Phase II Study Evaluating HPV-16 vaccination (ISA101b) and Pembrolizumab plus Cisplatin Chemoradiotherapy for "Intermediate Risk" HPV-16 associated Head and Neck Squamous Cell Carcinoma

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Commercial Agent:	Cisplatin
Radiation Therapy:	Intensity Modulated Radiotherapy (IMRT)

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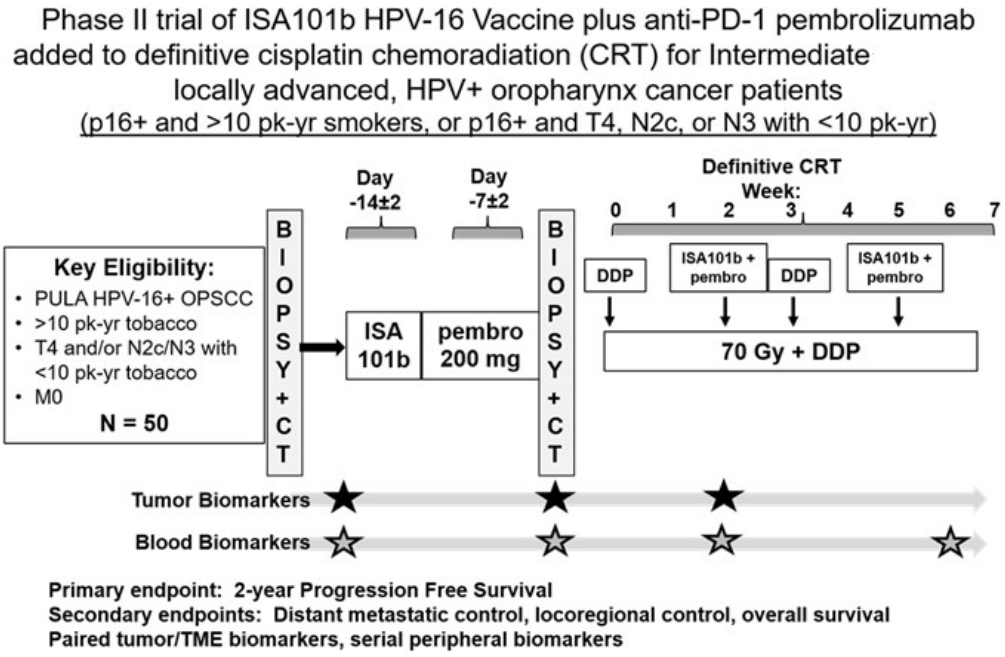
STUDY SCHEMA



***DDP Diamminedichloroplatinum (cisplatin)**

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1. BACKGROUND AND RATIONALE

1.1 Disease Background

Head and neck squamous cell carcinoma (HNSCC) is the most common cancer arising in the upper aerodigestive tract. HNSCC is the sixth leading cancer worldwide with 600,000 cases annually.[1] Despite advances in multimodality therapy, 5-year overall survival (OS) is 40-60%, and has increased only modestly in the past three decades.[2] The current standard of care for nonsurgical management of previously untreated locally advanced (PULA) HNSCC is concurrent cisplatin-radiotherapy (RT), which improved overall survival (OS), progression-free survival (PFS), and locoregional control (LRC) compared with RT alone in the landmark Intergroup 0126 trial.[3,4] Although LRC and OS are improved with concurrent cisplatin-RT, a meta-analysis indicated disappointing local and distant failure rates of 50% and 15% respectively, and an absolute survival benefit of only 6.5% compared to conventionally fractionated RT alone.[5] Immunotherapy for HNSCC has proven not only promising but recently clinically effective[6].

1.2 HPV and Head and Neck Cancer Survival

HPV(+) HNSCC is rapidly increasing in incidence.[7] In the US, two-thirds of patients with oropharynx cancer have HPV(+) tumors. HPV status and pack-years of tobacco exposure are the major determinants of OS in HNSCC, followed by nodal stage.[8] Based upon HPV status, pack-years and nodal stage, patients with HNSCC can be classified patients into three risk groups having low, intermediate, or high risk of death. This clinical risk classification has framed national clinical trial priorities in PULA HNSCC. Specifically, de-intensification strategies are being tested in patients with low-risk HPV(+) HNSCC whereas intensification strategies are the major unmet need for high-risk HPV(-) and intermediate-risk HPV(+) disease.[9,10]

Tumor HPV status has been shown to be a strong, independent prognostic biomarker for patients with HNSCC treated with cisplatin- or cetuximab-based RT. Patients with HPV-positive OPSCC have a 58% (HR 0.42, 95%CI 0.27-0.66) reduction in risk of death when compared to patients with HPV-negative OPSCC, after adjustment for other prognostic factors. HPV-positive cancers have been shown to have higher response rates to chemotherapy and CRT, resulting in higher local-regional disease control. Furthermore, the combination of HPV status, pack-years of tobacco smoking, and tumor stage has been shown to classify patients as having a low, intermediate, or high risk of death. Three-year rates of OS were 93% (95%CI, 88.3-97.7) in the low-risk group, 70.8% (95%CI 60.7-80.8) in the intermediate-risk group, and 46.2% (95%CI 34.7-57.5) in the high-risk group. Novel therapies are thus needed in both intermediate and high-risk patients to improve both local-regional control and reduce risk of distant metastases.

Additional analyses conducted by O'Sullivan, et al. (2013) have indicated that patients with p16-positive OPSCC with T4 or N2C-N3 disease have a high-risk of disease progression, even if tobacco exposure is < 10 pack-years. Therefore, these patients are also considered eligible for this clinical trial. The sample size calculations presented in this protocol consider the outcome of all of these eligible patients based on observations from RTOG 0522. It is important to note that many of the clinical trials investigating the impact of tumor HPV status on survival have utilized a validated surrogate; tumor p16-expression status as measured by immunohistochemistry. P16-expression has been shown to have ~92% sensitivity and ~90% specificity in comparison to the gold standard of expression of high-risk HPV oncogenes E6 and E7. For this trial, to ensure enrollment of productive HPV-infected HNSCC patients, we will require both p16 IHC (>70% positive tumor cells) AND HPV DNA measured by ISH or PCR.

1.3 Primary CRT for Head and Neck Cancer

The majority (~60%) of HNSCC patients is diagnosed with local-regionally advanced disease, and the 5-year survival for this subset is heavily dependent on HPV status. The current nonsurgical standard of care for the majority of patients with local-regionally advanced (Stage III-IV) oropharynx, larynx, hypopharynx, and unresectable oral cavity cancers is organ preservation CRT. Cisplatin-based CRT has been shown to improve local-regional control, PFS, and OS when compared to radiotherapy alone. Altered fractionation of radiation does not seem to eliminate the benefit of the addition of concurrent chemotherapy. A recent meta-analysis estimated the absolute benefit of concomitant chemoradiotherapy over radiotherapy to be 6.5% at 5-years for all head and neck cancer patients. A recently completed, large randomized phase III trial (RTOG 1016) is testing whether CRT with cisplatin vs. cetuximab results in equivalent survival or whether one or the other regimen is superior in p16-positive oropharynx cancer patients. Surveys of community oncology practices indicate approximately equivalent use of cisplatin- and cetuximab-based CRT for the primary treatment of HNSCC.

While high-dose cisplatin (75-100 mg/m^2) every 21 days is the most common schedule used, bolus cisplatin (75-100 mg/m^2 every 21 days) is associated with frequent toxicities such as nausea and vomiting, renal impairment, cytopenias, and ototoxicity. As a result of these toxicities, ~ 30% of individuals in randomized clinical trials in RTOG do not receive all 3 cycles of chemotherapy. To overcome these challenges, several clinical trials have investigated weekly cisplatin schedules. Weekly schedules have ranged in dose between cisplatin 20-50 mg/m^2/week. A randomized study of 20 mg/m^2/week failed to show improvement vs. radiation alone. However, other schedules of higher dose weekly cisplatin (30-40 mg/m^2 with radiation) have proven to be effective, feasible, and tolerable in HNSCC. For nasopharyngeal cancer, randomized clinical trials have demonstrated a survival advantage for cisplatin 30 mg/m^2/week in comparison to radiotherapy alone for stage II disease and for cisplatin 40 mg/m^2/week for stage II-IV disease (Chan 2004). For HNSCC, a French randomized trial also demonstrated a survival benefit from cisplatin 50 mg/week (~30 mgs/m^2) in comparison to RT alone and doses as low as cisplatin 6 mg/m^2/day of RT have also shown a survival benefit, with high treatment delivery and reduced toxicity. Whatever the regimen, a formal analysis of cisplatin dosing indicated that a cumulative dose of cisplatin 200 mg/m^2 concurrent with radiation is most often associated with meaningful improvement in tumor control and/or survival. Interestingly, data exist that cisplatin CRT in an HPV-positive murine HNSCC model requires the adaptive immune system to mediate the optimal therapeutic effects, supporting the combination with anti-PD-1 mAb blockade to enhance this effect, as proposed here.

1.4 **HPV-specifi**c Therapeutic Vaccination

Kenter et al [11] investigated the immunogenicity and efficacy of a synthetic long-peptide vaccine in women with HPV-16-positive, high-grade vulvar intraepithelial neoplasia. Twenty women with HPV-16-positive, grade 3 vulvar intraepithelial neoplasia were vaccinated three or four times with a mix of long peptides from the HPV-16 viral oncoproteins E6 and E7 in incomplete Freund's adjuvant. The end points were clinical and HPV-16-specific T-cell responses. The most common adverse events were local swelling in 100% of the patients and fever in 64% of the patients; none of these events exceeded grade 2 Adverse Events (AE). At 3 months after the last vaccination, 12 of 20 patients (60%; 95% confidence interval [CI], 36 to 81) had clinical responses and reported relief of symptoms. Five women had complete regression of the lesions, and HPV-16 was no longer detectable in four of them. At 12 months of follow-up, 15 of 19 patients had clinical responses (79%; 95% CI, 54 to 94), with a complete response in 9 of 19 patients (47%; 95% CI, 24 to 71). The complete-response rate was maintained at 24 months of follow-up. All patients had vaccine-induced T-cell responses, and post hoc analyses suggested that patients with a complete response at 3 months had a significantly stronger interferon-gamma-associated proliferative CD4+ T-cell response and a broad response of CD8+ interferon-gamma T cells than did patients without a complete response. They concluded that clinical responses in women with HPV-16-positive, grade 3 vulvar intraepithelial neoplasia can be achieved by vaccination with a synthetic long-peptide vaccine against the HPV-16 oncoproteins E6 and E7. Complete responses appeared to be correlated with induction of HPV-16-specific immunity. These data support the application of HPV-specific vaccination with anti-PD-1 immunotherapy with pembrolizumab to enhance the efficacy of primed and

expanded HPV-16 E6/E7-specific cellular immunity against intermediate risk HPV+ HNSCC. The vaccine published in Kenter et al. has been developed further and licensed to ISA Pharmaceuticals and thus makes a useful combination partner with pembrolizumab and chemoradiation (CRT) in this trial. More recently, in a pilot trial in patients with recurrent or metastatic HPV16+ cervical cancer, a single dose of this vaccine was found to synergize with myelosuppressive chemotherapy in the induction of robust HPV16-specific T cell responses. The vaccine was administered 14 days after the second cycle of chemotherapy, at the time point when increased levels of myeloid suppressor cells among PBMC had declined to the levels of normal donors, coincident with arise in T cell immunocompetence [12]. Unpublished data indicate that the current version of this vaccine, ISA101, also induces a robust immune response to HPV16 in patients with advanced cervical cancer when first dosed at the same time point after chemotherapy, followed by two booster doses spaced by three weeks. In both studies the treatment had an acceptable safety profile.

1.5 Pembrolizumab

Pembrolizumab (formerly MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between programmed cell death protein 1 (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 ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

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Please refer to the pembrolizumab Investigator Brochure (IB) for descriptions of all preclinical and clinical available data.

These data support a hypothesis that checkpoint inhibitors administered prior to or concomitant with radiotherapy can induce clinically significant anti-tumor immune responses induced by “vaccination” to tumor-specific antigens exposed during radiation-induced cell death (Formenti 2012). Such a phenomenon may be particularly relevant to viral-induced tumors, such as HPV-positive HNSCC.

<u>Anti-PD-1 in Combination with Cisplatin</u>

In terms of safety and efficacy of combining anti-PD-1 mAb with cisplatin, administration of anti-PD-1 in combination with high-dose cisplatin has been shown to have acceptable toxicity in a phase I clinical trial in patients with NSCLC. A phase I clinical trial evaluating the safety of anti-PD-1 (10 mgs/kg q3 weeks) in combination with platinum-based doublets (gemcitabine/cisplatin, pemetrexed/cisplatin, carboplatin/paclitaxel) has been completed in 43 patients with chemotherapy-naïve advanced NSCLC. Importantly, no dose limiting toxicities (DLTs) were observed across all 3 regimens. Grade 3-4 toxicity was observed in 49% of patients, and grade 3-4 toxic events likely attributable to anti-PD-1 (pneumonitis, rash, nephritis, and colitis) were observed in 7 (16%) patients. One death due to pneumonitis was observed. Single agent anti-PD-1 has demonstrated clinical activity in refractory squamous NSCLC. A phase III study comparing single agent anti-PD-1 to docetaxel in patients with metastatic NSCLC has been stopped early because of superior OS in the anti-PD-1 arm, per a Bristol-Myers Squibb press release. Anti-PD-

1 is now FDA approved for second line use in patients with squamous lung cancer based upon these results.

Checkpoint Inhibitors and Radiotherapy

In addition to direct cytotoxic effects, radiotherapy may induce an immune effect important to tumor cell death [14]. Preclinical data support synergy between checkpoint inhibitors and radiotherapy. Mouse models of poorly immunogenic tumors have demonstrated that concomitant administration of anti-CTLA-4 antibodies and radiotherapy results in antitumor T cell responses both in the radiation field as well as outside of it (an abscopal effect) [14]. PD-1 blockade after completion of radiotherapy also has been shown to induce rejection of persistent tumors in mouse models. Combination of PD-1 blockade and anti-CD137 stimulation increased the response to radiotherapy in a mouse model of triple negative breast cancer, and PD-L1 blockade concomitant with radiotherapy improved survival in comparison to either therapy alone in mouse models of glioma (Zeng 2013). In human subjects, case-reports support the existence of a clinically significant abscopal effect for patients with melanoma who have received ipilimumab prior to radiotherapy [15]. These data support a hypothesis that checkpoint inhibitors administered prior to or concomitant with radiotherapy can induce clinically significant anti-tumor immune responses induced by "vaccination" to tumor-specific antigens exposed during radiation-induced cell death [14,16]. Such a phenomenon may be particularly relevant to viral-induced tumors, such as HPV-positive HNSCC and to highly genetically unstable tumors such as HPV-negative HNSCC. In addition radiotherapy can synergize with therapeutic vaccination as demonstrated in a mouse model of HPV16-induced tumors.

Checkpoint Inhibitors and Chronic Viral Infections

PD-1/PD-L1 interactions may contribute to T cell exhaustion in the setting of chronic viral infections such as HPV. Blockade of PD-1/PD-L1 interactions has been shown to restore proliferation and cytokine secretion of functionally impaired CD8+ T cells and to restore their ability to lyse cells and decrease viral load in a model of chronic lymphocytic choriomeningitis (LCMV) infection (Barber 2006). PD-1 also has been shown to be upregulated in HIV infection and blockade enhanced HIV-specific T cell responses in vitro (Kaufmann 2008). PD-1 blockade also has been observed to result in clearance of chronic, persistent hepatitis B infection in a mouse model (Tzeng 2012). PD-1 and PD-L1 have recently been shown to be upregulated in the tumor microenvironment in a mouse model of hepatitis-C induced hepatocellular carcinoma, contributing to tumor-antigen-specific tolerance. Preliminary studies also suggest that patients with pre-existing antibodies to tumor-specific antigens (e.g. NY-ESO-1, an antigen expressed in 30-40% of patients with advanced melanoma) have an increased response to ipilimumab. Notably, approximately 70% of patients with HPV16-positive HNSCC have high titers of antibodies to the E6 or E7 oncoproteins at diagnosis, indicating immune exposure to these tumor-specific antigens. Nonetheless, potential immune exhaustion of HPV-16 E6/7-specific T cells exist, which are often driven into a terminally differentiated phenotype [17]. These data suggest that PD-1 blockade has promise for the enhancement of standard of care therapies for HPV-positive cancers as well. Similar promising data with another virus associated cancer, Merkel Cell Carcinoma caused by merkel cell polyomavirus (MCV) is exquisitely responsive to pembrolizumab anti-PD-1 immunotherapy [18].

1.5.1 Rationale for Dose Selection/Regimen/Modification

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

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral

target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

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

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

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe. A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage. Given the safety of ISA HPV-16 E6/E7 “long peptide” vaccine+adjuvant [11] and tolerability of pembrolizumab (reviewed above), we do not anticipate unexpected toxicities of adding these sequentially to standard chemoradiation. Thus, we will employ a Bayesian approach to adding the two investigational agents sequentially to standard chemoradiation, carefully analyzing for any delays in SOC therapy, and suspending the trial if the investigational agent(s) appear to generate unexpected toxicities that prevent administration of this chemoradiation (see statistics section for Continuous Monitoring Plan).

1.6

ISA101b

The original HPV-16-SLP vaccine (which was produced at the LUMC and is a predecessor to both ISA101 and ISA101b) was tested in several investigator-sponsored studies in patients with HPV-16 positive gynecological premalignant and malignant diseases [19]. The combined studies of the LUMC research group have shown that in the majority of patients with premalignant CIN or VIN lesions and in slightly more than half of the patients with recurrent or metastatic cervical cancer, a positive T cell response can be induced by vaccination with HPV-16-SLP in Montanide adjuvant compared with a weak or non-demonstrable T cell response in patients who are not vaccinated[11,20-2511,19-2411,19-2411,19-24] These results were recently confirmed in an independent study with VIN patients [12,21,26] Moreover, in a pilot study in patients with late stage cervical cancer, the T cell immune responses induced by a single dose of HPV16-SLP were considerably augmented by timing the vaccination at 15 days after the second cycle of carboplatin/paclitaxel chemotherapy, due to the much lower levels of myeloid cells, but intact T cell numbers and improved T cell function at this time point [12].

1.6.1
Pharmacokinetics and Product Metabolism in Humans

The pharmacokinetic characteristics of ISA101/ISA101b have not been studied since pharmacokinetics of a vaccine are not considered clinically relevant and do not correlate with the pharmacodynamic or immunologic actions. Pharmacodynamic effects are best assessed by the immunogenicity studies that have been incorporated in the clinical studies. Studies with other synthetic long peptides, however, indicate that the distribution of the peptides is mainly restricted to dendritic cells (DC) in the draining lymph nodes.

Furthermore, the investigational product is not metabolized in the conventional sense: the peptides are taken up by antigen presenting cells, which process the peptides into smaller entities, called T-cell epitopes, which are presented on the surface of the antigen presenting cell (APC). In the meantime, the APC traffics to a draining lymph node, where naive T cells recognize the T-cell epitopes and are activated by these epitopes. The peptides are degraded within APCs through normal degradation pathways.

1.6.2
ISA101b Rationale for Dose Selection/Regimen

ISA101 is currently being studied in a company-sponsored Phase 1 trial, CervISA in patients with recurrent or metastatic HPV16+ cervical cancer. The CervISA trial, a Phase 1 dose escalation study, has enrolled 77 subjects as of May 27, 2016, of which 60 are expected to be evaluable for both safety and immunologic responses. In addition to routine safety monitoring and reporting, the sponsor and the Data Monitoring Committee (DMC), have reviewed the emerging safety data on 4 separate occasions, prior to each dose escalation decision and again prior to the amendment to expand the 40 and 100 μg/peptide cohorts. Preliminary safety data were reviewed with a data cut-off of May 31, 2016. The key findings related to the safety of ISA101 in the CervISA trial can be summarized as follows:

- Most of the AEs in the study were expected toxicities reported to be related to chemotherapy or to complications associated with progression of cervical cancer.
- Dose-related local injection site reactions (ISRs) to ISA101 were the most frequent AEs reported to be related to ISA101 with ISRs occurring in most subjects who received ISA101. Most of the local ISRs were reported to be Grade 1 to 2 in severity. One local ISR was reported to be Grade 3 in severity at a dose of 300 μg/peptide. The frequency of ISRs appeared to be dose related. At the 100 μg/peptide dose level (to be used in the bridging study of ISA101b) most of the patients had local ISRs.
- As of May 31, 2016, there have been 4 systemic allergic reactions reported as SAE to possibly related to ISA101. Three of these reactions occurred at a dose level of 300 μg/peptide and were reported to be probably related to ISA101. One of these events was reported as Grade 4 and the others were reported to be Grade 3. In addition there was one report of a Grade 3 systemic allergic reaction reported at the 100 μg/peptide dose level, though the event was determined to have occurred within 30 minutes after receiving IV contrast material but 2-3 hours after ISA101.While the temporal relationship to IV contrast material was clear, a relation to ISA101 could not be absolutely excluded so this reaction was reported to be possibly related to ISA101. No additional systemic allergic reactions have been reported to date in the extension cohorts with the addition of 6 patients each at both the 40 and 100 μg/peptide levels.

Thus, based on the assessment of treatment emergent adverse events in the initial dose escalation cohorts and the 2 expansion cohorts, it was determined that the 100 μg/peptide dose level had an acceptable safety profile and generated a robust HPV16 specific immune response (see Section 1.6) in the majority of patients with advanced cervical cancer. For a cumulative summary tabulation of treatment emergent adverse events, see Appendix E. Additional details on the safety of vaccine can be found in the Investigator Brochure.

Thus, the available safety data with ISA101 indicate it has an acceptable safety profile for use at dose levels up to 100 μg per peptide in the target patient population. The most frequent toxicities are local injection site reactions (predominantly Grade 1-2) and the AEs do not appear to overlap with those of the standard chemotherapy used in the treatment of this disease. Systemic allergic reactions are potentially the most serious adverse reactions to ISA101 so

appropriate precautions should be observed as described in the Investigator Brochure.

ISA101b is a modified product formulation of the original ISA101 vaccine that has two minor modifications compared to ISA101:

- One difficult to produce peptide (G-3980-R) has been eliminated from the HPV16 vaccine. As a result there are a total of 12 HPV16 peptides in ISA101b compared to 13 peptides in ISA101. This peptide covers amino acids 59-63 of E7 so no significant loss of immunogenicity is anticipated, because it leaves only a gap of 7 amino acids in the otherwise contiguous sequence of HPV16 E6 and E7 of the ISA101b vaccine. Nevertheless we wished to directly evaluate the immune response to each of the 13 individual SLP's in the precursor vaccine HPV-16-SLP, to directly assess which contribution the response to peptide G-3980-R had in the overall response to the 13 peptides. To this end we evaluated the immune response from VIN patients vaccinated with the HPV-16-SLP vaccine in the interferon-γ ELISPOT assay. The results show that peptide G-3980-R was immunogenic in most patients, however it did not contribute inordinately more to the immunogenicity of the 13 peptides than any of the other 12 peptides. As expected from the high level of HLA polymorphism, individual patients responded better or less well to individual peptide components of the HPV-16-SLP vaccine.
- The reconstitution solution has been modified to ensure a more reproducible and stable preparation. The details are described in the ISA1901b Investigator Brochure.

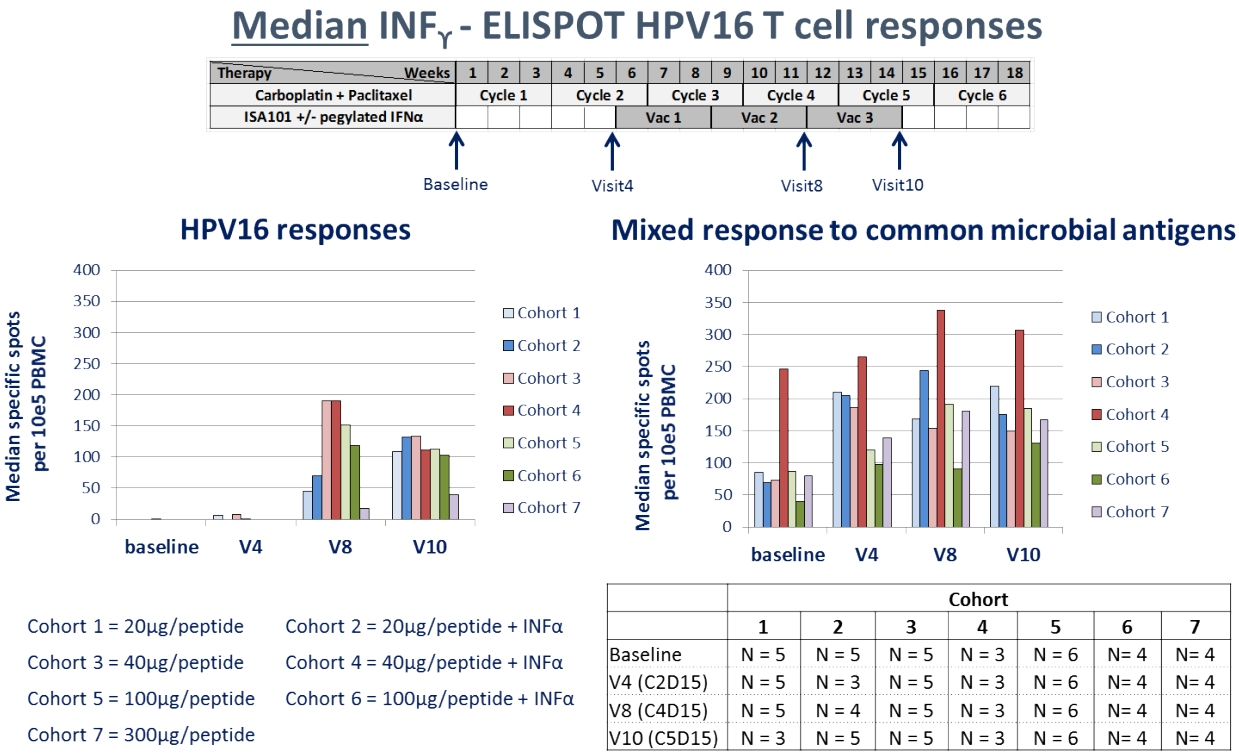
ISA101b is expected to be a more consistent vaccine product that will induce similar immunologic responses and have a similar safety profile to ISA101. This will be verified in a cohort of patients receiving ISA101b in the ongoing CervISA study.

1.7

Immunologic Responses to ISA101

Standard chemotherapy for late stage cervical cancer depletes immunosuppressive mononuclear myeloid cells without depleting T cell numbers and without affecting T cell function, thereby allowing a more robust T cell response to therapeutic vaccination [12].

In a pilot study with the predecessor vaccine, HPV-16-SLP, it was observed that administration of a single dose of the vaccine at the time of the myeloid nadir following carboplatin and paclitaxel chemotherapy, resulted in a more robust T cell immune response to HPV16 antigens in 11/12 patients with recurrent/ metastatic cervical cancer than has been previously observed without timed vaccination in the same category of patients [12]. This appeared to be due to the fact that carboplatin and paclitaxel chemotherapy depletes immunosuppressive mononuclear cells that are increased in late stage cervical cancer patients, without depleting T cell numbers and function [12]. In a preclinical mouse study on the effect of carboplatin and paclitaxel on HPV-specific vaccination, the myeloid cell depletion was also manifest in the tumor micro-environment and led to synergy in tumor eradication between this chemotherapy and HPV-16-SLP vaccination [12]. Confirming the pilot study, to date, the majority of patients in the CervISA study who received as least 2 doses of ISA101 vaccine, had strong immune responses to HPV16 antigens (Fig.1)



	Cohort						
	1	2	3	4	5	6	7
Baseline	N = 5	N = 5	N = 5	N = 3	N = 6	N= 4	N= 4
V4 (C2D15)	N = 5	N = 3	N = 5	N = 3	N = 6	N= 4	N= 4
V8 (C4D15)	N = 5	N = 4	N = 5	N = 3	N = 6	N= 4	N= 4
V10 (C5D15)	N = 3	N = 5	N = 5	N = 3	N = 6	N= 4	N= 4

Figure 1 Measurement of Interferon-γ ELISPOT responses in PBMC taken at various times before and after chemotherapy and/or ISA101 vaccination

Venous blood samples (45 mL in heparinized tubes and 8.5 mL in clot activator tube) were taken for immunomonitoring prior to chemotherapy (baseline), 2 weeks after the second cycle just before the first vaccine dose (visit 4), 2 weeks after the 4th chemo cycle, coinciding with 3 weeks after the second vaccine dose (visit 8) and 2 weeks after the 5th cycle of chemotherapy, coinciding with 3 weeks after the third vaccine dose (visit 10).

Figure 1 shows the response so far in the ongoing CervISA study to common microbial antigens and to HPV16 E6/E7 before and at two time points after ISA101 vaccination, expressed as median specific spots in the validated Interferon-γ ELISPOT assay. The baseline response to HPV16 E6/E7 is weak or not demonstrable before ISA101 vaccination, whereas the control response to common microbial antigens (Candida Albicans, PPD, Tetanus toxoid), the so-called memory mix (MRM) is robust. Chemotherapy alone causes a slight increase in the spontaneous T cell response to HPV16 E6/E7 and a marked increase in the T cell response to the MRM (data from visit 4, just before the first ISA101 vaccine dose). At visit 8 (after 2 vaccine doses) and at visit 10 (after 3 vaccine doses), robust vaccine-induced T cell responses to HPV16 E6/ E7 are evident. The data indicate that the immune response to the 40 µg and 100 µg cohorts is quite strong, responses to 20 µg/peptide being lower. These responses, like in the pilot study,[12] were again substantially higher than in a previous study in the same category of patients without timing of vaccination with respect to chemotherapy cycles [20]. Moreover, the data indicate that the immunologic responses were at least the same high levels of T cell immune response against HPV16 E6/E7 in many patients as in previous complete clinical responding patients with premalignant high grade VIN disease [11] (van Poelgeest et al. 2016).

The combined immunological and clinical results indicate that in patients with pre-malignant disease more robust immune responses are induced than in patients with cervical cancer. This appears to be due to the abnormally high numbers of myeloid derived suppressor cells in the systemic circulation in patients with cancer as shown in the pilot study [12]. In this study, all of 18 patients with advanced cervical cancer (recurrent or metastatic) had elevated levels in PBMC of immunosuppressive myeloid cell populations. A first cohort of 6 patients receiving standard carboplatin

and paclitaxel chemotherapy was studied longitudinally regarding these myeloid cell numbers and lymphocyte numbers in the course of this chemotherapy and without receiving SLP vaccination. It was observed that the elevated levels of immature myeloid cells had returned to those of healthy donors 10-15 days after the second course of chemotherapy, associated with non-diminished levels of T lymphocytes. T cell responses to common recall responses had also returned to the higher levels seen in healthy donors. Based on these data, in this time window in a subsequent cohort of 12 donors, a single vaccine dose of HPV16-SLP was delivered at a dose of 300 micrograms per peptide in Montanide adjuvant. Compared to the weak responses observed previously without timed vaccination in late stage cervical cancer patients, 11 out of 12 patients now showed much more robust T cell immune responses to HPV16 E6/E7, comparable to or even higher than the levels seen in clinically responding VIN patients [12].

Thus, properly timed vaccination during ongoing courses of chemotherapy can help to elevate vaccine-induced T cell immune responses in late stage cancer patients. In agreement with the observed strength and breadth of T cell immune responses, more meaningful clinical responses were observed in patients with VIN than in patients with cervical cancer upon HPV16-SLP monotherapy, including durable complete and partial responses. Properly timed vaccination during chemotherapy courses (i.e. at the myeloid nadir, approximately 14 days after administration of chemotherapy) in the ongoing CervISA study is intended to improve the clinical outcome for patients with cervical cancer. Based on both pre-clinical and clinical studies, these effects of properly timed vaccination at the myeloid nadir are considered relevant for the design of this trial using cisplatin.

In melanoma and lung cancer adjuvant trials, single agent anti-PD-1 Ab is administered for up to a year after primary therapy. This time frame is based upon observations in recurrent metastatic disease demonstrating prolonged time to steady state antibody levels, as well as delayed responses to therapy beyond 4 months of therapy. In theory, continued immunotherapy during this time frame may facilitate immune clearance of micro-metastatic disease and prevent or prolong disease progression. The optimal duration of adjuvant immunotherapy is unknown both in animal models and in human subjects. Also unknown are the potential long-term toxicities of this therapy in head and neck cancer patients. Historically, head and neck cancer patients are poorly inclined toward adjuvant therapies, and rates of refusal or discontinuation of adjuvant therapies included in clinical trials are high. Therefore, in the lead in component we will evaluate the safety and feasibility of adjuvant immunotherapy in the patient population. Should this prove safe and feasible, the phase III clinical trial design may be modified to extend the period of adjuvant administration of anti-PD-1 Ab for a total timeframe of therapy of as long as one year (In this protocol, the term “adjuvant therapy” will be defined as administration of anti-PD-1 Ab starting 3 months after completion of chemoradiotherapy for 7 months).

1.8

Correlative Studies Background

This study will permit *ex vivo* analysis of immune-inflammatory responses in both the TME and the peripheral circulation, including activation and proliferation markers on both tumor-infiltrating as well as circulating lymphocytes and NK cells, in pre- and post-treatment biospecimens, taking advantage of the viral oncogene tumor antigens expressed by HPV-16+ OPSCC. Priority correlative studies may include the following:

1) Baseline tumor PD-L1 expression. Tumor PD-L1 expression will be classified according to the combined positive score (CPS). A planned retrospective subgroup analysis will be conducted, correlating baseline PD-L1 status with 2-year PFS in both arms.
2) Paired analysis of immune-inflammatory markers in tumor and tumor infiltrating lymphocytes in pre- and post-treatment biopsies. Changes in immune-inflammatory markers will reflect immunomodulation by concurrent cisplatin and IMRT, the standard of care for this patient population. Immune-inflammatory markers will include: A) IHC or immunofluorescence to include: CD3, CD8, CD45RO, CD4/FOXP3, PD-L1 (co-stain on Macs), Ki-67; location of cells. B) RNAseq will be done where fresh-frozen tumor is

available.

3) Specific immunophenotypic and functional comparion of HPV-16 E6/E7 specific T cells responses using tetramer (for ~ 50% of enrolled patients who express HLA-A2.1), or overlapping E6/E7 peptides, using flow cytometry and IFNγ ELISPOT or cytolytic assays. Analyses will compare HPV specific vs bulk T cell phenotypes and function.
4) Seropositivity (circulating antibodies) and cytokines. Circulating antibodies against HPV E6/E7 and other viral tumor antigens, and circulating cytokines will be analyzed using multiplex (Luminex) ELISA assays.
5) Paired analysis of peripheral blood immune-inflammatory markers pre- and post-treatment with pembrolizumab. Markers will include: multi-color flow cytometry, including T cell activation panel, NK cells, myeloid-derived suppressor cells (MDSC), Treg subsets, phenotypic subsets, expression of PD1 and other co-stimulatory/co-inhibitory markers on these specimens.
6) Paired analysis of TCR clonality will be conducted in pre- and post-treatment TILs.
7) Next generation sequencing (exomes) for genomic epitope mapping with HLA allelic typing. Further detail is given in Section 9.4.5.

Summary of Rationale

- Data from the RTOG 0522, inclusive of the patients who meet the eligibility criteria for this trial, indicates that 30-40% of patients with intermediate risk, local-regionally advanced HPV+ HNSCC will experience disease progression within 5 years of CRT. Novel therapeutic approaches are needed to improve disease control (Ang 2010, O'Sullivan 2013).
- Anti PD-1 targeted therapy (pembrolizumab, Merck Inc.) demonstrated activity in metastatic recurrent HNSCC, with a response rate of 20% in patients with HNSCC (Seiwert 2014) Eligibility criteria included 1% or greater PD-L1 expression on tumors. Response rates were different for patients with high versus low PD-L1 expression (45% versus 11%).
- Over 60% of HPV-positive HNSCC express PD-L1, and tumor infiltration by PD-1+CD8+ T cells is frequently present in HPV-positive HNSCC.
- Anti-PD-1 mAb can be safely administered with high dose cisplatin.
- PD-1 blockade has demonstrated synergistic activity with radiotherapy in vivo.
- PD-1 blockade can reverse T cell exhaustion to chronic viral infections such as HPV and therefore, may be particularly effective in viral-induced tumors.
- ISA101b vaccination has demonstrated clinical and immunologic responses in HPV-16+ cancers.

2. STUDY OBJECTIVES

Primary Hypothesis and Endpoints

The addition of pembrolizumab and HPV-16 E6/E7 specific therapeutic vaccination (ISA101b) to cisplatin-based chemoradiotherapy will improve PFS for patients with newly diagnosed, local-regionally advanced, intermediate risk HPV-associated HNSCC.

2.1

Primary

The primary objective of this study is to evaluate treatment intensification using fixed-dose pembrolizumab (concurrent) plus ISA101b added to the standard, concurrent cisplatin-IMRT in patients with PULA HPV-16+, "intemediate risk" HNSCC in order to recommend survival estimates in a subsequent definitive randomized trial as determined by the endpoint of 2-year PFS rate (please see Section 10.2 for Statistical Methods).

2.2

Secondary

Secondary objectives for this study include:

- To evaluate the toxicity of the combination of pembrolizumab and ISA101b and concurrent cisplatin-IMRT in patients with PULA HPV-16+ "intermediate risk" HNSCC

2.3 Exploratory

- To evaluate associations between tumor PD-L1 expression and PFS and OS in patients treated with pembrolizumab and ISA101b; PD-L1 status will not be used during this trial for treatment decisions
- To evaluate the safety, feasibility and patient compliance with continued administration of single agent pembrolizumab 200 mg IV q21 days for 14 further doses after completion of chemoradiation with concomitant pembrolizumab
- To evaluate associations between HPV E6/E7 seroreactivity and PFS in p16-positive patients treated with and without pembrolizumab and therapeutic ISA101b
- To evaluate the frequency and functional phenotype of HPV-16 E6 and E7 specific circulating activated, PD-1+ T cells in patients treated with and without pembrolizumab
- To evaluate the dynamics of PD-L1 expression in the tumor and TILs via a repeat biopsy after 2-3 weeks after pembrolizumab and ISA101b vaccination in patients with accessible tumor.
- To evaluate the tumor microenvironment, activation, and proliferation markers on TIL and NK cells in pre- and post-treatment specimens, including CD3, CD8, CD45RO, CD4/FOXP3, PD-L1, and Ki-67
- To evaluate the dynamic expression of TA-specific T cells in serial peripheral blood mononuclear cells (PBMCs) by flow cytometry

3.

PATIENT SELECTION

3.1

Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

- Patients must have histologically-confirmed head and neck squamous cell carcinoma with no evidence of distant metastasis. The primary site will be the oropharynx. Patients with squamous cell carcinoma of unknown primary, metastatic to cervical lymph nodes, are permitted to enroll provided all other eligibility criteria are met.
- Patients must have intermediate risk disease, defined below. Staging evaluation should be determined by imaging studies and complete head and neck exam in accordance with the American Joint committee on Cancer Staging Manual, 7th edition.
    - Patients must meet one of the following criteria:
        - Oropharynx: p16(+) PLUS HPV ISH(+) AND one of the following
        - T3 OR ≥N2a AND ≥ 10 pack-years tobacco exposure (see Tobacco Assessment Form, Appendix A)
        - T4 or N2c/N3 disease irrespective of tobacco exposure
        - Unknown primary: p16(+) PLUS HPV ISH(+) AND one of the following
        - ≥ N2a AND ≥ 10 pack-years tobacco exposure

- N2c/N3 disease irrespective of tobacco exposure

  Note: for patients with oropharyngeal or unknown primary tumors, p16 status must be known, and HPV-16 ISH should also be performed. p16-positive disease is defined as ≥70% of tumor cells demonstrating diffuse nuclear and cytoplasmic staining by p16 immunohistochemistry (IHC).

- Patients should be considered not a candidate for curative-intent surgery with diagnosis of AJCC 7th edition Stage III, IVa, or IVb disease. Diagnostic excisional biopsy of primary tumor and/or nodal sites is permitted.
  - Diagnostic simple palatine or lingual tonsillectomy is permitted, provided patient has RECIST-measurable nodal disease.
  - Patients with a second HNSCC primary tumor are eligible for this study, provided more than 2 years have elapsed since the first diagnosis of HNSCC, the original tumor was managed with surgery only (no adjuvant chemotherapy or radiotherapy), and has not recurred.
- Patients with simultaneous primaries are excluded, with the exception of patients with bilateral tonsil/base of tongue HPV+ cancers or patients with T1-2, N0, M0 differentiated thyroid carcinoma (resected or management deferred), who are eligible.
- No prior systemic (chemotherapy or biologic/molecular targeted therapy) or radiation treatment for head and neck cancer.
  - Patients may have received chemotherapy or radiation for a previous, curatively treated non-HNSCC malignancy, provided at least 2 years have elapsed.
  - Patients must be untreated with radiation above the clavicles.
- Patients with a history of curatively-treated non-HNSCC malignancy must be disease-free for at least 2 years except for excised and cured: carcinoma-in-situ of breast or cervix; non-melanoma skin cancer; T1-2, N0, M0 resected differentiated thyroid carcinoma; superficial bladder cancer; T1a or T1b prostate cancer comprising < 5% of resected tissue with normal prostate specific antigen (PSA) since resection.
- The patient must undergo a MANDATORY research biopsy at baseline. There will be 2 other optional biopsies that the patient will be asked to consent to, the first isduring week 2 of pembrolizumab/ISA101b vaccination, prior to start of cisplatin-IMRT (this correlates to week -1), the second one will be during week 2 after the start of IMRT (this correlates to week 1). All patients will be evaluated for the feasibility of research biopsy at the time of enrollment, as a condition of eligibility. The performing physician must agree that a cup forceps biopsy or an 18 to 14 gauge core needle biopsy can be safely performed. Every effort will be made to couple the baseline research biopsy to a standard of care diagnostic or staging procedure.

  NOTE*: Patients who have had research tissue procured under an omnibus tissue consent, who are determined to have sufficient tissue for analysis of immune- inflammatory biomarkers per the study PI, may substitute the archived tissue and do not need to undergo baseline research biopsy. Such tissue must have been obtained within the prior 24 weeks and no interval anti-cancer therapy administered.*
- Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1 (See Appendix B)
- Age ≥ 18
- Patients must have clinical evidence of disease
- Patients must demonstrate adequate organ function as defined in Table 1; all screening labs should be performed within 10 days of treatment initiation.

  - Table 1. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mm^3
Platelets	≥100,000 / mm^3
Hemoglobin	≥9.0 g/dL

System	Laboratory Value
Renal	
Serum creatinine **OR**	≤1.5 X upper limit of normal (ULN) **OR**
Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	Creatinine clearance ≥60 mL/min (for subject with creatinine levels > 1.5 X institutional ULN) determined by 24-hour collection or estimated by Cockcroft-Gault formula: [a] Calculated creatinine clearance = [(140-age) X (actual body weight in kg) X (0.85 if female)]/(72 X serum creatinine)
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

- Written informed consent must be obtained from all patients prior to study registration. Patients should have the ability to understand and the willingness to sign a written informed consent document.
- If a woman of childbearing potential, documentation of negative pregnancy within 4 weeks prior to first dose (see section 8.1.1). Sexually active patients must agree to use adequate contraceptive measures, while on study and for 120 days after the last dose of study drug. All fertile female subjects (and their partners) must agree to use a highly effective method of contraception. See Appendix E for approved contraceptive methods. Should a woman become pregnant or suspect she is pregnant while in this study, she should inform her treating physician immediately. A negative pregnancy test should be documented during screening and then within 72 hours of first dose.
- Male participants:
  A male participant must agree to use a contraception during the treatment period and for at least 120 days after the last dose of study treatment and refrain from donating sperm during this period. See Appendix E for approved contraceptive methods.

3.2

Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study:

- Oral Cavity, Larynx, Hypopharynx or Nasopharyngeal primary site
- Current participation in or previous participation in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of study treatment.
- Prior treatment with anti-HPV agents except prevention HPV vaccines
- History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in the investigational agents (pembrolizumab and ISA101b (e.g. Montanide and components of reconstitution solution for ISA101b).
- Distant metastatic disease including CNS or leptomeningeal metastases is not allowed.
- Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition; note: HIV testing is not required for entry into this protocol. The need to exclude patients with AIDS from this protocol is necessary

because the cisplatin and IMRT involved in this protocol may be significantly immunosuppressive.

- Received prior monoclonal antibody within 4 weeks prior to study Day 1 or who has not recovered (i.e. ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
- Active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study.
- Known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
- Received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).
- Significant pulmonary disease, including pulmonary hypertension, history of (non-infectious) pneumonitis that required steroids, evidence of interstitial lung disease or active, non-infectious pneumonitis
- History or current evidence of any other medical or psychiatric condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
- Peripheral neuropathy ≥ Grade 2
- Significant cardiovascular disease, including:
    - Cardiac failure New York Heart Association (NYHA) class III or IV.
    - Myocardial infarction, severe or unstable angina within 6 months prior to Study Day 1.
    - History of serious arrhythmia (i.e., ventricular tachycardia, or ventricular fibrillation).
    - Ventricular cardiac arrhythmias requiring anti-arrhythmic medications.
    - Known left ventricular ejection fraction (LVEF) ≤ 50%.
- Significant thrombotic or embolic events within 3 months prior to Study Day 1. Significant thrombotic or embolic events include but are not limited to stroke or transient ischemic attack (TIA). Catheter-related thrombosis is not a cause for exclusion. Diagnosis of deep vein thrombosis or pulmonary embolism is allowed if the patient is clinically stable and has completed or is on stable anti-coagulation therapy.
- Major surgery within 6 weeks prior to Study Day 1 (subjects must have completely recovered from any previous surgery prior to Study Day 1). Biopsy, diagnostic tonsillectomy, airway tumor debulking or excisional lymph node biopsy do not constitute major surgery.
- Active infection requiring antibiotics or antifungals within 7 days prior to first dose of study drug.
- Significant electrolyte imbalance prior to enrollment (note that patients may be supplemented to achieve acceptable electrolyte values):
    - Hypomagnesemia <1.2 mg/dL or 0.5 mmol/L.
    - Hypokalemia < 3.0 mmol/L.
- Women must not be pregnant or breastfeeding because chemotherapy and/or pembrolizumab may be harmful to the fetus or the nursing infant. Pregnant women are excluded from this study because chemotherapy and/or pembrolizumab have the potential for teratogenic or abortifacient effects.
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Has had an allogenic tissue/solid organ transplant.

4. PATIENT REGISTRATION

For questions regarding the eligibility of subjects, please contact the study PI, Dan Zandberg, MD at (412) 864-7955.

Registration will require the following information: 1) your name, telephone and email; 2) protocol name and number; 3) date treatment begins; 4) subject name; 5) date of birth; 6) primary study physician; 7) primary treatment institution; 8) confirmation of eligibility; 9) copies of the informed consent signature page; 10) verification that the informed consent was signed.

Following registration (*prior* to any study treatment), patients are required to undergo baseline research biopsy. Every effort will be made to couple the baseline research biopsy to a standard of care diagnostic or staging procedure. NOTE: Patients who have had research tissue procured under an omnibus tissue consent, who are determined to have sufficient fresh, fresh-frozen, and paraffin tissue for analysis of immune-inflammatory biomarkers per the study PI, may substitute the archived tissue and do not need to undergo baseline research biopsy.

Please see section 9.2 for biopsy, processing and shipping details.

5. TREATMENT PLAN

The first ISA101b vaccine will be initiated 2 weeks prior to cisplatin-IMRT, and one week prior to the first dose of pembrolizumab. Vaccines will continue for 2 additional administrations at weeks 2 and 5, +/- 1 days from the successive pembrolizumab infusions. Pembrolizumab will be initiated 1 week prior to cisplatin-IMRT at the dose of 200 mg IV q3 weeks (+/- 3 days). Pembrolizumab will be continued concurrently through cisplatin-IMRT (weeks 2, 5, and continued for a 42 week maintenance period after completion of cisplatin-IMRT for a total pembrolizumab treatment period of 50 weeks (17 doses). NOTE: Per discretion of the treating investigator, the week 8 dose of pembrolizumab may be delayed up to 3 weeks for recovery of acute toxicities from chemoradiotherapy. This dose would be considered delayed, and all subsequent doses would be administered at a q3 week interval (+/- 3 days) until completion.

Table 2. Treatment Plan

- **IMRT 70 Gy (35 fractions, 2Gy/fraction)**
- **Cisplatin 100 mg/m^2 q21 days X 2 doses**
- **Pembrolizumab 200 mg q3week IV (flat dose) X 17 doses**
- **ISA10b Vaccine (Three vaccinations given 2 weeks prior to CRT and one week prior to pembrolizumab, then in weeks 2 and 5 during CRT on the same days (+/- 1 day) as pembrolizumab)**
- **PD-L1 status will not be used during this trial for treatment decisions**

Patients will be administered pembrolizumab 200 mg q3 weeks starting week -1 for a total of 17 doses.

Table 2.	**Week of Treatment**									
	-2	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**8-47**
IMRT			**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Cisplatin			**X**			**X**				
Pembrolizumab**		**X**			**X**			**X**		**X**
ISA101b	**X**				**X***			**X***		

*ISA101b may be administered +/- 1 day of pembrolizumab administration for Weeks 2 and 5.
**Pembrolizumab 200mg IV q 3 weeks is given starting week -1 and continued for a total of 17 doses.

5.1 Study Drug Administration: Pembrolizumab

5.1.1 Route of Administration

Pembrolizumab will be administered as an IV infusion.

5.1.2 Doses to be Administered

The dose of pembrolizumab will be 200 mg (fixed dose) IV every 3 weeks (+/- 3 days), beginning one week (week -1) prior to concurrent cisplatin-IMRT for a total of 17 doses.

5.1.3 Dosing Schedule

Subjects will receive pembrolizumab once every 3 weeks (+/- 3 days) as IV infusion over 30 minutes for up to 1 year.

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

5.1.4 Order of Administration

Pembrolizumab may be given either before or after the radiation therapy fraction that is given the same day. If pembrolizumab and ISA101b are to be administered on the same day, pembrolizumab should be administered first with an observation period of 30 minutes prior to SC injection of ISA101b. Patients should then be observed for 4 hours after the administration of ISA101b. Patients may receive radiation therapy during observation period but must be accompanied by staff during transport.

5.1.5 Prohibited Concomitant Medications

- Colony stimulating factors, including G-CSF, pegylated G-CSF, GM-CSF as well as erythropoietin stimulating agents
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Radiation not specified in this protocol
- Investigational agents other than pembrolizumab
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Glucocorticoids for any purpose other than the following two clinical situations:
    - To control cisplatin-related nausea, if viewed as absolutely necessary for symptom control by the treating medical oncologist. NOTE: every effort will be made to minimize glucocorticoid administration either as premedication or as prophylaxis/treatment of delayed nausea from cisplatin (up to 10 mg or equivalent will be allowed).
    - To modulate symptoms from an event of clinical interest of suspected immunologic etiology.

Subjects who, in the assessment of the treating 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 or Follow-up phases of this trial.

5.2 Study Drug Administration: ISA101b

5.2.1 Route of Administration

ISA101b will be administered as two subcutaneous (SC) injections. These injections will be given in separate limbs

(arms or legs). It is recommended to rotate between limbs e.g. first vaccination in the upper-legs, second vaccination in the upper-arms and third vaccination in the upper-legs. This clarification of recommended injection site selection will assure more uniformity of ISA101 administration and allow for more systematic assessment of injection site reactions (ISRs).

5.2.2 Doses to be administered

Patients will receive a fixed dose of ISA101b at a dose of 100 µg/peptide. The Pharmacy Manual contains specific instructions for the reconstitutions, preparation and administration of the ISA101b therapeutic vaccine.

5.2.3 Dosing Schedule

Patients will receive three rounds of vaccination 3-4 weeks apart via two SC injections per vaccination round, after pembrolizumab treatment. Vaccination #1 will be administered 1 week before pembrolizumab (+/- 2 days).

5.2.4 Order of Administration

When possible, ISA101b will be given on the same day of pembrolizumab for vaccines #2 and #3 (+/- 1 day). When administered on the same day, ISA101b should be given after the administration of pembrolizumab.

Patients should then be observed for 4 hours after the administration of ISA101b.

5.2.5 Special Precautions and Prohibited Concomitant Medications

Systemic immunosuppressive therapy (e.g. chronic *systemic* steroids) use is not allowed. Local application (i.e. stable doses of topical or inhaled corticosteroids) *is allowed*.

5.3 Drug Administration – Cisplatin

5.3.1 Route of Administration

Cisplatin will be administered as an IV infusion.

5.3.2 Doses to be Administered

Patients will receive cisplatin, 100 mg/m^2, administered intraveneously on days 1 (Week 0) and 22 (Week 3) of the treatment course (see Table 2). A window of +/- 2 days is permissible. Weekends counts as days. The actual body weight will be used for all patients. There should be no dose modifications because of obesity.

5.3.3 Dosing Schedule

Patients will receive cisplatin once every 21 days as an IV infusion over 60-120 minutes, for a total of 2 doses. Cisplatin should be administered on Monday, Tuesday, or Wednesday of each treatment week and may be given either before or after the radiation therapy fraction that is given on the same day. If radiation is held for more than 2 days (for any reason), cisplatin will be held until radiation resumes.

Patients will receive pembrolizumab 200 mg q3 weeks starting week -1 for a total of 17 doses.

5.3.4 Concomitant Medications during Cisplatin Infusion

5.3.4.1 Nausea

Cisplatin, 100 mg/m^2, is a highly emetogenic drug with the potential for both acute and delayed nausea and vomiting. Institutional guidelines for highly emetogenic chemotherapy regimens should be followed. In the absence of such guidelines, the following are suggested regimens:

For acute nausea and vomiting, premedication should include fosaprepitant and a 5-HT3 antagonist, such as granisetron 1 mg IV; ondansetron, up to 16 mg IV; or palonosetron, 0.25 mg IV. Palonosetron has a longer half-life (40h) than the first generation 5-HT3 antagonists. NOTE: The use of a corticosteroid premedication, such as dexamethasone 10 mg IV, is typically combined with a 5-HT3 antagonist and fosaprepitant when administering high

dose cisplatin. In this protocol, omitting scheduled corticosteroid premedication, due to the immunosuppressive properties, is strongly encouraged. However, if necessary in the judgment of the treating investigator, a corticosteroid may be administered as a premedication for acute nausea/vomiting.

Breakthrough nausea and vomiting may be managed at the discretion of the treating investigator. Delayed nausea and vomiting (greater than 24 hours after chemotherapy administration) may be managed per investigator discretion, including use of the following potential nausea regimens: oral metoclopramide 10 mg qid x 2-4 days; prochlorperazine 10 mg qid x 2-4 days; a 5HT3 antagonist (e.g. granisetron, ondansetron) may also be given for up to 3 days (only if palonosetron was not given prior to chemotherapy).

5.3.4.2 Hydration and Electrolyte Support

Patients must receive vigorous hydration and diuresis.A suggested regimen is prehydration with 1 liter of D5N over 2-4 hours and mannitol, 12.5 grams IV bolus immediately prior to cisplatin. Cisplatin 100 mg/m^2 in 500-1000 mL NS is administered over 1-2 hours followed by an additional 1-1.5 liters of IV fluid. Any pre-existing dehydration must be corrected prior to cisplatin administration. Institutional guidelines should be followed in the event of extravasation. Additional IV fluid, potassium chloride, and/or magnesium sulfate may be administered at the discretion of the medical oncologist.

5.4 Dose Modifications

5.4.1 Pembrolizumab Dose Modifications

Retreatment criteria for pembrolizumab and cisplatin are independent (retreatment criteria for cisplatin are specified in section 5.4.3 below). There are no dose reductions for pembrolizumab. If the subject meets retreatment criteria, the full dose of 200 mg will be administered. If subjects do not meet retreatment criteria, then the dose of pembrolizumab will be skipped. The sole exception to this rule is the first dose of pembrolizumab following completion of chemoradiotherapy. Although scheduled for Week 8 of protocol treatment, the first post-chemoradiotherapy dose of pembrolizumab may be delayed up to 3 weeks to allow for recovery from acute toxicities, in accordance with the treating investigator's judgment. In the case of such delay, the following three doses of pembrolizumab will then be scheduled every 3 weeks (+/- 3 days) from the first post-chemoradiotherapy dose.

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 Table 4 below and in greater detail below. 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 to pembrolizumab, the investigator is instructed to follow the Events of Clinical Interest (ECI) reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Please see section 12.4.2 for ECI reporting guidance.

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.

Table 4 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab and IO combination partners

General instructions:

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

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab + all IO Drugs	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab + all IO Drugs	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
				feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab + all IO Drugs	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Asymptomatic cardiac enzyme elevation with clinical suspicion of myocarditis (previously CTCAE v4.0 Grade 1)	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab + all IO Drugs	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
	Grade 3	Withhold or discontinue based on the event [e]	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

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

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

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

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

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

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

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

Dose modification and toxicity management of infusion-reactions related to pembrolizumab
Pembrolizumab or other drugs in combination may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 5. For other drugs, refer to specific infusion treatment guidelines in Table 5**.**

Table 5. Pembrolizumab Infusion Reaction Dose modification and Treatment Guidelines

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

Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov

Other allowed dose interruption for pembrolizumab

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

5.4.2 ISA101b Dose Modifications, Timing and Precautions

ISA101b dose modifications are not allowed.

In order to assure the maximal immune response to ISA101b, the second 2 doses of ISA10b should each be given at the expected myeloid nadir, i.e. 14 days after administration of cisplatin. This timing should be maintained if the dose of cisplatin is delayed for any reason.

Patients receiving ISA101 in Montanide should be observed for at least 4 hours after vaccination to provide means for immediate treatment of allergic reactions should that be necessary (precautions include availability of staff well-trained in resuscitation, intravenous access for administration of fluids, antihistamines and corticosteroids, and epinephrine for intramuscular injection).

The dose of ISA101b will not be adjusted.

However, ISA101b will be discontinued in case of severe systemic allergic reaction, characterized by dyspnea, urticarial, severe generalized skin rash or other signs of severe systemic allergic reaction.

Local injection site reactions are expected with the administration of ISA101b. Severe ISRs requiring discontinuation of ISA101b have been reported. The investigator should use clinical judgement in administering further ISA101b if the reactions are particularly severe and/or increasing with successive doses of ISA101b. If ISA101b has to be discontinued for toxicity related to ISA101b, patient may continue treatment on trial with Cisplatin, IMRT, and pembrolizumab with PI approval. If a patient does not meet criteria to discontinue ISA101b, however declines further vaccine for any reason the patient can only stay on the trial if at least 2 of the vaccine doses have been given.

5.4.3 Cisplatin Dose Modifications – CTCAE v. 5.0

Neutropenia:

- If on the day of scheduled treatment with cisplatin the absolute neutrophil count (ANC) $<1200/mm^3$, hold the second chemotherapy treatment but not the radiation until ANC $\geq 1200/mm^3$, then treat at 100% dose.
- Neutropenic fever (ANC $<1000/mm^3$ with a single temperature of >38.3 degrees C [101 degrees F] or a sustained temperature ≥38.0 degrees C for more than 1 hour) will require a 25% dose reduction of the second cisplatin dose.

Thrombocytopenia:

- If on the day of the scheduled treatment with cisplatin the platelet count is < 75,000/mm^3, hold the second chemotherapy treatment but not the radiation until platelets are ≥ 75,000/mm^3 then treat at 100% dose.
- Thrombocytopenia that results in bleeding will require a 25% dose reduction of the second cisplatin dose.

Neurotoxicity:

- If Grade 2 neurotoxicity developed, hold cisplatin but continue radiation until toxicity improves to < Grade 1, then reduce the second cisplatin dose to 80 mg/m^2.
- Is any signs of Grade 3 or greater neurotoxicity occur, discontinue cisplatin but continue radiation.

Renal Adverse Events:

- Cisplatin dose should be based on the serum creatinine or creatinine clearance immediately prior to the second cisplatin dose using the following guidelines: Note: If creatinine is > 1.5 mg/dL creatinine clearance must be calculated [Cockcroft-Gault] in order to make dose adjustment. If the calculated creatinine clearance is 50 mL/min or above, a 24-hour urine collection is not needed. If the calculation is < 50 mL/min, a 24-hour urine collection is mandated, and the cisplatin dose will be determined as follows:

Serum Creatinine		Creatinine Clearance	Cisplatin Dose
≤ 1.5 mg/dL	or	>50 mL/min	100 mg/m^2
>1.5 mg/dL	and	40-50 mL/min	50 mg/m^2
>1.5 mg/dL	and	<40 mL/min	Hold drug*

- *Cisplatin should be held but radiation continued and the creatinine measured weekly until it is < 1.5 mg/dL or the creatinine clearance is > 50 mL/min, and then the second dose of cisplatin should be given at the reduced dose of 50 mg/m^2.

Nausea and Vomiting:

- Maximum supportive therapy be will given and cisplatin will be continued at full dose for ≤ Grade 2 nausea and vomiting. For Grade 3 nausea and vomiting refractory to supportive therapy, cisplatin will be held until recovery to < Grade 2. No dose reductions will be made.

Mucositis:

- Significant mucositis of Grade 3 or Grade 4 is expected from radiation, and cisplatin should not be a reason for a treatment break unless it significantly interferes with fluid intake or nutrition. Aggressive supportive care is encouraged.

Ototoxicity:

- For clinical hearing loss not requiring a hearing aid, reduce cisplatin to 50 mg/m^2. For hearing loss requiring a hearing aid, discontinue cisplatin. For Grade 2-3 tinnitus, at the time of the second dose, hold cisplatin until improvement to Grade 1 or less and then reduce the 2nd dose of cisplatin to 50 mg/m^2. If tinnitus does not improve to Grade 1 or less by the last day of radiation therapy, discontinue cisplatin.
- An audiogram us strongly recommended when there is any report of significant change in hearing and/or an increase in tinnitus.

For any other grade 3-4 non-hematologic adverse events possibly related to cisplatin, hold cisplatin until toxicities have recovered to grade 1 or less. As noted in Secton 5.4.2, in order to assure the maximal immune response to ISA101b, the second 2 doses of ISA10b should each be given at the expected myeloid nadir, i.e. 14 days after administration of cisplatin. This timing should be maintained if the dose of cisplatin is delayed for any reason.

If the second dose of the cisplatin is delayed more than 21 days because of hematologic, neurologic, renal, or other adverse events, that dose will be omitted. If a weight change of ≥10% occurs, the second cisplatin dose should be adjusted.

5.5 Intensity Modulated Radiotherapy (IMRT)

5.5.1 Dose specifications

IMRT will be delivered in 35 fractions over 7 weeks (five fractions per non-holiday week) in one plan (SIB). Concomitant boost using separate IMRT plans is not allowed.

Missed treatments due to holidays or logistic reasons can be compensated for by delivering an additional BID treatment during the week, OR treating on the Saturday or Sunday of that week, OR adding to the end of treatment.

5.5.2 Immobilization and Simulation

Patients must have an immobilization device (e.g. Aquaplast mask) made prior to treatment planning CT scan.

Use of an immobilizing mouthpiece is strongly encouraged for all patients.

The treatment planning CT scan should be performed with IV contrast so that the major vessels of the neck are easily visualized. The treatment planning CT scan must be performed with the immobilization device and in the treatment position. Slice thickness should be 0.3 cm or thinner.

5.5.3 Target Delineation

The Gross Tumor Volume (GTV) is defined as all known gross disease determined from CT, PET/CT, clinical information, endoscopic findings and/or potentially MRI.

The Clinical Target Volume (CTV) is defined as the GTV plus areas considered to contain potential microscopic disease, delineated by the treating physician. The margin between each GTV and its CTV will typically be a minimum of 0.5 cm except in those areas where the GTV is immediately adjacent to structures known to be uninvolved such as bone and adjacent muscle. In this instance, the CTV should be modified to exclude structures that serve as a boundary that prevents the natural spread of disease (bone, muscle, etc).

The Planning Target Volume (PTV) will provide a margin around each CTV (i.e. both the primary tumor and the lymph nodes containing clinical or radiographic evidence of metastases) to compensate for the variation of treatment set up and internal organ motion. Studies should be implemented by each institution to define the appropriate magnitude of the uncertainty components of the PTV; however, an unedited expansion of 0.3 - 0.5 cm is recommended.

In general, the PTV should not go outside of the skin surface; if it does exceed the skin surface, the application of bolus material over this portion of the PTV may be considered if it is judged clinically that the skin is at risk but is generally not recommended.

For those institutions that are using daily IGRT for margin reduction, the minimum CTV-to-PTV expansion is 3 mm (a larger expansion may be necessary for a target volume subject to significant intra- fraction variability, such

as the non-immobilized oral tongue).In general, the CTV-to-PTV expansion (with IGRT) should not exceed 5 mm.

The primary tumor and involved nodes (CTV1) will typically consist of a 0.5-1.5 cm expansion of the gross tumor volume (GTV) to cover potential local invasion and will be prescribed 2 Gy/fraction, total 70 Gy (see Section 5.5.8. for details of prescription for PTV1).

High-risk sub-clinical disease sites, which include possible local subclinical infiltration at the primary site (primary site CTV2) and first echelon nodes, which are not clinically or radiographically involved (nodal CTV2), should be expanded by 3-5 mm to create PTV2. PTV2 should receive a total dose of 60 Gy/35 fractions.

Lower-risk targets (PTV3) (such as neck nodal levels which are not first echelon nodes and are not adjacent to levels containing grossly involved nodes) will be prescribed 54 Gy/35 fractions.

Treatment of the low neck: see details in Section 5.4.7. If the low neck is treated, the preferred technique is to treat with isocentric matching AP or AP-PA fields with larynx block, matched to the IMRT portals just above the arytenoids. The dose will be 2 Gy per fraction prescribed to 3 cm depth to a total dose of 50 Gy in 25 daily fractions. Whole-neck IMRT is allowed. Involved low neck nodes will receive total 70 Gy in 35 fractions. This can be achieved by either boosting the low neck field with an additional 16 Gy in 8 fractions, by an AP or AP-PA fields, or by planning the whole neck using IMRT. In cases of gross involvement of the vallecula or low neck, whole-neck IMRT should be considered. Whole-neck IMRT may also be considered if level VI is considered to be at risk due to gross involvement of level IV nodes.

All plans must be normalized such that 95% of the volume of the PTV1 is covered with prescription dose of 70 Gy. Additionally: At 1 cc PTV1 volume on the DVH curve, the dose should not be > 110% of the prescribed dose. At a volume of 0.03 cc within the PTV1 volume on the DVH curve, the dose should not be < 95% of the prescribed dose. For any volume of tissue outside the PTVs that has a size of 1 cc, the dose should not be > 74 Gy.

Lymph Node Regions

a. Submental nodes *(level IA)*: In cases where the floor of mouth, oral tongue, or level IB are involved.
b. Submandibular nodes (*level IB)*: To be covered when the ipsilateral level II has nodal disease of at least 3 cm or the oral cavity is involved.
c. Upper deep jugular *(junctional, parapharyngeal)* nodes: all cases *(at the neck side ipsilateral to the primary tumor).*
d. Subdigastric *(jugulodigastric)* nodes, midjugular, lower neck, and supraclavicular nodes *(levels II through IV)*: all cases, bilaterally; unilateral neck treatment can be considered for well lateralized T1/2 primary tumors.
e. Posterior cervical nodes *(level V)*: all cases, at the neck side where there is evidence of jugular nodal metastases.
f. Medial retropharyngeal nodes: all cases involving the pharyngeal axis (i.e. not required for oral cavity and larynx cancers not involving pharyngeal structures) should be contoured from the level of C1 through the superior border of the hyoid body.

Critical Normal Structures

The normal tissue volume to be contoured will include the skin surface, brainstem, spinal cord, mandible, glottic larynx, supraglottis, esophagus, trachea, oral cavity, lips, and parotid and submandibular salivary glands. The PRV (planning risk volume) spinal cord contours will be defined at least 0.5 cm larger in the radial dimension than the spinal cord *(i.e. the cord diameter on any given slice will be 1.0 cm larger than the cord itself*). The normal tissues will be contoured and considered as solid organs. The tissue within the skin surface and outside all other critical normal structures and PTVs is designated as unspecified tissue.

5.5.4 Treatment Planning and Delivery

Megavoltage energy photon beam irradiation is required. Any treatment planning and delivery system that has been credentialed for head and neck IMRT for previous RTOG trials is acceptable.

5.5.5 Image Guidance for IGRT When Using Reduced Margins

Daily image guidance of IMRT may be achieved using any one or more of the following techniques:
Orthogonal kilovoltage (KV) images, e.g. ExacTrac;
Linear-accelerator mounted kV and MV helical conebeam CT images;
Linear-accelerator mounted MV CT images (e.g., Tomotherapy);
Other mechanism, after discussion with the Radiation Oncology Co-Chair, David A. Clump, MD, PhD.

The institution's procedure for registering daily treatment imaging datasets with a reference dataset should comply with the following recommendations:

Region-of-Interest (ROI) or "clip box" for fusion should be set to encompass the high dose PTV and adjacent spinal cord; if the supraclavicular region is a part of the target volume the ROI should extend to the C6 level; If the fusion software allows the user to create an irregular ROI (e.g., ExacTrac), treatment room objects seen on in-room x-rays should be excluded from the registration; manual (e.g., based on bony anatomy) and automatic (e.g., based on mutual information) types of registration can be used; the result of the fusion must be visually checked for the alignment of the bony anatomy, such as vertebral bodies and applicable soft tissue structures (e.g., optic nerves and/or optic chiasm).

Following the registration, the translational and (if the appropriate technology is available) rotational corrections should be applied to the treatment couch. If all the variances are less than 2.5 mm (this typically corresponds to one half of the usual PRV margin), the treatment can proceed without correction (however, the physician/team may elect to perform adjustments even for a variance < 2.5 mm). If one or more corrections are 2.5-5 mm, adjustment is necessary prior to treatment; however, re-imaging is not mandatory. If one or more of the corrections are larger than 5 mm, the imaging must be repeated in addition to performing table/positioning adjustments. However, the use of numerous repeat IGRT studies should be avoided (see next section).

Management of Radiation Dose to the Patient from IGRT
Estimates of patient doses per imaging study for various imaging systems vary considerably. The doses are in the range of 1 mGy for Cyberknife's and BrainLab's ExacTrac planar kV- systems. The doses from helical MV CT scan on a tomotherapy unit were estimated to be in the range of 1 to 3 cGy for head and neck studies, similar to doses reported for kV cone beam CT on the Elekta Synergy machine. The doses for MV cone beam CT are in the range of 10 cGy for a pelvis study to 6 cGy for a head and neck study. Thus, the doses for 3D imaging systems are in the range from 1 to 6 cGy for head and neck imaging and can contribute from 0.5 to 3% to the daily dose of 2.0 Gy. These dose estimates apply to a single imaging procedure, and the 2 cGy dose is used as a typical fraction size for comparison purposes within the treated region. It is important to point out that the imaging dose typically covers parts of the patient's anatomy that are outside the high-dose region that is treated therapeutically, and that it is sometimes necessary to repeat the procedure a number of times during, before, or after a single fraction delivery. The imaging dose to nearby critical structures may become significant when repeated IGRT procedures are performed for patients with severe set up problems (e.g., requiring frequent corrections of more than 5 mm). It is recommended that patients demonstrating severe set up problems during the first week of treatment be moved to a treatment with larger margins.

5.5.6 Definition of Normal Tissues/Organs at Risk (OARs)

NOTE: **Only the parts of the normal tissues/organs at risk outside the PTVs will be considered for dose optimization purposes.**

Spinal Cord: The cord begins at the cranial-cervical junction (i.e. the top of the C1 vertebral body). Superior to this is brainstem and inferior to this is cord. The inferior border of the spinal cord is at approximately T3-4

(i.e., just below the lowest slice level that has PTV on it). The spinal cord shall be defined based on the treatment planning CT scan. In addition, however, a Planning Risk Volume (PRV) spinal cord shall be defined. The PRVcord = cord + 5 mm in each dimension. This is irrespective of whether or not IGRT is used.

Brainstem: The inferior most portion of the brainstem is at the cranial-cervical junction where it meets the spinal cord. For the purposes of this study, the superior most portion of the brainstem is approximately at the level of the top of the posterior clinoid. The brainstem shall be defined based on the treatment planning CT scan. In addition, however, a Planning Risk Volume (PRV) brainstem shall be defined. The PRVbrainstem = brainstem + 3 mm in each dimension.

Lips and Oral Cavity: These should be contoured as 2 separate structures as the goal is to keep the lip dose much lower than the oral cavity dose. The definition of lips is self-explanatory. The oral cavity will be defined as a composite structure consisting of the anterior ½ to $^{2}/_{3}$ of the oral tongue/floor of mouth, buccal mucosa, and palate.

Parotid Glands: Parotid glands will be defined based on the treatment planning CT scan.

OARpharynx: This will be defined as the "uninvolved" posterior pharyngeal wall plus adjacent constrictor muscles. This extends from the superior constrictor region (the inferior pterygoid plates level) to the cricopharyngeal inlet (posterior cricoid cartilage level).

Cervical Esophagus: This will be defined as a tubular structure that starts at the bottom of Oropharynx and extends to the thoracic inlet.

Glottic/Supraglottic Larynx (GSL): This will be defined as a "triangular prism shaped" volume that begins just inferior to the hyoid bone and extends to the cricoid cartilage inferiorly and extends from the anterior commissure to include the arytenoids. This includes the infrahyoid but not suprahyoid epiglottis.

Mandible: This includes the entire boney structure of the mandible from TMJ through the symphysis.

Unspecified Tissue Outside the Targets: This will be defined as tissue located between the skull base and thoracic inlet that is not included in either the target volumes or the normal tissues described above.

In cases of weight loss > 10% or significant shrinkage of lymphadenopathy during therapy, it is recommended that the immobilization mask will be adjusted or re-made in order to preserve adequate immobilization, and that a repeated simulation CT be performed to assess the dose distributions in the current anatomy. Whether or not a new IMRT plan will be generated is at the discretion of the treating physician. If a new plan is made, the targets should be the same as those used for the initial plan. The new CT dataset should be used for IGRT image registration when the patient's shape changes significantly.

5.5.7 Management of the Low Neck/Supraclavicular Region (Match versus No Match)

It is recognized that comprehensive head and neck irradiation incorporating IMRT can be done in several ways, any of which is permitted for this study. Patient-specific QA measurements are required for all IMRT treatments. When a field "match" technique is used for treating the lower neck, patient-specific measurements should include a verification of the dose coverage in the gap region for each patient.

1. Match: The upper cervical lymphatics and primary tumor bed are treated with IMRT. The lower cervical lymphatics and supraclavicular region are treated with a single AP (or occasionally APPA for larger patients with posterior neck at high risk) non-IMRT technique. The latter non-IMRT field(s) is matched to the upper neck IMRT fields. This technique requires comprehensive mid-line spinal cord blocking in the lower neck

fields. This technique also allows for a simultaneous blocking of portions of the larynx, hypopharynx, and cervical esophagus in the lower neck fields. Matching 2 IMRT plans is allowed.

2. No Match: The entire clinical target volume (CTV) [upper and lower neck and primary tumor bed] is irradiated with IMRT. There is no match line between upper and lower portions of the regions at risk. In this technique, limiting radiotherapy dose to organs at risk (OARs), e.g., the cervical esophagus, is entirely achieved by inverse treatment planning via IMRT algorithms.

5.5.8 Dose Prescription

Doses to PTVs

See Sections above for definitions of CTVs and PTVs and their prescribed doses. The goal is for 95% of the PTV70 to receive ≥ 2 Gy with a minimum dose (cold spot) of no less than 66.5 Gy. It is recognized that portions of the PTV70 close to the skin may receive significantly less than 66.5 Gy. This is acceptable as long as cold spots within PTV1 do not exist at a depth deeper than 8 mm beneath the skin.

For planning prioritization and priorities in dose coverage, in the final plan, PTV1 will be the highest priority target structure. PTV2 and PTV3, if applicable, will be ranked in the IMRT planning as lower priority than PTV1 although usually at a higher priority than normal structures other than spinal cord and brain stem.

Doses to Normal Structures

Spinal Cord: The PRVcord (as defined in Section 5.3.6.) should not exceed ≤ 50 Gy to any volume in excess of 0.03 cc (approximately 3 mm x 3 mm x 3 mm). In treatment planning, the spinal cord PRV should be given the highest priority.

Brainstem: The PRVbrainstem (as defined in Section 5.3.6.) should not exceed 52 Gy to any volume in excess of 0.03 cc (approximately 3 mm x 3 mm x 3 mm). In treatment planning, the PRVbrainstem should be given less priority than the PRVcord but more priority than the other critical structures listed below.

Lips: Reduce the dose as much as possible. The mean dose should be < 20 Gy. This may be exceeded in oral cavity cancers.

Oral Cavity: Reduce the dose as much as possible. The mean dose should be < 30 Gy for the non-involved oral cavity. Efforts should also be made to avoid hot spots (> 60 Gy) within the non-involved oral cavity.

Parotid Glands: In most cases, it will be easier to spare one parotid than the other. The treatment planning goal will be for this individual parotid gland to receive a mean dose of <26 Gy.
Taking into account new data suggesting monotonous improvement in saliva as dose is reduced, without a threshold (Dijkema 2010), the objective will be to reduce the mean doses to both parotid glands as much as possible.

Contralateral submandibular gland: If contralateral level I is not a target, aim to reduce mean contralateral submandibular gland to < 39 Gy.

OARpharynx: Reduce the dose as much as possible. Some recommended (but not mandatory) treatment goals include: 1) No more than 33% of the OARpharynx exceeds 50 Gy; 2) Mean dose < 45 Gy; 3) No more than 15% of the OARpharynx exceeds 60 Gy. *Cervical Esophagus*: Reduce the dose as much as possible. Some recommended (but not required) treatment goals include: Mean dose < 30 Gy.

Glottic and Supraglottic larynx (GSL): Reduce the dose as much as possible. The glottic larynx m e a n dose is recommended to be ≤20 Gy. If whole-neck IMRT is used, under- dosage of PTV2/PTV3 adjacent to the glottic larynx will be limited to <10% receiving < 95% prescribed dose (this under-dosage is similar to that caused by the laryngeal block inserted in the split- field IMRT; Webster 2009).

Mandible: Reduce the dose as much as possible. Hot spots within the mandible should be avoided. It is recommended that maximum dose within the mandible be < 66 Gy, except in areas overlapping PTV.

Unspecified Tissue Outside the Targets: No more than 1cc of unspecified tissue outside the targets can receive 74 Gy or more.

5.5.9 Planning Priorities and Goals

1. Spinal Cord
2. Brainstem
3. PTV1
4. PTV2 (if applicable)
5. PTV3 (if applicable)
6. a. OARpharynx
   b. Parotid gland contralateral to primary tumor site
7. a. GSL
   b. Esophagus
8. a. Lips
   b. Oral Cavity
9. a. Parotid gland ipsilateral to primary tumor site
   b. Mandible
10. Unspecified tissue outside the targets

5.5.10 Critical Structures

Note: All required structures must be labeled as listed in the table below.

Table 8. Standard Nomenclature for Normal and Critical Structures

Standard Name	Description	Reference Dose
GTV	Primary tumor and involved nodes	70
CTV70	Primary tumor and involved nodes	70
PTV70	CTV to PTV expansion should be 5 mm minimal margin without IGRT; 3 mm minimal margin with Daily IGRT	70
CTV60	First Echelon nodal regions	60
PTV60	CTV to PTV expansion should be 5 mm minimal margin without IGRT; 3 mm minimal margin with Daily IGRT	60
CTV50	Lower risk nodal regions	50
PTV50	CTV to PTV expansion should be 5 mm minimal margin without IGRT+ mm minimal margin with Daily IGRT	50
CTV54	Lower risk nodal regions	54
PTV54	CTV to PTV expansion should be 5 mm minimal margin without IGRT; 3 mm minimal margin with Daily IGRT	54
SpinalCord	Spinal Cord	≤ 48
SpinalCord_05	Planning risk Volume of 5 mm	≤ 50
BrainStem	Brain Stem	≤ 50
BrainStem_03	Planning Risk Volume of 3 mm	≤ 52
Parotid	Mean doses to one Parotid	≤ 26
OralCavity (excluding PTV's)	Oral Cavity	Mean dose ≤ 32

Mandible (excluding PTV's)	Mandible	D0.03cc < 66
OARPharynx	Uninvolved posterior pharyngeal wall plus adjacent constrictor muscles; should not include PTVs	Mean dose ≤ 40
Esophagus_Up	Cervical Esophagus	Mean dose ≤ 30
Larynx	Glottic/Supraglottic Larynx	Mean dose ≤ 35
NonPTV_7000	Maximum dose (hot spot > 1cc outside the PTVs)	D1cc < 63

5.5.11 Image-Guided Radiotherapy

Daily image guidance of IMRT may be achieved using any one or more of the following techniques:

- Orthogonal kilovoltage (KV) images
- Linear-accelerator mounted kV and MV cone-beam CT images (CBCT)

5.5.12 Radiation Therapy Treatment Interruptions

Treatment interruptions are strongly discouraged. Treatment breaks must be clearly indicated in the treatment record when they occur. Patients who have treatment interruptions for >3 weeks will be taken off study. The interruption of radiation therapy for grade 4 mucositis / dermatitis / dysphagia is at the discretion of the treating radiation oncologist. Treatment breaks, if necessary, ideally should not exceed five treatment days at a time and ten treatment days total. Treatment breaks should be allowed only for resolution of severe acute toxicity and/or for intercurrent illness and not for social or logistical reasons. Cisplatin, pembrolizumab and ISA101b will not be administered during radiotherapy treatment breaks.

5.5.13 Radiation Therapy Adverse Events

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 will be utilized for grading all adverse events. All appropriate treatment areas should have access to a copy of the CTCAE, v. 5.0. A copy of the CTCAE, v. 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Grade 3 therapy-induced mucositis and/or dysphagia are expected to develop in about one third to one half of patients. Nutritional evaluation prior to the initiation of therapy for a prophylactic gastrostomy (PEG) tube placement is highly recommended. Placement of a feeding tube should be recorded on the appropriate case report form, as should use of a feeding tube during and after treatment (e.g., greater than or less than 50% of nutrition by tube). Other common radiation adverse events include: fatigue, weight loss, regional alopecia, loss of teeth or cavities, xerostomia, hoarseness, transient ear discomfort, dysgeusia, and skin erythema and desquamation within the treatment fields.

Less common long-term treatment adverse events include: hypothyroidism, loss of hearing, chronic swallowing dysfunction requiring permanent feeding tube, brachial plexopathy, and cervical fibrosis. Much less common radiation adverse events include: mandibular osteoradionecrosis (< 5% incidence with attention to dental recommendations), and cervical myelopathy (< 1% with restriction of spinal cord dose to ≤ 45 Gy).

5.6 Duration of Treatment and Follow-up

Complete treatment includes the course of definitive radiotherapy with concurrent chemotherapy and a total of 17 doses of pembrolizumab. In the absence of treatment delays due to adverse event(s), treatment may continue until disease progression or until one of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),

- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

Patients will be administered pembrolizumab 200 mgs q 3 weeks starting week -1 for a total of 17 doses.

Patients will be followed for progression and survival using Standard of care intervals. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.7 Participant Discontinuation Criteria

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

As certain data on clinical events beyond study intervention discontinuation may be important to the study, they must be collected through the participant's last scheduled follow-up, even if the participant has discontinued study intervention. Therefore, all participants who discontinue study intervention prior to completion of the protocol-specified treatment period will still continue to be monitored in this study and participate in the study visits and procedures as specified in Section 2.2 unless the participant has withdrawn from the study.

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

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study intervention
- After prolonged study intervention interruption that prohibits restarting study intervention if agreed upon with the Sponsor
- Radiographic disease progression outlined in Section 6.1.4.
- Any progression or recurrence of malignancy, or any occurrence of another malignancy that requires active treatment
- Any study intervention-related toxicity specified as a reason for permanent discontinuation as defined in the guidelines for dose modification due to AEs in Section 5.2.4.
- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, placed the participant at unnecessary risk from continued administration of study treatment.
- The participant has a confirmed positive serum pregnancy test

5.8 Dose Limiting Toxicity (DLT) Monitoring

Dose Limiting Toxicity will be continuously monitored and is explicitly intended for the purposes of safety monitoring of the concurrent combination of ISA101b, pembrolizumab and cisplatin-IMRT. As defined, excess DLT is not expected during standard of care cisplatin-IMRT. Excess DLT which delays SOC CRT greater than 2 weeks, however, would trigger protocol suspension and determination of causality attribution and whether the

protocol can proceed with all or only one of the investigational agent(s). Please see Section 10.3 for details of the Continuous Monitoring Plan.

5.8.1 DLT

DLT is defined as the occurrence of a severe adverse event (AE) listed below that is *at least possibly* related to ISA101b and pembrolizumab and occurs within 24 weeks of the initiation of ISA101b or pembrolizumab and leads to at least a two week delay in CRT. AEs will be graded according to NCI CTCAE version 5.0. The Data Safety and Monitoring Committee of the H&N Program at UPMC Hillman Cancer Center (HCC) will determine causality and whether to remove the suspension and permit the trial to proceed, in consultation with the Principal Investigator, key co-Investigator(s), ISA and Merck.

DLT Definition:

Since autoimmune/inflammatory events may occur at any time during the course of treatment, and may occur with evidence of clinical benefit, the following criteria will be used to define DLT:

- Any ≥ Grade 3 non-hematologic toxicity **except**:
  - Grade 3 in-field radiation dermatitis for which IMRT is held ≤ 1 week (5 fractions)
  - Grade 3 mucositis for which IMRT is held ≤ 1 week (5 fractions)
  - Asymptomatic Grade 3 hypomagnesemia or hypokalemia, which corrects to Grade ≤ 2 with replacement therapy
  - Grade 3 dysphagia
  - Grade 3 pain
  - Grade 3 weight loss
  - Grade 3 fatigue
- An alanine or aspartate amino transaminase elevation of greater than three times the upper limit of normal with concurrent elevation of bilirubin two times the upper limit of normal attributable to pembrolizumab should be considered a DLT. Patients meeting this criterion should be permanently discontinued from pembrolizumab.
- Autoimmune toxicity of any grade requiring systemic corticosteroids or other anti-inflammatory that cannot be tapered off in less than 12 weeks.
- Delay in completion of radiation therapy >10 fractions, or inability to complete prescribed IMRT course, due to immune toxicity at least possibly attributed to pembrolizumab or ISA101b.
- Grade ≥ 3 neutropenia with fever (oral temperature > 39°C)
- Grade ≥ 3 thrombocytopenia with bleeding
- Grade ≥ 3 local injection site reaction at the site of ISA101b injection
- Grade ≥ 3 systemic allergic following ISA101b injection

Patients who experience a DLT to pembrolizumab will receive no additional doses of pembrolizumab and will be withdrawn from the protocol. Patients who experience a DLT to ISA101b will receive no additional doses of ISA101b but may continue on protocol treatment (cisplatin, IMRT, Pembrolizumab) with PI approval.

For any grade 5 event (death), the study will suspend if possibly related to the administration of investigational products. In such a case, a thorough assessment of the safety profile must be conducted, and the risk benefit consideration must be justified for any additional subject enrollment.

6. RESPONSE ASSESSMENT

Patients will undergo clinical neck and chest exam and CT scan with IV contrast at baseline and at 12-14 weeks following completion of IMRT. Note, an integrated PET/CT with contrasted diagnostic CT scan is recommended if

feasible at the participating site; however, only a diagnostic CT scan is required. Rare patients may require an MRI for tumor measurements, due to allergy to iodinated contrast despite premedication. In these patients, MRI of the neck should be performed at baseline and at first response assessment.

Responses will be coded for:

- Clinical examination response, primary and nodes
- CT response, primary and nodes (see modified RECIST criteria version 1.1, Section 6.1)
- If applicable: Integrated PET/CT response, primary and nodes (see Integrated PET/CT response criteria, Section 6.2.4)

6.1 Solid Tumor Response Criteria (modified RECIST Criteria version 1.1)

6.1.1 Malignant Disease Evaluation

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion.

All measurements should be recorded in metric notation by use of a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than four weeks before treatment registration.

The term unevaluable in reference to measurability will not be used because it does not provide additional meaning or accuracy.

At baseline, the primary tumor and pathologic neck lymph nodes will be characterized as either measurable or non-measurable.

6.1.1.1 Measurable Disease

6.1.1.1.1 Primary Tumor

Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm (2.0 cm) using conventional techniques or as ≥10 mm (10 cm) using spiral CT scan.

6.1.1.1.2 Neck lymph nodes

- Neck lymph nodes are considered pathologic and measurable if short axis ≥ 15 mm.
- Neck lymph nodes are considered pathologic but non-measurable if short axis ≥ 10 mm but < 15 mm.
- Neck lymph nodes are considered non-pathologic and non-measurable if short axis < 10 mm.

6.1.1.2 Non-measurable Disease

- All other lesions, including small lesions [longest diameter < 20 mm (2.0 cm) with conventional techniques or < 10 mm (1.0 cm) with spiral CT scan] are truly non-measurable lesions.
- Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

6.1.2 Definition of a Response

6.1.2.1 Target Lesions

All measurable lesions, including the primary tumor, and up to a maximum of five neck lymph nodes, should be measured at baseline.

The sum of the longest diameter of the primary tumor, and the short axis diameter of target pathologic lymph nodes, will be calculated at baseline and reported as the *baseline sum diameter*.

6.1.2.1.1 Complete Response (CR)

The disappearance of the primary tumor and the resolution of pathologic neck adenopathy. For the definition of

radiographic complete response in the neck, the resolution of neck lymph nodes to < 10 mm in short axis diameter with non-pathologic appearance is sufficient.

6.1.2.1.2 Partial Response (PR)

At least a 30% decrease in the sum of target lesion diameters (longest diameter of the primary tumor; short axis diameter of the target lymph nodes), taking as reference the *baseline sum diameter*.

6.1.2.1.3 Progressive Disease (PD)

At least a 20% increase in the sum of target lesion diameters (longest diameter of the primary tumor; short axis diameter of the target lymph nodes), taking as reference the *smallest sum diameter* recorded since the baseline sum diameter measurements, or the appearance of one or more new lesion(s).

6.1.2.1.4 Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

6.1.2.2 Non-target Lesions

All other lesions or sites of disease. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

6.1.2.2.1 Complete Response (CR)

The disappearance of all nontarget lesions and normalization of tumor marker levels, if applicable.

6.1.2.2.2 Partial Response (PR)/Stable Disease (SD)

The persistence of one or more nontarget lesion(s).

6.1.2.2.3 Progressive Disease (PD)

The appearance of one or more new lesion(s) and / or unequivocal progression of existing non target lesions.

6.1.2.3 Symptomatic Deterioration

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having *symptomatic deterioration.*

6.1.3 Evaluation of Patient's Best Overall Response

The best overall response is the best response recorded from registration until disease progression/recurrence, taking as reference for progressive disease the smallest measurements recorded since registration. Table 9 below provides overall responses for all possible combinations of tumor responses in target and nontarget lesions, with or without new lesions.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Table 9. Overall Response for all Possible Combinations of Tumor Response

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

NOTE: BOTH CLINICAL (by ENT examination) AND RADIOGRAPHIC RESPONSE (by CT or MRI scan) will be recorded. Response in the primary and the neck will be reported separately.

6.1.3.1 First Documentation of Response

The time between initiation of therapy and first documentation of PR or CR.

6.1.3.2 Confirmation of Response

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed no less than four weeks after the criteria for response are first met.

6.1.3.3 Duration of Response

Duration of overall response - the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded since treatment started.

6.1.3.4 Duration of Overall Complete Response

The period measured from the time measurement criteria are met for complete response until the first date that recurrent disease is objectively documented.

6.1.3.5 Duration of Stable Disease

A measurement from registration until the criteria for disease progression is met, taking as reference the smallest measurements recorded since registration. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of six weeks.

6.1.3.6 Survival

Survival will be measured from the date of entry on study.

6.1.3.7 Time to Progression and Progression-free survival

This interval will be measured from the date of entry on the study to the appearance of new metastatic lesions or objective tumor progression.

Progression-free survival (PFS) will be calculated from treatment initiation to disease progression or death from any cause or last follow up.

6.2 Methods of Measurement

Imaging based evaluation is preferred to evaluation by clinical examination. The same imaging modality must be used throughout the study to measure disease.

6.2.1 CT and MRI

CT and magnetic resonance imaging (MRI) are the best currently available and most reproducible methods for measuring target lesions. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be performed by use of a 5 mm contiguous reconstruction algorithm. This specification applies to tumors of the chest, abdomen, and pelvis, while head and neck tumors, and those of the extremities require specific procedures.

6.2.2 Clinical Examination

Evaluation of measurable disease in the primary site as well as nodal sites by ENT examination should be recorded at baseline. Determination of complete response will occur through radiologic imaging as well as the post-protocol endoscopic examination.

6.2.3 Cytology and Histology

Cytologic and histologic techniques can be used to differentiate between complete and partial response in rare cases (e.g., after treatment to differentiate residual benign lesions and residual malignant lesions in germ cell tumors). Cytologic confirmation of the neoplastic nature of any effusion that appears or worsens during treatment is required

when the measurable tumor has met response or stable disease criteria.

Should primary site biopsy, primary site salvage, and/or neck dissection be conducted, the pathologic response will be recorded.

6.2.4 FDG-PET Scan

The ideal post-chemoradiotherapy response assessment method would accomplish two goals: 1) accurately detect viable residual disease amenable to surgical consolidation and 2) be prognostic for longer term clinical outcomes including PFS. Head and neck clinical examination alone has an accuracy of less than 50% for residual neck nodal disease,[27] while post-radiation diagnostic CT has higher sensitivity than clinical exam but unsatisfactory specificity.[28,29] An increasing body of literature suggests that post-chemoradiotherapy positron emission tomography (PET), performed 8-12 weeks following completion of radiotherapy has improved accuracy relative to CT for classification of complete response in PULA HNSCC. The negative predictive value (NPV) for determination of residual neck disease, in particular, ranges from 92-100%.[30-33] The University of Pittsburgh institutional experience indicates that serial PET/CT scans permit safe deferral of planned neck dissection following chemoradiotherapy.

Moreover, a PET scan added to a diagnostic contrasted CT is superior to clinical examination or diagnostic CT alone (utilizing RECIST criteria 1.0) with regard to correlation with PFS.[34] However, RECIST 1.0 criteria were developed primarily for response assessment in the metastatic solid tumor setting, where patients are treated with palliative systemic therapy. RECIST 1.0 criteria inadequately address the post-chemoradiotherapy setting. Specifically, a CR in accordance with RECIST 1.0 requires disappearance of all target lesions, including pathologic lymph nodes. However, non-pathologic, subcentimeter lymphadenopathy can persist after chemoradiotherapy. RECIST 1.1 now incorporates new guidelines for the measurement of nodal disease, and for determination of CR, a short axis of < 1 cm is no longer considered residual disease. The accuracy of the RECIST 1.1 criteria for assessment of residual disease or as a predictor of PFS following chemoradiotherapy for PULA HNSCC is not reported.

To date, the majority of PET/CT literature conducted has been retrospective. The sole prospective study of PET/CT versus CT for first response assessment following chemoradiotherapy was performed at MDACC.[30] This study found that PET/CT was not superior to diagnostic CT alone for the accuracy of response assessment in unselected PULA HNSCC patients. However, in high risk patients defined as HPV-negative, non-oropharynx, and/or tobacco-exposed, PET/CT outperformed CT. The study has been criticized for lack of integration of a diagnostic contrasted CT into the PET/CT acquisition algorithm, although PET/CT results were interpreted with knowledge of a separate contrasted diagnostic CT; such integration enhances per-lesion sensitivity and specificity in head and neck cancer staging[35] and is of theoretical utility in the post-treatment patient. The CT criteria used in the MDACC study to classify nodal disease were clinical and related to RECIST. However, long axis nodal measurements were utilized with the following categorization:

- Pathologic (residual disease) nodes: > 15 mm in longest diameter and/or necrotic appearance
- Indeterminate nodes: 10-15 mm in longest diameter and non-specific CT findings
- Non-pathologic nodes: < 10 mm in longest diameter and non-necrotic (CR)

At the University of Pittsburgh, the standard of care following definitive chemoradiotherapy for PULA HNSCC has been integrated PET/CT. Following PET/CT patients are placed into four clinical categories: Negative PET/CT (Complete Response); Probably Negative PET/CT (Low clinical concern; follow up indicated); Probably Positive PET/CT (Clinical concern; tissue sampling indicated); Positive PET/CT (Definite residual disease). This categorization has been found to correlate with clinical outcomes, specifically PFS.[36]

Definition of CR following chemoradiotherapy therefore has two relevant, literature-based definitions which may be of clinical utility: 1) Quantitative anatomic criteria (RECIST 1.1); 2) Integrated PET/CT criteria.

6.3 Complete Response: Modified RECIST 1.1 (see section 6.1)

- Disappearance of primary tumor. Irregular contour of the tumor bed is consistent with radiation change
- Decrease in measurable pathologic neck lymph nodes to short axis measurement < 10 mm and non-necrotic appearance with normalization of nodal configuration. Increased enhancement may be attributable to radiation change.
- No new pathologic lesions

7. DRUG INFORMATION

7.1 Pembrolizumab

7.1.1 Study Drug Materials

Pembrolizumab drug substance is produced at two locations to yield: 1) a partially formulated aqueous solution stored under refridgerated conditions (2-8˚C) at a concentration of 40-50 mg/mL in 10 mM histidine buffer, pH 5.2-6.8, and 2) a fully formulated aqueous solution stored frozen (-40˚C) at a concentration of 22.5-27.5 mg/mL in 10 mM histidine buffer, pH 5.2-5.8 containing 7% sucrose and 0.02% polysorbate 80. The drug products are sterile filtered into Type I glass vials intended for single use.

Two drug product dosage forms are available for pembrolizumab: 1) a white to off-white lyophilized powder, 50 mg/vial, and 2) a liquid, 100 mg/vial.

- Pembrolizumab Powder for Solution for Infusion, 50 mg/vial (manufactured using the partially formulated drug substance), is reconstituted with sterile water for injection prior to use. It is formuated with L-histidine as buffering agent, polysorbate 80 (surfactant), sucrose as stabilizer/tonicity modifier, and hydrochloric acid (HCl) and/or sodium hydroxide (NaOH) for pH adjustment (if necessary).
- Pembrolizumab Solution for Infusion, 100 mg/vial is a liquid drug product (manufactured using the fully formulated drug substance), and has the identical formulation as that of the reconstituted lyophilized vial.

The product after reconstitution with sterile water and the liquid drug product are a clear to opalescent solution which may contain proteinaceous and extraneous particulates.

Both the pembrolizumab reconstituted product and the liquid product are intended for IV administration and both can be further diluted with normal saline in IV containers made of polyvinyl chloride (PVC) or non-PVC material.

7.1.2 Pembrolizumab Study Drug Storage

Both pembrolizumab drug product dosage forms are to be stored under refrigerated conditions (2^{o} C– 8^{o}C) and in a secure location.

Note: No other use of pembrolizumab study drug intended for use in this trial is authorized by the sponsor. The investigator (or designee) will be responsible for the appropriate handling and disposition of residual study drug in partially used vials.

Vial Labels: Pembrolizumab vial labels will bear the appropriate label text for investigational agents, as required by governing regulatory agencies.

Complete study drug information (including packaging, labeling, storage and disposition) is provided in the Pembroliuzumab Investigator's Brochure (IB).

7.2 ISA101b

7.2.1 Study Drug Materials

ISA101b is a vaccine and consists of nine overlapping long E6 peptides (five 32-mer and four 25-mer E6 peptides) and three 35-mer E7 peptides. These peptides overlap by 10 to 18 residues and cover the complete sequence of HPV16 E6 oncoprotein. Most of the E7 oncoprotein sequence is covered by the peptide sequences (only amino acids 57-63 are not covered).

7.2.2 ISA101b Study Drug Storage and Use

The drug product ISA101b will be presented in two vials, HPV-DP-5P containing five peptides and HPV-DP-7P containing seven peptides. Lyophilized powder of the two peptides/drug products are stored in glass vials in the dark at -20°C and in a secure location.

Before SC injection, the peptides of each two drug products are reconstituted with Reconstitution Solution and mixed with Montanide ISA 51 VG to obtain two solutions which are administered separately in different anatomic locations. **The maximum hold time after preparation is 2 hours.** Instructions for ISA101b preparation and administration are described in the ISA101b pharmacy manual.

Vial Labels: ISA101b vial labels will bear the appropriate label text for investigational agents, as required by FDA.

Complete study drug information (including packaging, labeling, storage and disposition) is provided in the ISA101b Investigator's Brochure (IB).

7.3 Cisplatin

Refer to the package insert for additional information.

Formulation: Each vial contains 10 mg of DDP, 19 mg of sodium chloride, 100 mg of mannitol, and hydrochloric acid for pH adjustment. One vial is reconstituted with 10 ml of sterile water. The pH range will be 3.5 to 4.5. Cisplatin injection also is available from the manufacturer in aqueous solution, each ml containing 1 mg cisplatin and 9 mg NaCl and HCL or NaOH to adjust pH.

Mechanism of Action: The dominant mode of action of cisplatin appears to be inhibition of the incorporation of DNA precursors, although protein and RNA synthesis are also inhibited. Although this drug seems to act as an alkylating agent, there are data to indicate that its mode and sites of action are different from those of nitrogen mustard and the standard alkylating agents.

Administration: Cisplatin will be given as a bolus, infused over 1-2 hours along with appropriate hydration and anti-emetics.

Storage and Stability: Reconstituted solution of cisplatin is stable for 20 hours when stored at 27°C and should be protected from light if not used within 6 hours. The vials and injection should not be refrigerated. Cisplatin has been shown to react with aluminum needles, producing a black precipitate within 30 minutes.

Adverse Events: Human toxicity includes nausea, vomiting, renal toxicity (with an elevation of BUN and creatinine and impairment of endogenous creatinine clearance, as well as renal tubular damage, which appears to be transient), ototoxicity (with hearing loss that initially is in the high-frequency range, as well as tinnitus), and hyperuricemia. Much more severe and prolonged toxicity has been observed in patients with abnormal or obstructed urinary excretory tracts. Myelosuppression, often with delayed erythrosuppression, is expected.

Supply: Cisplatin is commercially available. The use of drug(s) or combination of drugs in this protocol meets the criteria described under Title 21 CFR 312.2(b) for IND exemption.

8. CLINICAL AND LABORATORY EVALUATIONS

Schedule of Assessments. See Appendix C for Study Calendar.

8.1 Pre-Registration Evaluations

NOTE: This section lists baseline evaluations that are required for registration. Evaluations should be performed within 4 weeks of Day -14 unless otherwise indicated. *Labs will need to be collected within 10 days of Day -14.

8.1.1 Required Pre-Registration Evaluations

- History and physical examination, including vital signs, weight, height and ECOG performance status

determination

- Complete blood count, including platelets and differential*
- Blood chemistry studies (may be obtained from whole blood or serum/plasma samples), including BUN, creatinine, electrolytes (K^+, Na^+, CI^-, CO_2), glucose, calcium, Mg^{++} and liver function tests (total protein, albumin, total bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase), uric acid, and lactate dehydrogenase*
- Thyroid function tests, including thyroid stimulating hormone (TSH), free thyroxine, (FT4), anti-thyroglobulin antibody, thyroid peroxidase antibody, thyroid stimulating immunoglobulin, and ANA. Establishing baseline thyroid function and pre-existing thyroid auto-immunity is required, as an appropriate clinical standard prior to chemoradiotherapy for HNSCC as well as anti-PD1 therapy for HNSCC. However, normal thyroid function and absence of subclinical thyroid auto-immunity (example detectable anti-thyroglobulin antibody or detectable thyroid peroxidase antibody) are not eligibility criteria. If subclinical hypothyroidism is detected, treatment should be initiated according to standard practice. If subclinical thyroid auto-immunity is detected, the patient is still eligible.
- For women of child bearing potential: blood pregnancy test within 4 weeks of registration to rule out pregnancy. (Note, pregnancy test will be repeated within 72 hours of initiating protocol treatment.)
- Baseline tumor measurements within 4 weeks prior to registration. Contrasted diagnostic CT scan for staging and baseline tumor measurements. In the rare case that a patient is not a candidate for contrasted diagnostic CT scan (e.g. history of allergic reaction to iodinated contrast despite premedication), MRI of the neck may be conducted for baseline tumor measurements. **Note**: when feasible, a baseline integrated PET/CT with diagnostic contrasted CT scan is preferred. Any scans or x-rays used to document measurable disease should be done as close to study entry as possible and within 4 weeks prior to treatment.
- Evaluation by an otolaryngologist with endoscopy as indicated, within 8 weeks prior to registration. Endoscopy for oropharyngeal carcinoma may be performed as clinically indicated.
- HPV status must be established and positive in accordance with protocol definition
- Tobacco history assessment form (See Appendix A)
- Dental evaluation, Nutrition consult, SLP consult and audiogram, are strongly encouraged but not mandatory.
- Correlatives: submission of tumor tissue and research blood collection

8.2 Evaluations during treatment

8.2.1 Week -2 to 6 (During concurrent cisplatin-IMRT)

Weekly:

Note: The following assessments will be performed on the day of or the day prior to treatment administration.

- Targeted physical examination
- ECOG Performance Status
- Vital signs, including weight
- Toxicity assessment with attribution
- Update of concomitant medications
- Complete blood count, including platelets and differential
- Blood chemistries (may be obtained from whole blood or serum/plasma sample), including BUN, glucose, uric acid, lactate dehydrogenase, creatinine, electrolytes (K+, Na+, CI-, CO2), calcium, Mg++ and liver function tests [bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase, total protein, albumin]
- Research biopsy of primary or nodal tumor tissue will be obtained at screening (mandatory), week 2 of Pembrolizumab (optional) and week 2 of IMRT (optional). Primary site biopsy is preferred, although a lymph node biopsy is acceptable in the event of inaccessible primary tumor. All effort should be made to biopsy the same site originally biopsied (i.e. primary tumor

or pathologic lymph node), if accessible. Although the research biopsy may occur on any of the four days following cisplatin administration, it is preferred to occur on the first or second day following cisplatin administration. It is also preferred that the research biopsy precede the radiotherapy fraction on the day of research biopsy.
- Research blood on the same day as biopsy

8.2.2 Week 2 and Week 5:

Note: The following assessments will be performed every 3 weeks during concurrent cisplatin-IMRT, and may be performed on the day of or the day prior to treatment administration. It is recommended that research blood should be coupled to the standard of care blood draw, to minimize needle sticks to the patient.

- Thyroid function tests (TSH, FT4)
- Research blood
- Anti-thyroglobulin antibody, thyroid peroxidase antibody, thyroid stimulating immunoglobulin, and ANA tests

8.2.3 Week 7

- Targeted physical examination
- ECOG Performance Status
- Vital signs, including weight
- Toxicity assessment with attribution
- Update of concomitant medications
- Complete blood count, including platelets and differential
- Blood chemistries (may be obtained from whole blood or serum/plasma sample), including BUN, glucose, uric acid, lactate dehydrogenase, creatinine, electrolytes (K+, Na+, CI-, CO2), calcium, Mg++ and liver function tests [bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase, total protein, albumin]
- Thyroid function tests (TSH, FT4)
- Research blood collection

8.2.4 Weeks 8-50 (During continued pembrolizumab treatment)

8.2.4.1 Every 3 weeks (up to 17 total doses of pembrolizumab to total 1 year of therapy)

Note: The following assessments will be performed every 3 weeks, and may be performed on the day of or the day prior to pembrolizumab administration.

- Targeted physical examination
- ECOG Performance Status
- Vital signs, including weight
- Toxicity assessment with attribution
- Update of concomitant medications
- Complete blood count, including platelets and differential
- Blood chemistries (may be obtained from whole blood or serum/plasma sample), including BUN, glucose, uric acid, lactate dehydrogenase, creatinine, electrolytes (K+, Na+, CI-, CO2), calcium, Mg++ and liver function tests [bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase,

total protein, albumin]
- Thyroid function tests (TSH, FT4)
- Research blood collection

8.2.4.2 Every six weeks

- Anti-thyroglobulin antibody, thyroid peroxidase antibody, thyroid stimulating immunoglobulin, and ANA

8.3 Post-treatment Evaluations

8.3.1 Response Assessment

8.3.1.1 RECIST 1.1.

Response assessment by RECIST 1.1 is required; integrated PET/CT interpretation is strongly recommended where available.

Twelve to 14 weeks following completion of cisplatin-IMRT (week 20-22 of protocol treatment), patients will undergo diagnostic contrasted CT scan of the neck and chest, to assess treatment response according to RECIST 1.1. In the case where MRI of the neck was required for baseline tumor measurements, MRI of the neck should be repeated for response assessment, with non-contrasted diagnostic CT of the chest. **Note: where available, an integrated PET/CT with contrasted diagnostic CT scans of the neck and chest is strongly preferred for response assessment.**

8.3.1.2 Evaluation by an Otolaryngologist using Endoscopy as Indicated

The patient will be evaluated by the otolaryngologist 12-14 weeks following completion of cisplatin-IMRT (during weeks 20-22). Endoscopy for oropharyngeal or unknown primary carcinoma will be performed for response assessment as clinically indicated and is at the discretion of the surgeon. The surgeon will evaluate the need for primary site biopsy and/or neck dissection.

8.3.2 End of Treatment Visit(s)

Patients will undergo the following procedures at the end of treatment visit:

- History and physical examination and performance status
- Update of concomitant medications
- Vital signs, including weight
- Assessment for resolution of toxicities, including irAE, with attribution
- Thyroid function testing (TSH, T3, FT4)
- Complete blood count, including platelets and differential
- Blood chemistries (may be obtained from whole blood or serum/plasma sample), including BUN, glucose, uric acid, lactate dehydrogenase, creatinine, electrolytes (K+, Na+, CI-, CO2), calcium, Mg++ and liver function tests [bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase, total protein, albumin]

8.3.3 Surgical Consolidation

The necessity for and timing of surgical consolidation will be determined by the surgeon. If patients undergo surgical consolidation, tumor tissue will be submitted for corelative study.

Primary site

Surgery will be performed in the setting of resectable disease if the patient is considered a surgical candidate and:
1) Definitive radiotherapy was not completed, and radiographic and clinical evidence of persistent or progressive disease is established
2)Biopsy-proven disease is demonstrated at the primary site and/or neck at least 8-12 weeks after completion of

IMRT. In cases of residual disease at the primary site, the neck will always be dissected at the time of surgical salvage 9if neck dissection was not previously conducted at post-protocol biopsy).

Surgical Management of the Neck
Neck dissection will be performed if there is a concern for residual disease. The extent of the neck dissection will be determined by the surgeon. The consideration to perform less than radical procedures is strongly encouraged if oncologically safe. Note: the presence of residual disease at surgical consolidation, if performed within 24 weeks of completion of IMRT, will not constitute a progressive event.

8.4 Long Term Follow Up

After completion of IMRT, patients will be evaluated radiographically and clinically as per standard of care. This would be every 3 months for 12 months (+/- 2 weeks), then every 6 months (+/- 1 month) for one year, then annually (+/- 1 month) for three years, which will represent a total of 5 years from completion of IMRT. Other evaluations will start approximately 6 months after completion of IMRT, and will include the following:

- History and physical examination and performance status
- Vitals signs, including weight
- Assessment for late toxicity, including dysphagia, xerostomia, gastrostomy tube dependence, pain, fibrosis and lymphedema within the head and neck, hypothyroidism, late autoimmune events
- Thyroid function testing (TSH, FT4)
- Complete blood count, including platelets and differential
    - Blood chemistries (may be obtained from whole blood or serum/plasma sample), including
    - BUN, glucose, uric acid, lactate dehydrogenase, creatinine, electrolytes (K+, Na+, CI-, CO2), calcium, Mg++ and liver function tests [bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase, total protein, albumin]

- Research blood collection (6, 9, 12, 18, 24, 36, 48, and 60 months after completion of radiotherapy +/- one month)
- During the long term follow-up period, tumor assessments using anatomic imaging (CT or MRI) will be performed 6, 9, 12, 18, 24, 36, 48, and 60 months after completion of radiotherapy (+/- one month). The minimum protocol imaging requirement is: contrasted, diagnostic neck and chest CT at intervals specified above. More intensive imaging may be conducted per local practice.
- Swallowing assessments per local practice and as indicated by post-radiotherapy evaluation and patient-reported symptoms
- Note: patients who have documented progression may discontinue scheduled study assessments and will be followed only for survival. Survival assessments will be performed every 6 months (+/- 2 months) by telephone contact and/or by evaluation of the Social Security Death Index or other public records.
- Note: should tumor recurrence be pathologically documented at any time, a sample of the diagnostic specimen should be submitted for correlative studies.

9. BIOMARKER, CORRELATIVE, OR SPECIAL STUDIES

9.1 Biomarker Sampling Schedule

Table 10. Biomarker Sampling Schedule

Collection timing	**Serum**	**PBMC**		**Tumor Tissue**		**Whole Blood**	
Study Day	**Soluble Biomarkers**	**Immuno-phenotyping**	**Ex vivo Functional Assay**	**Tumor Biopsy**		**Gene Expression**	**SNP**

Baseline (with biopsy, prior to registration)	X	X	X	X		X	X
Week -2, Week -1, CRT Weeks 0-3, Week 5, Week 7	X	X	X				
Optional biopsy: Week 1, Week 2				X			
Prior to every third maintenance pembrolizumab treatment (C4, 7, 10, 13, 16)	X	X					
Long term follow up	X	X					
At Progression, if applicable	X	X	X			X	

9.2 Research Biopsies

Patients will undergo research biopsies of accessible primary tumor or a pathologic lymph node at up to 3 timepoints during protocol treatment:

1) Baseline research biopsy will occur after documentation of eligibility, and prior to initiation of protocol treatment. All efforts will be made to couple the baseline research biopsy to a standard of care procedure. NOTE: Patients who have had research tissue procured under an omnibus tissue consent, who are determined to have sufficient fresh, fresh-frozen, and paraffin tissue for analysis of immune-inflammatory biomarkers per the PI, may substitute the archived tissue and do not need to undergo baseline research biopsy.

2) Patients will be asked to undergo an optional second research biopsy at the second week of cisplatin-IMRT.

3) Patients will be asked to undergo another optional research biopsy at the second week of Pembrolizumab.

NOTE: All effort should be made to biopsy the same site originally biopsied (i.e. primary tumor or pathologic lymph node), if accessible. The research biopsy will occur either in the outpatient office of the otolaryngologist/head and neck surgeon, or in the interventional radiology suite of the radiologist. Imaging guidance with ultrasound or CT may be used as necessary. Local anesthetic will be administered per standard of care.

9.2.1 Research Biopsy Methodology

All patients will be evaluated for the feasibility of research biopsy at the time of enrollment, as a condition of eligibility. The performing physician must agree that a cup forceps biopsy or an 18 to 14 gauge core needle biopsy can be safely performed. Tissue estimated to be equivalent to at least two 4 mm punch biopsies or four 18 to 14 gauge core needle biopsies will be collected. Tumor samples are ideally to be stabilized with the addition of media (formaline, sterile saline, PBS, or RPMI as indicated below) within 5-15 minutes of surgical excision. The tumor samples will be separated into two parts and processed as follows: 1) A tissue sample will be placed in a formalin filled plastic container, then embedded into paraffin for further analysis (FFPE). The FFPE sample will be stored in the UPMC Head and Neck Tissue Core with study identifiers. 2) A fresh tissue specimen will be placed in sterile media (such as: saline, RPMI, or PBS) and transported immediately to the laboratory of Robert Ferris, MD PhD for processing of tumor-infiltrating lymphocytes (TIL).

9.2.2 Sample Preparation and Shipment Instructions

For the research biopsies, the tissue samples will be processed as follows:

FFPE: For head and neck tumors, construction of tissue microarrays (TMAs) and immunohistochemical staining of the patient specimens will be performed at the UPMC/UPCI Head and Neck Tissue Core. These studies will be supervised by our head and neck pathologist, Dr. Raja Seethala, who directs the Tissue Core of the UPCI Head and Neck Cancer SPORE (PI, Ferris). Dr. Seethala is experienced in TMA construction and analysis of protein expression. Paraffin embedded tissue blocks will be sectioned at 5μm and stained with H&E for morphologic characterization and to serve as guide slides for TMA construction.

TIL and Isolated Tumor Cells: Samples should be weighed and added to 9X volume of lysis buffer (100 mg of tissue per 900 μL lysis buffer). The recommended lysis buffer is 60mM Tris-HCL with 2mM EDTA, pH 7.4. Samples should be homogenized immediately following collection; however, if the samples are not homogenized immediately then the samples should be frozen in liquid nitrogen and stored at -80°C. In order to minimize protease activity the following inhibitors should be added: aprotinin, antipain, leupeptin, and pepstatin A (all at 1 μL/mL) and 2mM PMSF (phenylmethylsulfonyl fluoride). Tissues may be homogenized using a Potter-Elvehjem homogenizer (Teflor pestle and glass mortar) attached to a variable-speed drill, a polytron or a tissuemizer. During the homogenization process, the tubes should be submersed in an ice bath to maintain the sample at 2–8°C. Following homogenization, the tissue preparation will be centrifuged for 2 minutes in a microfuge at 13,000xg. Ensure the cell pellet is not disturbed; aspirate the supernatant. A total volume of 350μL is required.

For intracellular flow cytometry, isolated tumor cells will be fixed with 1.5% paraformaldehyde for 10 minutes, then permeabilized with 100% methanol for at least 24 hours. Cell will be washed with fetal calf serum, then stained with primary antisera directed towards TAP1, TAP2, LMP2, or calreticulin, then with a fluorochrome-bound secondary antibody. Both incubations will be for 30 minutes at room temperature. FACS analysis will then be performed immediately after staining using an EPICS XL cytometer (Beckman Coulter). A minimum of 10,000 cells will be analyzed per test. At least 3 independent tests will be performed for each condition, and protein expression reported as a mean ± standard error of the mean.
Shipment Instructions:

University of Pittsburgh

1) The formalin-fixed tissue will be delivered to the UPMC/UPCI Head and Neck Tissue Bank as follows:

Raja Seethala, M.D.

University of Pittsburgh

Head and Neck Tissue Core

A-724 Scaife Hall

200 Lothrop Street

Pittsburgh, PA 15213

2) Fresh tumor will be delivered immediately to the laboratory of Robert Ferris, MD, PhD for processing:
Carly Reeder

Room 2.19 - Ferris lab
Hillman Cancer
Center 5117 Centre
Ave
Pittsburgh, PA 15213
Phone: 412-623-7738

The formalin-fixed tissue will be paraffin-embedded at the local site, then shipped at room temperature to the UPMC Head and Neck Tissue Bank:

Raja Seethala , M.D. University of Pittsburgh

Head and Neck Tissue Bank

A-724 Scaife Hall

200 Lothrop Street
Pittsburgh, PA 15213

Fresh tumor specimens will be placed in normal saline and shipped at room temperature by overnight (fedex) shipping for morning delivery to the laboratory of Robert Ferris, MD, PhD:

Carly Reeder
Room 2.19 - Ferris lab
Hillman Cancer Center
5117 Centre Ave
Pittsburgh, PA 15213
Phone: 412-623-7738

9.3 Research Blood

Research blood will be collected at the following time points on the study:

- Baseline
- Week -2, Week -1, CRT Weeks 0-3, Week 5, Week 7
- Optional biopsy: Week 1, Week 2
- Prior to each dose of pembrolizumab during radiation
- Prior to every third maintenance pembrolizumab treatment (C4,7,10,13,16)
- During long term follow up visits, and at recurrence if applicable.

Peripheral blood obtained by venipuncture will serve as the source for laboratory testing. Up to 80 mL of blood may be obtained at each draw. Two red top tubes for serum collection and 5 green top tubes of blood for separation and collection of plasma and mononuclear cells will be collected at each time point. At baseline only, a lavender top tube (EDTA) for DNA will be collected.

Blood Collection Procedures, Green and Red Top:

Before venipuncture, prepare five green top (heparin) plastic 10 ml tubes (green top #1, #2, #3, #4, #5) and one red top plastic (no gel) 10 ml blood collection tubes (red top #1) and affixes a label to each blood collection tube. Information to be encoded on the label includes the clinical trial study number, subject's unique identification (ID) number, tube number (1-5), and date. The tubes are placed in a test tube rack, in order of collection (green tops, then red tops).

The phelobotomist uses standard venipuncture techniques. Following collection of blood, green top tubes will be inverted 5 times to prevent clotting and platelet clumping.

Approximate blood volume to be collected in each tube is as follows: 8 ml in green top tubes and 8 ml in red top tube.

The phlebotomist affixes the Subject ID on to 2 blood collection forms (one for green top, one for red top).

The phlebotomist uses the blood collection forms to record date and time of phlebotomy and approximate amount of blood collected into each tube.

Green top tubes may be kept at room temperature and should be shipped by same-day courier (Pittsburgh sites) or overnight (non-Pittsburgh sites) to Dr. Ferris's lab at the address specified below. Enclose the blood collection form with the shipment.

Red top tube:

- To allow clot formation, allow red top tube to sit at room temperature (22° to 25° C) for 15 to 30 minutes.
- If further processing cannot be accomplished immediately after clot retraction, red top tube should be placed at 4° C.
- Centrifuge red top tube at 2,000 rpm for 10 min to sediment the clot.
- Aspirate the serum into 1-1.5 mL aliquots and transfer to 1.8 mL freezing tubes (4-8 tubes depending upon volume of serum).
- Information to be encoded on each freezing tube includes the clinical trial study number, the subject's unique ID number, the tube # (1-8), the contents of the freezer tube (serum), and date.
- Freeze and store at -80° C.

Ship on dry ice to Dr. Ferris's lab at the address specified below (for baseline blood draw, ship with the lavender top tube, detailed below). Enclose the blood collection form with the shipment.

Blood Collection Procedure, Lavender Tube (DNA for SNP Analysis)

- At the baseline blood draw only, one lavender top tube will be collected. It will be collected first, prior to collecting green and red top tubes. An additional (3rd) blood collection form will be used for the lavender top tube.
- Approximate blood volume to be collected is 5 ml.
- Divide the lavender tube contents into four 1-1.5 ml aliquots (in 1.8 ml freezing tubes). Information to be encoded on each freezing vial includes the clinical trial study number, the subject's unique ID number, the tube # (1-4), the contents of the freezer tube (whole blood EDTA), and date.
- Freeze at -80°C.
- Ship on dry ice to Dr. Ferris's lab at the address specified below (ship with the baseline red top tubes, detailed above). Enclose the blood collection form with the shipment.

Carly Reeder
Room 2.19 - Ferris lab
Hillman Cancer Center
5117 Centre Ave
Pittsburgh, PA 15213
Phone: 412-623-7738

9.4 Correlative Science Studies

9.4.1 Tumor PD-L1 Expression

Baseline and on-treatment PD-L1 expression will be tested; exploratory analysis will be conducted to correlate PD-L1 (double staining for PD-1 and other markers) with clinical response and immunogenicity.

Correlative study design: The effect of tumor PD-L1 expression on treatment response to anti-PD-1 targeted immunotherapy is currently unclear and a matter of ongoing investigation. Identification of predictive biomarkers of response to therapy protects patients from exposure to risks of ineffective therapies and improves cost-effectiveness. FFPE tumor samples (blocks) will be available for all patients, from dedicated research biopsies at the three timepoints specified. Analysis of PD-L1 expression will be performed by the 22-C3 antibody to PD-L1 by the Qualtex laboratory. PD-L1 membrane staining will be assessed by light microscopy. Either complete circumferential or partial linear plasma membrane staining will constitute positive PD-L1 staining. While cytoplasmic staining may be observed, it will not be used in the evaluation of tissue sample status. Two cut-off values of ≥ 1% and ≥50% tumor cell membrane staining will be reported as positive.

Hypothesis: Patients with PD-L1 "positive" or "high H-score" tumors will have an improved PFS in comparison to patients with PD-L1 negative tumors. Double staining of PD-1/PD-L1 will correlate better with outcome than single marker(s).

9.4.2 Tumor Antigen (EGFR, p53, E6, E7) Seroreactivity

Correlative study design: Patients who are seropositive to tumor-specific antigens may derive greater benefit from checkpoint inhibitors. In the case of melanoma, preliminary data indicate that patients with pre-existing antibodies to tumor-specific antigens (e.g. NY-ESO-1, an antigen expressed in 30-40% of patients with advanced melanoma) have an increased response to ipilimumab.[37] Approximately 30-40% of patients with HNSCC have detectable titers to EGFR, p53 etc. at diagnosis, indicating immune exposure to these tumor-specific antigens. Seropositivity therefore may be a biomarker for PD-1/PD-L1-mediated suppression of the T cell immune response to these tumor-specific antigens that also are necessary for the malignant phenotype of HPV-negative tumors. Likewise, in patients with HPV-positive tumors, antibodies against E6 and E7 can be observed. Serum samples at enrollment and follow up will be evaluated for the presence of EGFR, p53, E6, E7, etc. seroreactivity by use of a multiplex luminex assay utilizing GST N-terminal tagged proteins covalently bound to microspheres. The assay has been developed as a collaboration between the Ferris laboratory and that of Pawlita/Waterboer group (German Cancer Research Institute, Heidelberg). Results will be reported as positive or negative based on mean fluorescent values (MFI) in comparison to positive and negative controls.

Hypothesis: Patients who are seropositive to EGFR, p53, E6, E7 and other tumor antigen-specific antibodies will have an improved PFS when treated with pembrolizumab in comparison to patients who are seronegative.

9.4.3 Peripheral Blood CD69/137+ or ICOS+ Activated T cells

Correlative study design: Activated T cells expressing markers CD69 and/or CD137 have been identified in the circulation of head and neck cancer patients but at a low level.[17,38,39] These cells would be expected to increase after pembrolizumab, reversing T cell "exhaustion" and enabling their reactivation. This detectable population will be measured at baseline and longitudinally in the control and pembrolizumab-treated cohorts. Within this subset of activated, circulating T cells, we will phenotype them by memory/naïve status (using markers CD45Ra and CCR7) according to the patient's HLA type. Activated T cells expressing markers CD69, ICOS, and/or CD137 will be measured at baseline and longitudinally by use of flow cytometry. We also will secondarily measure memory status, EGFR and p53, and other antigen specificity and PD1-positivity within this population of circulating T cells using flow cytometry.[17,38-40] Pre-post comparison of these frequencies will be performed for each patient during therapy,

using each patient's baseline as the control time point, and comparing the kinetics of these values in patients treated with sequential vs. concurrent pembrolizumab during CRT.

Hypotheses: Intra-patient increase in the number of activated, CD69+ and/or CD137+ T cells will increase during CRT, between baseline and end-of-CRT blood draw. We also hypothesize that a significant intra-patient increase in the activation markers CD69 and CD137 will be observed.

9.4.4 Tumor Cell Immune Escape, Extent of Tumor Infiltrating Lymphocytes ("Immunoscore"), and Activating/Suppressive Ligands

HLA and antigen loss variants and expression levels will be investigated in tumor tissues, focused on HLA class I, antigen processing machinery (APM) components.[41] Next generation sequencing of tumors will analyze for HLA, beta-2-microglobulin, TAP1/2 and other APM component mutations as recently published.[42] Immunohistochemistry (IHC) will be used to assess the number and composition of immune infiltrates ("immunoscore") in order to define the immune cell subsets present within FFPE tumor tissue before exposure to therapy. These IHC analyses will include, but not necessarily be limited to, the following markers: CD4, CD8, FOXp3, PD-1, PD L1, and PD-L2.

9.4.5 Whole Exome Sequencing and RNA-Seq Analysis

To define the neo-epitope landscape and to delineate alterations in transcriptomic signatures we will complete whole exome sequencing (WES) and RNA-Seq on all protocol patients. WES will be performed on baseline tumor samples and DNA from corresponding peripheral blood samples. Somatic mutations and candidate neo-antigens generated from these mutations will be characterized. Neo-antigen prediction will be performed using HLA binding algorithms.[43,44] RNA-Seq analysis will be performed in matched baseline and Week 2 of CRT biopsies, so the patient's baseline tissue serves as his/her own control. The goal of these studies is to describe the baseline mutational burden and neo-epitope landscape, and to identify changes in RNA-Seq defined transcriptomic signatures. Associations with clinical response and PFS will be evaluated. In particular, RNA-Seq data will highlight which of the predicted neo-epitopes are expressed during CRT alone as compared to patients treated with CRT and concurrent pembrolizumab. These data will describe the immunomodulatory role of CRT distinct from the addition of pembrolizumab and will provide powerful biomarker insight into the potential value of concurrent vs. sequential treatment.

9.4.6 Paired Analysis of TCR Clonality in Pre- and Post-Treatment Tumor Specimens

To define the alterations in TCR clonality between matched baseline and Week 2 CRT biopsies from this trial, we will use the ImmunoSeq assay from Adaptive Biotechnologies to sequence TCR beta chains (as performed previously by the Ferris laboratory). The goal of these studies is to examine oligoclonal expansion of T cell subsets and correlate these with clinical outcomes. Clonotypic expansion is a biomarker for a tumor-specific immune response in the absence of knowing tumor antigens of interest a priori.

9.4.7 Germline DNA Analysis for SNPs

To evaluate for and compare variations in DNA that may increase likelihood of prolonged progression-free survival in the context of pembrolizumab plus cisplatin-IMRT for PULA HNSCC, we will isolate DNA from whole blood samples drawn at baseline for single-nucleotide polymorphism (SNP) analysis.

9.4.8 Cryopreserved PBMC will be kept in 10% DMSO in liquid nitrogen for analysis of strength and breadth of HPV16 E6/E7-specifc T cell responses by a validated interferon γ Elispot assay. This assay will be performed to assess responses to ISA101b. In addition it will be informative to evaluate differences in HPV16 E6/E7-specific immune responses between long term clinical responders and low/non clinical responders. In HLA-A2+ patients the level of tetramer+ CD8+ T cells will be evaluated. In addition, if suffcient cryopreserved PBMC are available, poistive IFN γ Elispot assyas will be followed by intracellular cytokine staining assayed by intracellular cytokine staining in flow cytometry of CD4+ and CD8+ T cells.

10. STATISTICAL METHODS

This is a single arm phase 2 trial evaluating the efficacy of the addition of the ISA101b to pembrolizumab plus standard cisplatin-RT in intermediate risk HPV-16+ PULA HNSCC. This study is being performed in order to evaluate the oncologic efficacy of ISA101b with pembrolizumab and CRT and to characterize the safety profile of the combination.

10.1 Objectives

Primary Objective:

To estimate two year progresssion-free survival of the combination of ISA101b, pembrolizumab and cisplatin-based CRT in intermediate risk HPV positive patients and to decide if the outcome warrants further investigation of the combination when compared to the current standard of care (CRT) for this patient population.

Secondary Objective:

- To evaluate the toxicity of the combination of pembrolizumab and ISA101b and concurrent cisplatin-IMRT in patients with PULA HPV-16+ "intermediate risk" HNSCC

10.2 Sample Size

Sample size is determined by the primary objective of testing whether the addition of ISA101b+ pembrolizumab improves two year progression-free survival in HPV-16+ OPSCC patients treated with cisplatin CRT. The historical control for the study is based part upon the results of O'Sullivan and Huang[45] in which the probability of 3 year OS was approximately 65% for high risk (Class III, T4 or N3, age < 70) HPV+ patients treated with chemoradiation and that 3 year OS is an approximation of two year PFS. For the combination of pembrolizumab, ISA101b, and chemoradiation to be considered for further study it is desirable to demonstrate a 25% relative improvement in 2 year PFS from 65% to 81%. We plan to devote 2 years to patient accrual with 1 additional year of follow-up after closing accrual. Using a one-tailed exponential test (using the maximum likelihood estimator for the hazard rate of the exponential distribution) at $\alpha = .10$, 45 patients will provide 87% power to reject the null hypothesis of 65% two year PFS when the underlying two year PFS is 81%. In order to obtain 45 evaluable patients, we will oversample through a total accrual of 50 fully treated patients. Thus, the planned enrollment to this trial is 50 patients.

10.3 Continuous Monitoring for Treatment Delay

A Phase I trial of pembrolizumab plus cisplatin chemoradiation in HNSCC has been reported, demonstrating safety of this combination. Thus, unexpected toxicities are not anticipated. While the ISA101b vaccine may lead to allergic type reactions, it can be readily discontinued in this case, with little harm to the patient as long as overall standard of care therapy continues. Thus, the primary monitoring is for delay in delivery of potentially curative chemoradiation therapy.

A Bayesian continuous monitoring schema will be implemented to protect against unexpected delays of two weeks or greater in the post-vaccine administration of radiation + DDP due to adverse effects of ISA and/or pembrolizumab. A 30% rate of treatment delay is considered unacceptable. We will suspend the trial pending DSMB review if the posterior probability that the 2 week treatment delay rate exceeds 30% is greater than 50%. In addition, the study will be suspended for any death that is possible related to the administration of investigational products. In such a case, a thorough assessment of the safety profile must be conducted, and the risk benefit consideration must be justified for any additional subject enrollment. Monitoring will commence with the third patient completing ISA101b + pembrolizumab and continue, if needed, through 50 patients. If π denotes a random variable representing the proportion of subjects who experience a delay of two weeks or more, we assume π has a prior beta distribution with a mean of .15 and parameters a = 1.5 and b = 8.5.

Selected values of the posterior probability PP ($\pi \geq .30$ |# delays and prior) are shown in the table below. Also shown are selected binomial probabilities of suspending the study for observed count of observation failures.

Number Patients	Number Delays	Min PP($\pi \geq .30$)	P(x > # delays | $\pi = .30$
3 - 4	3	.541	.084
5 – 7	4	.558	.126
8 – 11	5	.513	.210
12 -14	6	.530	.219
15 – 17	7	.545	.225
18 – 21	8	.511	.277
22 – 24	9	.525	.275
25 - 27	10	.539	.272
28 – 31	11	.509	.312
32 – 34	12	.522	.307
35 – 37	13	.534	.302
38 – 41	14	.508	.335
42 – 44	15	.520	.328
45 – 47	16	.531	.322
48 – 50	17	.541	.316

10.4 Proposed Data Analysis

At study completion a one-sided one sample exponential test will be conducted to detect an increase above the presumed historical control rate of 65% PFS at two years. If this test is rejected at $\alpha = .10$, the treatment regimen will be considered sufficient for further investigation. Probabilities of times to local recurrence, regional recurrence and death will be estimated by the Kaplan-Meier method with 95% confidence intervals as well as by cumulative incidence if needed to adjust for the competing risk of death. Treatment-related toxicities will be summarized by frequency and grade. If intervening deaths are observed cumulative incidence plots and analysis of competing risks may be conducted.

A series of correlative studies will be conducted based on blood and tissue samples collected prior to treatment among patients treated with pembrolizumab + ISA101b. The first item below is a planned analysis with alpha spending of 10% to be distributed among the biomarker endpoints.

1. Test the association of baseline H score of PD-L1 tumor expression, circulating antibodies against HPV, EGFR and p53 and other tumor antigens upon PFS using proportional hazards regression adjusting if needed for confounding variable such as T stage, N stage and smoking history.
2. Estimate and test the effect of ISA101b using paired (pre/post pembrolizumab +/- ISA101b) values of immune and inflammatory makers will be compared with the Wilcoxon test.
3. Compare differences in circulating HPV, EGFR, p53 and other tumor antigen antibodies using baseline versus 3-6 weeks or 6 months post-treatment. Linear mixed effect models will be used to evaluate the longitudinal response.
4. Estimate and characterize sequential series of blood samples pre- and post-treatment for a series of immune and inflammatory markers. These will be specified by the investigator and due to exploratory nature of these additional tests, p values will be adjusted for false discovery.

The change in CD4, CD8, FOXp3, PD-1, PD L1, and PD-L2 over time will be described graphically. An exploratory analysis of the relationship between those changes and PFS will be conducted by proportional hazards regression.

To define the neoepitope landscape and to delineate alterations in transcriptomic signature, we will complete whole exome sequencing (WES) and RNA-Seq on all 45 patients who receive pembrolizumab. Fisher's Exact test will be used to compare mutational burdens and differences in the frequency of nucleotide changes. Differentially

expressed genes will be identified and differences between treatment groups and clinical response groups will be explored. Log-rank tests will be used to compare Kaplan-Meier curves, and the relationship with PFS will be further characterized by proportional hazards (Cox) regression.

To define the alterations in TCR clonality between matched baseline and Week 2 CRT biopsies from this trial, we will use the ImmunoSeq assay from Adaptive Biotechnologies to sequence TCR beta chains. Differences in TCR clonality between treatment groups and clinical response groups will be explored using Fisher exact test.

10.5 Bayesian Continuous Monitoring for Futility

We will continuously monitor PFS data for futility. Under the null hypothesis of no improvement in PFS due to the combination of pembrolizumab and ISA1-1b, the expected 2-year PFS is assumed to be 65%. Under the alternate hypothesis, PFS would improve to 75%. Assuming an exponential distribution, 65% PFS at two years is equivalent to an annual hazard rate of .215. To monitor efficacy, we will use Bayesian monitoring. We have selected a conjugate prior distribution for the annual hazard rate that has a Gamma distribution with a shape parameter of 4 and a rate of 18.6. The provides a mean of .215 and 80% confidence limits of (.09 - .36), equivalent to a 2 year PFS between 49 and 83%. We will close the trial for futility if there is low probability that the combination therapy will be effective. Specifically, if the posterior probability that the 2-year progression-free survival is greater than 65%, is less than 10%, the trial will be closed for futility. Monitoring and updating the posterior probability can occur at any time at the discretion of the investigator, either at regular intervals or as each patient completes follow-up.

11. LABELING, PACKAGING, STORAGE, AND RETURN OF CLINICAL SUPPLIES

11.1 Investigational Products

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

Clinical Supplies will be provided by Merck as summarized in Table

7. Table 12. Product Descriptions

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

Product Name & Potency	Dosage Form
ISA101b	Sterile Lyophilized Powder for Subcutanous Injection after reconstitution (-20°C and in a secure location)

11.2 Packaging and Labeling Information

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

11.3 Clinical Supplies Disclosure

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

11.4 Storage and Handling Requirements

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

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

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

11.5 Storage and Handling Requirements

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

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

12. ASSESSING AND REPORTING ADVERSE EVENTS

12.1 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related.

Suspected adverse reaction. Any adverse event for which there is a reasonable possibility that the drug caused the adverse event (considered "possibly related"). For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of suspected adverse reactions where there is reason to conclude that the drug caused the event.

Serious Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Specifically, results in death, is life-threatening, requires inpatient hospitalization or causes prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Any subject death within 30 days of the last dose of study drug, regardless of the causality or a secondary malignancy should also be recorded as a serious adverse event.

Life-threatening, suspected adverse reaction. A suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator), its occurrence places the patient or

research subject at immediate risk of death. It does not include a suspected adverse reaction that had it occurred in a more severe form, might have caused death.

Unexpected, suspected adverse reaction. A suspected adverse reaction is considered "unexpected" if it is not listed in the general investigational plan or clinical protocol; or is not listed at the specificity or severity that has been previously observed and/or specified. If an investigator brochure is not required or available, suspected adverse reaction is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation. Any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure can also be considered unexpected. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) will be recorded in the subjects' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a serious adverse event) and; 2) an assessment of the casual relationship between the adverse event and the study drug(s). All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 5.0.

Adverse events or abnormal test findings felt to be associated with the study drug(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Principal Investigator.

In the event of an adverse event the first concern will be for the safety of the subject.

12.2 Review of Safety Information

The principal investigator/sponsor must promptly review all information **relevant to the safety of the drug obtained or otherwise received from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States. The study sponsor must notify all participating investigators of potential serious risks, from clinical trials or any other source, as soon as possible. Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, to Merck and to ISA.**

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

For purposes of this trial, an overdose of ISA101b will be defined as any dose of 1000 mcg per peptide or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of ISA101b. 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 pembrolizumab or ISA101b, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of pembrolizumab or ISA101b meeting the protocol definition of overdose is administered 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 of pembrolizumab with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220) using the departmental SAE form or on a Form FDA 3500 MedWatch.

All reports of overdose of ISA101b with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days to the ISA contact: visscher@isa-pharma.com , +31 71 3322311 using the departmental SAE form or on a Form FDA 3500 MedWatch.

12.3 Reporting of Pregnancy and Lactation to the Sponsor, to ISA, and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy 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 within 24 hours to the Sponsor and within 2 working days to **the Merck Global Safety facsimile number: +1-215-993-1220**

AND

to the ISA contact: visscher@isa-pharma.com , +31 71 3322311.

using the departmental SAE form or on a Form FDA 3500

MedWatch.

12.4 Immediate Reporting of Adverse Events to the Sponsor, Institutional IRB, to ISA and to Merck

12.4.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any time during protocol treatment with ISA101b,, pembrolizumab, cisplatin and IMRT that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization (NOTE: Elective outpatient procedures for feeding tube placement do not constitute SAEs in this protocol.)
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Refer to Table 13 below for additional details regarding each of the above criteria.

For the time period beginning when the first dose is administered, until 90 days after last dose, any serious adverse event, or follow up to a serious adverse event, including death due to any cause that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety and ISA using the CRS departmental SAE form if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning when the first dose is administered through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to pembrolizumab, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety and ISA using the departmental SAE form. Elective outpatient procedures for feeding tube placement do not require expedited Adverse Event Reporting.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and to ISA, 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 pembrolizumab or ISA101b that is brought to the attention of the investigator at any time following fist administration of treatment through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported to the Sponsor and to Merck Global Safety and to ISA101b using the departmental SAE form.

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

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220 AND
to the ISA contact: visscher@isa-pharma.com , +31 71 3322311.

12.4.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to **Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220) and** to the **ISA contact: visscher@isa-pharma.com , +31 71 3322311** using the departmental SAE form

For the time period beginning from when the first dose is administered, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety and to ISA if it causes the subject to be removed from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or placebo treatment.

For the time period beginning from when the first dose is administered through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety and to ISA.

Events of clinical interest for this trial include:

1. an overdose of pembrolizumab or ISA101b, as defined in Section 12.2 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. for pembrolizumab only, 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.

Table 13. Reporting Time Periods and Time Frames for Adverse Events and Other Reportable Safety Events

Type of Event	**Reporting Time Period:** **Consent to Randomization/ Allocation**	**Reporting Time Period:** **Randomization/ Allocation through Protocol-specified Follow-up Period**	**Reporting Time Period:** **After the Protocol-specified Follow-up Period**	**Time Frame to Report Event and Follow-up Information to Merck:**
Serious Adverse Event (SAE)	Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all	Report if: - drug/vaccine related. (Follow ongoing to outcome)	Within 2 business days but no longer than 3 calendar days of receipt of information
Pregnancy/Lactation Exposure	Report if: - participant has been exposed to any protocol-specified intervention (eg, procedure, washout or run-in treatment including placebo run-in) Exception: A positive pregnancy test at the time of initial screening is	Report all	Previously reported – Follow to completion/termination; report outcome	Within 2 business days but no longer than 3 calendar days of receipt of information

Type of Event	Reporting Time Period: Consent to Randomization/ Allocation	Reporting Time Period: Randomization/ Allocation through Protocol-specified Follow-up Period	Reporting Time Period: After the Protocol-specified Follow-up Period	Time Frame to Report Event and Follow-up Information to Merck:
	not a reportable event.			
Event of Clinical Interest (require regulatory reporting)	Report if: - due to intervention - causes exclusion	Report - potential drug-induced liver injury (DILI) - require regulatory reporting	Not required	Within 2 business days but no longer than 3 calendar days of receipt of information
Cancer	Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all	Not required	Within 2 business days but no longer than 3 calendar days of receipt of information
Overdose	Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all	Not required	Within 2 business days but no longer than 3 calendar days of receipt of information

Evaluating Adverse Events

Table 14. Evaluating Adverse Events
An investigator who is a qualified physician, will evaluate all adverse events as to:

V5.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of pembrolizumab that:	
	†**Results in death**; or	

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

	The following components are to be used to assess the relationship between the test drug and the AE: (continued)	
Relationship to pembrolizumab, cisplatin, and/or IMRT (continued)	**De-challenge**	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive de-challenge. If no, this is a negative de-challenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Re-challenge	Was the subject re-exposed to pembrolizumab in this study? If yes, did the AE recur or worsen? If yes, this is a positive re-challenge. If no, this is a negative re-challenge. (Note: This criterion is not applicable if the initial AE resulted in death or permanent disability. NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY PEMBROLIZUMAB, OR IF REEXPOSURE TO PEMBROLIZUMAB POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE PRINCIPAL INVESTIGATOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding pembrolizumab or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of pembrolizumab relationship).**	
Yes, there is a reasonable possibility of pembrolizumab relationship.	There is evidence of exposure to pembrolizumab. The temporal sequence of the AE onset relative to the administration of pembrolizumab is reasonable. The AE is more likely explained by pembrolizumab than by another cause.	
No, there is not a reasonable possibility of pembrolizumab relationship	Subject did not receive pembrolizumab OR temporal sequence of the AE onset relative to administration of pembrolizumab is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	
Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding ISA11b or class pharmacology or toxicology as described in the ISA101b Investigator Brochure?	
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	Use the following scale of criteria as guidance (not all criteria must be present to be indicative of ISA101b relationship).	
Yes, there is a reasonable possibility of ISA101b relationship.	There is evidence of exposure to ISA101b. The temporal sequence of the AE onset relative to the administration of ISA101b is reasonable based on the information in the ISA101b Investigator Brochure. The AE is more likely explained by ISA101b than by another cause.	

No, there is not a reasonable possibility of ISA101b relationship	Subject did not receive ISA101b OR temporal sequence of the AE onset relative to administration of ISA101b is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

Timelines for Reporting of Suspected Adverse Events

All events meeting the definition of a serious adverse event should be reported according to the departmental SAE checklist and SAE form. Serious adverse events are collected from the date of the subject's first dose of treatment until 90 days after the final dose. The initial SAE form should be sent to the following within 24 hours/1 business day of the Sponsor-Investigator becoming aware:

1. Dan Zandberg, MD
2. crssafetysubmissions@upmc.edu
3. Local Institutional Review Board when reporting requirements are met.

Merck Global Safety (Attn: Worldwide Product Safety; FAX 215 993-1220) – within 2 working days of the Investigator learning of the event

ISA operational contact (visscher@isa-pharma.com; FAX +31 71 33 22 311) – within 2 working days of the Investigator learning of the event.

In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the Sections B and C of the departmental SAE form:

- CTCAE term(s) and grade(s)
- current status of study drug
- all interventions to address the AE (testing and result, treatment and response)
- hospitalization and/or discharge dates
- event relationship to study drug

Follow-up reports:

All SAEs should be followed to resolution or stabilization. Additional information may be added to a previously submitted report by adding to the original departmental SAE form and submitting it as follow-up or creating supplemental summary information and submitting it as follow-up with the original departmental SAE form. All follow-up forms must include the date the form is revised.

12.4.3 Review of Safety Information: Sponsor-Investigator Responsibilities

The sponsor must promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States.

Note : The requirements of the Sponsor for the reporting of suspected adverse drug reactions to the FDA differ from the requirements of the Investigator (see below and Investigator Responsibilities on the O3IS website) for the reporting of adverse events to the Sponsor. Sponsor-investigators of IND applications are subject to compliance with both the adverse reaction reporting requirements of the Sponsor and the adverse event reporting requirements of the Investigator.

12.4.4 IND safety reports

The sponsor must notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under Sections 1.5.1 to 1.5.4 below. In each IND safety report, the sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction, and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

12.4.5.1 Serious and Unexpected Suspected Adverse ReactionS

The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

12.4.5.2 Findings From Other Studies

The sponsor must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies (other than those reported under section 1.5.1), whether or not conducted under an IND, and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug. Ordinarily, such a finding would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

12.4.5.3 Findings from Animal or In Vitro Testing

The sponsor must report any findings from animal or in vitro testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity at or near the expected human exposure. Ordinarily, any such findings would result in a safety-related change in the protocol, informed consent, investigator brochure (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

12.4.5.4 Increased rate of occurrence of serious suspected adverse reactions

The sponsor must report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

12.4.5 Submission of IND safety reports

The sponsor must submit each IND safety report in a narrative format or on Form FDA 3500A or in an electronic format that FDA can process, review, and archive. FDA will periodically issue guidance on how to provide the electronic submission (e.g., method of transmission, media, file formats, preparation and organization of files). The sponsor may submit foreign suspected adverse reactions on a Council for International Organizations of Medical Sciences (CIOMS) I Form instead of a Form FDA 3500A. Reports of overall findings or pooled analyses from published and unpublished in vitro, animal, epidemiological, or clinical studies must be submitted in a narrative format. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division in the Center for Drug Evaluation and Research or in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. Upon request from FDA, the sponsor must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

12.4.6.1 Unexpected fatal or life-threatening suspected adverse reaction reports

The sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

12.4.6.2 Reporting format or frequency

FDA may require a sponsor to submit IND safety reports in a format or at a frequency different than that required under this paragraph. The sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the FDA review division that has responsibility for review of the IND.

12.4.6.3 Investigations of marketed drugs

A sponsor of a clinical study of a drug marketed or approved in the United States that is conducted under an IND is required to submit IND safety reports for suspected adverse reactions that are observed in the clinical study, at domestic or foreign study sites. The sponsor must also submit safety information from the clinical study as prescribed by the post marketing safety reporting requirements.

12.4.6.4 Reporting study endpoints

Study endpoints (e.g., mortality or major morbidity) must be reported to FDA by the sponsor as described in the protocol and ordinarily would not be reported under Section 1.7 third bullet of this section. However, if a serious and unexpected adverse event occurs for which there is evidence suggesting a causal relationship between the drug and the event (e.g., death from anaphylaxis), the event must be reported under Serious and unexpected suspected adverse reaction as a serious and unexpected suspected adverse reaction even if it is a component of the study endpoint (e.g., all-cause mortality).

12.4.6 Follow-up

- The sponsor must promptly investigate all safety information it receives.
- Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Follow-up IND Safety Report."
- If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable under section IND safety reports of this section is so reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

12.4.7 Disclaimer

A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse event. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse event.

The principal investigator must promptly review all information relevant to the safety of the drug obtained or otherwise received from foreign or domestic sources, including information derived from any clinical or epidemiological investigations, animal or in vitro studies, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities and reports of foreign commercial marketing experience for drugs that are not marketed in the United States. The study sponsor must notify all participating investigators of potential serious risks, from clinical trials or any other source, as soon as possible.

13. DATA SAFETY AND MONITORING PLAN

The study will be monitored by the UPCI/UPMC Data Safety and Monitoring Committee (DSMC). In addition, Investigator/Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in the head and neck cancer disease center Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- serious adverse events

- subject safety issues

- recruitment issues
- accrual
- protocol deviations
- unanticipated problems
- breaches of confidentiality

Minutes from the discease center DSMB meetings are available to those who are unable to attend in person.

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 5.0. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB and FDA, as per guidelines. Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPMC Hillman Cancer Center DSMC which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed bi-annually.

Both the UPMC Hillman Cancer Center DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

14. ADMINISTRATIVE AND REGULATORY DETAILS

14.1 Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically (i.e., at a minimum of annually) by qualified staff of the Education and Compliance Office – Human Subject Research, Research Conduct and Compliance Office, University of Pittsburgh.

The Sponsor-Investigator and the University of Pittsburgh and UPMC will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

14.2 Data Handling and Record Keeping

The Sponsor-Investigator will maintain records in accordance with Good Clinical Practice guidelines.

The Sponsor-Investigator will retain the specified records and reports for at least 7 years after the marketing application is approved for both investigational drugs; or, if a marketing application is not submitted or approved for the investigational drug, until 7 years after investigations under the IND have been discontinued and the FDA so notified.

15. APPENDICES

15.1 Appendix A: Tobacco Use Assessment Form

1. Have you ever smoked a total of 100 cigarettes (approximately 5 packs) or more over your life-time?
   ○ Yes ○ No
2. Have you ever smoked cigarettes regularly, that is, at least one cigarette per day for six months or longer?
   ○ Yes ○ No
3. How old were you when you first started smoking at least one cigarette per day?
   _____years old
4. Do you currently smoke cigarettes?
   ○ Yes ○ No
   If no, how old were you when you last smoked a cigarette?
   _____years old
5. Thinking about all the years that you have smoked, how many cigarettes do you (or did you) usually smoke in a day?
   ○ 1-9 cigarettes per day
   ○ 10 to 19 cigarettes per day
   ○ 20 to 29 cigarettes per day
   ○ 30 to 39 cigarettes per day
   ○ 40 to 49 cigarettes per day

6. Have you ever smoked cigars regularly, that is, at least one cigar per day for six months or longer?
   ○ Yes ○ No

7. How old were you when you first started smoking at least one cigar per day?
   _____years old

8. Do you currently smoke cigars?
   ○ Yes ○ No
   If no, how old were you when you last smoked a cigar?
   _____years old
9. How many cigars did you usually smoke in a day?
   _____cigars per day

10. Have you ever smoked a pipe regularly, that is, at least one pipe per day for six months or longer?
    ○ Yes ○ No
11. How old were you when you first started smoking at least one pipe per day?
    _____years old
12. Do you currently smoke a pipe?
    ○ Yes ○ No
    If no, how old were you when you last smoked a pipe?
    _____years old
13. Thinking about all the years that you have smoked, how many pipes do you (or did you) usually smoke in a day?
    _____pipes per day

15.2 Appendix B: Performance Status Criteria

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

15.3 Appendix C: Study Calendar

		Study Treatment (weeks)											End of Tx[s]	Long Term Follow Up[n]
	Pre-study[a]	-2	-1	0	1	2	3	4	5	6	7	Q3 Weeks (8 – 47)		(at 6, 9, 12, 18, 24, 36, 48, 60 months)
Medical History, PE, Vital Signs[b]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Height	x													
Weight	x	x	x	x	x	x	x	x	x	x	x	x	x	x
ECOG PS	x	x	x	x	x	x	x	x	x	x	x	x	x	x
*CBC with Differential [c]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
*Serum chemistry[d]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
*Liver function[e]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant meds	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Pregnancy test[o]	x	x												
Oropharyngeal cases, p16 plus HPV ISH status[p]	x													
Thyroid function	x					x			x		x	x		x
aPTT, PT/INR	x													
ANA, anti-Tg Ab, TPO, TSI[f]	x					x			x			x[f]		x
H&N/endoscopic evaluation[q]	x[q]											x[q]		
Tobacco history	x													
Tumor measurements, RECIST[g]	x											x[g]		x
PET/CT[h]	x											x[h]		x
CT neck[i]	x											x[i]		x
CT chest[j]	x											x[j]		x
Dental evaluation[k]	x													
Nutrition consult[k]	x													
SLP consult[k]	x													x
Audiogram[k]	x													x

		Study Treatment (weeks)											End of Tx[s]	Long Term Follow Up[n]
	Pre-study[a]	-2	-1	0	1	2	3	4	5	6	7	Q3 Weeks (8 – 47)		(at 6, 9, 12, 18, 24, 36, 48, 60 months)
Cisplatin[r]				x			x							
Pembrolizumab			x			x			x			x		
ISA101b		x				x			x					
IMRT				x	x	x	x	x	x	x				
Adverse event evaluation	X	x	x	x	x	x	x	x	x	x	x	x	x	x
Correlatives: submission of tumor tissue[l]	x				x	x								
Correlatives: blood[m]	x	x	x	x	x	x	x		x		x	x		x

a. Pre-treatment evaluations will be performed within 4 weeks prior to Day -14, unless otherwise specified.
b. Targeted Physical Exam may be performed prior to each treatment visit.
c. Complete blood count with differential (CBCDs) should include WBC, ANC, PLT, Hb, Hct. CBCDs required for protocol therapy may be performed on the day of or the day prior to cisplatin/pembrolizumab administration.
d. Serum chemistry should include Na, K, Cl, CO_2, BUN, Cr, Glucose, Ca, Uric Acid, LDH, and Magnesium.
e. Liver function tests should include SGOT (AST), SGPT (ALT), total bilirubin, total protein, albumin, alkaline phosphatase.
f. ANA, anti-Tg Ab, TPO, TSI will be collected at screening, Week 2, Week 5, and q 6 weeks starting at Week 8 (i.e. Week 8, 14, 20, etc.).
g. A diagnostic contrast CT of the neck and chest will be conducted for staging and tumor measurements at baseline, and 12-14 weeks after completing IMRT. If feasible at participating sites, an integrated PET/CT with diagnostic contrasted CT is recommended at both time points. Tumor measurements will be conducted in accordance with RECIST 1.1. Rare patients may require an MRI for tumor measurements, due to allergy to iodinated contrast despite premedication. In these patients, MRI of the neck should be performed at baseline and at first response assessment. During long term f/u, neck MRI should continue to be performed in the place of neck CT.
h. Integrated PET/CT with diagnostic contrasted neck CT is recommended at pre-study baseline and 12-14 weeks after completing IMRT. However, PET/CT is not required for study participation.
i. CT of the neck (or MRI in those with iodinated contrast allergy) will be conducted at the following intervals post IMRT: 6 mos, 9 mos, 12 mos, 18 mos, 24 mos, 36 mos, 48 mos, 60 mos. More intensive imaging may be conducted per local practice.
j. Chest CT (without contrast in those with iodinated contrast allergy) will be conducted at the following intervals post IMRT: 6 mos, 9 mos, 12 mos, 18 mos, 24 mos, 36 mos, 48 mos, 60 mos. More intensive imaging may be conducted per local practice.
k. These evaluations are strongly encouraged, but not mandatory.

l. A baseline research biopsy for correlative studies will be performed and submitted at baseline prior to any protocol treatment. An optional research biopsy of tumor tissue may be obtained during week 1 (Week 2 of IMRT) and at week 2 of pembrolizumab. It is preferred that the research biopsy precede the radiotherapy fraction on the day of research biopsy.
m. Blood for correlative studies will be taken according to the schedule outlined in section 9.1.
n. Long term follow up assessments should occur every 3 months for 1 year, then every 6 months for 1 year, and yearly for 3 years for a total of 5 years from completion of IMRT. No specific requirements if patient is more than 5 years from study entry. If patient dies during follow-up, cause of death should be recorded.
o. Women of childbearing potential are required to have a pregnancy test within 4 weeks of registration. Repeat pregnancy testing is required within 72 hours of treatment initiation. Pregnancy testing is required as clinically indicated during study treatment.
p. p16 plus HPV ISH status must be established at the participating site, if not previously known before the screening process.
q. Evaluation by an otolaryngologist with endoscopy may be performed as clinically indicated.
r. Cisplatin should be administered on Monday, Tuesday, or Wednesday of each treatment week and may be given either before or after the radiation therapy fraction that is given on the same day.
s. 3 weeks (+/- 1 week) from last dose of Pembrolizumab

*Lab work is to be collected within 10 days prior to Day -14.

15.4 Appendix D: CervISA: Cumulative Summary Tabulation of Treatment Emergent Adverse Events Related to ISA101

With onset date from start vaccination; C2D15

System Organ Class Preferred Term	20µg N=9 n (%)	20µg+INF N=8 n (%)	40µg N=6 n (%)	40µg+INF N=8 n (%)	100µg N=11 n (%)	100µg+INF N=7 n (%)	300µg N=6 n (%)	300µg+INF N=6 n (%)
Any Related Treatment Emergent Adverse Event	4 (44.4)	5 (62.5)	5 (83.3)	6 (75.0)	9 (81.8)	7 (100)	5 (83.3)	6 (100)
Blood and lymphatic system disorders		1 (12.5)		2 (25.0)		1 (14.3)		
Anaemia		1 (12.5)		1 (12.5)		1 (14.3)		
Febrile neutropenia		1 (12.5)		1 (12.5)				
Leukopenia		1 (12.5)						
Neutropenia		1 (12.5)				1 (14.3)		
Thrombocytopenia						1 (14.3)		
Gastrointestinal disorders				1 (12.5)	2 (18.2)			2 (33.3)
Abdominal pain								1 (16.7)
Diarrhoea				1 (12.5)				1 (16.7)
Nausea				1 (12.5)	1 (9.1)			2 (33.3)
Vomiting				2 (18.2)				1 (16.7)
General disorders and administration site conditions	2 (22.2)	3 (37.5)	3 (50.0)	5 (62.5)	8 (72.7)	7 (100)	5 (83.3)	5 (83.3)
Chills						1 (14.3)		
Fatigue				1 (12.5)	1 (9.1)	1 (14.3)	1 (16.7)	3 (50.0)
Influenza like illness		1 (12.5)		2 (25.0)		2 (28.6)	1 (16.7)	1 (16.7)
Infusion site extravasation					1 (9.1)			
Injection site erythema					1 (9.1)	1 (14.3)		
Injection site induration								1 (16.7)
Injection site pruritus	1 (11.1)							
Injection site reaction		1 (12.5)		3 (37.5)	5 (45.5)	4 (57.1)	3 (50.0)	2 (33.3)
Malaise		1 (12.5)			2 (18.2)	1 (14.3)		
Pyrexia	1 (11.1)	2 (25.0)		1 (12.5)	2 (18.2)	2 (28.6)		2 (33.3)
Systemic inflammatory response syndrome							1 (16.7)	
Vaccination site erythema			1 (16.7)					
Vaccination site induration			2 (33.3)		1 (9.1)		1 (16.7)	
Vaccination site nodule						1 (14.3)		
Vaccination site pain						1 (14.3)		
Vaccination site pruritus			1 (16.7)					
Vaccination site reaction		1 (12.5)					1 (16.7)	2 (33.3)
Vaccination site swelling			1 (16.7)	1 (12.5)				
Immune system disorders								2 (33.3)
Hypersensitivity								2 (33.3)
Infections and infestations		1 (12.5)				1 (14.3)		2 (33.3)
Influenza						1 (14.3)		
Nasopharyngitis								1 (16.7)
Oral herpes								1 (16.7)
Vaccination site infection		1 (12.5)						
Injury, poisoning and procedural complications					1 (9.1)	1 (14.3)		4 (66.7)
Infusion related reaction					1 (9.1)			
Injection related reaction						1 (14.3)		1 (16.7)
Vaccination complication								3 (50.0)
Investigations		1 (12.5)						
Platelet count decreased		1 (12.5)						
Metabolism and nutrition disorders			1 (16.7)	1 (12.5)				
Decreased appetite			1 (16.7)	1 (12.5)				
Musculoskeletal and connective tissue disorders	1 (11.1)				1 (9.1)			1 (16.7)
Back pain								1 (16.7)
Muscle spasms					1 (9.1)			
Myalgia								1 (16.7)
Pain in extremity	1 (11.1)							
Nervous system disorders								1 (16.7)
Headache								1 (16.7)
Psychiatric disorders				1 (12.5)				
Bradyphrenia				1 (12.5)				
Respiratory, thoracic and mediastinal disorders					1 (9.1)			1 (16.7)
Dyspnoea exertional					1 (9.1)			
Pulmonary embolism								1 (16.7)
Skin and subcutaneous tissue disorders					1 (9.1)			
Pruritus					1 (9.1)			
Vascular disorders			1 (16.7)					
Haematoma			1 (16.7)					
Not yet coded	2 (22.2)	2 (25.0)	1 (16.7)		1 (9.1)	1 (14.3)		1 (16.7)

Appendix E Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*

• Combined (estrogen- and progestogen- containing) hormonal contraception [b, c]
  ○ Oral
  ○ Intravaginal
  ○ Transdermal
  ○ Injectable

• Progestogen-only hormonal contraception [b, c]
  ○ Oral
  ○ Injectable

Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
• Progestogen- only contraceptive implant [b, c] • Intrauterine hormone-releasing system (IUS) [b] • Intrauterine device (IUD) • Bilateral tubal occlusion
• **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
• **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e. when used consistently and correctly). b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least [X days, corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential] after the last dose of study treatment. c) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

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