Mayo Clinic Cancer Center

MC200710 Stimulating Immune Response with Neoadjuvant Human Papilloma Virus (HPV)-16 specific Vaccination in HPV-Oropharyngeal Squamous Cell Carcinoma (HPV-OPSCC)

Sponsor/Principal Investigator: [redacted]

Co-Principal Investigators: [redacted]

Co-Investigators: [redacted]

Statistician: [redacted]

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√Study contributor(s) not responsible for patient care

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Drug Company Supplied: PDS0101 (ImmunoMAPK-RDOTAP/HPV-16 E6 & E7 peptides)

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Protocol Resources

Questions:	Contact Name:
Patient eligibility*, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion and submission	[redacted]
Clinical coordinators (lead site first)	[redacted]
Clinical data coordinators (lead site first)	[redacted]
Protocol document, consent form, regulatory issues	[redacted]
Laboratory Contact for Correlative Studies	[redacted]
Serious Adverse Event Reporting	[redacted]

*No waivers of eligibility allowed

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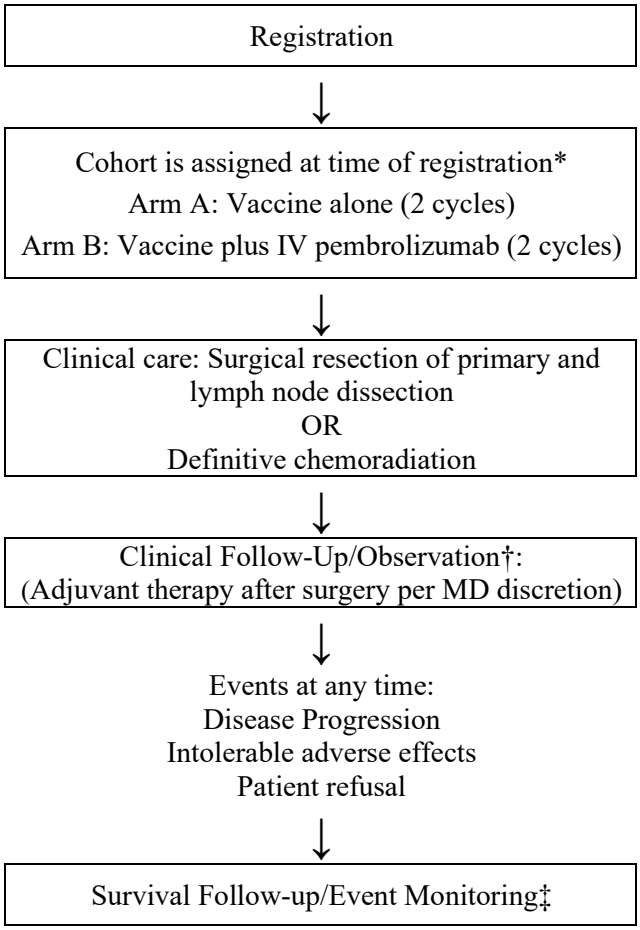
Schema

Prior to discussing protocol entry with the patient, contact the Mayo Clinic Research Site Management Office [redacted] for dose level and to ensure that a place on the protocol is open to the patient.



*Cohorts are enrolled sequentially in a cohort of 3 design: Arm A 3 patients, followed by Arm B 3 patients, followed by Arm A 3 patients, followed by Arm B 3 patients, until 18 patients are enrolled. Then enrollment will alternate between Arms A and B with each patient until up to 10 evaluable patients per arm are enrolled. The goal is to ensure an equal number of evaluable patients on each arm. If one arm achieves 10 evaluable patients, we will continue enrollment exclusively to the incomplete arm, with a total enrollment of 24 patients maximum.

†See Section 13.4

‡See Section 4.2

Cycle = 21 ±3 days for two cycles

Generic names: Pembrolizumab, MK3475 Brand name(s): KEYTRUDA Availability: Commercial (covered by study funds)	Generic names: PDS0101 (ImmunoMAPK-RDOTAP/HPV-16 E6 & E7 peptides) Brand name(s): NA Availability: provided by PDS Biotechnology Corp.

1.0 Background

1.1 Head and neck cancers

Human papillomavirus-associated oropharynx cancer (HPV-OPSCC) is the most common type of head and neck cancer and has a much better prognosis compared to tobacco-related head and neck squamous cell carcinoma (HNSCC) with 5 year survival of approximately 90% versus 40%, respectively (Ang, Harris, Wheeler, & et al., 2010) [1]. As many patients with HPV-OPSCC are younger with fewer comorbidities and could potentially live for years with the sequelae of cancer treatment, there is great interest in treatment "de-intensification." We and others have investigated de-intensification of standard therapy for the HPV-OPSCC.

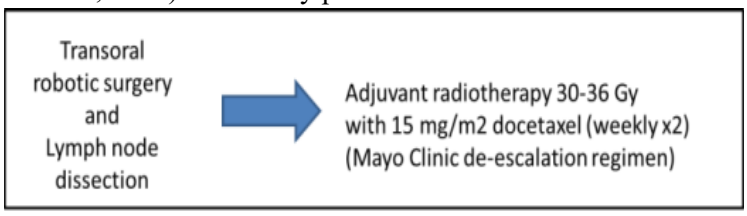


Figure 1. Schema of MC1273 phase 2 study

The published "Mayo regimen" (**Figure 1**) investigated a decreased dose of adjuvant radiotherapy (RT) to 30 -36 Gy with chemotherapy following curative intent, margin-clearing transoral robotic surgery (TORS) for HPV-OPSCC, instead of the standard of care (SOC) adjuvant RT dose of 60 Gy. Our study demonstrated overall survival (OS) and loco-regional recurrence free survival (LRFS) comparable to historical controls.

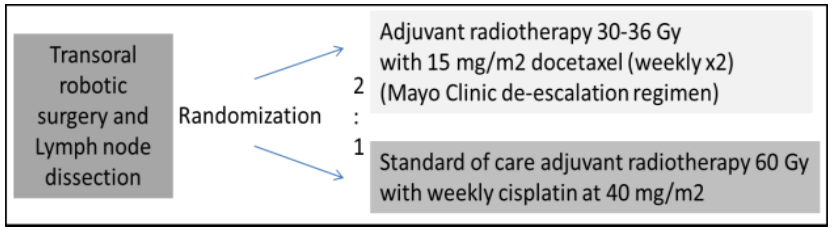


Figure 2. Schema of MC1675, randomized phase 3 study

The 2-year locoregional tumor control rate was 96.2% (Ma, Price, Moore, & et al., 2019) [2].

Our group has subsequently completed a randomized phase 3 study comparing this "Mayo regimen" to the SOC (60 Gy adjuvant RT, respectively). The trial has completed accrual and the results are maturing. This was a Mayo Clinic only study and accrued approximately 250 patients (**Figure 2**).

We have also pioneered the use of HPV cell-free tumor DNA (ctHPVDNA) in patients undergoing TORS. It is detectable in 90% of patients at diagnosis and 95% of the patients are HPV16. In

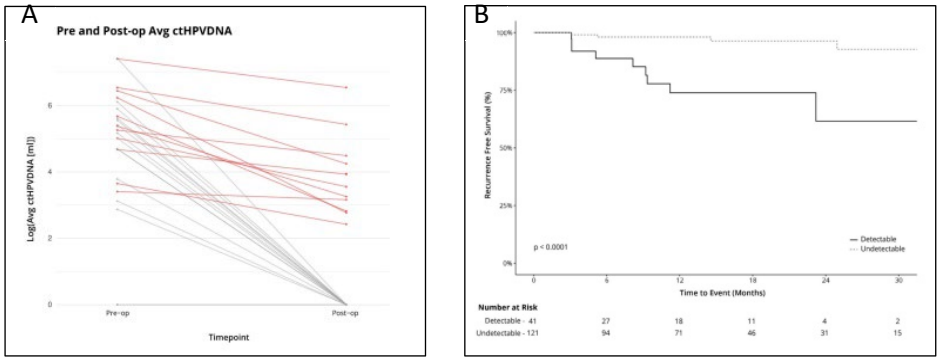


Figure 3. (A) Pre and post-op ctHPVDNA, red lines indicate patients with positive PCR post-op. (B) Post-op ctHPVDNA was associated with worse recurrence-free survival (HR 4.919, p0.012)

addition to clinical factors such as nodal and tumor staging, ctHPVDNA is an independent predictor of recurrence-free survival (RFS) (**Figure 3**) (Routman, et al., 2019, full manuscript under review).

1.2 Rationale for "intensification" of HPV-OPSCC at high risk of recurrence

However, contrary to the expectation, not all patients with HPV-OPC have a good prognosis. Approximately 10-20% of patients with HPV-OPSCC suffer from locoregional recurrence (LRR) or distant metastasis (DM) despite standard therapies (Weller, Ward, Berriochoa, & et al., 2017) (Guo, Rettig, & Fakhry, 2016) [3] [4]. In addition to clinical predictors such as number of lymph nodes involved or extranodal extension (ENE), we have identified ctHPVDNA as predictor of recurrence (see above). These select patients may need intensification of systemic therapies to reduce their risk of recurrence. In Keynote-048, a phase 3 randomized study, pembrolizumab with or without cytotoxic chemotherapy has demonstrated improved OS in recurrent/metastatic (R/M)-HNSCC (Burtness, Harrington, Greil, & et al., 2019) [5]. PDS0101 has been shown to induce HPV-specific immune responses alone and in combination with checkpoint inhibitors in preclinical studies and is currently being investigated in recurrent/metastatic setting for HPV-related cancers (Investigators brochure PDS0101).

1.21 PDS0101 Clinical Experience and Dose Selection

A phase I clinical study of PDS0101 demonstrating strong clinical HPV16-specific T-cell responses and safety has been completed. The results of the study suggest effective induction of HPV16-specific T-cell immunogenicity over a broad dose range and no SAEs.

Twelve female subjects entered the study: 3 subjects in Cohort 1 (1.0 mg R-DOTAP dose and 2.4 mg HPVmix), 3 subjects in Cohort 2 (3.0 mg R-DOTAP dose and 2.4 mg HPV mix), and 6 subjects in Cohort 3 (10 mg R-DOTAP dose and 2.4 mg HPVmix). All subjects received all 3 doses of PDS0101 administered by SC injection, as scheduled on Days 1, 22, and 43. Per protocol, all subjects were women with confirmed high-risk HPV infection and biopsy-proven CIN1. Ages ranged from 24 to 51 years; the median age was 33.0 years. All subjects had blood draws pre-vaccination and 14 days (±5 days) after each vaccination, and once at 90 days after vaccination 3 for immunogenicity testing.

1.211 T-cell Immunogenicity:

Except for 2 subjects (Subject 005-002 in the 1.0 mg R-DOTAP cohort and Subject 002-002 in the 3.0 mg cohort), outliers who had unusually high baseline HPV16 T cell responses, all remaining 10 subjects had a positive vaccine-induced response (a ≥3- fold increase over baseline at ≥1 of the 4 post-vaccination visits by either the IFN-γ or granzyme B ELISpot assays, with background counts subtracted).

By IFN-γ assay (HPV16-specific T-cells), 9 subjects (9/10, 90%) had a positive vaccine-induced response; 6 subjects (6 of 9, 67%) had responses by granzyme B (1 additional subject had insufficient specimen for granzyme B testing).

Results from both the IFN-γ and the granzyme B assays strongly suggest that there could potentially be an increase in HPV16-specific T-cell responses with the 3.0 mg R-DOTAP dose over the 1.0 mg dose but showed no evidence suggestive of an increased response of the 3.0 mg R-DOTAP dose over the 10.0 mg R-DOTAP dose. Greather than 20-fold increases in HPV16-speccific CD8+ T cell responses over pre-treaatment levels were observed at the recommemded phase 2 R-DOTAP dose of 3.0mg.

No clear trend in T-cell response relative to post-vaccination timepoint (14 days post-vaccination 1, 2, or 3, or 90 days post-vaccination 3) was observed. All doses were active in inducing HPV16-specific T-cell responses.

IFN-γ (all T-cells) and the granzyme B (CD8+ T-cells) responses were elicited across all R-DOTAP doses tested in the study subjects who had various HLA types.

Documented high levels of circulating Granzyme-B CD8+ T-cells prompted an IRB-approved retrospective evaluation of clinical outcomes for 24 months following the 3rd dose of PDS0101. Clinical responses (reported regression / elimination of CIN) determined by cytology and/or colposcopy as available, were observed in 60% of evaluable patients across the three tested doses, with the timing of these responses documented as early asa 1 to 3 months in some patients.

1.212 Safety

All subjects reported treatment-emergent adverse events (TEAEs); the majority were administration site reactions.

All administration site reactions were deemed treatment-related.

Most administration site reactions were mild or moderate in severity. Some severe administration site reactions were reported in the 3.0 and 10.0 mg R-DOTAP cohorts (grading of serious as per the FDA Toxicity Grading scale for Healthy Volunteers in Preventative Vaccine Trials September 2007). Most administration site reactions resolved the same day or within a few days, although skin discoloration at the injection site took up to several weeks to resolve in some subjects.

Per the Subject Symptoms Diary, administration site reactions were more serious and of longer duration in subjects in the 10.0 mg R-DOTAP cohort.

Administration site reactions (injection site reactions) included: Common events (>10%): site swelling, site pain, site skin discoloration or erythema (redness), and Uncommon events (1-10%) site fibrosis, site discomfort, site inflammation, site pruritis, site papule, site nodule, site paresthesia, site inflammation, site rash.

No clinically significant differences in the types and pattern of TEAEs were observed between vaccinations 1, 2, or 3.

No DLTs were observed; thus, the maximum tolerated dose (MTD) was the 10.0 mg R-DOTAP dose.

No serious adverse events (SAEs), study discontinuations due to adverse events (AEs), or deaths occurred.

No clinically relevant abnormal hematology, blood chemistry, urinalysis, or physical findings were observed.

Other adverse events reported by Oncology patients with relation to PDS0101 vaccine (Uncommon 1%-10%) include arthralgia, insomnia, tachycardia, diarrhea, lymphocyte count decreased, rash, headache.

The following adverse events were observed in Oncology patients but deemed unrelated to the combination (PDS0101+pembrolizumab): hypotension, lymphoedema, peripheral ischemia, pain in extremity, compartment syndrome, rhabdomyolysis, bradycardia, blood creatine phosphokinase increased, acidosis, hyperglycemia, hyperhidrosis, sepsis, acute kidney injury, oropharyngeal pain

Other adverse events reported in pre-neoplastic patients (healthy volunteers) included Very Common AEs (>10%) headache, and Uncommon AEs (1%-10%) fatigue, malaise, pyrexia, diarrhea, nausea, vomiting. myalgia. dizziness. lethargy, and insomnia.

The adverse event assessments are similar whether subjects receive PDS0101 monotherapy or in combination with pembrolizumab.

The PDS0101 formulation contains 10% v/v concentration of DMSO in the vaccine and was demonstrated to be safe and well-tolerated in both the preclinical and human safety studies. Three reported clinical studies of other HPV16 peptide vaccines used 20% v/v concentration of DMSO in the formulations (Uppaluri, Dunn, & Lewis Jr, 2008); (Chang & et al., 2016); (Preston, et al., 2013). One study also used 100% DMSO for 1 of the dose groups (Dunn, Dunn, & Curry, 2007).

1.3 Rationale for neoadjuvant immune-based therapy

Neoadjuvant therapy prior to surgery allows for biomarker and pathologic evaluation of disease with disease present and appears to be safe and feasible in cancers of the head and neck (Schoenfeld, Hanna, Jo, & et al., 2020). In the adjuvant setting, where the majority of patients have no discernable evidence of ctHPVDNA (see above) and where tumor antigen may not be present, the response to immune based therapies may be less robust and harder to determine. Furthermore, many oncologic paradigms utilize initial neoadjuvant response to dictate intensity of adjuvant therapies. PDS0101 has been shown to have minimal clinically meaningful toxicity (Investigators Brochure PDS0101). Therefore, PDS0101, pending response in the neoadjuvant setting, could likewise be used to potentially *de-intensify* therapy. With response, a patient that otherwise would not qualify for RT dose de-escalation or observation may be downstaged and qualify for de-intensification, on study.

1.31 Addition of pembrolizumab

Importantly, anti-tumor activity and pathologic regression have been documented using checkpoint inhibitors (CPIs) in neoadjuvant treatment of HPV-related and unrelated head and neck cancer with varying degrees of pathologic tumor response (pTR) reported (Ferrarotto, Bell, Rubin, & et al., 2020) (Uppaluri, Campbell, Egloff, & et al., 2020) (Schoenfeld, Hanna, Jo, & et al., 2020). In a study of neoadjuvant and adjuvant pembrolizumab in locally advanced, HPV-unrelated HNSCC, 22% (8 of 36) of subjects had pTR-2 ($\geq$ 50% regression) and 22% (8 of 36) had pTR-1 (10%-49% regression) (Uppaluri, Campbell, Egloff, & et al., 2020). A major limitation of the effectiveness of CPIs is the lack of pre-existing T cell immune infiltration in the tumor microenvironment (TME). PDS0101 has been shown to generate high levels of potent, tumor-specific CD8+ killer T-cell responses *in vivo* in both preclinical and human studies. Investigating this combination in the neoadjuvant treatment setting will provide an opportunity to further maximize anti-tumor responses over those seen with pembrolizumab monotherapy. In this study we are proposing to deliver two doses of PDS0101 alone or in combination with the immune checkpoint inhibitor pembrolizumab (KEYTRUDA®), prior to definitive surgery or chemoradiation with curative intent.

1.4 Hypothesis

We hypothesize that incorporating HPV vaccination with or without checkpoint inhibition into the neoadjuvant setting will result in augmentation of immunologic response and potentially anti-tumor responses as assessed by ctHPVDNA, imaging, and pathologic regression. We propose a proof of principle, pilot prospective study in locally advanced HPV-OPSCC investigating the efficacy of neoadjuvant PDS0101 and PDS0101 in combination with

pembrolizumab in patients who will undergo surgical resection for their HPV-OPSCC. This will be a prospective, two-arm, open-label, unblinded study.

1.41 Rationale for Definitive Chemoradiation Group

The majority of patients nationally receive chemoradiation for treatment of H&N oropharyngeal carcinoma. Many patients are not candidates for surgical resection. Given this situation, understanding of the response rates of PDS0101 with or without pembroluzimab in the neoadjuvant setting prior to definitive chemoradiation is important and may be more important in a patient population which typically has more advanced disease. For patients in this group, ctHPVDNA will be used as the sole primary endpoint. For patients without detectable ctHPVDNA at baseline these patients will not count towards the primary endpoint.

1.5 Correlative Studies

We will collect tissue and blood to use for correlative studies including tumor micro-environmental changes and pathologic response, evaluate ctHPVDNA as a marker for tumor response, determine HPV16-specific T-cell responses as well as other immune responses.

We are planning to use various potential methodologies and assessments including antibody titers, flow cytometry (e.g., Isoplexis Functional Proteomics), immune profiling (e.g., CyTOF), functional ELISPOT assay following exposure to known HPV16 antigens (to quantify responders), T-cell receptor (TCR) sequencing (e.g., Adaptive Technologies), and genomic analyses. When possible, we will compare pre-treatment and post-treatment results as applicable. We will also bank DNA, WBCs, and tissue (fresh and formalin-fixed) for future studies.

2.0 Goals

2.1 Primary Goal

To determine pathologic and/or ctHPVDNA response to PDS0101 or PDS0101 plus pembrolizumab in patients with high risk HPV-OPSCC.

NOTE: Pathologic response is defined as necrosis, keratinous debris, giant cell reaction, and T cell/immune cell infiltration as a percentage of total tumor bed area.

2.2 Secondary Goals

2.21 To determine tumor response by RECIST 1.1

2.22 To determine progression-free survival and overall survival

2.3 Tertiary Goals

2.31 To determine the safety of PDS0101 delivered alone or with pembrolizumab

2.4 Correlative Research

2.41 Determine the changes in tumor microenvironment (TME) with PDS0101 alone or with pembrolizumab

2.42 Determine circulating ctHPVDNA as a biomarker for tumor response

2.43 Determine HPV16-specific T-cell response utilizing multiplex flow cytometry and other parameters

3.0 Patient Eligibility

Prior to discussing protocol entry with the patient, contact the Mayo Clinic Research Site Management Office [redacted] for dose level and to ensure that a place on the protocol is open to the patient.

3.1 Registration – Inclusion Criteria

3.11 Age ≥18 years

3.12 Disease characteristics

3.121 Locally advanced HPV-OPSCC and high-risk HPV-specific testing with at least **one** of the following:

- Radiology ENE OR
- cN2 (AJCC 8th Edition) disease (contralateral/bilateral nodes) OR
- cN3(AJCC 8th Edition) disease (LN >6 cm) OR
- Radiographic evidence of 2 or more involved lymph nodes

3.122 Candidate for curative intent surgery or chemo-radiation

3.13 Measurable or unmeasurable disease as defined by RECIST 1.1 criteria (See Section 11.0)

3.14 Eastern Cooperative Oncoloogy Group (ECOG) performance status of 0 or 1.

3.15 Adequate organ function as defined below ≤15 days prior to registration:

- White blood cell (WBC) count ≥3,000/mm^3
- Platelet count ≥75,000/mm^3
- Hemoglobin ≥9.0 g/dL (5.6mmol/L)
  NOTE: Transfusions are not allowed ≤7 days prior to registration
- Total bilirubin ≤1.5 X upper limit of normal (ULN)
  (or total bilirubin ≤3.0 X ULN with direct bilirubin ≤1.5 X ULN in patients with well-documented Gilbert's Syndrome)
- Aspartate transaminase (AST/SGOT) ≤2.5 X ULN
- Creatinine ≤1.5 mg/dL (133 µmol/L) OR
  Calculated creatinine clearance ≥30mL/min/1.73m^2 for patients with creatinine levels above ULN

  Cockcroft-Gault Equation:

  Creatinine clearance for males = $\frac{\textbf{(140 - age)(weight in kg)}}{\text{(72)(serum creatinine in mg/dL)}}$

  Creatinine clearance for females = $\frac{\textbf{(140 - age)(weight in kg)(0.85)}}{\text{(72)(serum creatinine in mg/dL)}}$

- PT/INR/PTT ≤1.5 X ULN OR if patient is receiving anticoagulant therapy and PT or PTT is within therapeutic range of intended use of anticoagulants

3.16 Negative pregnancy test done ≤3 days prior to registration for persons of childbearing potential only.

3.17 Persons of childbearing potential or able to father a child must be willing to use an effective method of contraceptionfor the course of the study starting with the

first dose of study therapy through 120 days after the last dose of study medication. (See Section 9.7)
NOTE: Abstinence is acceptable if this is the usual lifestyle and preferred method of contraception for the patient.

3.18 Provide written informed consent.

3.19a Willing to return to enrolling institution for follow-up (during the Active Monitoring Phase of the study).

3.19b Willingness to provide mandatory blood specimens for correlative research (see Section 14.0).

3.19c Willingness to provide mandatory tissue specimens for correlative research (see Section 17.0).

3.2 Registration - Exclusion Criteria

3.21 Active autoimmune disease requiring systemic treatment, documented history of severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents.

NOTE: Exceptions are allowed for:

- Vitiligo
- Resolved childhood asthma/atopy
- Intermittent use of bronchodilators or inhaled steroids
- Daily steroids at dose of ≤10mg of prednisone (or equivalent)
- Local steroid injections
- Stable hypothyroidism on replacement therapy
- Stable diabetes mellitus
- Sjögren's syndrome

3.22 Any prior head or neck chemotherapy, radiotherapy, and/or immunotherapy.

3.23 Any of the following prior therapies:

- Live vaccine <30 days prior to registration, including intranasal flu vaccine (e.g. Flu-Mist®) (Note: Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed). Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette-Guerin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
- Chemotherapy or targeted small molecule therapy <21 days prior to registration
- Investigational therapy or investigational device <30 days prior to registration
- Any prior investigational HPV-specific therapeutic vaccine

3.24 Current or prior use of immunosuppressive medication <14 days prior to registration
The following are exceptions to this criterion:

- Intranasal, inhaled, topical steroids, or local steroid injections (e.g., intraarticular injection)

- Systemic corticosteroids at physiologic doses not to exceed 10 mg/day of prednisone or its equivalent
- Steroids as premedication for hypersensitivity reactions (e.g., premedication for CT scans)

3.25 Uncontrolled intercurrent illness including, but not limited to:
- Ongoing or active infection requiring systemic therapy
- Interstitial lung disease
- Serious, chronic gastrointestinal conditions associated with diarrhea (e.g., Crohn's disease or others)
- Known active hepatitis B (i.e., known positive HBV surface antigen (HBsAg) reactive)
- Known active hepatitis C (i.e., positive for HCV RNA detected by PCR)
- Known HIV (Note: Patients on stable HAART for ≥6weeks with CD4 counts ≥200 cells/mm^3 undetectable HIV viral load by quantitative PCR and no opportunistic infections Castlemaan's Disease ≤12 months prior to enrollment are allowed)
- Known active tuberculosis (TB)
- Symptomatic congestive heart failure
- Unstable angina pectoris
- Unstable cardiac arrhythmia or
- Psychiatric illness/social situations that would limit compliance with study requirements (e.g., substance abuse)

3.26 History of allogeneic hematopoietic transplant or any solid organ transplant

3.27 Receiving any other investigational agent which would be considered as a treatment for the primary neoplasm.

3.28 Other active malignancy <2 years prior to registration.
EXCEPTIONS: Non-melanotic skin cancer (SCC/BCC), micropapillary thyroid cancer, Gleason 6 prostate cancer, carcinoma-in-situ of the breast or cervix

3.29a Any of the following conditions ≤6 weeks prior to registration:
• Cerebrovascular accident (CVA)
• Admission for unstable angina
• Cardiac angioplasty or stenting or coronary artery bypass graft surgery
• Untreated pulmonary embolism or untreated deep venous thrombosis (DVT)
• Arterial thrombosis

3.29b Receipt of immunotherapy/immunomodulatory or immunosuppressive agents (e.g., IFNs, tumor necrosis factor, interleukins, immunoglobulins or other biologic response modifiers [GM-CSF, GCSF] ≤6 weeks prior to registration.

4.0 Study Schedule

4.1 Test schedule for HPV-OPSCC (all treatment arms)

			Active Monitoring Phase					Observation
Tests and procedures	≤28 days prior to Registration	≤15 days prior to Registration	Baseline Prior to Treatment on C1D1	End of C1 Prior to Treatment on C2D1	End of C2[1]	End of treatment[2]	Safety follow-up[3]	Clinical follow-up[4]
Window			-3 days	-3 days	-7 days		+7 days	
History and exam, Wt, PS	X		X	X	X	X	X	X
Adverse event assessment			X	X	X	X	X	X
Consultation with Head and Neck Surgery or Radiation Oncology[5]	X							
Height		X						
Pregnancy test[6]		X						
Hematology: CBC with 5-part differential		X		X	X	X	X	
Chemistry group: Complete metabolic panel (CMP)		X		X	X	X	X	
Thyroid testing (TSH; reflex per clinical care)		X		X	X	X	X	X
Coagulation test (PT/INR, aPTT)		X						
ctHPVDNA *Naveris* assay (send out)[R]			X	X	X		X	
Disease Evaluation/Tumor measurement[7]	X				X[8R]			X[9]
Biopsy[10]	X							

Cycle = 21 ±3 days; R = Research funded

[1] NOTE: Surgery if done must be at least 2 weeks after second dose and within 8 weeks after second dose. Definitive radiation therapy if done must be ≥2 weeks after second dose and ≤8 weeks after second dose.

[2] End of treatment on this study will be surgery for surgical patients and end of chemo-radiation for chemo-radiation patients (may be combined with Safety Follow-up)

[3] Follow-up visit at least 30 days after last dose for safety monitoring and for surgical patients should be prior to start of adjuvant therapy. If the patient has adjuvant therapy visit ≥30 days after the second dose, then it could be considered 30-day safety follow up **and** end of treatment visit

[4] Clinical follow-up beginning approximately 2-6 weeks after surgery for first post-op visit; then 3-6 months post-surgery for surgical patients; and 6 weeks after end of chemo-radiation for chemo-radiation patients, then 3-6 months post completion of chemoradiation.

[5] See Section 6.9b

[6] For persons of childbearing potential only. Must be done ≤3 days prior to registration and repeated prior to C1D1 if >3 days between registration and treatment.

[7] Standard clinical imaging should be used; CT neck, chest, abdomen, or FDG-PET/CT to rule out distant metastasis. Tumor measurement will happen prior to surgery (end of C2).

[8] If there is no evidence of distant metastasis on baseline screening, then neck CT scan is sufficient at this time point. Tumor measurement will happen prior to surgery (end of C2).

[9] Any imaging done during clinical follow-up period per institutional standards will be used for event monitoring

[10] If clinical biopsy is done, then research tissue can be obtained at the same time. If clinical biopsy is not needed or not done, then research biopsy can be offered.

			Active Monitoring Phase					Observation
Tests and procedures	≤28 days prior to Registration	≤15 days prior to Registration	Baseline Prior to Treatment on C1D1	End of C1 Prior to Treatment on C2D1	End of C2[1]	End of treatment[2]	Safety follow-up[3]	Clinical follow-up[4]
Mandatory research blood specimens[11R]			X	X	X	X	X	X
Mandatory research tissue specimens[12,R]	X				X[13]			
Patient post-vaccine diary and gauge[14]				X	X			

[11] Blood samples are collected per Section 14.0 and Lab Manual

[12] Tissue specimens are collected per Section 17.0 and Lab Manual

[13] Research tissue specimens will be collected from surgical sample on the day of the surgery, no separate biopsy needed. For definitive chemoradiation patients, a research biopsy may be offered. If patient refuses, there is no protocol violation.

[14] Post-vaccine diary and gauge (Appendices 2-3) should be given to patient on day vaccine is given (C1D1, C1D2). Instruct patients to complete for 7 days after the vaccine using the provided gauge for reference. Completed diary must be submitted at the next visit. Please note diary compliance in medical record and confirm AE and conmed data are transferred to Medidata Rave.

4.2 Survival Follow-up

	Survival Follow-up*				
	q. 3 months until PD	At PD	After PD q. 6 months	Death	New Primary
Survival Follow-up	X	X	X	X	At each occurrence

If a patient is still alive 2 years after registration, no further follow-up is required.

NOTE: All timepoints are ±1 month

5.0 Grouping Factor

5.1 Group Assignment: Arm A (PDS0101 alone) vs. Arm B (PDS0101 with pembrolizumab)

6.0 Registration Procedures

6.1 Registration by Cohort

Cohorts will switch every 3 patients for first 18 patients. Then for the last few patients, cohorts will switch with every patient until 10 evaluable patients per arm are enrolled to ensure an equal number of evaluable patients on both arms. If one arm achieves 10 evalu-able patients, we will continue enrollment exclusively in the incomplete arm, until a maximum of 24 patients have been enrolled. Patients will be registered to the current open cohort.

6.11 Prior to discussing protocol entry with the patient, call the Mayo Clinic Research Site Management Office [redacted] for dose level and to ensure that a place on the protocol is open to the patient.

6.12 To register a patient, fax [redacted] a completed eligibility checklist to the Mayo Clinic Research Site Management Office between 8 a.m. and 4:30 p.m. Central Time, Monday through Friday.

6.2 Verification of Materials

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.3 Documentation of IRB Approval

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) with Research Site Management [redacted] If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted, and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.4 Correlative Research - **Mandatory**

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.0, 14.0 and/or 17.0).

6.5 Patient Permissions

At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research on cancer at Mayo Clinic.
- Patient has/has not given permission to store and use his/her sample(s) for future research at Mayo Clinic to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.
- Patient has/has not given permission to store coded genetic and coded medical information in secured databases for research analyses.

6.6 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic in Rochester, Minnesota, under the supervision of a medical oncologist

6.7 Treatment start

Treatment cannot begin prior to registration and must begin ≤14 days after registration.

6.8 Pretreatment

Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.9a Baseline symptoms

All required baseline symptoms (see Section 10.6) must be documented and graded.

6.9b Head and neck surgery or radiation oncology consult required

A head and neck surgeon or radiation oncologist has seen the patient and confirmed the patient is a suitable candidate for this study. (Email or note in medical record will suffice.)

6.9c Study drug

Study drug is available on site.

6.9d Blood draw kits

Blood draw kit is available on site.

6.9e Study Conduct

The clinical trial will be conducted in compliance with regulations (21 CFR 312, 50, and 56), guidelines for Good Clinical Practice (ICH Guidance E6), and in accordance with general ethical principles outlined in the Declaration of Helsinki; informed consent will be obtained from all participating patients; the protocol and any amendments will be subject to approval by the designated IRB prior to implementation, in accordance with 21 CFR 56.103(a); and subject records will be stored in a secure location and subject confidentiality will be maintained. The investigator(s) will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

7.0 Protocol Treatment

7.1 Treatment Schedule

7.11 Pretreatment medication table (as needed)

Agent	Dose	Route	Day (±3 days)
Acetaminophen	650 mg	PO	1
Diphenhydramine	25 mg	PO	1

7.12 Treatment medication table

Arm	Agent	Dose Level	Route	Day **(±3 days)**
A	PDS0101	1 mL	SC	D1*
B	PDS0101	1 mL	SC	D1*
	Pembrolizumab	200 mg	IV	D1*

*Cycle = 21 ±3 days for two cycles

7.2 Pembrolizumab (Arm B only)

Pembrolizumab will be administered as a 30-minute IV infusion once every 3 weeks for two cycles on Day 1 (±3 days). Every effort should be made to target infusion timing to be as close to 30 minutes as possible. However, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). Pembrolizumab will be administered first.

7.3 PDS0101 (Arms A and B)

Each subject will receive PDS0101 (2.7 mg HPV-16 E6 and E7 peptides (400 μg/peptide) mixed with 3.0 mg R-DOTAP) administered as a divided dose of two 0.5mL SC injections in the upper anterior arm of the same arm. Injection sites should be separated by a minimum of 6 centimeters. The first PDS0101 vaccine dose should be administered in the non-dominant arm and the second dose should be delivered in the alternate arm.

For individuals with upper extremity lymphedema, PDS0101 should be delivered in the alternate unaffected arm or the anterior thighs (if both upper extremities are affected), alternating as previously described.

For patients receiving both drugs: PDS0101 will be administered between 30-60 min after completion of the pembrolizumab infusion.

For all patients: Following the first vaccination of PDS0101, subjects will have vital signs and symptoms monitored for 1 hour (at approximately 0, 15, 30 and 60 minutes). If no significant immediate AEs are identified with the first vaccination, patients should be monitored after vaccination for at least 15 minutes (at approximately 0 and 15 minutes) with subsequent vaccinations.

Patients should record the occurrence of any AEs, including injection site reactions daily, for 7 days post study treatment (monotherapy or combination). A diary is provided in Appendix II. Copies of the gauge (Appendix III) printed on acetate will be provided for each patient. Please note patient compliance with diary completion in medical record and confirm AE and concomitant medication data are transferred to Medidata Rave.

7.4 Return to consenting institution

For this protocol, the patient must return to the consenting institution for evaluation during treatment per the study calendar in Section 4.0 (Active Monitoring Phase).

7.5 Treatment by local medical doctor (LMD)

Treatment for this trial by a local medical doctor (LMD) is not allowed. Treatment by a local medical doctor for routine and emergent care is allowed.

7.6 Surgery or Chemoradiation per clinical standard of care

Patients must be candidate for either curative-intent surgery or chemoradiation.

If the patient is a surgical candidate, definitive surgery will be performed per institutional guidelines and procedures at the discretion of the treating oropharyngeal surgeon.

Treatment with radiation therapy with or without chemotherapy will be with curative intent and per enrolling institutional standards at the discretion of the treating radiation oncologist.

8.0 Dosage Modification Based on Adverse Events

There will be no dose modification for both PDS0101 and Pembrolizumab. Guidelines for the management of the adverse events is presented in Tables 8.2 and 8.3. However, if clinically indicted the treating physician may hold, omit or discontinue one of the drugs at any time per discussion with Study Investigator or Co-Investigator. This information must be documented in the patient clinical record.

→ ***ALERT:*** ***ADR reporting may be required for some adverse events (See Section 10.0)*** ←

8.1 Dose Levels (Based on Adverse Events in Tables 8.*2* and 8.3)

There are no dose reductions for PDS0101 or pembrolizumab

NOTE:

For Arm A if clinically indicated, treatment may be discontinued for an adverse event, and patient may proceed to surgery and/or chemoradiation therapy as indicated.

In Arm B, if either pembrolizumab or PDS0101 is discontinued or omitted, the patient can continue the other drug.

If one of the drugs is held or delayed, the other drug is delayed as well and always administered on the same day.

If both are discontinued, the patient will proceed to surgery and/or chemoradiation therapy as indicated.

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (November 27, 2017)* unless otherwise specified*** ← ←

* Located at [redacted]

8.2 Dose Modification Guidelines for Second Dose (Cycle 2, Day 1) of Pembrolizumab

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	ACTION**
Cardiac disorders	Myocarditis	Hold treatment for Grade 1-2 until it resolves, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation; permanently discontinue for Grade 3-4
Gastrointestinal disorders	Diarrhea or colitis	Grade 2-3 hold treatment until Grade 0-1, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation; Grade 4 permanently discontinue
Investigations	Elevated AST, ALT, or total bilirubin	Hold treatment for Grade 2 until it resolves to 0-1, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation; permanently discontinue for Grade 3-4
Endocrine disorders	Endocrine disorders – Other, specify: Hypophysitis	Hold treatment for Grade 2-3 until it resolves to 0-1, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation; permanently discontinue for Grade 4
Endocrine disorders	Hyperthyroidism	Hold treatment for Grade 3-4 until it resolves to 0-2, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation
Endocrine disorders	Hypothyroidism	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	ACTION**
Metabolism and nutrition disorders	Glucose intolerance (Type 1 diabetes mellitus [if new onset]) or Hyperglycemia	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure. Resume pembrolizumab when patients are clinically and metabolically stable. If treatment is delayed ≥2 weeks proceed to surgery or chemoradiation.
Respiratory, thoracic and mediastinal disorders	Pneumonitis	Hold treatment for Grade 2 until it resolves to 0-1, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation; Permanently discontinue for Grade 3-4
	All Other Immune-Related Adverse Events	Hold for Grade 2-3 until AE resolves to Grade 0-1, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation or discontinue based on type of event. Events requiring discontinuation include but are not limited to: Anaphylaxis, Guillain-Barre syndrome, Encephalitis. Grade 4 permanently discontinue

8.3 Dose Modification Guidelines for Second Dose (Cycle 2, Day 1) of PDS0101

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	ACTION**
General disorders and administration site conditions	Injection site reaction	Permanently discontinue for Grade 3-4
Immune system disorders	Anaphylaxis	Permanently discontinue
	All Other Adverse Events considered related to PDS0101	Hold for Grade 2-3 until AE resolves to Grade 0-1, if treatment is delayed ≥2 weeks proceed to surgery or chemoradiation or discontinue based on type of event. Grade 4 permanently discontinue

9.0 Ancillary Treatment/Supportive Care

9.1 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.2 Blood products and growth factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the Journal of Clinical Oncology, Volume 33, No 28 (October 1), 2015: pp. 3199-3212 (WBC growth factors) AND Journal of Clinical Oncology, Volume 28, No 33 (November 20), 2010: pp. 4955-5010 (darbepoetin/epoetin).

9.3 Antiemetics

Antiemetics may be used at the discretion of the attending physician except for steroids. Steroids should be used only if necessary for adverse immune-related events.

9.4 Diarrhea

Patients should be monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood, or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, infectious etiologies should be ruled out, and if symptoms are persistent and/or severe, endoscopic evaluation should be considered.

All patients who experience diarrhea should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

NOTE: Loperamide/diphenoxylate/atropines should NOT be used for diarrhea symptoms unless: (1) it is believed that pembrolizumab-related enterocolitis is unlikely to be present.

9.5 Immunotherapy-related toxicities

Immunotherapy related toxicities including but not limited to pneumonitis, diarrhea, hypophysitis, hyper or hypothyroidism, hepatitis and nephritis will be managed with local, systemic steroids or other immunosuppressive agents according to the severity and discretion of the treating MD and multidisciplinary team managing the patient.

9.6 Concomitant Medications/Vaccination

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the patient’s primary physician.

9.61 Acceptable Concomitant Medications

9.611 General Guidelines

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 10.

9.612 Coordination and timing of COVID-19 vaccination

COVID-19 vaccination timing should be based on local investigator clinical assessment and judgment. (Note: Whenever possible, it is recommended to avoid COVID vaccination on the day of PDS0101 dosing with or without pembrolizumab because it may be difficult to attribute certain AEs (e.g., fever, infusion reactions) to the study agent(s) or the COVID vaccine if they are both administered on the same day.

9.62 Prohibited Concomitant Medications

The following medications are **not** permitted during the screening and treatment phase (including retreatment for post-complete response relapse) of this trial:

- Anti-neoplastic systemic cytotoxic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy 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, varicella/zoster, yellow fever, intranasal influenza, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology.

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

9.7 Contraception

PDS0101 and pembrolizumab may have adverse effects on a fetus *in utero*. Furthermore, it is not known if any there are any transient adverse effects on the composition of sperm.

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

Female patients will be considered of non-reproductive potential if they are either:
(1) postmenopausal (minimum age 50 years with complete absence of menstruation for at least 2 continuous years); in females <45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state

in females not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.)
OR

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

OR

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

All patients of reproductive potential must agree to avoid becoming pregnant or impregnating a partner while receiving study drug and for 120 days after the last dose of study drug. (Some suggested methods are included in Appendix IV.)

Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study patients of childbearing potential must adhere to the contraception requirement from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a patient of childbearing potential will not reliably comply with the requirements for contraception, that patient should not be entered into the study.

9.8 Use in pregnancy

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

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

9.9 Use in nursing adults

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

10.0 Adverse Event (AE) Monitoring and Reporting

The site Principal Investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The Sponsor/Sponsor-Investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, 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
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

10.01 Summary of SAE Reporting for this study

(please read entire section for specific instructions):

WHAT form:	WHERE to send:
Pregnancy Reporting [redacted] Attach to MCCC Electronic SAE Reporting Form [redacted]	**Manually send copies to PDS Biotech at:** [redacted] NOTE: The MCCC form will automatically be sent to [redacted]
Mayo Clinic Cancer Center SAE Reporting Form: [redacted]	**Manually send copies to PDS Biotech at:** [redacted] NOTE: The MCCC form will automatically be sent to [redacted]

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a 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.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days, or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0 A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site:
[redacted]

a. Identify the grade and severity of the event using the CTCAE version 5.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events – are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the Investigator Brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite – The AE *is clearly related* to the agent(s)/procedure.

Probable – The AE *is likely related* to the agent(s)/procedure.
Possible – The AE *may be related* to the agent(s)/procedure.
Unlikely – The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated – The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME (Combination) Arm

NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the **entire combination (arm) is then considered an investigational intervention for reporting.**

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for **investigational** agents/interventions.
- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report, unless hospitalization is required. Refer to Section 10.4 for specific AE reporting requirements or exceptions.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

- An increased incidence of an expected adverse event (AE) is based on the patients treated for this study at their site. A list of known/expected AEs is reported in the package insert or the literature, including AEs resulting from a drug overdose.
- Commercial agent expedited reports must be submitted to the FDA via MedWatch 3500A for Health Professionals (complete all three pages of the form).

[redacted]

10.32 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

*Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be reported in an expedited manner[1]
General disorders and administrations site conditions	Injection site reaction	≤Grade 2
Skin and subcutaneous tissue disorders	Pain of skin	≤Grade 2

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations and surgery required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.

10.4 Expedited Reporting Requirements for IND Agents

10.41 Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the sponsor within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥24 hrs	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" – A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 10.32 for exceptions to Expedited Reporting

10.42 General reporting instructions
The Mayo IND and/or MCCC Compliance will assist the Sponsor-Investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form:
[redacted]

[redacted] for investigational agents or commercial/investigational agents on the same arm.

Submit copies of Mayo Expedited Event Report Form by email to PDS Biotech at: [redacted]

10.43 Reporting of re-occurring SAEs
ALL SERIOUS adverse events that meet the criteria outlined in Table 10.41 MUST be immediately reported to the Sponsor within the timeframes detailed in the corresponding table. This reporting includes but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)
Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the appropriate documentation and use the Mayo Clinic Cancer Center Expedited Event Report form [redacted] o submit to [redacted] The Mayo Clinic Compliance Unit will review and process the submission to the Mayo Clinic IRB and work with the IND Coordinator for submission to FDA.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease that cannot be attributed to a CTCAE term associated with Grade 5 should be reported as **Grade 5 "Disease progression"** under the system organ class (SOC) of "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) inadvertently becomes pregnant while on treatment with PDS0101 and pembrolizumab (when applicable) the subject will immediately be removed from the study. Any pregnancy occurring after the subject receives the first PDS0101 vaccine and until 30 days after second final PDS0101vaccine must be reported to the Investigator within 24 hours of learning of its occurrence. The Investigator will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the industry partners without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The Study Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the industry partner.

Prior to obtaining private information about a pregnant patient and the infant, the investigator must obtain consent from the pregnant patient and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form:

[redacted]

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions – Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions – Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration – Other (neonatal loss)"** under the General disorders and administration SOC.

10.56 Adverse Events of Special Interest (AESIs) for PDS0101

Events of clinical interest related to PDS0101 include:

1) Possible temporary discoloration (other than redness) of skin at local injection sites. Description (hyper- or hypopigmentation) and duration will be captured.
2) Any overdose with either of the compounds.

10.6 Required Routine Reporting

10.61 Baseline and Adverse Events Evaluations

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v5.0 **unless** alternate grading is indicated in the table below:

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Blood and lymphatic system disorders	Lymph node pain	X	X
Ear and labyrinth disorders	Ear pain	X	X
General disorders and administration site conditions	Fatigue	X	X
	Injection site reaction		X
Respiratory, thoracic and mediastinal disorders	Sore throat	X	X
Skin and subcutaneous tissue disorders	Rash, maculo-papular	X	X

10.62 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 1 and 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

10.8 Reporting for PDS Biotech

Submit copies of all SAE reports on the same day report is completed to PDS Biotech at: [redacted]

11.0 Treatment Evaluation/Measurement of Effect

This study utilizes ctHPVDNA and pathologic response for treatment response. Objective response rate is a secondary endpoint and will be assessed prior to surgery or definitive chemoradiation.

Response and progression will be evaluated in this study using the international criteria proposed by the revised **Response Evaluation Criteria in Solid Tumors** (**RECIST**) guidelines (**version 1.1**)[15]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the short axis measurements in the case of lymph nodes are used in the RECIST guideline.

11.1 Schedule of evaluations

- **Screening**:
  - Baseline ctHPVDNA, research biopsy and scan to assess the baseline levels of ctHPVDNA, baseline pathology and disease status should have been performed within 14 days prior to registration.
  - CT scan of neck, chest and abdomen should be obtained. CT scan obtained as part of FDG-PET-CT is acceptable, but a at least a dedicated CT neck is preferred.

  NOTE: MRI of the neck is acceptable in lieu of CT-neck but not preferred. If MRI of the neck is used at baseline, then MRI should be used to assess the response prior to surgery or chemoradiation.
- **Cycle 2, Day 1**: The ctHPVDNA levels will be collected again prior to the second dose of study treatment.
- **Prior to surgery or definitive chemoradiation (end of Cycle 2)**:
  - The ctHPVDNA levels will be collected again prior to the surgical resection.
  - The first scan after treatment initiation will be performed prior to surgical resection or chemoradiation. If there is no evidence of distant metastasis on baseline screening, then CT scan of the neck is sufficient at this time point.

  NOTE:If MRI of the neck is used at baseline, then MRI should be utilized to assess the response prior to surgery or chemoradiation.

  NOTE: Following surgical resection, the scans are performed per institutional standard of care and at the discretion of treating MD.

At the time of surgical resection: A research surgical pathology sample will be collected during surgery to assess for pathologic response.

For patients not undergoing surgery, an optional research biopsy may be performed to assess for pathologic response.

11.2 Definitions of Measurable and Non-Measurable Disease

11.21 Measurable Disease

11.211 A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

11.212 A superficial non-nodal lesion is measurable if its longest diameter is ≥1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color

[15] Eisenhauer EA, Therasse P, Bogaert J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 45(2): 228-247, 2009.

photography, including a ruler to estimate the size of the lesion, is recommended.

11.213 A malignant lymph node is considered measurable if its short axis is >1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

NOTE: Please collect lymph node level (1, 2, 2a, 2b, 3, 4, 5 and retropharyngeal [RP])

11.22 Non-Measurable Disease

11.221 All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/ pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions. In addition, lymph nodes that have a short axis <1.0 cm are considered non- pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.31 Measurement Methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.
- Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

11.32 Acceptable Modalities for Measurable Disease:

- **Conventional CT and MRI**: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.
- As with CT, if an **MRI** is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.
- **PET-CT**: CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

- **FDG-PET**: FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease [PD] and particularly possible 'new' disease. A 'positive' FDG-PET scanned lesion is defined as one which is FDG avid with an update greater than twice that of the surrounding tissue on the attenuation corrected image; otherwise, an FDG-PET scanned lesion is considered 'negative.' New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:
  a. Negative FDG-PET at baseline with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
  b. No FDG-PET at baseline and a positive FDG-PET at follow-up:
     i. If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
     ii. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT at the same evaluation, additional follow-up CT scans (i.e., additional follow-up scans at least 4 weeks later) are needed to determine if there is truly progression occurring at that site. In this situation, the date of PD will be the date of the initial abnormal FDG-PET scan.
     Iii If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, it is not classified as PD.

11.33 Measurement at Follow-up Evaluation:

- There is only one follow up evaluation as part of the study (evaluation prior to surgery or after chemoradiation). Further follow up after surgical resection is as per institutional guidelines.
- Histologic techniques could be used to differentiate between PR and CR in rare cases (e.g., residual lesions prior to surgical resection could represent fibrotic tissue)

11.4 Measurement of Effect

11.41 Target Lesions & Target Lymph Nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions, representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. These lesions can be non-nodal or nodal (as defined in 11.21), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

  Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.
- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.
- Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The

BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.

- Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.
- The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.42 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.22) are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. These lesions and lymph nodes should be followed in accord with Section 11.433.

11.43 Response Criteria

11.431 All target lesions and target lymph nodes followed by CT/MRI/PET-CT/ must be measured on re-evaluation at evaluation times specified in Section 11.1. Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response.

11.432 Evaluation of Target Lesions

- Complete Response (CR): All of the following must be true:
  a. Disappearance of all target lesions.
  b. Each target lymph node must have reduction in short axis to <1.0 cm.
- Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (*see* Section 11.41).
- Progression (PD): At least one of the following must be true:
  a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.
  b. At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.

11.433 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

- Complete Response (CR): All of the following must be true:
  a. Disappearance of all non-target lesions.
  b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.
- Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.
- Progression (PD): At least one of the following must be true:
  a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.
  b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)
  c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

11.44 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following tables:

11.441 For Patients with Measurable Disease

Target Lesions & Target Lymph Nodes	**Non-Target Lesions & Non-Target Lymph Nodes**	**New Sites of Disease**	**Overall Objective Status**
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR**
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

** NOTE: This study uses the protocol RECIST v1.1 template dated 2/16/2011. For data collection and analysis purposes the objective status changed from SD to PR in the protocol RECIST v1.1 template as of 2/16/2011 and to match RECIST v1.1 requirements.

11.442 For patients with Non-measurable disease Only

Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR	No	CR
Non-CR/Non-PD	No	Non-CR/Non-PD
Not All Evaluated*	No	Not Evaluated (NE)
Unequivocal PD	Yes or No	PD
Any	Yes	PD

*See Section 11.431

11.45 Symptomatic Deterioration
Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration.

11.5 Treatment Evaluation after Completion of Surgery or Definitive Chemoradiation

11.51 Definition of Recurrence
The term "recurrence" is used if cancer returns either in the locoregional area of the primary tumor or at distant sites following R0 or R1 resection

11.52 How Recurrence is Determined
Recurrence is determined radiographically and biopsy could be performed at the discretion of treating physician except as detailed below or when CT findings are equivocal in the determination of the enrolling physician.

Locoregional recurrence: Identified by a new soft-tissue mass in the operated thyroid bed or regional cervical lymph nodes. In some cases post-radiation changes may make it difficult to distinguish from early tumor progression. In cases in which the diagnosis is ambiguous, discussion with the PI should occur. Performance of tissue biopsy should be considered prior to assigning a diagnosis of locoregional recurrent disease.

12.0 Descriptive Factors

12.1 History of tobacco use: Yes vs. No
If yes,
Current tobacco user: Yes vs. No
If yes
Type: Smoking vs. smokeless tobacco
If smoking: Number of pack years: ____

12.2 AJCC 8 clinical staging: I vs II vs III

12.3 Clinical TNM staging (AJCC 8th Edition HPV-associated): specify T ___ N____ M____

12.4 Planned treatment type: Surgery vs. chemo-radiation

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Continuation of treatment

Patients who are CR, PR, or SD will continue treatment per protocol.

13.2 Recurrence/Progressive Disease (PD)

Patients who develop PD or recurrence while in clinical follow-up will go to the survival follow-up phase.

13.3 Off protocol treatment

Patients who go off protocol treatment for reasons other than PD will go to the survival followup/event-monitoring phase per Section 4.0.

13.4 Observation (Clinical Follow-up)

If the patient has achieved CR, PR, or SD, the patient will proceed with surgery as indicated or definitive chemoradiation. Adjuvant therapy will be per the discretion of the treating team. Patients will be observed every 3 months for max of two years after surgery. Once patients have PD or go off treatment for other reasons, they'll go to survival follow-up.

13.5 Duration of therapy for CR

Patients who achieve a CR will still only receive the max of 2 cycles. After 2 cycles, the patient will proceed with surgery or chemoradiation. Patients will be observed after surgery or chemoradiation (see 13.4 above) every 3 months for 2 years or until PD and then they will go to survival follow-up.

13.6 Duration of therapy for PR or SD

Patients who are in PR or SD will receive a maximum or total of 2 cycles. After 2 cycles, the patient will proceed with surgery or chemoradiation. After surgery or chemoradiation, they should be observed (see 13.4 above) for 6 months post-surgery or chemoradiation until PD and then they will go to survival follow-up/event monitoring.

13.7 Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. If the patient received treatment, the patient may continue treatment at the discretion of the physician as long as there are not safety concerns. The patient will continue in the Active Monitoring/Treatment phase of the study, as per section 4.0 of the protocol.

- If the patient never received treatment, on-study material must be submitted. Survival Follow-up will be required per Section 4.0 of the protocol.

13.8 Major violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. If the patient received treatment, the patient may continue treatment at the discretion of the physician as long as there are not safety concerns. The patient will continue in the Active Monitoring/Treatment phase of the study, as per section 4.0 of the protocol.

13.9 Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood and Body Fluid Specimens to be Collected for this Protocol

Research (Section for more information)	**Specimen Purpose**	**Mandatory or Optional**	**Volume to collect per tube (# of tubes to be collected)**	**Timepoint 1** Prior to treatment on C1D1 (Baseline)	**Timepoint 2** Prior to treatment on C2D1 (End of C1)	**Timepoint 3** End of C2[16]	**Timepoint 4** Safety Follow Up[17]
ctHPVDNA Section 14. 41 MC200710-C1	Correlative	Mandatory	10mL (1)	X	X 10mL	X 10mL	X 10mL
DNA Section 14.43	Banking	Mandatory	varies	X 4mL + 10mL	X 10mL	X 10mL	X 10mL
PBMC analysis of immune biomarkers Section 14.42 MC200710-C2	Correlative	Mandatory	10 mL (3)	X	X	X	X
Immunogenic Markers Section 14.42 MC200710-C3	Correlative Banking	Mandatory	10 mL (1)	X	X	X	X

NOTE: See Lab Manual for additional information on collection, processing and shipping

[16] Prior to surgery for surgical patients and prior to chemo-radiation for chemo-radiation patients.
[17] For surgical patients first post-surgical visit (may be safety follow-up plus end of treatment plus observation); for chemo-radiation patients during Weeks 4-5 assessments

14.2 Collection and Processing

See lab manual for details on collection and processing

14.3 Shipping and Handling

14.31 Kits will be used for this study.

14.311 Kits will be supplied by the study team and Naveris Inc.

14.312 The kit contains supplies and instructions for collecting, processing, and shipping specimens.

14.313 Naveris: Obtain kits by contacting Naveris (see Lab Manual).

14.314 **All specimens must be collected and shipped Monday – Friday ONLY.**

14.32 Shipping Specimens

One tube per timepoint will be shipped to Naveris per lab manual. Naveris will supply the tubes and kits for this shipment.

Three tubes to go to [redacted] laboratory – page laboratory for expedited pickup per Protocol Resources.

Remaining tubes to be processed/stored in BAP

14.33 Handling Specimens

See lab manual for details on specimen handling

14.4 Background and Methodology

Correlative analyses will be performed on blood collected

14.41 ctHPVDNA

ctHPVDNA will be assessed prospectively at all timepoints using a previously validated multiplex ctHPVDNA panel including HPV type 16 (Routman, et al., 2019). This ctHPVDNA assay has been previously characterized, with approximately 90% pretreatment sensitivity, and marked response to intervention including surgery and radiation therapy. ctHPVDNA will be reported at each timepoint as copies/mL. Detectable threshold for the purposes of this study will be 5 copies/mL or greater. A significant response to induction therapy will be considered decrease in absolute copies of ctHPVDNA ≥50% from baseline prior to surgery or definitive chemoradiation. Given increased shedding with cell kill documented in both oropharyngeal and anal cancer including with fractionated RT, an increase of ctHPVDNA at the interim timepoint after cycle 1 will not be considered progression. Patients with undetectable ctDNA will continue with blood draws for assessment. Patients with undetectable pretreatment ctDNA will be considered non-evaluable.

14.42 Immunophenotyping

Immunophenotyping will be used to characterize correlatives of response within and across patients. PBMCs will be analyzed to characterize specific CD8+ and CD4+ T cell populations of responders and non-responders by high flow cytometry, functional assays, and identification of HPV16-specific T cells. PBMCs will be processed per the BAP standard as above and frozen.

14.43 Banking

WBCs and plasma will be banked for future genomic analysis. Serum will be banked for future analyses.

15.0 Drug Information

15.1 PDS0101 (R-DOTAP (Versamine®) + HPVmix)

15.11 Background

PDS0101 is a novel nanoparticle-based immunotherapy (therapeutic vaccine) developed to treat genital cancers such as anal, cervical, vulvar cancer etc. and their precancers (late-stage dysplasia/neoplasia), as well as head and neck cancers caused by infection with the human papilloma virus (HPV). PDS0101 is a subcutaneously administered therapy consisting of two vialed components mixed at the time of administration. The immunotherapy is composed of liposomal nanoparticles of the pure immunologically active R enantiomer of the cationic (positively charged) lipid DOTAP (dioleoyl-trimethylammonium propane) mixed with 6 lipidated antigenic peptides derived from the HPV-16 E6 and E7 proteins.

15.12 Formulation

PDS0101 is a 2-vial formulation which is mixed 1:1 v/v just prior to administration. Active vials:

- Vial #1: R-DOTAP (Versamine®) – 2 mg/mL, 6 mg/mL, and 20 mg/mL ± 10% concentrations in 280 mM sucrose/Sterile Water for Injection. Fill volume is 1.8 mL.
- Vial #2: HPVmix – 0.8 mg/mL per peptide ± 25% Peptide injectable formulation of 6 HPV-16 lipidated peptides in 280 mM sucrose and 20:80 DMSO/Sterile Water for Injection. Fill volume is 0.6 mL.

15.13 Preparation and storage

PDS0101 (R-DOTAP (Versamine®) and HPVmix) is stored at -70 degrees Celsius ± 10 degrees Celsius. The two vial formulation should be thawed and mixed as outlined in the current PDS0101 Investigator's Broochure and pharmacy manual provided by PDS Biotech.

15.14 Administration

The PDS0101 mixture (1.2 mL which contains overfill) is divided and administered as two subcutaneous injections of 0.5 mL in the upper anterior arm of the same arm. The two injections should be at least 6 cm apart for each vaccination. The first vaccination (two subcutaneous injections of 0.5 mL) should be given in the non-dominant arm. Each subsequent vaccination should be alternated between the non-dominant versus dominant arm.

15.15 Pharmacokinetic information

No pharmacokinetic testing in humans to date.

15.16 Potential drug interactions

None known to date.

15.17 Known potential adverse events

In Oncology patients (N= 15) receiving the combination of PDS0101 and pembrolizumab the following adverse events were observed.

Very Common known potential adverse events >10%

Injection site reactions (ISR): with or without swelling, pain, skin discoloration, or erythema. Fatigue; chills; hypertension; Pain in extremities; anxiety; nausea

Common known potential adverse events, ≥10%: **None**

Uncommon known potential adverse events, 1% - 10%:

Injection site reactions (ISR): fibrosis; site discomfort; site inflammation; site pruritus.

Arthralgia, Insomnia, Tachycardia, Diarrhea, Lymphocyte count decreased, Rash, Headache

The following adverse events were observed but deemed *unrelated* to the combination [PDS0101+pembrolizumab]:
Hypotension, Lymphoedema, Peripheral ischemia, Pain in extremity, Compartment syndrome, Rhabdomyolysis, Bradycardia, Blood creatine phosphokinase increased, Acidosis, Hyperglycemia, Hyperhidrosis, Sepsis, Acute kidney injury, Oropharyngeal pain

In pre-neoplastic patients (N=12) evaluated as healthy volunteers the following adverse events were observed:

Very Common known potential adverse events >10%

Injection site reactions (ISR): with or without swelling, pain, skin discoloration, site hemorrhage, induration, or erythema. Headache

Common known potential adverse events, ≥10%: **None**

Uncommon known potential adverse events, 1% - 10%:

Injection site reactions: site papule, site nodule, site paresthesia, site inflammation. Site pruritus, site rash, site redness.

Fatigue, Malaise, Pyrexia, Diarrhea, Nausea, Vomiting. Myalgia. Dizziness. Lethargy, and Insomnia.

The adverse event assessments are similar whether subjects receive PDS0101 monotherapy or in combination with pembrolizumab.

15.18 Drug procurement:
Drug will be provided free of charge by PDS Biotech

Outdated or remaining drug is to be destroyed on-site as per procedures in place at each institution.

15.19 Nursing guidelines

15.191 PDS0101 should be administered as two injections in the upper anterior of the same arm. They should be given approximately 6 cm apart. Subsequent injections should be administered in the opposite arm on a rotating basis.

15.192 Due to the early investigational nature of this agent not all side effects can be known at this time. Monitor patients for about one hour after first vaccine, and at least fifteen minutes after second dose and report any side effects to the study team.

15.193 The most common side effect in early studies was injection site reaction. This consisted of erythema, pain and swelling at the site.

15.194 Gastrointestinal side effects have been noted including diarrhea, nausea and vomiting. Treat symptomatically and assess for effectiveness of intervention.

15.195 Fatigue, lethargy and malaise have been reported. Instruct patient in energy conserving lifestyle.

15.196 Patients have reported myalgia. Treat symptomatically and monitor for effectiveness.

15.2 **Pembrolizumab** (MK-3475, SCH 900475, Keytruda®)

15.21 Background
Pembrolizumab is a potent humanized IgG4 monoclonal antibody with high specificity of binding to the PD-1 receptor, thus inhibiting its interaction with PD-L1 and PD-L2. Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1.

15.22 Formulation
Pembrolizumab is available as a liquid 25 mg/mL, 100 mg/vial.

15.23 Preparation and storage
Vials should be stored in the refrigerator at temperatures between 2-8°C.

Drug concentrate is further diluted with normal saline (or 5% dextrose in the concentration range of 1 to 10 mg/mL) in IV containers made of polyvinyl chloride (PVC) or non-PVC material. The infusion solution in the IV bag should be immediately administered. Diluted pembrolizumab solutions may be stored at room temperature for a cumulative period of up to 4 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion. In addition, IV bags may be stored at 2-8°C for up to a cumulative time of 20 hours. This 24-hour total hold time from dilution may include up to 6 hours at room temperature

15.24 Administration
Pembrolizumab is administered by intravenous infusion over 30 minutes via a 0.22 micron in-line filter. The final infusion volume must be between 1 and 10 mg/mL. Maximum rate of infusion should not exceed 6.7 mL/minute through a peripheral or indwelling catheter. Flush the line with 0.9% NaCL following the completion of the infusion.

15.25 Pharmacokinetic information
a) **Absorption** – Because pembrolizumab is administered intravenously, it is immediately and completely bioavailable. Steady-state concentrations of pembrolizumab are reached by16 weeks of repeated dosing with a Q3W regimen, and the systemic accumulation is 2.1-fold. The peak concentration, trough concentration, and area under the plasma concentration versus time curve at steady state of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg Q3W.
b) **Distribution** – Pembrolizumab has a limited volume of distribution.
c) **Excretion** – CL is approximately 23% lower after achieving maximal change at steady state compared with the first dose. The terminal elimination half-life (t½) is estimated to be 22 days at steady state.
d) **Metabolism** – Pembrolizumab is catabolized through non-specific pathways; metabolism does not contribute to its CL.

15.26 Potential Drug Interactions

There are no known significant drug interactions.

15.27 Known potential adverse events:

Very common known potential adverse events, ≥10%:
Skin and subcutaneous tissue disorders: Pruritus, skin rash
Gastrointestinal disorders: Diarrhea, nausea, abdominal pain
General disorders and administration site conditions: fatigue

Common known potential adverse events, >10%:
Blood and lymphatic system disorders: anemia
Immune system disorders: infusion related reaction
Endocrine disorders: hyperthyroidism, hypothyroidism
Metabolism and nutrition disorders: decreased appetite
Nervous system disorders: headache, dizziness, dysgeusia
Respiratory, thoracic, and mediastinal disorders: pneumonitis, dyspnea, cough
Gastrointestinal disorders: colitis, vomiting, constipation, dry mouth
Skin and subcutaneous tissue disorders: severe skin reactions, vitiligo, dry skin, erythema
Musculoskeletal and connective tissue disorders: arthralgia, myositis, musculoskeletal pain, arthritis, pain in extremity
General disorders and administration site conditions: asthenia, edema, pyrexia, influenza like illness, chills
Investigations: alanine aminotransferase increased, aspartate aminotransferase increased, blood alkaline phosphatase increased, blood creatinine increased

Uncommon known potential adverse events, 1% - 10%:
Infusion related reactions
Blood and lymphatic system disorders: neutropenia, thrombocytopenia, leukopenia, lymphopenia, eosinophilia
Endocrine disorders: hypophysitis, adrenal insufficiency, thyroiditis, hypopituitarism
Metabolism and nutrition disorders: type I diabetes mellitus, hyponatremia, hypokalemia, hypocalcemia
Psychiatric disorders: insomnia, confusional state
Nervous system disorders: epilepsy, lethargy, peripheral neuropathy
Eye disorders: uveitis, dry eye
Cardiac disorders: myocarditis, atrial fibrillation
Vascular disorders: hypertension
Gastrointestinal disorders: pancreatitis
Hepatobiliary disorders: hepatitis
Skin and subcutaneous tissue disorders: lichenoid keratosis, psoriasis, alopecia, dermatitis, dermatitis acneiform, eczema, hair color changes, papule
Musculoskeletal and connective tissue disorders: tenosynovitis
Renal and urinary disorders: nephritis, acute kidney injury
Investigations: blood bilirubin increased, amylase increased, hypercalcemia

Rare known potential adverse events, <1% (Limited to important or life-threatening):
Blood and lymphatic system disorders: immune thrombocytopenic purpura, hemolytic anemia
Immune system disorders: sarcoidosis
Nervous system disorders: Guillain-Barre syndrome, myasthenic syndrome

Gastrointestinal disorders: small intestinal perforation
Skin and subcutaneous tissue disorders: toxic epidermal necrolysis, Stevens-Johnson syndrome, erythema nodosum

The risk profile for pembrolizumab also includes two important potential risks: a) myasthenic syndrome, and b) an increased risk of severe complications (such as early severe graft versus host disease and veno-occlusive disease) of allogeneic transplant in patients with hematologic malignancies who have previously been treated with PD-1 inhibitors.

15.28 Drug procurement

Commercial supply will be purchased by the study.

15.29 Nursing Guidelines

15.291 Pembrolizumab side effects vary greatly from those of traditional chemotherapy and can vary in severity from mild to life threatening. Instruct patients to report any side effects to the study team immediately. Side effects may be immediate or delayed up to months after discontinuation of therapy. Most side effects are reversible with prompt intervention of corticosteroids.

15.292 Diarrhea can be seen, however is less common than that seen with anti-CTLA-4 agents. However, it can be severe, leading to colonic perforation. Instruct patients to report ANY increase in the number of stools and/or change in baseline, blood in the stool, abdominal pain to the study team immediately.

15.293 Rash/pruritis/dermatitis is seen. Patients should report any rash to the study team. Treat per section 9.0 and monitor for effectiveness.

15.294 Monitor LFTs closely as elevations in these levels could indicate early onset autoimmune hepatitis. Patients should also be instructed to report any jaundice, or right upper quadrant pain to the study team immediately.

15.295 Pneumonitis can be seen and may be mild (only seen on imaging) to severe. Patients should be instructed to report any SOB, dyspnea, cough, chest pain, etc. to the study team immediately. Patients reporting these symptoms should have a pulse ox checked and consider immediate imaging per the treating MD.

15.296 Endocrinopathies (including hypopituitarism, hypothyroidism, hypophysitis, and adrenal insufficiency) are seen with this agent. Patients may present only with the vague sense of fatigue and “not feeling well.” Additional symptoms may be that of nausea, sweating and decreased activity tolerance. Instruct patients to report these signs or symptoms immediately and obtain appropriate labs as ordered by MD.

15.297 Patients who are started on steroid therapy for any side effects of pembrolizumab toxicity should be instructed to take the steroids as ordered, and not to discontinue abruptly as symptoms may return and be severe. Patients may be on steroid therapy for weeks. Instruct patients to report any increase or change in side effects with any dosage decrease as patients may need a slower taper.

15.298 Fatigue is common and may or may not be associated with immune-related side effects. Assess patient's fatigue level prior to each cycle of therapy and report any changes to the study team.

15.299a Patients should avoid receiving live vaccines within 30 days of study drug administration or per other study guidelines.

15.299b Patients who have undergone an allogenic bone marrow transplant, have an increased risk of severe complications including early GVHD, and veno-occlusive disease, if they have previously been treated with pembrolizumab

15.299c Myocarditis has been reported and associated with pembrolizumab. Instruct patients to report chest pain, SOB, or dyspnea to study team immediately and/or seek emergency medical attention.

15.299d Autoimmune hematologic disorders including ITP and hemolytic anemia have been reported. Monitor blood counts closely and report any abnormalities to the study team.

15.299e Rare neurologic disorders including Guillain-Barre syndrome and myasthenia gravis have been reported. Instruct patients to report any neurologic symptoms including weakness, paresthesias or numbness, tingling to the study team immediately.

16.0 Statistical Considerations and Methodology

16.1 Overview and Primary Endpoint Analysis:

This pilot study will determine the initial clinical benefit of the PDS0101 vaccine alone (Arm A) and PDS0101 vaccine plus IV pembrolizumab (Arm B) for patients with high risk of HPV-OPSCC. The primary endpoint is to determine the pathologic and/or ctHPVDNA response rate in each arm separately, where pathologic response (pTR) is defined as necrosis, keratinous debris, giant cells/histiocytes as percentage of total tumor bed area (Uppaluri, Campbell, Egloff, & et al., 2020). Patients with a pTR-1 (10-49%) or greater will be considered pathologic responders. Each arm will be assessed separately.

An observed response rate of at least 20% for either pathologic response or ctHPVDNA response ≥50% decrease from baseline) will be deemed worthy of further investigation for each arm. If the primary endpoint is negative, then secondary endpoints will be assessed to see if any of the treatment arms are promising enough for further study in this exploratory pilot study. All endpoints will be assessed. Secondary endpoints will consist of progression-free survival (PFS), overall survival (OS), objective response rate, and adverse events. These endpoints will be assessed in each cohort separately. In addition, this study will also assess a couple of translational endpoints as well.

Note: 11% of patients may be unevaluable for the primary endpoint due to undetectable ctHPVDNA at baseline. These patients in the definitive chemo-radiation cohort will not be evaluated for the primary endpoint. All secondary endpoints, including adverse event reporting and correlative analyses, will be performed for these patients.

16.11 Sample Size

Study will enroll in a cohorts of 3 design, where the first 3 patients will be accrued to Arm A, then the next 3 to Arm B, then the next 3 to Arm A, etc. until we have 18 patients enrolled. Then the study will enroll alternating arms with each patient until we have 10 patients per arm. We will continue to enroll up to 24 total patients (12 per arm) or until 10 evaluable patients per arm are enrolled. If we reach 10 evaluable patients in one arm but not the other, we will enroll exclusively to the incomplete arm for the remainder of the study, up to 24 patients maximum.

16.12 Accrual Time and Study Duration

The anticipated accrual rate is approximately 2 patients per month. Therefore, the accrual period for this pilot study is expected to be approximately 12 months. The final analysis can begin approximately 15 months after the trial begins, i.e., as soon as the last patient has completed surgery or definitive chemoradiation and all tissue has been evaluated for response.

16.2 Data and Safety Monitoring

The Principal Investigator(s) and the study statistician will review the study at least twice a year to identify accrual, adverse event, and any endpoint problems that might be developing. The trial is monitored continually by the study team who are notified of every Grade 4 and 5 event in real time. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office. Any safety issues requiring protocol changes are communicated through protocol amendments.

16.21 Adverse Event Stopping Rules

Based on previous experience with this disease, we expect approximately

20% of patients to experience Grade 4+ adverse events.

If at any time, 3 of the initial 10 patients or 30% of all patients (i.e., when accrual is greater than 10 patients), have experienced a Grade 3 or higher injection site reaction or infusion related reaction or any Grade 4 or 5 adverse event (at least possibly related to the study treatment), accrual to the study will be suspended to allow for a full review of the data.

Each Grade 5 event (death) will be reviewed on a case-by-case basis in a real time fashion to determine whether study accrual should be suspended.

Study accrual will be suspended for:

- Any Grade 5 event that is at least possibly related to the administration of the investigational product
- Any Grade 5 event within 30 days of receiving investigational product that is at least possibly related to the administration of the investigational product
- If two or more patients fail to proceed to planned surgery or chemo-radiation due to disease progression or adverse events that prevent or delay surgical resection or chemo-radiation within the allotted timeline per protocol (>2 weeks or ≤8 weeks after the last dose of study drug)

After consideration by the study team [i.e., Study Chair(s), Statistician, Operations Office, etc.] and consultation with representatives at the primary Institutional Review Board (IRB) affiliated with the Operations Office, and our MCCC DSMB, a decision will be made as to whether and how the study will proceed.

16.3 Analysis Plan:

16.31 Primary Endpoint:
The proportion of responses (pathologic response or ctHPVDNA responses done separately) will be estimated by the number of successes divided by the total number of evaluable patients. Ninety-five percent confidence intervals for the true success proportion will be calculated according to the exact binomial method for each arm separately.

16.32 Definitions and Analyses of Secondary Endpoints (Note: These endpoints will be analyzed separately for each arm)

16.321 Progression-Free survival (PFS) is defined as the time from registration to the first of either disease progression or death from any cause. Patients who receive the study drug, but then never return for an evaluation will be censored on their last follow-up date. PFS will be estimated using the method of Kaplan-Meier. PFS rates at 12 and 24 months will also be reported.

16.322 Overall survival (OS) is defined as the time from registration to death from any cause. OS will be estimated using the method of Kaplan-Meier.

16.323 The response rate will be estimated using RECIST 1.1 criteria. A tumor response is defined to be either a CR or PR noted up until surgery or definitive chemoradiation. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be

evaluable for response. Patients who go off study early before having a tumor assessment performed will be considered a failure.

16.33 Adverse Events
All patients that have intitiated treatment will be considered evaluable for adverse event (AE) analyses. The maximum grade for each type of AE will be recorded for each patient, and frequency tables will be reviewed to determine AE patterns. AEs will be analyzed separately by arm.

16.34 Translational Endpoints
Determine the changes in tumor microenvironment (TME) with PDS0101 and PDS0101 ± pembrolizumab, assess circulating ctHPVDNA as a marker for tumor response, determine HPV16-specific T-cell response, and finally assess immunologic response utilizing multiplex flow cytometry. All of these translational endpoints are considered exploratory and hypothesis generating due to the small proposed sample size for this study with only 12 patients per arm. All analyses will be done separately for each arm. Associations of the categorical biomarkers with clinical data like response will be done via Fisher's Exact tests. Continuous biomarker data will be associated with response using Wilcoxon Rank-Sum tests.

16.35 Over Accrual:
If more than the target number of patients are accrued, the additional patients will be included in final point estimates and confidence intervals as though they were accrued for the final analysis.

16.4 Inclusion of Women and Minorities

16.41 Study Availability
This study will be available to all eligible patients, regardless of gender, race, or ethnic origin.

16.42 Differential effects
There is no information currently available regarding differential effects of this regimen in subsets defined by gender, race, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analysis will, as always, look for differences in treatment effect based on racial groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.43 Area Population

Based on prior studies involving similar disease sites, we expect about 20% of patients will be classified as minorities by race and around 50% will be women. Expected sizes (per study design) of racial by gender subsets are shown in the following table:

Accrual Targets			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	2	2	4
Not Hispanic or Latino	10	10	20
Ethnic Category: Total of all subjects	**12**	**12**	**24**
Racial Category			
American Indian or Alaskan Native	1	0	1
Asian	0	1	1
Black or African American	1	1	2
Native Hawaiian or other Pacific Islander	1	0	1
White	9	10	19
Racial Category: Total of all subjects	**12**	**12**	**24**

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa.

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

17.1 Summary Table of Research Tissue Specimens to be Collected for this Protocol

Research Study	Specimen Purpose	Mandatory or Optional	Type of Tissue to Collect	Archived tissue or biopsy	At time of surgery or research biopsy[18]
IHC for TILs and imaging mass cytometry	☒ Correlative	Mandatory	Formalin fixed paraffin embedded (FFPE)	X	X
TCR clonality and sequences, flow cytometry	☒ Correlative	Mandatory	Frozen tissue	X[19]	X

NOTE: See Lab Manual for additional information

[18] Biopsy is requested for chemoradiation patients
[19] If available

17.2 Diagnostic Slides from Original and /or Recurrent Tissue

Diagnostic slides from the time of surgical resection for the primary site and involved lymph nodes will be provided to Dr Joaquin Garcia or another pathologist specializing in Head and Neck Pathology, for review and characterization of pathologic response. Along with original diagnostic slides, include pathology reporting form, surgical pathology report and operative report. Pathologist will be blinded to study treatment arm.

17.3 Correlative Tissue Collection

17.31 Tissue Kits will not be provided for this protocol.

17.32 Paraffin Embedded Tissue

Available tissue from diagnostic biopsy will stored for future correlative analyses. See Lab Manual for further information.

17.33 Frozen Tissue

At time of research biopsy 1-2 cores should be flash frozen.

At the time of surgery, tissue from the primary site and up to 2 involved lymph nodes will also be frozen. See Lab Manual for further information.

17.4 Background and Methodology

17.41 Immunohistochemistry, tumor infiltrating lymphocytes, and imaging mass cytometry

We will quantify tumor infiltrating lymphocytes and compare responders and non-responders. We will determine spatial locations of T cells and other immune cells in tumor and lymph node tissues of both responders and non-responders using imaging mass cytometry, as well as at the primary tumor site in comparison to involved lymph nodes, noting differences by pathologic response.

17.42 Frozen Tissue Analyses

We will compare paired analysis of TCR clonality and sequences in tumor and peripheral samples. Additionally, we will utilize multi-parameter flow cytometry combined with single cell omics to characterize immune infiltrate and function of responders and non-responders.

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18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Survival Follow-up

See Section 4.

18.3 CRF completion

This study will use Medidata Rave® for remote data capture (rdc) of all study data. Data collection for this study will be done exclusively through the Medidata Rave® clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active account and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on the organization roster at the enrolling site.

18.4 Site responsibilities

Each site will be responsible for ensuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation for diagnosis and progression prior to study entry, as well as for evidence of response to study therapy and progression after study therapy. These documents should be submitted within 14 days of registration (for prior to study entry materials) or within 14 days after the visit at which response or progression is determined. Pathology and operative reports from clinical and research biopsy as well as from surgery should be entered into Rave.

Upload patient vaccine diaries into Rave when available.

18.6 Labeling of materials

Each site will be responsible for ensuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Overdue lists

A list of overdue forms and outstanding queries will be available in Rave through the Rave Task Summary. In addition to this, the Overdue Materials report is available on the Cancer Center Systems homepage.

19.0 Budget

19.1 Costs charged to patient:

Routine clinical care

19.2 Tests to be research funded:

- Research biopsy
- PDS0101 administration
- Pembrolizumab administration
- Collection, processing and storage of blood and tissue samples for future, unspecified research

19.3 Other budget concerns:

- PDS Biotech will provide Mayo Clinic with funding to support the costs of running this study.
- PDS Biotech will provide study drug PDS0101 and funding for pembrolizumab for use in this study

20.0 References

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

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

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

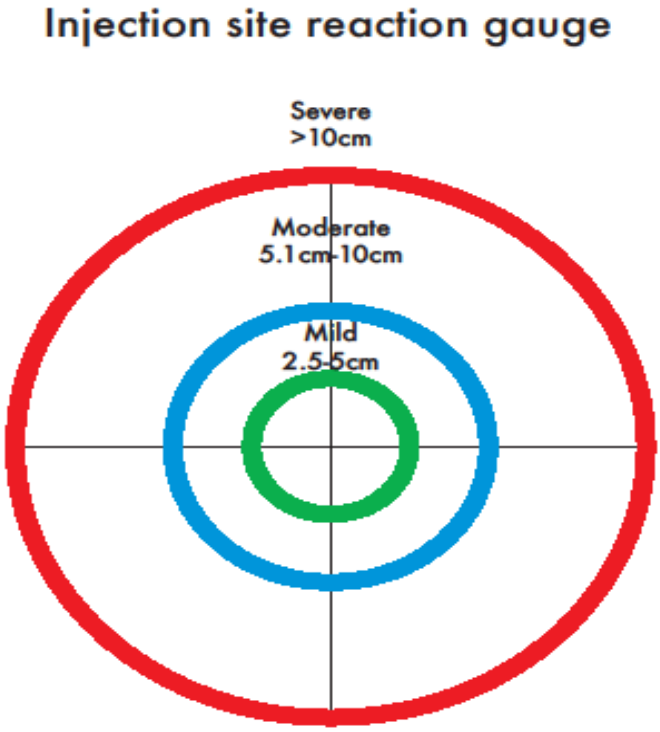
[redacted]

Appendix II Patient PDS0101 Vaccine Diary

This document is provided separately per Mayo Clinic IRB requirement.

Appendix III Injection Site Reaction Gauge

NOTE: Sample only – not to scale. (Will be printed on acetate)



Example only. Please use the clear version provided by the study team to check vaccine site.

Appendix IV Suggested Contraception Options

NOTE: Final decision for contraception method is between the provider and the patient.

(1) practice abstinence† from heterosexual activity.

OR

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

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):
• intrauterine device (IUD)
• vasectomy of a female subject's male partner
• contraceptive rod implanted into the skin

Combination method (requires use of two of the following):
• diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
• cervical cap with spermicide (nulliparous women only)
• contraceptive sponge (nulliparous women only)
• male condom or female condom (cannot be used together)
• hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for patients participating at sites in this country/region.