registration

5. TREATMENT PLAN

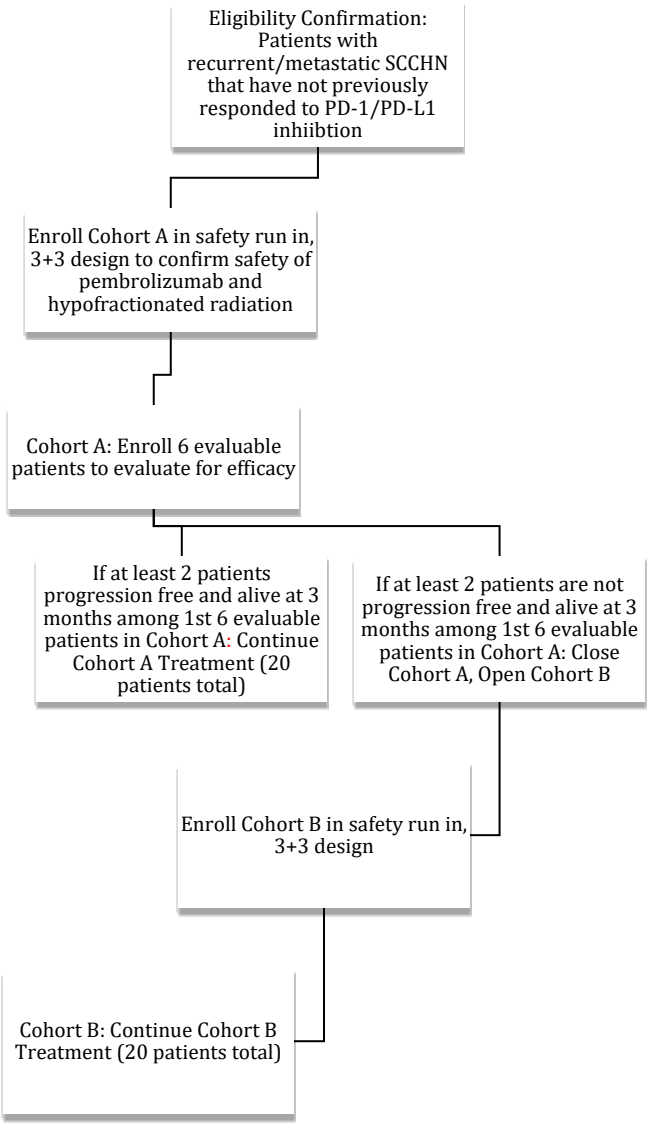
5.1 Treatment regimen

This is a phase 2 study with one or two cohorts depending on response rate assessing systemic response in patients receiving pembrolizumab in combination with palliative therapy for metastatic SCCHN. Pembrolizumab will be started day 1-10 of radiation, or up to 3 days prior to radiation start, and then administered each 21 day cycle. Radiation in each cohort (A and B) is described in more detail below. Although the toxicities of pembrolizumab and radiation are established, because these two agents are being combined in this protocol each cohort will begin with a 3 to 6 patient safety run in to confirm safety of the combined regimen.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy during the course of treatment.

The study and treatment schema are shown below:

Trial Enrollment – Study Schema



TREATMENT SCHEMA (21 Day Cycles)

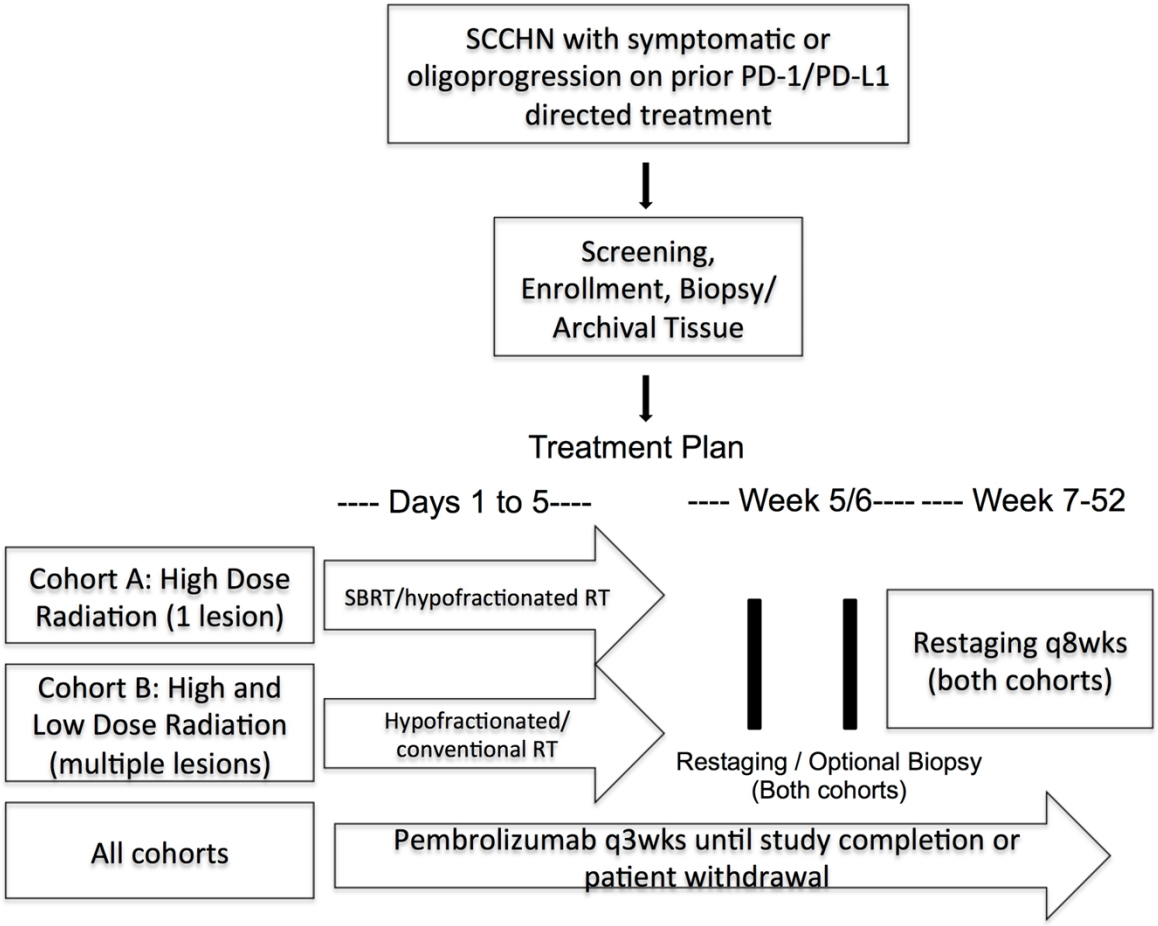


Table 1: Pembrolizumab Regimen Description

Agent	Premedication ; Precautions	Dose	Route	Schedule	Cycle Length
Pembrolizumab	Not routinely necessary unless prior infusion reaction.	200 mg, *q3w, iv*	IV over approximately 30 minutes (range: 20-75minutes). Please refer to Section 8 for compatible infusion set materials including in-line filter.	q3w	21 days (3 week)

Cohort A: Pembrolizumab and hypofractionated radiation

Pembrolizumab will be given within 10 days after starting radiotherapy treatment, or up to 3 days prior to radiotherapy start. Patients in cohort A will receive hypofractionated palliative radiation starting on day 1 to a dose of 8 Gy in each of 3-5 fractions no more frequently than every other day to a single lesion. The specific dose will be determined by the treating radiation oncologist based on clinical indication. Radiation should be completed in the minimum amount of time possible provided radiation is not given on consecutive days, and shall in no instances extend over a period of more than 21 calendar days.

Following this first cycle, pembrolizumab will be continued every 3 weeks. Radiation will, in general, not be repeated during subsequent cycles, except as at the discretion of the Principal Investigator in the case of patients thought to be deriving overall benefit from treatment.

Cohort B: Pembrolizumab and hypofractionated and low dose radiation to multiple lesions

In contrast to Cohort A where a single lesion is irradiated, the goal of cohort B is to irradiate a significant, if not total, volume of visible disease in order to predispose for a systemic immune response. Pembrolizumab will be given within 10 days after starting radiotherapy treatment, or up to 3 days prior to radiotherapy start. Patients in cohort B will receive hypofractionated palliative radiation starting on day 1 to a dose of 8 Gy in each of 3-5 fractions no more frequently than every other day to 1-3 lesions. If the treating radiation believes a dose of 8 Gy is not indicated due to the clinical situation, the dose of radiation may be reduced to 5-6 Gy per fraction. In patients where there are other potential lesions to irradiate, these subjects may also receive palliative radiation to a dose of 20 Gy in 5 fractions to an additional 1-3 lesions, with the goal of treating a significant burden of the patient's measurable disease. Radiation should be completed over a period of approximately 14-21 calendar days, or if more than 3 lesions are irradiated may be partially delivered during the second cycle given logistical radiation planning and treatment constraints. 8

Gy radiation doses will not be given on consecutive days; however, low dose radiation may be administered on subsequent days or on the same days as high dose radiation is administered. In general, a maximum of 2-3 lesions will be treated on any single day, although treating up to 4 lesions is allowed.

Following this first cycle, pembrolizumab will be continued every 3 weeks. Radiation may be administered during subsequent cycles to other lesions using either 5-8 Gy in each of 3-5 fractions or 20 Gy in 5 fractions as deemed appropriate.

5.2 Pre-treatment Criteria

5.2.1 Screening for trial eligibility

Day -28 to Day 1: Screening Visit
Eligibility and exclusion criteria are provided in Section 3. These criteria will be assessed within 28 days of the first dose of study treatment (Pembrolizumab) to establish eligibility and baseline values.

Informed consent will be obtained after the study has been fully explained to the subject and before the conduct of any screening procedures or assessments. If screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 visit and cycle 1 day 1 labs do not need to be performed.

Demographic information and baseline characteristics will be collected at the Screening Visit. Standard demographic parameters include age, sex, and race/ethnicity (recorded in accordance with prevailing regulations). Baseline characteristics will include ECOG PS (Appendix A), disease status, medical histories, and prior and concomitant medications. P16 / HPV status will also be collected when available.

Additional testing required, as per Section 3, is: hematology panel (see Table 2), chemistry panel (see Table), coagulation panel, urine or serum HCG (in women of childbearing potential; see Section 3 for when serum HCG testing is required), TSH, and EKG.

Patients will also need to undergo a radiation planning appointment prior to the start of treatment.

Archival tumor sample should be collected (block or if not possible, 20 unstained slides) from both most recent biopsy, as well as any tumor tissue obtained prior to starting initial PD-1 or PD-L1 directed therapy, if available. If a tumor deposit is safely accessible, a baseline tumor biopsy is also suggested. A second optional biopsy will be repeated if a tumor remains accessible (close to the end of cycle 2, and within 14 days before the third cycle of pembrolizumab).

Further details about collection and handling of tumor biopsy specimen can be found in Section 9 and appendices.

Table 2: Clinical Laboratory tests:

Category	Tests
Hematology panel	
	• Hematocrit, hemoglobin, platelet count, WBC with differential (bands, basophils, eosinophils, lymphocytes, monocytes, neutrophils), ANC
Chemistry Panel	
	• Chloride, potassium, sodium, BUN, serum creatinine, phosphorus, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin (NOTE: the frequency of checking magnesium levels is left up to the treating provider)

Abbreviations: ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; β-hCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; WBC = white blood cells, ANC=absolute neutrophil count

5.3 On treatment visits

Reasonable effort should be made to conduct study visits on the day scheduled (+/- 3 days).

Patients will also see the treating radiation oncologist as they are receiving radiation as per standard clinical practice.

Any changes from screening clinical evaluation findings that meet the definition of an AE will be recorded on the AE page of the eCRF.

<u>Cycle 1, Day 1</u>
If screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 visit and screening tests do not need to be repeated.
Draw blood sample for:
- Hematology panel (Table 2)
- Chemistry panel (Table 2)

Record:
- ECOG PS
- Weight
- Vital signs
- Physical exam
- Concomitant medication use

Review all laboratory results before administering study treatment.

Criteria to treat at cycle 1 day 1:

- **Absolute neutrophil count ≥1000/µl**
- **Platelets ≥75,000/µl**
- **ALT and AST ≤3.0 x ULN in a patient with no documented liver metastases; ALT and AST ≤5.0 x ULN in a patient with documented liver metastases**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert's syndrome)**

Every-3-week assessments, day 1 of every cycle:
The following assessments should be performed on the indicated weeks.

Draw blood/collect urine sample for:
- Hematology panel
- Chemistry panel
- TSH (every 3 cycles)

Record:
- ECOG PS
- Weight
- Vital signs
- Physical exam
- Concomitant medication use
- AEs or SAEs

Criteria to treat at day 1:

- **Absolute neutrophil count ≥1000/µl**
- **Platelets ≥75,000/µl**
- **ALT and AST ≤3.0 x ULN in a patient with no documented liver metastases; ALT and AST ≤5.0 x ULN in a patient with documented liver metastases**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert's syndrome)**

Review all laboratory results before administering study treatment.

5.3.1 Additional On-Treatment Assessments

Tumor Assessments
Tumor assessments will be performed according to RECIST 1.1 and immune related response criteria (irRC) (see Section 11). Response evaluations will be performed week 5 (+/- 1 week) and then every 8 weeks or as clinically indicated. In case of systemic response, confirmatory scans will be performed 4 weeks after the scan that documented response.

Research Blood Sample Collection

Research blood sample collections should be collected at the following time points:
1. At baseline, within 14 days prior to cycle 1 day 1 of protocol therapy (ideally as close to administration of cycle 1 day 1 therapy as possible)
2. At approximately week 4 (following the completion of all radiation therapy) or cycle 2 of pembrolizumab, then again at week 7 or cycle 3, and at then optionally at later pembrolizumab cycles as applicable.

Specific instructions for research blood draw handling are described in Section 9 and appendices.

Tumor biopsy.
If a tumor is readily accessible, a tumor biopsy is suggested at the following time points:
1. At baseline, ideally as close to administration of cycle 1 day 1 therapy as possible
2. After cycle 2, within 14 days prior to cycle 3 day 1 of protocol therapy and generally after restaging scans to be performed on week 5

Further details about collection and handling of tumor biopsy specimen can be found in Section 9 and appendices.

5.3.2 End-of-Treatment Procedures

End-of-Treatment Visit
All subjects will be asked to return to the site for a final, End-of-Treatment visit, if possible. This visit must be performed within 30 days of final administration of study treatment. End-of-treatment assessments will not have to be repeated if the same assessments were performed within 7 days of this visit.

Record:
- For patients who come off for reasons other than progressive disease: Tumor assessments to be performed every 6 weeks if patients come off within one year of therapy. If study treatment is discontinued due to PD, then tumor assessments are not required for that subject.
- ECOG PS
- Physical exam
- Vital signs and weight
- Concomitant medication use
- AEs or SAEs

Draw blood sample for:
- Hematology (Table 2)
- Chemistry panel (Table 2)

5.3.3 Follow-up Period Procedures

Subjects who do not have PD upon discontinuation of study treatment are required to have tumor assessments during the post-treatment follow-up period (using the same methodology and acquisition techniques as were used for previous assessments). Response evaluations will be

performed every 6 weeks. Further details about collection and handling specimens can be found in Section 9 and appendices.

5.4 Agent Administration

5.4.1 Pembrolizumab

Pembrolizumab administration
Sponsor Merck will provide each investigator with adequate supplies of pembrolizumab. Pembrolizumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Pembrolizumab will be administered in clinic every 21 days (+/- 5 days) during each cycle. It will be administered as a 30 minute IV infusion. Given the variability of infusion pumps from site to site, a window of -10 and +45 minutes is permitted (i.e., infusion time is 30 minutes: -10 min/+45 min).

The Investigator's Brochure contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.4.2 Radiation treatment

5.4.2.1 Prioritizing lesions for irradiation

In addition to providing palliative benefit, this study attempts to use radiation to engender a systemic immune response in the context of PD-1 inhibition. Therefore, the choice of site or sites irradiated will be based on a combination of safety, palliative benefit and, if feasible, correlative studies will in parallel evaluate whether there is an association between abscopal responses and personalized stratification based on hypothetical T-cell trafficking[61]. Prior to radiation simulation, initial staging scans will be analyzed in detail. Lesions will be prioritized for treatment as follows (in order of priority): 1) Lesions must be safe to irradiate at the dose prescribed by the protocol as deemed by the treating radiation oncologist and able to meet standardly accepted radiation dose constraints and as specified below; 2) Lesions must be irradiated for potential palliative benefit as determined by the treating physicians; 3) Lesions that have not responded or progressed on prior immune checkpoint blockade. 4) The largest lesion that meets the criteria states above determined using bidirectional measurements. Based on this algorithm, the top rated lesion will receive 8 Gy in each of 3-5 fractions. In cohort B, the goal is to irradiate a significant percentage, if not all of the total visible tumor burden.

In parallel, we will investigate the feasibility of ranking all metastatic lesions in terms of the theoretic potential of these lesions to stimulate an immune response at a distant, unirradiated metastatic site as described in more detail in the correlative studies section.

5.4.2.2 Treatment simulation: The treatment planning process will include CT based simulation with 1.25- 2.5-mm cuts to cover the area(s) of interest.

5.4.2.3 Contours: Several tumor contours will be defined by the physician:

CTV1-4 (CTV): Tumor volumes to be treated. CTV1-3 will be treated to 5-8 Gy in each of 3-5 fractions. In cohort B, CTV2-6 will be treated to 20 Gy in 5 fractions. These volumes include gross disease; elective irradiation to other areas is generally not permitted. An optional 0-2 cm margin on gross disease (GTV) is allowed when determining the CTVs at the treating radiation oncologist's discretion to account for any uncertainty or microscopic disease. For targets that have significant motion (e.g. lung or liver targets), 4D-CT planning with delineation of an internal target volume (ITV) is recommended, and other motion management (e.g. gating/compression systems) may be used. The exact margins are patient- specific. A 2mm – 1cm planning tumor volume (PTV) margin added to the CTV will also be included to account for set up variation, (and internal target motion), as appropriate to each individual lesion.

5.4.2.4 Several contours of the organs at risk will be defined by the physician and/or treatment planner if visible within the axial slices covered by CTV1.

- Spinal cord
- Brainstem
- Brachial plexus
- Cauda equina
- Cochlea
- Esophagus
- Heart/pericardium
- Trachea and ipsilateral bronchus
- Skin
- Stomach
- Duodenum
- Jejunum/ilieum
- Liver
- Kidneys
- Lung
- Optic structures (Globes, right and left optic nerves and optic chiasm)

5.4.2.5 Prescriptions:

Target1-3 (CTV1-3+ individualized PTV margin) will be treated to a dose of 8 Gy in each of 3-5 fractions.
Target2-6 (CTV2-6 + individualized PTV margin) will be treated to a dose of 20 Gy in 5 fractions for patients in cohort B.

Photons or electron treatments are allowed, but graphic plans are mandated. Planning may be electron based, 3D conformal, IMRT or SBRT, as appropriate to each individual case / lesion to achieve coverage of the PTV target and maintain normal tissue tolerance. The percentage PTV covered to 100% of the prescription dose will be reported; reasonable effort should be made to

keep this greater than 95% while maintaining normal tissue dose constraints as indicated below. Bolus may be used as appropriate.

5.4.2.6 Timing of radiation treatment

- Cohort A: Three to five treatments of 8 Gy will be delivered no more frequently than every other day and over a period that does not extend over more than 21 days. Pembrolizumab will be given within 10 days after starting radiotherapy treatment, or up to 3 days prior to radiotherapy start.

- Cohort B: Three treatments of 8 Gy will be delivered no more frequently than every other day and over a period that does not extend over more than 21 days. Pembrolizumab will be given within 10 days after starting radiotherapy treatment, or up to 3 days prior to radiotherapy start. 20 Gy in 5 fractions will be delivered to an additional 1-3 lesions that do not require urgent palliation.

5.4.2.7 Organs at risk (OAR) – Treating radiation oncologists will abide by accepted radiation dose constraints, especially in regards to 8 Gy fraction treatment. For patients in Cohort B, a cumulative dose calculation is recommended when irradiated lesions are in close proximity. Guidance for dose constraints are provided in the table 3 below.

Table 3:

Constraints for 3 fraction treatment

Structure	**Volume (mL)**	**Volume Max (Gy)**	**Max Point Dose (Gy)**
Spinal cord	<0.25	18	19.5
Brainstem	<1	18	23
Brachial plexus	<3	22.5	24
Cauda equina	<5	21.9	24
Cochlea			20
Esophagus	<5	21	27
Heart/pericardium	<15	24	30
Trachea and ipsilateral bronchus	<4	15	30
Skin	<10	22.5	24
Stomach	<10	21	24
Duodenum	<5	15	24
Jejunum/ileum	<5	16.2	27

Liver	700mL spared from volume max	17.1	
Kidneys	200mL spared from volume max	14.4	
Lung	1000mL spared from volume max	11.4	
Optic structures	<0.2	15	19.5

Constraints for 5 fraction treatment

Structure	**Volume (mL)**	**Volume Max (Gy)**	**Max Point Dose (Gy)**
Spinal cord	<0.25	22.5	D0.035<30
Brainstem	<0.5	23	30
Brachial plexus	<3	27.5	D0.035<35
Cauda equina	<5	30	35
Cochlea			25
Esophagus	<5	20	35
Heart/pericardium	<15	32	38
Trachea	<4	18	35
Skin	<10	30	D0.035<32
Stomach	<10	18	32
Duodenum	<5	18	32
Jejunum/ileum	<5	20	32
Liver	700mL spared from volume max	21	
Kidneys	200mL spared	17.5	

	from volume max		
Lung	1500mL spared from volume max	13	
Optic structures	<0.2	22	25

Note: If there is any overlap with prior radiation treatment fields, max point dose administered to the brainstem and spinal cord and optic structures in the area of prior overlap should be limited to 12 Gy.
PTV coverage may be compromised to ensure an acceptable plan.

5.5 Definition of Dose-Limiting Toxicity

Pembrolizumab and radiation have been evaluated previously specifically in patients with SCCHN. However, although this is not a phase I protocol, the use of pembrolizumab in combination with high and low dose radiation is relatively novel, and therefore we plan to include a safety-run-in to ensure that treatment is well tolerated in this setting.

Dose-limiting toxicity (DLT) is based on the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE). DLT refers to toxicities experienced between the start of treatment and cycle 3, day 1 of pembrolizumab (the DLT evaluation period). A DLT will be defined as follows:

DLTs will be graded according to the National Cancer Institute's CTCAE v4.0 (http://ctep.cancer.gov/forms/CTCAEv4.pdf). A DLT is any event that: a) occurs during the DLT evaluation period; and b) is possibly, probably, or definitely related to the administration of pembrolizumab and radiation; and c) fulfills any of the following criteria:

1. Any Grade ≥ 3 pneumonitis, neurological event or uveitis.
2. Any Grade 2 pneumonitis, neurological event or uveitis, with the *following exception*:

- Grade 2 pneumonitis, neurological event or uveitis that downgrade to Grade ≤ 1 within 3 days after onset, whereby maximal supportive care, including systemic corticosteroids, is permitted.

3. Any *other* Grade ≥ 3 toxicity, with the *following exceptions*:

- Grade 3 irAEs that downgrade to Grade ≤ 2 within 3 days, or to Grade ≤ 1 or baseline within 14 days after onset, whereby maximal supportive care, including systemic corticosteroids, is permitted.
- Grade 3 colitis that downgrades to Grade<= 2 within 7 days after onset, whereby maximal supportive care, including systemic corticosteroids, is permitted.

- Grade 3 asymptomatic endocrinopathy, managed with or without systemic corticosteroid therapy and/or hormone replacement therapy.
- Grade 3 inflammatory reaction attributed to a local antitumor response (e.g., inflammatory reaction at sites of metastatic disease, lymph nodes, etc.).
- Grade 3 fatigue for ≤ 7days.
- Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management.
- Liver transaminase elevation ≤ 8 times ULN that downgrades to Grade ≤ 2 (≤ 5 times ULN) within 7 days after onset, whereby maximal supportive care, including systemic corticosteroids, is permitted.
- Total bilirubin ≤ 5 times ULN that downgrades to Grade ≤ 2 (≤ 3 times ULN) within 7 days after onset, whereby maximal supportive care, including systemic corticosteroids, is permitted.
- Grade ≥ 3 neutropenia that (1) is not associated with fever or systemic infection, (2) does not require medical intervention, and (3) improves to Grade 2 within 7 days.
- Grade 3 and 4 lymphopenia.
- Grade 3 thrombocytopenia that (1) is not associated with clinically significant bleeding, (2) does not require medical intervention, and (3) improves to Grade 2 within 7 days.
- Isolated Grade 3 electrolyte abnormalities that are not associated with clinical signs or symptoms and are reversed with appropriate maximal medical intervention within 3 days.
- Any pre-existing laboratory abnormality that deteriorates to Grade 3/4, but where the increment of deterioration is considered not clinically significant by both investigator and sponsor.

Immune-related AEs (irAEs) are defined as AEs of immune nature (i.e., inflammatory) in the absence of a clear alternative etiology. In the absence of clinical abnormality, repeat laboratory testing will be conducted to confirm significant laboratory findings prior to designation as a DLT.

While rules for adjudicating DLTs are specified above, an AE that is Grade < 3 or listed as exempt above may also be defined as a DLT after consultation with the sponsor and Investigators, based on the emerging safety profile of pembrolizumab. Likewise, subjects who become not evaluable for DLT because they discontinued or interrupted treatment due to toxicities other than DLTs may be counted as DLT subjects.

5.6 General Concomitant Medication and Supportive Care Guidelines

5.6.1 Concomitant Medication Guidelines

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

Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All

concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

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

Prohibited Concomitant Medications
Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy not specified in this protocol
- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from radiation or an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

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

5.6.2 Supportive Care Guidelines – general medications

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy below. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. Antiemetics and anti-diarrheal medications should not be administered prophylactically before initial treatment with study drugs. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may

be used as per standard clinical practice before subsequent doses of study drugs or before, during or after radiation treatment.

- Anticoagulants - Anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Coagulation parameters should be checked at least once monthly, or more frequently at discretion of treating physician.
- Pain medications administered per standard clinical practice are acceptable while the patient is enrolled in the study.

Patients who experience toxicities should be treated symptomatically as clinically indicated. Medications that are considered necessary for the subject's welfare and that are not expected to interfere with the evaluation of study treatment or be restricted may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

5.7 Contraception, Pregnancy and Nursing considerations

5.7.1 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 post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);
OR
(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;
OR
(3) has a congenital or acquired condition that prevents childbearing.
Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:
(1) practice abstinence[†] from heterosexual activity;
OR
(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):
- intrauterine device (IUD)
- vasectomy of a female 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: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) 14 days prior to the initiation of study medication throughout the study period up to 4 months 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.

5.7.2 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the 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 Merck. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to Merck and followed as described above.

5.7.3 Use in Nursing Women

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

5.8 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression. In the case of progressive disease, confirmatory scans are recommended between 4-8 weeks following the date of initial progression along with continued treatment, provided the patient is thought to be deriving clinical benefit and is counseled regarding the risks and benefits of continued treatment. If progression is confirmed, date of progression is dated at the time of the original scans. If progression is confirmed, continued treatment is only allowed if irRC demonstrates a response or stable disease, again provided the patient is thought to be clinically benefitting and is counseled regarding the risk and benefits of remaining on treatment.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

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

A Treatment Ended/Off Study Form will be filled out within OnCore, when a participant is removed from protocol therapy.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Jonathan Schoenfeld at 18874 (pager #).

5.9 Duration of Follow Up

Participants removed from protocol therapy will be followed for overall survival, every 6 months, until death (by phone).

Given pembrolizumab has become standard of care for patients with recurrent / metastatic SCCHN, patients on study who proceed with treatment locally outside of a study center should have medical records and scans obtained on a regular basis to monitor for date of progression / response.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. For these patients, scans should be performed every 6 weeks for 1 year.

5.10 Criteria for Taking a Participant Off Study

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

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

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

For Centralized Subject Registrations, the research team submits a completed Off Treatment/Off Study form to ODQ when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Jonathan Schoenfeld, MD, MPH at Partners pager 18874.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and within 30 days of the last study intervention. Participants continuing to experience toxicity at the last scheduled study visit may be kept on the study until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

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

No dose reductions are allowed for pembrolizumab. For toxicities that are attributable to pembrolizumab, this drug should be held as directed.

6.2 Management of toxicities attributable to pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 4 below.

Table 4: Dose modification guidelines for Pembrolizumab for drug-related adverse events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
			less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Myocarditis	1-2	Toxicity resolves to 0	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
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

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

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
[1] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.
[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Supportive care for pembrolizumab toxicity, particularly suspected immune-mediated toxicity
Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined in Section 6 and in greater detail in the ECI guidance document, which is available from the study PI upon request. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids.

Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance (see Section 7) but does not need to follow the treatment guidance (as outlined in the ECI guidance document).
It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **Pneumonitis**:

o 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.

o For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
o 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).

o All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
o For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
o For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
o When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

- **Hypophysitis**:

o 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.
o 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.
o **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.
o **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**:

o For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).

▪ Treat with IV or oral corticosteroids

o For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.

o 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**:

o For **Grade 2** events, treat with corticosteroids.

o For **Grade 3-4** events, treat with systemic corticosteroids.

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

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

Table 5: Infusion Reaction Treatment Guidelines for pembrolizumab

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 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to:	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
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	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.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Adverse Events Lists

7.1.1 Adverse Event List(s) for pembrolizumab

Refer to the current version of Investigator's Brochure for detailed pembrolizumab safety/toxicity information.

7.1.2 Adverse Event List(s) for radiation

Adverse events for radiation are variable and depend on the site that is being irradiated. General side effects include fatigue, radiation dermatitis, and a low long term risk of a radiation induced malignancy. Other side effects include possible damage to normal tissues potentially included in the radiation treatment field (e.g. muscle, bone, nerve, mucosal surfaces, stomach, bowel, bladder, liver

or heart). Doses to these normal structures are limited to the extent possible to minimize these risks as per section 5.4.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

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

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

7.5 Expedited Reporting to Hospital Risk Management

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

7.6 Routine Adverse Event Reporting

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

7.7 Adverse Event Reporting

7.7.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the PI

7.7.2 Investigators **must** report to the PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.7.3 **Adverse Event Reporting Guidelines**

All participating sites will report AEs to the Sponsor-Investigator per DF/HCC requirements, and the IRB of record for each site as applicable per IRB policies. The table below indicates which events must be reported to the DF/HCC Sponsor-Investigator.

Attribution	DF/HCC Reportable Adverse Events(AEs)				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, events must be reported within 1 business day of learning of the event.					

7.8 Immediate Reporting of Adverse Events and Events of Clinical Interest to Merck

7.8.1 Serious Adverse Events

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

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

7.8.2 Reporting Requirements for Participating Investigators

Each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

It is the responsibility of each participating investigator to report adverse events within 48 hours to the Overall PI, Dr. Jonathan Schoenfeld, MD, MPH (at the contact given below), or representative personnel; and submit to DFCI IRB within 10 working days.

Jonathan Schoenfeld, MD, MPH
617-632-3591
jdschoenfeld@partners.org

7.8.3 Reporting Requirements for DFCI IRB

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.8.4 Reporting Requirements for Merck

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 30 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

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

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

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

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

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

7.8.5 Events of Clinical Interest (ECIs)

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

1. an overdose of Merck product, as defined in Section 7.7.3 - Definition of an Overdose for This Protocol and Reporting of Overdose to Merck, that is not associated with clinical symptoms or abnormal laboratory results.

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

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

Additional adverse events:

A full document entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"), which provides guidance regarding identification, evaluation and management of ECIs and irAEs, is in the possession of the PI and can be accessed upon request.

ECIs (both non-serious and serious adverse events) identified from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

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

7.8.6 Definition of an Overdose of pembrolizumab for This Protocol and Reporting of Overdose to Merck

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

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

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

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

7.8.7 Reporting of Pregnancy and Lactation to Merck

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

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

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational and other agents administered in this study can be found in Section 7.1.

8.1 Pembrolizumab

Please refer to the Investigator's Brochure for detailed agent information, and to the FDA label for additional information.

8.1.1 Description

Pembrolizumab is a humanized monoclonal antibody of the IgG4/kappa isotype. Other name: MK-3475, Keytruda. Pembrolizumab blocks negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

The molecular weight of Pembrolizumab is 148.9-149.5 KDa.

8.1.2 Form

Clinical supplies will be manufactured and provided by Merck as summarized in Table 7.

Table 7: Product Description

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

8.1.3 Storage and Stability

Store intact vials between 2°C-8°C (36°F-46°F). Do not freeze. Protect from light by storing in the original box.

Stability testing of the intact vials is ongoing.

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for up to 20 hours. PEMBROLIZUMAB solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the IV bag, and the duration of infusion.

8.1.4 Compatibility

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin.

8.1.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 Availability

Pembrolizumab is an investigational agent and will be supplied free of charge from Merck.

8.1.7 **Preparation**

Pembrolizumab solution for infusion must be diluted prior to administration. Allow the required number of vials to equilibrate to room temperature. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of Pembrolizumab to an infusion bag containing 0.9& Sodium Chloride Injection, USP of 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL.**

8.1.8 **Administration**

Route of administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of administration: Infuse over approximately 30 minutes (range: 20-75 minutes) using an infusion set containing a low-protein binding 0.2 to 5 m in-line filter make of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

8.1.9 **Ordering**

Pembrolizumab will be obtained directly from Merck Pharmaceuticals.

8.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 **Destruction and Return**

At the end of the study, unused supplies of pembrolizumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Background information on the pre-clinical and clinical rationale for these investigations is discussed in Section 2.

Correlative studies to be performed on tissue collected are described in section 9.1.

In all patients, either archival tissue or baseline biopsy is required for enrollment. Even if archival tissue is available, in all patients in whom a tumor deposit is safely accessible, a baseline tumor biopsy is suggested. In terms of archival tissue, we will attempt to obtain archival tissue taken from biopsies performed prior to the start of any immune therapy as well as after progression on immune therapy. We plan to use archival / baseline biopsy tissue to perform a number of immune profiling assays, detailed below. On baseline tumor biopsies, we will perform characterization based on immunohistochemistry for PD-L1 and other immune markers, and mRNA expression. Additionally, we plan future DNA analysis, and associated testing including whole exome sequencing.

An optional sequential biopsy will also be performed after cycle 2, within 14 days prior to cycle 3 of therapy. We will perform immunohistochemical and mRNA analyses on these specimens to determine the impact of combined radiation PD-1 blockade on immunologic parameters.

Serial blood draws for correlative science should be obtained at baseline, at week 4 or cycle 2, then again at week 7 or cycle 3, and optionally at later cycles. On each blood draw, we will perform flow cytometry and / or CyTOF analyses to characterize protein expression of immune mediators, detailed below, and additional blood will be banked for future testing, including Luminex cytokine profiling.

Sequential imaging is included in this trial for staging purposes and to monitor subsequent response to treatment. De-identified images will also be used for correlative imaging studies evaluating the potential of irradiated lesions to stimulate distant abscopal responses based on hypothetical T-cell trafficking as described above and by Poleszczuk et al. [61].

When adequate tissue is available, we will also assess PD-L1 expression via QualTek external services using the 22C3 antibody. Tumor will be considered positive if >5% (PD-L1) or >10% (PD-L2) of the tumor cell population demonstrates unequivocal staining. PD-1 positivity will be defined as >3% positive cells/high power field. All IHC stained slides will be evaluated and scored by a pathologist. A subset of slides will be reviewed by a second pathologist to ensure concordance of interpretation.

9.1 Characterizing the immunologic microenvironment of SCCHN

9.1.1 Hypotheses

- This study will enroll patients who have previously failed to benefit or progressed on PD-1 pathway directed therapy. We will attempt to identify mechanisms of resistance to PD-1 pathway inhibitors by interrogating the tumor microenvironment. Specifically, we hypothesize that resistant tumors will:
  - Demonstrate low levels of PD-L1 expression compared to an unselected population of historical controls or previously reported populations of responding patients
  - Have relatively few numbers of tumor infiltrating infiltrates compared to an unselected population of historical controls or previously reported populations of responding patients

- This study will also obtain sequential biopsies before and after radiation to evaluate whether targeted radiation can change the local tumor microenvironment, specifically, whether:
  - Radiation increases PD-L1 expression on tumor cells
  - Radiation increases T-cell infiltration into tumor deposits

9.1.2 Collection, handling and transportation of biopsy specimens

Biopsies should not be performed on Friday afternoons, as there may not be time for processing of the fresh tissue.

If feasible, the protocol will require 2 cores, additional cores (up to 7) will be obtained using an optional consent form.

The order of specimen collection should be:
- First core: 10% neutral buffered formalin
- Second core: Sterile DMEM or RNAlater

Optional cores are alternated between formalin and sterile DMEM/ RNAlater.

After being obtained, processing of the cores is as follows:
- All samples should be de-identified
  - Formalin specimens will be processed into Formalin-fixed paraffin embedded block(s) and or unstained/unbaked slides (15-20), stored at room temperature, as per institutional procedure.
  - Tissue stored in DMEM or RNAlater will be stored at -70 to -80 degrees C.

The study coordinator should be notified before a biopsy specimen is taken / sent to arrange for transfer to the Center for Immuno-Oncology and Core Pathology Laboratories at Dana-Farber Cancer Institute / Brigham and Women's Hospital and to provide most up to date contact and shipping information.

As general guidance , samples should be transported or shipped to:
(DMEM/RNAlater samples):
Center for Immuno-Oncology
Dana-Farber Cancer Institute
1 Jimmy Fund Way, JF0406
Boston, MA 02215
Phone: (617) 632-2421

Formalin samples:
Brigham & Women's Hospital
20 Shattuck Street
Thorn building 603B
Boston, MA 02215

Note: DMEM/RNAlater samples should be transported / shipped on dry ice.

9.1.3 Tissue banking

All leftover tissue will be banked in either the Center for Immuno Oncology, lab of Ravindra Uppaluri, MD PhD or the lab of Kai Wucherpfennig PhD as per standard lab protocol, such that it can be used for additional or optional future analyses as needed.

These analyses may include RNA analyses using the most current and informative methodologies at the time that correlative science is performed on all specimens. NanoString signatures and comprehensive RNA sequencing may be used.

Additionally, available tissue, may be used for whole exome sequencing to determine mutational burden and neoantigen detection, T-cell receptor sequencing to determine intratumoral TCR diversity and flow cytometry to quantify immune populations.

9.2 Characterizing immune markers in serum and peripheral blood mononuclear cells (PBMCs) prior to and after therapy with pembrolizumab plus radiotherapy

9.2.1 Hypotheses

- We hypothesize that the immune marker profile in the peripheral blood will change over the course of pembrolizumab plus radiation.
- We hypothesize that a larger increase in markers of immune activity in the peripheral blood will correlate with a better disease response as assessed on concurrent restaging scans, and in terms of best radiographic response at any time on trial.
- We hypothesize that an immune marker or composite of markers in the peripheral blood at baseline will correspond to TIL percentage in baseline tumor biopsy

9.2.2 Collection, handling, and shipping of blood specimens

Research blood collection is performed for cytokine analyses, CyTOF, flow cytometry and potential DNA isolation. The samples will be banked in the DFCI Center for ImmunoOncology laboratorites and/or laboratory of Ravindra Uppaluri MD PhD and/or the lab of Kai Wucherpfennig PhD for these and future research purposes. These specimens will become the property of the DF/HCC.

Blood draws should not be performed on Friday afternoons, as there may not be time for processing of the blood. If a blood draw must be performed on Friday morning, the lab of Mariano Severgnini must be notified ahead of time to ensure that there will be adequate time for processing the blood, since it cannot be stored over the weekend. PBMC processing can occur as below at the DFCI or may be performed locally and then PBMC subsequently shipped for future analyses.

The following research blood samples are obtained at the correlative timepoints:

- Five 10 mL green-top (heparin) tubes for PBMC analyses

All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of collection and time point (e.g., "Baseline" or "Cycle 2" or "Cycle 3").

All samples will then be hand carried at ambient temperature or immediately mailed to Mariano Severgnini at:
Center for Immuno-Oncology
Dana-Farber Cancer Institute
1 Jimmy Fund Way, JF0406
Boston, MA 02215
Phone: (617) 632-2421
Pager: 42093

Blood must be processed within 3-4 hours of its being drawn.

9.2.3 Assays on serum and isolated PBMCs

Studies of immune function performed on blood will include:
- Analysis of antibody response (both quantitative antibody titer and qualitative antibody binding and functionality)
- Study of T-cell composition and functioning
- Analysis of cytokine response
- Identification of circulating antigen
- Comparison of immune responsiveness in serum
- Analysis of blood cell composition using flow cytometry
- Effect of immune status on circulating tumor cells
- Analysis of genomic or protein polymorphisms that may affect immune functioning and inflammation
- Analysis of inflammatory status in correlation to toxicity response
- Analysis of intracellular signaling pathways

9.3 Correlative studies to be performed on staging PET-CT and/or CT chest, abdomen and pelvis, and/or staging MRI scans

9.3.1 Hypotheses

The likelihood of abscopal responses following radiotherapy in the setting of immunotherapy may depend on the likelihood that activated T-cells will traffic from the irradiated site or relevant draining lymph nodes to other, unirradiated tumor deposits. We will test this hypothesis by correlating an algorithm incorporating tumor location and size to predict T-cell trafficking with abscopal responses seen in the context of this trial. We will also test our ability to predict which particular distant metastatic lesions will respond following targeted radiation therapy.

9.3.2 Collection of imaging data

Imaging studies will be performed according to the study schedule. No investigational imaging modalities will be performed as part of this study. Initial staging studies will be deidentified and saved as DICOM data on a password protected server for analyses by study personnel.

9.3.3 Analysis of imaging data

Identification of potential target lesions will be performed by treating physicians, study investigators, and the TIMC, as appropriate. Prioritization of lesions will occur as previously described. We will then rank the lesions according to an algorithm previously described that may predict the likelihood of generating an abscopal response [61]. We will correlate the rankings with abscopal responses, as well as attempt to predict which distant lesions respond. We will also explore whether hypothesis guided targeting of irradiated lesions is feasible and consistent with palliative radiation therapy by calculating the likelihood that a top ranked lesion would be a safe and feasible target for treatment.

9.4 Additional analysis

The above-mentioned analyses may be altered based on novel developments in the field of cancer immune profiling at the time of correlative science. Additional markers or alternative technologies (based on scientific developments and/or novel technologies) may also be used, to explore potential prognostic or predictive candidate markers/panels or markers related to treatment benefit and/or safety, to improve diagnostic tests, or to understand SCCHN cancer biology.

9.5 Site Performing Correlative Studies

Pathological studies will be conducted by the Center for Immune Oncology at the DFCI. QualTek, Nanostring, and other external fee-for-service services may also be performed on deidentified specimens and used for PD-L1, RNA and other analyses as specified above. Flow cytometry assessment of both tissue and blood will be performed by the Center for Immune Oncology at the DFCI. Other analyses on tumor tissue and collected samples will be performed in the laboratory of Dr. Uppaluri, a surgical oncologist at the Dana Farber Cancer Institute, who has performed similar analyses on other human tissues. Deidentified data related to correlative analyses will be shared with NYU Langone Health as a collaborator to assist with data analysis and manuscript writeup, with an agreement in place prior to sending any deidentified data.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within **28 days** prior to start of protocol therapy (except for pregnancy test and baseline tumor biopsy, as detailed). If these screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values. Scans must be done within **28 days** prior to the start of therapy. Radiation simulation scan can provide more recent tumor measurements prior to the start of study treatment when available.

As detailed in the Study Calendar, a negative pregnancy test in women of child-bearing potential must be documented within **7 days** before the first dose of study medication.

A baseline tumor biopsy, obtained within **28 days** before starting protocol therapy, is also encouraged if tumor tissue is safely accessible. A second optional tumor biopsy sample will be obtained at the end of cycle 2, within 21 days of cycle 3, day 1.

In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted.

Table 8: Study Calendar

	Screening[a]	C1 D1	C1 D1-D21	C2 D1	C2 D14-D20	Cycle 3 and subsequent cycles	Off-Study[l]	Follow-Up
						D1		
Pembrolizumab[b]		X		X		X		
Radiotherapy[b]			X					
Informed consent	X							
Demographics	X							
Medical history	X							
Concurrent medications[c]	X	X----------X						
Physical exam	X	X		X		X	X	
Vital signs	X	X		X		X	X	
Weight	X	X		X		X	X	
Performance status	X	X		X		X	X	
Hematology panel (CBC with platelets)	X	X		X		X	X	
Chemistry panel[d]	X	X		X		X	X	
Coagulation panel (PT/PTT)	X							
Pregnancy test[e]	X							
EKG	X							
Tumor archival (optional)	X[f]							
Tumor biopsy (suggested)	X[g]				X[h]			
Adverse event evaluation	x	X----------X						
Tumor measurements	X	Tumor measurements are repeated at week 5(+/- week) and then every 8 weeks (+/- week). Documentation (radiologic) must be provided for participants removed from study for progressive disease. Confirmatory scans 4 weeks after documented response will be obtained						X[i]
Blood collection for correlative science[j]	X			X		X		
TSH[k]	X					X		
MD/NP/PA evaluation required		X		X		X	X	
Survival								X[m]
HIV Test[n]	X							

[a] Baseline evaluations are to be conducted within 28 days prior to start of protocol therapy. If these screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values. Baseline Laboratory tests must be completed within 14 days prior to first dose of the study drug.
[b] As described in section 5, pembrolizumab may start up to 3 days before and 10 days after radiotherapy start, although it is encouraged that pembrolizumab be started after at least several fractions of radiation have been administered. Radiation will be delivered as specified as per Cohort A (5-8 Gy in each of 3-5 fractions) or Cohort B (8 Gy in each of 3-5 fractions to 1-3 lesion, and 20 Gy in 5 fractions to 1-3 additional lesions). As specified in the protocol, radiotherapy during subsequent cycles is acceptable with permission of the PI.
[c] See Section 5 for concomitant medications guidelines
[d] Chloride, potassium, sodium, BUN, serum creatinine, phosphorus, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin (NOTE: the frequency of checking magnesium levels is left up to the treating provider)
[e] In female subjects with child-bearing potential, urine pregnancy test must be performed within **7 days** before the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, then a serum test is required to confirm the subject is not pregnant.
[f] Archival tumor samples should be collected (block or if not possible, 20 unstained slides) – both the most recent biopsy as well as prior to the start of any PD-1 directed therapy.
[g] Baseline tumor biopsy obtained within 28 days before starting protocol therapy is suggested for those with accessible tumor tissue. See Section 9.1.2 for biopsy handling and processing instructions.
[h] A second optional tumor biopsy will be performed in patients at the end of cycle 2, prior to cycle 3 day 1 of protocol therapy (ideally as close to administration of cycle 3 day 1 therapy as possible).
[i] For those taken off the study for toxicity tumor measurements should be repeated every 6 weeks. Patients with documented PD will be removed from the treatment and followed for overall survival every 6 months, until death (by phone).
[j] See Section 9 for blood handling and processing instructions.
[k] TSH is only required every 3 cycles
[l] Off-Study visit is to occur within 30 days of final administration of study treatment (week 52 at the latest). End of treatment assessments do not have to be repeated if the same assessments were performed within 7 days prior to the visit.
[m] Patients with documented PD will be followed for overall survival every 6 months or until death.
[n] A negative HIV test must be recorded prior to treatment

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect– Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

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

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

11.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in an irradiated area are not considered measurable.

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

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

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

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

11.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

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

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic

Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

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

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions (Note: irradiated target lesions are excluded from overall response determination)

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

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

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

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

11.1.4.2 Evaluation of Non-Target Lesions

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

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump

target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.3 Evaluation of Best Overall Response

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

Table 9: For Patients with Measurable Disease (i.e., Target Disease)

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

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as *"symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

Tbale 10: For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

11.1.6 Clinical Benefit rate

Clinical benefit rate: defined as CR, PR and stable disease (SD) ≥ 24 weeks.

11.2 Other response parameters

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

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

11.2.2 Impact of New Lesions on irRC

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

11.2.3 Definition of Target Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all target lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR):** Decrease, relative to baseline, or 50% or greater in the sum of the products of the two largest perpendicular diameters of all target and all new measurable target lesions (i.e., Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SBD increases by $\geq$25% when compared to SPD at nadir.
- **irStable Disease (irSD):** Does not meet criteria for irRC or irPR, in the absence of progressive disease.
- **irProgressive Disease (irPD):** At least 25% increase Percentage Change in Tumor Burden (i.e. taking SPD of all target lesions and any new lesions) when compared to SPD at nadir.

11.2.4 Definition of Non-Target Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all non-target lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** Non-target lesion(s) are not considered in the definition of PR; these terms do not apply.
- **irProgressive Disease (irPD):** Increases in number or size of non-target lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e. the SPD at nadir of the target lesions increases by the required amount).

11.2.5 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

- **Immune-Related Complete Response (irCR):** Complete disappearance of all tumor lesions (target and non-target) together with no new measurable/unmeasurable lesions for at least 4 weeks from the date of documentation of complete response.
- **Immune-Related Partial Response (irPR):** The sum of the products of the two largest perpendicular diameters of all target lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the SPD of the two largest perpendicular diameters of all target

lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline, of the irSPD compared to the previously SPD baseline of 50% or greater is considered an irPR.

- **Immune-Related Stable Disease (irSD):** irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease
- **Immune-Related Progressive Disease (irPD):** It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute PD:
  - At least 25% increase in the SPD of all target lesions over baseline SPD calculated for the target lesions.
  - At least 25% increase in the SPD of all target lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the target lesions.

Criteria for determining overall response by irRC are summarized as follows:

Immune-Related Response Criteria Definitions

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

11.2.6 Immune-Related Best Overall Response Using irRC (irBOR)

irBOR is the best confirmed overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

11.2.7 Local response is defined as irRC applied to the irradiated lesions. This is not applicable in the case of an irradiated bone lesion.

11.2.8 Abscopal response rate

Abscopal response is defined as described previously [62] to be a decrease in the longest diameter of at least 30% in any measurable (>1cm) non-irradiated lesion from baseline. A complete abscopal response is defined as the complete disappearance of a measurable non-irradiated lesion and a partial abscopal response was defined as at least a 3-% decrease in the longest diameter. Progressive disease in this context is defined as at least a 20% increase in the longest diameter of the best measurable non-irradiated lesion, whereas stable disease was defined as insufficient shrinkage or growth to qualify for a partial abscopal response or complete abscopal response or progressive disease.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

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

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary

provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multi-Center Guidelines

This protocol will adhere to DF/HCC Policy MULTI-100 and the requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Sponsor-Investigator, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix J.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This phase 2 study will enroll patients who have previously progressed on PD-1 directed therapy, for whom response to pembrolizumab monotherapy is unlikely. These paitents usually progress rapidly within a span of a few months and the expected progression-free survival rate at 3 months is about 35%. Therefore, the combined treatment will be considered promising and worthy of further evaluation if there is evidence that the 3-month progression-free survival (PFS) is approximately 65%. A two-stage design will be employed and a safety run-in phase will be conducted using a 3+3 design.

Safety Run-In:
The safety run-in phase will follow a 3+3 design. Initially 3 patients will be entered. If none of the 3 patients experiences dose-limiting toxicity (DLT), the safety run-in phase will end and the study will proceed. If 1 out of 3 patients experiences a DLT, an additional 3 patients will be entered. If none of the additional 3 patients experiences a DLT, the study will proceed. If at any time 2 (or more) patients experience a DLT in the run-in phase, the study will be terminated. Treatment safety will continue to be monitored after the run-in phase. If there are 33% or more of patients with a DLT at any time during the phase II portion, the study will pause and a detailed safety analysis will be conducted.

Phase II:
A two-stage design will be used in the phase II portion of this study. The first stage will accrue 6 patients and the patients in the run-in phase will be included in the stage I accrual. If at least 2 patients are progression-free and alive at 3 months, an additional 13 patients will be entered. If 10 or more patients are progression-free and alive at 3 months among 19 patients, the combination treatment will be considered worthy of further study for this group of patients. With this design, we will have 90% power to test the PFS rate of 65% vs. 35% based on a 0.08 level one-sided test.

If only 0 or 1 patient is progression-free at 3 months among the 6 patients in stage I accrual, the same design (safety run-in plus two-stage design) will be used to examine the combination treatment of pembrolizumab plus hypofractionated and low dose radiation (cohort B).

13.2 Sample Size, Accrual Rate and Study Duration

We plan to accrue 20 patients for a cohort in the phase II portion to allow for 1 unevaluable patient. The maximum sample size will be 26 patients if the enrollment to cohort B starts. The expected accrual rate is 1-2 patients per month, and the accrual of 26 patients is expected to complete in 18 months.

13.3 Stratification Factors

Patients will not be stratified; however, analyses will be performed on patients with HPV-associated and non-HPV associated disease.

13.4 Analysis of Primary Endpoint

The primary endpoint of this study is PFS rate at 3 months. Proportion of patients progression-free and alive at 3 months will be reported and the method of Atkinson and Brown will be used to compute the confidence interval of PFS rate for the cohort that has two stages of accrual. The Kaplan-Meier method will also be used to characterize PFS and PFS is defined as the time from registration to disease progression or death, whichever occurs first. Patients who are alive and progression-free will be censored at the date of last disease assessment.

13.5 Analysis of Secondary Endpoints

Efficacy Endpoints
The secondary endpoints of this study include local response within the irradiated fields, overall response per RECIST 1.1 and irRC, abscopal response and overall survival. Various types of response rates will be reported along with 90% exact binomial confidence intervals. The Kaplan-Meier method will be used to characterize overall survival.

Safety and tolerability
All patients will be evaluable for toxicity from the time of their first treatment with any study agent. Toxicity will be graded according to NCI CTCAE, Version 4.0 and summarized by maximum grade. Incidence rate of each toxicity will be reported with 90% exact binomial confidence intervals. Number of patents with DLTs will also be reported.

Correlative endpoints
Analyses of correlative endpoints are largely exploratory and hypothesis-generating and will be descriptive in nature. Any promising findings will be tested in future studies. Assuming 16 patients have baseline and post cycle 2 biospecimen available for analyses of tumor infiltrating lymphocytes (expressed as a percentage) and also for PD-L1 and PD-L2 expression (expressed as H-scores and also as percent positive), the study will have 80% power to detect a 0.65 SD change

in mean of a quantitative correlative parameter using Wilcoxon signed rank test with two-sided type I error of 0.1.

13.6 Reporting and Exclusions

Evaluation of Efficacy
For this Phase II trial, the efficacy evaluable population will be eligible patients who receive protocol therapy.

Evaluation of Safety

The safety population will be used in the safety data summaries. The safety population consists of all patients who took at least one dose of any protocol therapy and who have at least one post-baseline safety assessment. Note that a patient who had no adverse events constitutes a safety assessment. Patients who have received at least one dose of study drug but have no post-treatment safety data of any kind would be excluded.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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16. APPENDICES

Appendix A: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B: Pembrolizumab Events of Clinical Interest

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

APPENDIX C: Guidelines for collecting research biopsy tissue

Tissue specimens will be collected from metastatic lesions using standard institutional procedures. The amount of tissue collected may follow the guidelines listed below:

Skin/chest wall: A goal of 2 5-mm punch biopsies will be obtained.

Lymph node: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules are mandated on this protocol, unless they are clinically indicated.

Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 11-13 gauge needle.

Please note that the above are guidelines for the amount of tissue to be obtained, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue (roughly equivalent to the goal amount of tissue listed in the guidelines above, i.e. the equivalent of two 5-mm punch biopsies of the skin, or 3-6 18-gauge core biopsies) stored for research at the time of the procedure (provided that the tissue is processed as specified), in which case, the patient would not be required to undergo a separate research biopsy at baseline on this protocol.

Specimens will be shipped to the Clinical Research Coordinator who will then deliver tissues to pathology:

TBD

Coded laboratory specimens will be stored in the Tumor Bank of the DFCI. These specimens will become the property of DFCI. Patients will be informed that their specimens may be used for research by investigators at DF/HCC and other approved collaborators. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed.

Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:
Skin/chest wall (punch biopsy):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, or infection

Lymph node, liver, or bone (core needle biopsy):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, damage to adjacent organs. Additional risks may be present if intravenous conscious sedation is required

Breast (core biopsy):
- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.

Pleural fluid (thoracentesis):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs

Ascites fluid (paracentesis):
- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs. In order to minimize the risk of a biopsy, only qualified personnel will perform these procedures.

Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain separate procedure consent. Patients will be evaluated for comorbidities or concomitant medications that may increase the risk of potential complications. For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT or ultrasound will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. After lymph node biopsies, patients will be observed a minimum of 2 hours (range 2-4 hours) after the procedure, or according to standard institutional guidelines. After liver biopsies, patients will be observed a minimum of 4 hours (range 4-6 hours) after the procedure, or according to standard institutional guidelines. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

Risks of Anesthesia
Local Anesthesia
All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs. In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic

reactions to local anesthetics.

Intravenous Conscious Sedation
Certain biopsy procedures, such as lymph node, liver, or bone biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation and verbal commands.

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 patients undergoing IVCS during upper gastrointestinal endoscopies, the rate of immediate cardiopulmonary events was 2 in 1000.[63] The 30-day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia. In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (M.D. or R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Patients with active cardiac disease are excluded from this study. No local anesthetic spray to the oropharynx will be necessary, given that endoscopy is not a planned procedure. Following the procedure, patients will be observed closely in the recovery room for a minimum of 2 hours.

General Anesthesia
Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed *are not permitted* on this protocol, unless they are being done for clinical reasons, and excess tissue that otherwise would have been discarded is then banked for the purpose of this protocol.

For Biopsies of Soft Tissue, Liver, Bone, Breast, Etc:
1. After biopsy is performed, the tissue mass is placed on a sterile gauze
2. Using forceps, separate the tumor tissue
3. Place 2 pieces (cores) of tumor tissue in each cassette (typically end up with 3 cassettes per biopsy); the last cassette will contain many small pieces of tumor tissue
4. Fill cassettes with OCT
a. Completely cover tissue
b. Limit the amount of bubbles
5. Place cassettes on dry ice and prepare for transport by limiting OCT leakage
6. Return samples to the lab and complete freezing of samples in OCT with dry ice (about 10 minutes freezing time)
7. Once samples are frozen, place in plastic bag; label bag with date, protocol number, patient number, and number of initials included
8. Store in –80C freezer

For Effusions and Ascites
1. Fluid sample should be split into two equal aliquots
2. One aliquot should be spun down into a pellet and snap frozen in an ETOH/dry ice bath or in liquid N2
3. One aliquot should be fixed and processed as a standard cell block.
Note: if the sample preparation is done by a clinical cytopathology laboratory, it is important to explain that the sample is for research purposes only and that no thin prep should be performed as this uses up a significant portion of the sample.

For Fine Needle Aspiration Samples
A goal of 3 passes:
1. One pass should be evacuated and rinsed directly into 2mL of room temperature Trizol for RNA analysis.
2. One pass should be evacuated and rinsed directly into 2mL of room temperature Trizol for DNA analysis.
3. One pass should be evacuated and rinsed directly into 10-20mL of RPMI to prepare a cell block.

Fresh Tissue Shipping Procedures
Please ship frozen specimens over-night on dry ice to the following:
TBD

Please email TBD with the sample information and tracking information the day before shipping a frozen specimen.

APPENDIX D: Antibodies that can be used for immunohistochemistry in correlative studies

IHC Biomarkers	Priority	Clone/ Cat #	Source	Host species	Dilution	Optimized?
PD-L1	1	9A11	G. Freeman	Mouse	1/125	Yes
PD-L2	1	9E 6	G. Freeman	Mouse	1/10000	Yes
PD-1	1	EH33	G. Freeman	Mouse	1/600	Yes
CD3	1	IS503	Dako	Rabbit	1/250	Yes
CD4	1	4B12	Vector Labs	Mouse	1/200	Yes
CD8	1	144B	Dako	Mouse	1/100	Yes
FOXP3	1	206D	BioLegend	Mouse	1:50	Yes
TIM3	1	AF2365	R&D Systems	Goat	1:50	Yes
LAG3	1	17B4	LifeSpan BioSc	Mouse	1/200	Yes
Tie2	2	AF313	R&D Systems	Goat	1/500	Yes
ANGPT2	2	sc-74403	Santa Cruz Bio	Mouse	1/200	Yes
IDO1	2	ab55305	Abcam	Mouse	1/100	No
CD38	3	SPC32	Abcam	Mouse	1/300	Yes
CD56	3	123C3	Dako	Mouse	1/100	Yes
CD14	3	ab49755	Abcam	Mouse	1/100	Yes
CD16	3	ab183354	Abcam	Rabbit	1/100	No
CD11c	3	EP1347Y	Abcam	Rabbit	1/500	Yes

APPENDIX E: Antibodies that may be used for flow cytometry in correlative studies

Cell Type	Antibody	Color	Clone
T effector	CD4	FITC	SK3
		PC7	SK3
	CD62L	APC	DREG-56
	CD69	PE	FN50
T regs	CD4	FITC	SK3
		PC7	SK3
	CD25	PE	Bc96
		PC5	B1.49.9
	FOXP3	PE	PCH101
		FITC	PCH101
	CD127	APC	eBioRDR5
NK	CD3	FITC	UCHT1
		PC7	UCHT1
	CD56	PE	NCAM16.2
	CD57	PE	TB01
NKT	CD3	FITC	UCHT1
		PC7	UCHT1
	CD56	PE	NCAM16.2
	TCR a/b	APC	BW242/412
	CD314 (NKG2D)	PE	ON72
MDSC	HLA-DR	PC7	L243
		FITC	L243
	CD11b	FITC	Bear1
	CD14	APC	61D3
	CD33	PE	WM53
Cytotoxic	CD8	APC	BW135/80
		PE	BW135/80
	CD3	FITC	UCHT1
		PC7	UCHT1
Memory T	CD197 (CCR7)	PE	3D12
	CD45RO	FITC	UCHL1
	CD45RA	PC7	HI100
	CD4	FITC	SK3
		PC7	SK3
	CD8	APC	BW135/80
		PE	BW135/80
B cells	CD5	BV421	UCHT2
		FITC	UCHT2
	CD19	PC7	SJ25C1
		PE	SJ25C1

		APC	SJ25C1
	CD20	FITC	2H7
Classic Monocytes	CD14	APC	61D3
	CD16	FITC	eBioCB16
Dendritic	CD123	APC	6H6
	CD303a	FITC	201A
	CD11c	FITC	11-0116
	CD141	APC	M80
	CD1c	PE	L161
Macrophages	CD40	APC	5C3
Progenitors	CD34	PE	4H11
Intracellular Cytokines	IL-10	PE	JES3-9D7
	IL-17a	PercP Cy5	eBio64DEC17
	INFg	APC	B27
	TNFa	FITC	Mab11
Co-stimulatory and inhibitory markers	CD134 (OX40)	APC	ACT-35
	CD137 (4-1BB)	FITC	4B4
	CD154 (CD40L)	PercP 710	24-31
	CD223 (LAG3)	PercP 710	3DS223H
	CD252 (OX40L)	PE	11C3.1
	CD278 (ICOS)	FITC	ISA-3
	Tim-3	BV421	F38-2E2
	CD274 (PD-L1)	PE	MIH1
	CD279 (PD-1)	FITC	MIH4
		PE	MIH4
		APC	MIH4
	CD357 (GITR)	APC	621
	CD152 (CTLA-4)	efluor 660	14D3
Proliferation	Ki-67	FITC	20Raj1

APPENDIX F: Immunohistochemical staining assays

Design of immunohistochemical assay

The immunohistochemical assay for PD-L1 and PD-L2 is semi-quantitative while PD-1 stained slides will be scanned by an automated scanning microscope and quantitatively analyzed by Aperio image analysis system (Lecia Biosystems) after they are evaluated and positive cells are manually counted by a pathologist.

Standard EnVision two-step (indirect) staining method will be utilized. Four micrometer-thick sections will be cut, deparaffinized, rehydrated, and subjected to heat modified antigen retrieval in citrate buffer (pH 6) (Invitrogen) by steaming for 30 minutes. After cooling, tissue sections will be incubated with peroxidase block (DAKO, Carpinteria, CA) for five minutes, then serum free protein block (DAKO) for 20 minutes. Slides will be incubated at room temperature for one hour with a primary antibody. Antibodies will be diluted in Da Vinci Green Diluent (Biocare Medical, Concord, CA). EnVision™ anti-mouse HRP-labeled polymer (DAKO) will be applied to the sections for 30 minutes, followed by visualization using the chromogen 3,3-diaminobenzidine (DAKO). All the sections will then be counterstained with hematoxylin, dehydrated, mounted, and coverslipped. Positive and negative controls shall be included in each staining. Known positive stained Hodgkin Lymphoma (PD-L1), tonsil (PD-1), and melanoma (PD-L2) slides will be used as external control (separate slides). Stained slides will be stored at room temperature.

In a pilot study performed by our correlative scientists, immunoreactivity for PD-L1 was detected in the cytoplasm and membrane while PD-L2 and PD-1 expression was observed in the cytoplasm. Scoring for PD-L1 and PD-L2 will be semi-quantitative/ordered categorical. The percentage of the tumor cells staining positive for PD-L1 or PD-L2 and the intensity of the staining will be recorded (using the scale 0=no staining, 1=weak staining, 2=moderate staining, 3=intense staining). Absolute PD-1 positive cells will be counted under microscope lens x20 power field. Five representative areas will be chosen to count. The average number from 5 areas will be recorded and compared with data from image analysis.

For PD-1 staining, slides will be scanned by an automated scanning microscope and analyzed by Aperio image analysis system (Lecia Biosystems). Tumor areas will be marked by a pathologist to exclude non-neoplastic areas, such as stroma, normal epithelial, and necrotic regions. The software will be used to count the number of positive cells in each tissue. The percentage of PD-1 positive cells will be calculated. Data will be compared with that of manual counting by a pathologist to exclude tissue artifacts that cannot be recognized by computer image software.

Assay performance

Protocols of these three antibodies have been optimized and standardized to minimize staining variance. Positive control and negative controls were used and stained separately with each batch of slides. The IHC staining of three markers (PD-1, PD-L1, PD-L2) has been performed in two different labs by three different technicians on whole tissue sections of Hodgkin lymphomas, melanomas, lung cancers, and renal cell carcinomas. Three readers were involved, confirming the good reproducibility of the assay.

Thresholds of positivity

Tumor will be considered positive if >5% (PD-L1)[64] or >10% (PD-L2) of the tumor cell population demonstrates unequivocal staining. PD-1 positivity will be defined as >3% positive cells/high power field.[65] All IHC stained slides will be evaluated and scored by a pathologist. A subset of slides will be reviewed by a second pathologist to ensure concordance of interpretation.

APPENDIX G: TIL isolation from solid tumors

1. Prepare an enzyme solution of collagenase, hyaluronidase and deoxyribonuclease in advance:
2. Dissolve collagenase (collagenase type I, cat#17100-017, Invitrogen) in DMEM at a concentration of 1 mg/ml
3. Add hyaluronidase (hyaluronidase type V, cat#H6254, Sigma-Aldrich) to a final concentration of 1mg/ml (1,500 units/ml) and deoxyribonuclease (deoxyribonuclease I, type IV, cat#5025, Sigma-Aldrich) to a final concentration of 50 micrograms/ml (100 units/ml)
4. Filter the solution with a 10 ml sterile syringe, a sterile 23G needle, and a sterile 0.2 μm filter.
5. Record the date and time of the start of TIL isolation.*
6. Dissect patient tumor sample into pieces as small as possible with sterile scissor or scalpel. **Note**: Mincing of tumor may be facilitated by lining up two scalpels in parallel.
7. Submerge the pieces of tumor in 5 - 10 ml prepared enzyme solution in a 50 ml conical tube.
8. Enzymatically digest tumor pieces in 37°C waterbath for one to two hours; every 15 min., vigorously shake the tube.
9. Put a sterile cell strainer (100 μm, 352360, BD Falcon) on a 50 ml conical tube, and pass the digested tumor solution through the strainer; the flowthrough will be collected in the 50 ml conical tube. Rinse the strainer and undigested tumor once with PBS.
10. Add 2-5 ml complete DMEM medium (with 10% FBS + 50 μg/ml gentamycin) into flowthrough to stop digestion.
11. Spin the tube at 1500 rpm for 5 min in a centrifuge at room temperature.
12. In the meantime, put undigested tumor tissue into a sterile 50 ml conical tube, and add 5-10 ml enzyme solution and continue with digestion from step 4.
13. Repeat step 3 to step 8, based on tissue digestion

NOTE: For samples with lot of red blood cells and/or undigested debris that has passed through the cell strainer, the following is recommended before proceeding to step 11:

A. Resuspend the cell pellet in 10 ml complete DMEM medium

B. Add 10 ml Ficoll Paque Plus (Cat# 17-1440-03; GE Healthcare) in a 50 ml conical tube.

C. Slowly and gently layer the digested tumor suspension onto the Ficoll Paque Plus.

D. Centrifuge the tube at room temperature at 1500 - 2000 rpm (1000 g) with slow acceleration and deceleration for 20 - 30 min.

E. Pipette off the interface between complete DMEM and the Ficoll Paque Plus (lower part), and transfer the layer into a 50 ml conical tube. The bottom pellet will be composed of red blood cells and undigested debris.

F. Add 2 - 3X bed volume of PBS to dilute Ficoll.

G. Spin the tube at 1500 rpm for 5 min in a centrifuge at room temperature.

H. Aspirate supernatant and proceed to step 11.

1. Resuspend cell pellets in complete DMEM medium plus gentamycin 50ug/ml and combine cells-TIL into one sterile 50 ml conical tube.
2. Centrifuge at 1500 rpm for 5 min in a centrifuge at room temperature.
3. Aspirate off the supernatant and remove a small aliquot to record the cell count and viability, then place the tube on ice.
4. For each timepoint, collect the following parameters:
    a. Cell viability (%) before freezing*
    b. Total yield of TIL (x 10^6 cells/mL/vial) isolated prior to freezing*
5. Resuspend the cell suspension in pre-chilled PBMC freezing media (CTL-cryoABS kit, CTL cellular Technology)
    c. Transfer 1 ml aliquots of the cell suspension to a cryovial labeled with the **supplied Quintiles labels**. A minimum of one (1) cryovial should be obtained with a minimum concentration of cells at 1x10^6 cells/mL/vial.
    d. For each cryovial prepared, please record the total # of cells in the cryovial*. If there are more than 2x10^6 cells, then aliquot cells into as many cryovials as possible at a concentration of 1x10^6 cells/ml/vial.
6. Store in 1 ml aliquots in cryovials at -80^{0}C in a slow freeze container. Leave undisturbed overnight or for a **minimum of 12 hrs and a maximum of 24 hrs.**
7. Transfer into liquid nitrogen for long-term storage. Record the time, date, and location that the samples* were placed in liquid nitrogen storage.
8. At the sponsor's request, samples should be batch shipped at the end of study. Please, follow the shipping instructions provided. Samples will be shipped on liquid nitrogen.

APPENDIX H: Flow cytometry procedures

Prep without Permeabilization

KEEP EVERYTHING ON ICE

1. Thaw cell vial in 37 degree water bath until completely thawed.
2. Resuspend cells in 50 ml of RPMI medium (Gibco, 11A75-093) + 10% FBS + 1X final Anti-Anti (Gibco, 15240-062) in a 50 ml conical tube (Corning, 430290).
3. Culture cells in 2 T-150 culture flasks (Corning, 431465) overnight (25 ml per flask)
4. In one flask, activate cells by adding 0.4 ml (whole vial) of Dynabeads Human T- Activator (Gibco, 1161D). Before adding beads to flask, wash beads according to manufacturer's protocol.
5. Incubate cells for 24 hours at 37 degrees with 5% CO_2
6. Remove cells and media from flask and filter through 70 micron cell filter (Biologix, 15-1070) into 50 ml conical tube.
7. Spin conical tubes for 5 min at 1800 rpm in a Sorvall Legend XTR centrifuge.
8. Make wash/blocking media: PBS +2.5% FBS (Gibco, 14040).
9. Vacuum media off pellet, resuspend pellet in calculated volume of wash/blocking media according to calculations from cell density + number of wells and tubes for 700,000 cells/tube in 100 µl.
10. Pipet 100 µl/ well of cells + wash/blocking media containing FcR Blocking Reagent (Milteny Biotec, 130-059-901) into v-bottomed plate (Costar, 3894) according to well map (let sit for 20-30 minutes on ice).
11. Spin plate at 1800 rpm for 5 minutes at 4C in Sorvall Legend XTR centrifuge
12. Mix antibody cocktails in flat bottomed plate (amount according to manufacturer specifications or from previously developed assays)
13. After plate with cells is finished spinning, aspirate liquid off pellet by carefully tilting the plate. Add appropriate antibody cocktails from flat bottomed plate according to well map after pipetting up and down to mix at least three times.
14. Let plate with cells + antibodies sit for 45 minutes on ice in the dark.
15. Spin plate as previously described in step 11
16. Aspirate off liquid by tilting plate and wash with 150 µl/well of wash/blocking media, pipetting up at down to mix at least 3 times (described in step 6)
17. Resuspend cells in 150 µl/well in wash/blocking media
18. Keep plate and single tubes (single color controls) on ice, in the dark or covered with aluminum foil until read by Fortessa LTS II (Beckton- Dickinson).

APPENDIX I: Generation of PBMCs

1. Pour blood from green-cap tubes (heparin treated tubes) into two 50 ml conical tubes (Corning, 430290).
2. Spin tubes at 1500 rpm for 10 min (Sorvall Legend XTR centrifuge).
3. Aspirate 2 ml plasma/tube and aliquot into 4 tubes mircocentrifuge tubes (Fisherbrand, 05-408-138)
4. Spin plasma at 3000 RPM for 5 minutes (Sorvall Legend Micro 21R centrifuge)
5. Aspirate plasma into Cryogenic tubes 2 ml plasma/ tube (Corning, 430488).
6. Dilute blood 1:1 with PBS. (Blood amount should not exceed 25 ml per tube.)
7. Take 2 new 50 ml conical tubes and add 12 ml ficoll-paque (Cat# 17144003; GE Healthcare) per tube.
8. Slowly and gently layer the diluted blood on top of the ficoll-paque of the tube with a maximum volume of 35 ml.
9. Centrifuge the tube at 1900 rpm for 20 min at room temperature with slow acceleration (#7) and deceleration (#7) (Sorvall Legend XTR centrifuge).
10. Remove the PBMC layer from between the upper layer (diluted plasma) and middle layer (ficoll-paque) and transfer into a 50 ml conical tube. The lower layer is composed of red blood cells.
11. Completely fill conical tube containing isolated PBMC with PBS, mixing well.
12. Count viable cells by mixing 10 µl Trypan Blue with 10µl PBMC/PBS dilution in a microcentrifuge tube. Load 10 µl of mixture onto Countess Cell Counting Chamber Slide (Invitrogen, C10283) and read with Countess Automated Cell Counter (Invitrogen).
13. Centrifuge the tubes containing PBMC/PBS mixture at 1500 rpm for 5 min at room temperature (Sorvall Legend XTR centrifuge).
14. Remove PBS, and resuspend PBMC pellet in appropriate amount of freezing solution so that there are approx 5×10^6 cells/cryo vial in 300-500 µl of Fetal Bovine Serum (heat inactivated) plus 15% DMSO.
15. Put vials in CoolCell container (Biocision Inc.) and transfer to -80C freezer overnight.
16. Transfer cells to liquid nitrogen tank

DF/HCC Protocol #: 16-604

APPENDIX J

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

TABLE OF CONTENTS

1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP serves as a reference for any sites external to DF/HCC that are participating in a DF/HCC clinical trial.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Policies and Operations..

2. GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the following general responsibilities apply, in addition to those outlined in DF/HCC Policies for Sponsor-Investigators:

2.1 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- *Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).*
- *Distribute protocol and informed consent document updates to External Sites as needed.*
- *Oversee the data collection process from External Sites.*
- *Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by External Sites and provide to the DF/HCC Sponsor for timely review and submission to the IRB of record, as necessary.*
- *Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the reporting requirements for the IRB of record to all External Sites.*
- *Provide External Sites with information regarding DF/HCC requirements that they will be expected to comply with.*
- *Carry out plan to monitor External Sites either by on-site or remote monitoring.*
- *Maintain Regulatory documents of all External Sites which includes but is not limited to the following: local IRB approvals/notifications from all External Sites, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites*
- *Conduct regular communications with all External Sites (conference calls, emails, etc) and maintain documentation all relevant communications.*

2.2 External Site

An External Site is an institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC investigator. The External Site acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Each External Site is expected to comply with all applicable DF/HCC requirements stated within this Data and Safety Monitoring Plan and/or the protocol document..

The general responsibilities for each External Site may include but are not limited to:

- *Document the delegation of research specific activities to study personnel.*
- *Commit to the accrual of participants to the protocol.*
- *Submit protocol and/or amendments to their IRB of record. For studies under a single IRB, the Coordinating Center will facilitate any study-wide submissions..*

- *Maintain regulatory files as per ICH GCP and federal requirements.*
- *Provide the Coordinating Center with regulatory documents or source documents as requested.*
- *Participate in protocol training prior to enrolling participants and throughout the trial as required.*
- *Update Coordinating Center with research staff changes on a timely basis.*
- *Register participants through the Coordinating Center prior to beginning research related activities when required by the sponsor.*
- *Submit Serious Adverse Event (SAE) reports to sponsor, Coordinating Center, and IRB of record as applicable, in accordance with DF/HCC requirements.*
- *Submit protocol deviations and violations to the Sponsor, Coordinating Center, and IRB of record as applicable..*
- *Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.*
- *Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.*
- *Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.*
- *Notify the sponsor immediately of any regulatory authority inspection of this protocol at the External Site.*

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

Certain DF/HCC Policy requirements apply to External Sites participating in DF/HCC research. The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Revisions and Closures

The External Sites will receive notification of protocol revisions and closures from the Coordinating Center. When under a separate IRB, it is the individual External Site's responsibility to notify its IRB of these revisions.

- ***Protocol revisions:*** *External Sites will receive written notification of protocol revisions from the Coordinating Center. All protocol revisions should be IRB approved and implemented within a timely manner from receipt of the notification.*

- ***Protocol closures and temporary holds:*** *External Sites will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the External Sites on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.*

3.2 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for External Sites. The External Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each External Site upon request.

External Sites must send their version of the informed consent document to the Coordinating Center for sponsor review and approval. If the HIPAA authorization is a separate document, please submit to the sponsor for the study record. Once sponsor approval is obtained, the

External site may submit to their IRB of record, as applicable. In these cases, the approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each External Site will identify the appropriate members of the study team who will be obtaining consent and signing the consent form for protocols. External Sites must follow the DF/HCC requirement that for all interventional drug, biologic, or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

3.3 IRB Re-Approval

Verification of IRB re-approval for the External Sites is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received for the External Site on or before the anniversary of the previous approval date.

3.4. DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.5. Participant Registration and Randomization

To register a participant, the following documents should be completed by the External Site and faxed or e-mailed to the Coordinating Center **(fax: 617-582-8911)**

- Copy of required laboratory tests including: hematology panel (CBC with platelets), chemistry panel, coagulation panel (PT/PTT), pregnancy test (if applicable), EKG, TSH, and HIV test
- *Signed informed consent document*
- HIPAA authorization form (if separate from the informed consent document)
- Completed Eligibility Checklist

The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:

- *Register the participant on the study with the DF/HCC Clinical Trial Management System (CTMS).*
- *Upon receiving confirmation of registration, the Coordinating Center will inform the External Site and provide the study specific participant case number, and, if applicable, assigned treatment and/or dose level.*

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case

number. External Sites should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.3.1 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the External Site receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and IRB of record must be notified of any violations to this policy.

3.3.2 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without IRB approval will be permitted. All External Sites are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.5. **Data Management**

See protocol Section 12: Data Reporting / Regulatory Requirements

DF/HCC develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC provides a web based training for all eCRF users.

3.5.1. Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

If study forms are not submitted on schedule, the External Sites will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

3.6. **Protocol Reporting Requirements**

3.6.1. Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the

participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor and to the IRB of record.

3.6.2. Reporting Procedures

Requests to deviate from the protocol require approval from the IRB of record and the sponsor.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

3.6.3. Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements, and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

4. MONITORING: QUALITY CONTROL

The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

4.4. Ongoing Monitoring of Protocol Compliance

The External Sites may be required to submit participant source documents to the Coordinating Center for monitoring. External Sites may also be subject to on-site monitoring conducted by the Coordinating Center. The Coordinating Center will work with the Dana-Farber Clinical Trials Office to ensure trial requirements are met by all sites.

The Coordinating Center will implement ongoing monitoring activities to ensure that External Sites are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

External Sites will be required to participate in monthly Coordinating Center-initiated teleconferences. The conferences will review and document patient issues, review data entry, and share any pertinent updates. Conferences will include the study PIs, research nurses, and CRCs. Minutes are recorded and documented in the trial files. The Coordinating Center will provide regular and ongoing communication to External Sites about study-related information.

As part of the remote monitoring plan, External Sites will be required to forward de-identified copies of participants' medical record and source documents to the Coordinating Center to aid in source data verification. External sites will be required to submit source documentation for all participant Grade 3 or higher SAEs and source documentation pertaining to the primary endpoint of the study, PFS rate at 3 months. Source documentation should be submitted to the Coordinating Center monthly as applicable.

4.5. **Monitoring Reports**

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at External Sites that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

4.6. **Accrual Monitoring**

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each External Site. Accrual will be monitored for each External Site by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

For a Phase II trial, an accrual rate of at least 3 patients per site/annually is recommended:

5. **AUDITING: QUALITY ASSURANCE**

5.4. **DF/HCC Internal Audits**

All External Sites are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

5.5. **Audit Notifications**

It is the External Site's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

5.6. **Audit Reports**

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the IRB as applicable.

5.7. **External Site Performance**

The DF/HCC Sponsor and the IRB of record are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

External Sites that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be put on hold or closed.