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Title: Phase II Trial of Immunotherapeutic HPV vaccine PRGN-2009 with Pembrolizumab before Standard Treatment in Subjects with Newly Diagnosed HPV-Associated Oropharyngeal Cancer

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Drug Name:	PRGN-2009	Pembrolizumab
IND Number:	[redacted]	[redacted]
Sponsor:	CCR, NCI	CCR, NCI
Manufacturer:	[redacted]	[redacted]
Supplier	[redacted]	[redacted]

PRÉCIS

Background

- Human papilloma virus-associated oropharyngeal cancer (HPV-OPC) is the most common HPV-associated malignancy in the United States, with an increasing incidence. Although the prognosis for stage I HPV-OPC is favorable, about 20 percent of patients with stage II disease and 35 percent of patients with stage III disease will die within four years.
- The standard-of-care primary treatment for HPV-OPC without distant metastasis is definitive concurrent chemoradiotherapy (primarily) or surgery (which may be followed by adjuvant chemoradiotherapy).
- Neoadjuvant/induction immunotherapy is in clinical trials aiming to induce antigen-specific immunity prior to primary treatment and to reduce the risk of disease relapse. Pembrolizumab, an anti-PD-1 monoclonal antibody that is FDA-approved for first-line treatment of recurrent/metastatic head and neck squamous cell cancer (HNSCC) has been used in these trials and shown to be safe and active.
- PRGN-2009 is an anti-HPV immunotherapeutic vaccine. It has demonstrated induction of HPV antigen-specific responses and tumor growth inhibition in pre-clinical models of HPV-associated malignancy, with improved anti-tumor efficacy upon addition of T-cell immune checkpoint blockade. In a Phase I/II trial at the NCI it has demonstrated excellent safety and tolerability as monotherapy or in combination with checkpoint blockade in recurrent/metastatic disease but also as monotherapy in neoadjuvant/induction context for HPV-OPC.

Objective:

- To determine if the use of PRGN-2009 with pembrolizumab in participants with p16-positive OPC can result in a ≥ 2-fold increase in $CD3^+$ tumor infiltrating T cells post treatment compared with pre-treatment.

Eligibility:

- Age ≥ 18 years.
- Pathologically confirmed newly diagnosed Stage I (T1, T2; N1), II or III p16-positive OPC.

Design:

- This is a Phase II study to evaluate the effect of PRGN-2009 and pembrolizumab before definitive treatment in subjects with p16-positive OPC.
- Participants will receive PRGN-2009 and pembrolizumab prior to definitive treatment.
- Participants will receive two doses of PRGN-2009 $5x10^{11}$ viral particles (VP) subcutaneously (SC) approximately two weeks apart, and one dose of pembrolizumab 200 mg intravenously (IV) concurrently with the first vaccine dose.
- Up to 20 evaluable participants will be enrolled.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine if the use of PRGN-2009 with pembrolizumab in participants with p16-positive OPC can result in a ≥ 2-fold increase in $CD3^+$ tumor infiltrating T cells post treatment compared with pre-treatment

1.1.2 Secondary Objectives

- To determine if the use of PRGN-2009 plus pembrolizumab results in significantly prolonged survival as compared to the expected 80% three-year historical in p16-positive OPC participants
- To determine the 3-year overall survival (OS) and relapse-free survival (RFS) rate for PRGN-2009 plus pembrolizumab as neoadjuvant/induction therapy before definitive standard of care therapy in p16-positive OPC participants
- To assess the safety of PRGN-2009 plus pembrolizumab in p16-positive OPC participants
- To determine if the rate of ≥ 2-fold increase in tumor infiltrating lymphocytes by immunohistology following PRGN-2009 in combination with pembrolizumab is significantly more than the rate with PRGN-2009 alone

1.1.3 Exploratory Objectives

- To determine the overall response rate (ORR) per RECIST v1.1 in subjects receiving PRGN-2009 plus pembrolizumab

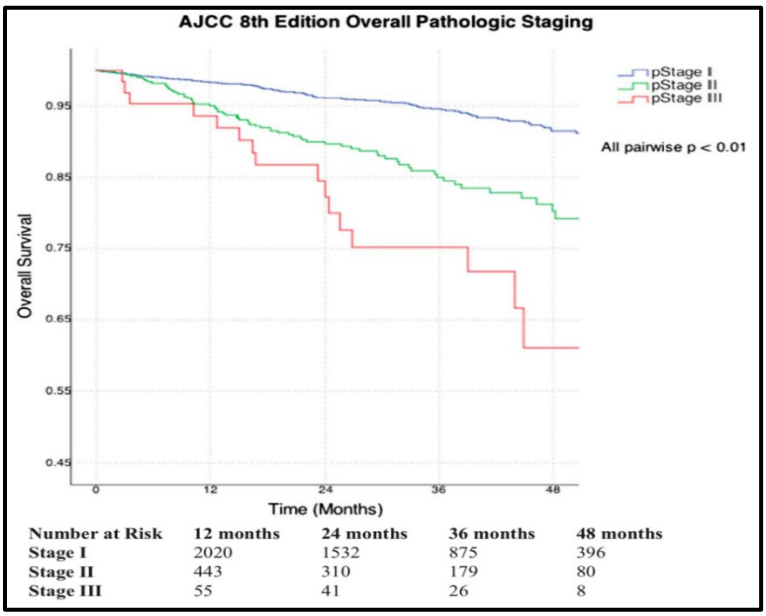
- To conduct exploratory immunologic studies to understand and improve the administered treatment, including but not limited to the following:
  - Standard immune cell types (CD4 and CD8 T cells, natural killer [NK] cells, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], and dendritic cells) as well as immune cell subsets
  - Function of specific immune cell subsets (CD4 and CD8 T cells, NK cells, Tregs, and MDSCs)
  - Immune responses to HPV antigens;
  - T-cell clonality
  - RNA expression
  - Exome sequencing
  - Soluble factors, cytokines, chemokines, antibodies, tumor-associated antigens, and/or other markers
  - Anti-vector antibodies (AVA)
  - Circulating free HPV 16/18 DNA
  - Immune markers
- To evaluate HPV status to assess HPV positivity and subtype
- To assess changes in salivary HPV DNA and salivary soluble factors
- To assess HPV 16/18-specific immune responses, activation status of the immune system, and anti-tumor specific immune responses induced by the exposure to PRGN-2009 with biomarker studies

1.2 BACKGROUND AND RATIONALE

1.2.1 HPV-associated Oropharyngeal Cancer

Human papilloma virus-associated oropharyngeal carcinoma (HPV-OPC) is the most common HPV-associated malignancy in the United States and the incidence is rising [1, 2]. Over 80% of OPC is HPV-associated, and nearly all HPV-OPC is caused by HPV 16 or 18.

Figure 1: Stage-specific overall survival based upon AJCC 8th Edition Classification



In stage II/III HPV-OPC primary treatment most commonly is concurrent chemoradiotherapy (CRT) [3]. Surgery is rarely utilized due to the morbidity associated with resection of oropharyngeal tissues. Despite maximum treatment, 20% of patients with stage II and 35% of patients with stage III disease will die within 4 years of diagnosis [4]. In patients cured of their disease, treatment often results in dysfunctional swallowing, taste, and speech, as well as in extensive tissue fibrosis and pain [5]. Patients with HPV-OPC are diagnosed at a younger age, making the long-term morbidity associated with concurrent CRT particularly concerning. Induction treatment strategies that have the goals of both decreasing disease recurrence and increasing survival permit the future study of definitive treatment de-intensification [6]. Attempts to de-intensify treatment of HPV-OPC by replacing cisplatin chemotherapy with the epithelial growth factor receptor (EGFR) monoclonal antibody (mAb) cetuximab have failed [3]. Of note, neoadjuvant/induction/ strategies in the context of HNSCC are not intended to downstage tumors and improve resectability, especially for OPC, where treatment of early disease is dominated by chemoradiotherapy.

Stage I (AJCC 8th edition) includes patients with T0-2 and N0-1 disease. Node-negative Stage I (T1-2 N0) disease is treated with radiotherapy or surgical resection (with or without neck lymph node dissection) and risk-stratified adjuvant treatment. Node-positive Stage I (T1-2 N1) disease with 1 ipsilateral lymph node not larger than 3 cm is treated with definitive chemoradiotherapy or radiotherapy or surgical resection (with or without neck lymph node dissection) and risk-stratified adjuvant treatment. Node-positive Stage I (T1-2 N1) with 1 ipsilateral lymph node larger than 3 cm or 2 or more ipsilateral lymph nodes (none larger than 6 cm) is treated like Stage II disease, with chemoradiotherapy or surgery and adjuvant treatment. Therefore, while the prognosis for Stage I patients is better than the prognosis Stage II and III, improvement of outcomes with primary treatment may allow for de-intensification of treatment in node positive Stage I (T1-2 N1) disease.

Consequently, novel strategies are required to improve outcomes following primary treatment in newly diagnosed early-stage HPV-OPC without incurring additional toxicity.

1.2.2 T cell Dysfunction in HPV-Positive OPSCC

HPV-positive OPSCCs are infiltrated by exhausted and functionally suppressed HPV-specific T cells [7-9]. This demonstrates that although an adaptive immune response against one or more HPV antigens develops, these T cells are ineffective at controlling the growth of antigen-expressing tumors. This also suggests that reversing exhaustion in or the suppression of existing HPV-specific T cells may induce HPV antigen-specific anti-tumor immunity. When ligated by programmed death-ligand 1 (PD-L1), signaling through programmed death-receptor 1 (PD-1) on T cells is a significant mechanism of T cell exhaustion [10], therefore PD-(L)1 blockade may reverse this exhaustion.

1.2.3 Neoadjuvant/Induction Immune Checkpoint Blockade in HNSCC

Patients with newly diagnosed early stage HNSCC eligible for definitive treatment with curative intent typically wait 4-6 weeks for initiation of treatment due to treatment planning logistics (radiotherapy mapping and simulation, scheduling for chemotherapy) [11]. During this "window of opportunity" participants receive no treatment for their cancer.

The clinical efficacy of anti-PD-1/ PD-L1 immune checkpoint blockade (ICB) in recurrent/metastatic HNSCC provided the rationale for ICB trials before definitive treatment in newly diagnosed locally advanced HNSCC. Induction or neoadjuvant immunotherapy for newly diagnosed HNSCC is an area of active study aiming to decrease locoregional failure and the development of distant metastasis after completion of definitive treatment. Uppaluri et al conducted a trial of pembrolizumab prior to surgery in HPV-unrelated HNSCC (coprimary endpoints of 1-year relapse rate and proportion of patients with pathologic response in the surgical specimen). In this trial, the pre-operative administration of pembrolizumab was shown to be safe, as no serious immune-related adverse events occurred in the interval between dosing and surgery and through 30 days after surgery, and there was no unexpected surgical delay or complication. With a median follow-up after surgery of 22 months, and 97% percent of patients having had 1 year or more of follow-up, the 1-year relapse rate was 16.7% for the high-risk pathology patients (lower than the historical rate of 35%), and 0% in the intermediate/low-risk pathology patients; pathologic tumor responses of >10% were observed in 16 of 36 patients (44%). Significant clinical tumor responses occurred in a minor proportion of patients, and downstaging of cancer occurred in 7 patients (19%) [12]. Ferrarotto et al conducted a trial of durvalumab with or without tremelimumab prior to surgery in OPC (primary endpoint of increase in the ratio of post-treatment to pre-treatment $CD8^+$ TIL density in surgical specimens by 0.5), showing it is feasible, safe, and results in pathologic tumor response and increase in $CD8^+$ tumor infiltrating T-lymphocytes [13]. Ferris et al conducted a trial of immunotherapy with nivolumab prior to definitive surgery in resectable HNSCC (primary endpoint the safety and tolerability of neoadjuvant nivolumab, with pathologic response evaluated in surgical specimens as a secondary endpoint), and demonstrated it is safe, and resulted in pathologic regressions, more so in HPV-positive tumors (23.5%) than HPV-negative tumors (5.9%) [14]. Hecht et al conducted a trial of chemoimmunotherapy (cisplatin, docetaxel, durvalumab, tremelimumab) prior to radiotherapy with concurrent immunotherapy in HPV-unrelated HNSCC, showing it is safe and resulted in promising progression free survival (PFS) [15]. Taken together, the results of these trials demonstrate that ICB is safe and has promising activity in both the neoadjuvant and the induction setting in newly diagnosed HNSCC.

1.2.4 PRGN-2009 Vaccine

In addition to reversing exhaustion and local immunosuppression of existing antigen-specific T cells, a complimentary approach to induce anti-cancer immunity is to induce *de novo* T cell responses with therapeutic vaccines [16]. The use of a therapeutic vaccine may be ideal for virally induced cancers as viral oncogenes required for malignant transformation are known (E6 and E7 for high-risk HPV). Human adenoviral vaccine platforms are effective at delivering nucleic acid payload to dendritic cells and can induce antigen-specific T cell immunity following as few as two treatments but can be limited by the presence of pre-existing immunity to the vaccine platform itself. Gorilla adenovirus vaccine platforms retain the advantages of human adenoviruses including the ability to delete genetic regions to ensure replication incompetence and allow for insertion of transgenes of interest, but are not recognized by human sera from healthy donors [17]. Whole genes or long synthetic peptides of interest can be inserted into adenoviral constructs to allow the processing of naturally processed and presented T cell antigens by antigen presenting cells. This allows for treatment of all patients with HPV-positive malignancy without HLA restriction. In collaboration with [redacted] a gorilla adenovirus vaccine that encodes HPV T cell antigen epitopes from HPV 16 and 18 (hereto referred to as PRGN-2009) has been developed.

PRGN-2009 was developed from the base gorilla adenoviral vector GC46. The adenovirus from which the GC46 adenovector is built was originally isolated from a healthy African gorilla. The wild type adenovirus was fully sequenced and the adenovector genome was isolated and inserted into plasmid DNA. Therefore, the GC46 adenovector is completely separated from the virus isolation process. The GC46 adenovirus vector is closely related to and clusters phylogenetically with the human species C adenovirus based on hexon, DNA polymerase, and E4 ORF6 protein sequence comparisons [18].

The seroprevalence of GC46 is less than 6% in the USA and the seropositive titers were low (below 100 IC_{90}). In comparison, the seroprevalence of Ad5 is 57% with most of the seropositive individuals having high titers (above 200 IC_{90}; [19]). In addition, comparative studies from human sera samples from Sub-Saharan Africa also confirmed the rare and weak pre-existing neutralizing activity in the human population. High level pre-existing seroprevalence to Ad5 has been shown to limit the effectiveness of Ad5 based adenovectors in clinical studies. Therefore, the rare and weak pre-existing neutralizing activity to GC46 provides an advantage for GC46 based adenovector use in clinical applications.

The cloned GC46 genome was engineered to have deletions of two essential regions of the adenovector, E1 and E4. Deletion of these regions removes several essential elements necessary for GC46 replication. Therefore, PRGN-2009 is not replication competent. PRGN-2009, as well as other GC46 adenovectors with E1 and E4 deletions, have been evaluated for growth in human cell lines and were found to only replicate in [redacted] complementing manufacturing cell lines, ORF6 and M2A. The multi-deleted, replication incompetent adenovector platform technology was first developed using Ad5 based adenovectors. These similarly deleted, constructed, and manufactured Ad5 adenovectors were used for clinical development of multiple molecular vaccine and gene therapy molecules administered to over 3,000 clinical study participants.

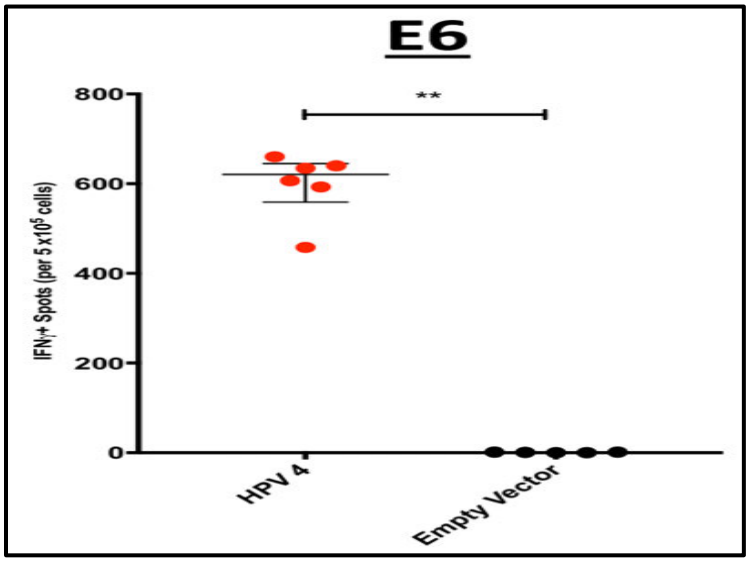
HPV-16 and HPV-18 are the genotypes of HPV most frequently associated with cervical, head and neck, and other cancers and, as such, infections with either genotype are considered “high risk” for the development of cancer. In HPV-16 and HPV-18, two primary oncoproteins, E6 and E7, are constitutively expressed by HPV-associated tumors and are critical for the induction and

maintenance of cellular transformation in HPV-infected cells. Recent evidence suggests the E5 protein also affects viral transformation. The HPV antigen expressed in PRGN-2009 was designed for optimum immunogenicity. Guided by bioinformatics analysis and in silico protein engineering, 32 key immunogenic peptides were selected which included cytotoxic T lymphocyte (CTL) specific peptides from E6 (HPV-16/-18), E7 (HPV-16/-18), and E5 (HPV16), and 3 unique agonist peptides for this multi-epitope antigen design [18]. These 35 CTL peptide sequences were grafted to a human ankyrin repeat protein scaffold, enabling protein linker sequences embedded between the peptides wherein this scaffold retains its tertiary structure displaying the HPV epitopes. Designed shuffling of the peptides prevents any reformation of E6 and E7 oncogenic protein potential and HPV protein viral function.

PRGN-2009 Drug Substance (DS) is manufactured by Advanced Bioscience Laboratories (ABL) utilizing a standardized adenoviral vector manufacturing process that was developed at [redacted] and transferred to ABL. The production cell line is [redacted] proprietary 293-ORF6 cell line, which complements for both adenoviral E1 and E4 ORF6 to enable efficient production of the replication incompetent GC46 adenoviral vector. The 293-ORF6 cells are cultured in serum free suspension in shaker flasks and infected with PRGN-2009 Master Virus Bank (MVB) at a multiplicity of infection (MOI) of 100 PU/cell. The PRGN-2009 culture harvest is downstream processed and purified using three rounds of cesium chloride (CsCl) density gradient ultracentrifugation to yield a highly purified PRGN-2009 DS material. Manufacture of the PRGN-2009 DP commences with the thaw of frozen PRGN-2009 DS, which is then sterile-filtered and filled into Diakyo Crystal Zenith (CZ) vials to create the DP, which is stored in freezer boxes in a secure freezer at -60ºC to -90ºC. PRGN-2009 has been tested in three independent *in vitro* analyses to assess oncogenic potential in mammalian cells. PRGN-2009 did not induce an oncogenic profile in any assay.

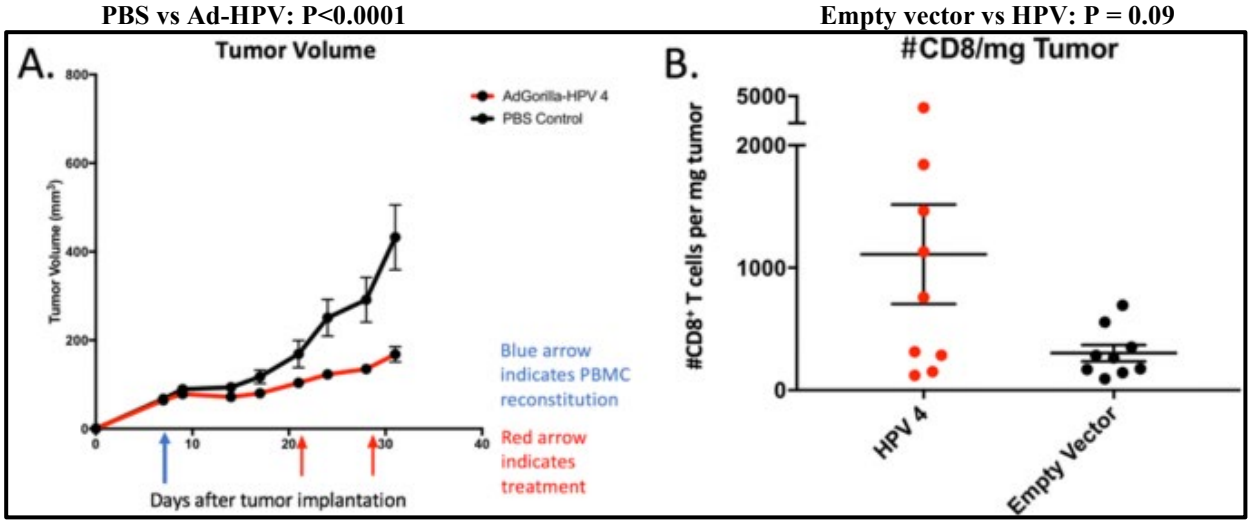
In pre-clinical models, PRGN-2009 induced the formation of peripheral blood HPV 16 E6 antigen-specific responses in wild-type C57BL/6 mice bearing HPV 16 E6/E7 positive lung SCCs (TC-1 tumors). No HPV 16 E6 antigen-specific T cell responses were measurable in the absence of PRGN-2009 (**Figure 2**).

Figure 2: Peripheral PBMC from WT B6 mice bearing TC-1 tumors were assessed for HPV 16 E6 antigen-specific responses following exposure to overlapping peptide. HPV4 = PRGN-2009.



Additionally, mice bearing HPV 16-positive SiHa cervical cancers were immune humanized with HLA-matched human PBMC and treated with PRGN-2009 (**Figure 3**). Treatment resulted in tumor growth inhibition and increased tumor infiltration of $CD8^+$ T cells. These data demonstrated that PRGN-2009 is capable of inducing HPV antigen-specific immune responses and anti-tumor immunity sufficient to inhibit tumor growth.

Figure 3: (A) NSG B2M-/- mice bearing SiHa tumors were immune humanized with HLA matched PBMC and mice were treated with PRGN-2009. (B) Following the second vaccine administration, SiHa tumors were assessed for $CD8^+$ T cell infiltration by flow cytometry.

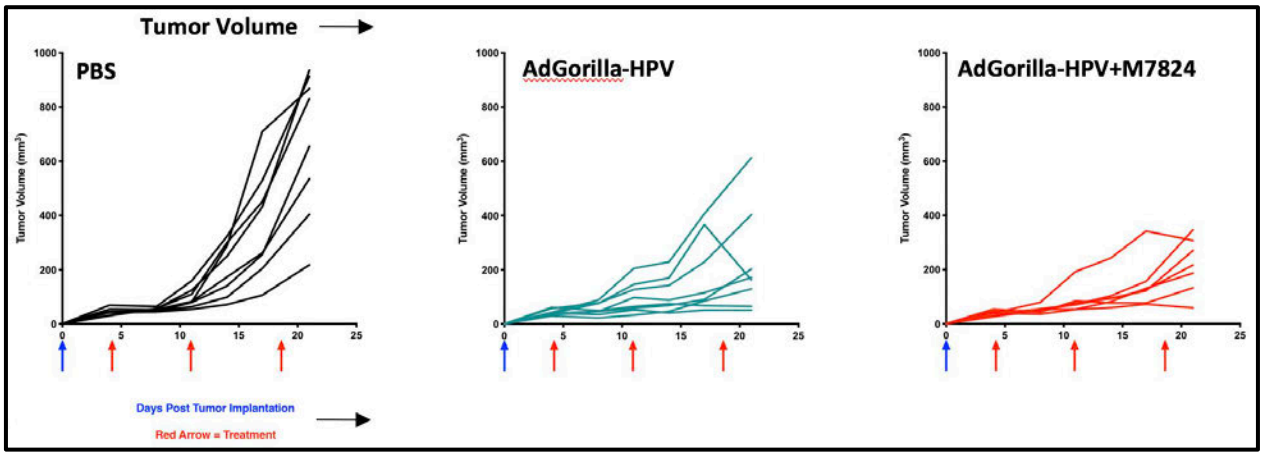


1.2.5 Rationale for Combination of PRGN-2009 with PD-1/PD-L1 Blockade

The rationale for combination therapy includes induction of antigen-specific T cell responses with vaccine that can then be fully activated with the addition of PD-1/PD-L1 immune checkpoint pathway blockade. In a phase II trial, ISA101, a synthetic long-HPV 16 E6/E7 peptide vaccine, was combined with nivolumab in patients with incurable HPV 16-positive cancer [20]. This study demonstrated an objective response rate of 33% and an acceptable safety profile. Two phase I studies have demonstrated the safety and feasibility of combining therapeutic vaccines targeting different antigens with PD-1 mAb treatment, along with correlative immunologic responses, in patients with melanoma [21, 22].

The combination of PRGN-2009 with PD-1/PD-L1 blockade was tested using with the anti-PD-L1/TGF-beta trap agent M7824 in wild-type C57BL/6 mice bearing TC-1 tumors (**Figure 4**). The addition of PD-1/PD-L1 blockade further enhanced tumor growth inhibition observed with PRGN-2009 alone. Cumulatively, these results indicate that the addition of PD-1/PD-L1 blockade to potentially therapeutic vaccination is both safe and feasible in the clinical setting as well as induces tumor growth inhibition in pre-clinical models of HPV-positive malignancy.

Figure 4: C57BL/6 mice bearing TC-1 tumors were treated with PRGN-2009 alone or in combination with PD-1/PD-L1 blockade (M7824) and assessed for primary tumor growth.



PBS vs Ad-HPV: P<0.0001 PBS vs Ad-HPV + M7824: P<0.0001

1.2.6 Clinical Experience with PRGN-2009

A Phase I/II clinical trial of PRGN-2009 alone or in combination with M7824 in HPV-associated malignancies is currently enrolling at the NCI (NCT04432597).

1.2.6.1 Phase I study of PRGN-2009 alone or with M7824 in advanced HPV-associated malignancies

The Phase I of this study addresses patients with recurrent/metastatic HPV-associated malignancies, and has two arms, with a sequential design: Arm 1A, a 3+3 dose-escalation and recommended phase 2 dose (RP2D) determining study of PRGN-2009 monotherapy (two dose levels, $1x10^{11}$ and $5x10^{11}$ viral particles [VP]), followed by Arm 1B, a study of the PRGN-2009 (RP2D) plus M7824 (fixed dose), with a primary endpoint of safety of the combination.

Early results of the ongoing trial as of the end of September 2021 were presented in the SITC21 Annual Meeting. Arm 1A of the study had been completed, having enrolled 6 participants, with

no dose limiting toxicities (DLTs) or dose expansion. Arm 1B was enrolling, having accrued 6 participants (10 evaluable for efficacy planned). PRGN-2009 as monotherapy was well tolerated and safe, with observed adverse events (AEs) (**Table 1**) being Grade 1-2 injection site reactions, flu-like symptoms, fatigue, and rash. Similar toxicity profile was noted in the combination with M7824, with the addition of G3-4 keratoacanthomas, duodenal hemorrhage and anemia, attributed to M7824. One patient in the combination arm died due to duodenal hemorrhage following refusal of blood transfusion support. The adverse events which were considered related to treatment with PRGN-2009 are summarized by system-organ class in **Table 1.**

Table 1: Treatment-Emergent Adverse Events Occurring in >1 Patient by System Organ Class and Preferred Term in Phase 1/2 Study in Patients with HPV+ Malignancies (NCT04432597)

System Organ Class Preferred Term	Phase 1						Phase 2	
	PRGN-2009, 1 x 10^{11} PU (n=3)		PRGN-2009, 5 x 10^{11} PU (n=3)		PRGN-2009, 5 x 10^{11} PU + M7824, 1200 mg (n=11)		PRGN-2009, 5 x 10^{11} PU (n=22)	
	# of events	# of patients	# of events	# of patients	# of events	# of patients	# of events	# of patients
Blood and lymphatic system disorders								
Anemia	10	2/3 (66.7%)	1	1/3 (33.3%)	5	4/11 (36.4%)	2	2/22 (9.1%)
Ear and labyrinth disorders								
Ear pain	0	0	0	0	0	0	2	2/22 (9.1%)
Gastrointestinal disorders								
Abdominal pain	1	1/3 (33.3%)	1	1/3 (33.3%)	3	3/11 (27.3%)	0	0
Constipation	1	1/3 (33.3%)	2	2/3 (66.7%)	1	1/11 (9.1%)	1	1/22 (4.5%)
Diarrhea	1	1/3 (33.3%)	0	0	2	2/11 (18.2%)	0	0
Dry mouth	0	0	0	0	2	2/11 (18.2%)	0	0
Duodenal hemorrhage	0	0	0	0	2	2/11 (18.2%)	0	0
Dyspepsia	0	0	0	0	0	0	1	1/22 (4.5%)
Mucositis oral	0	0	0	0	2	2/11 (18.2%)	1	1/22 (4.5%)
Nausea	2	2/3 (66.7%)	2	1/3 (33.3%)	3	2/11 (18.2%)	3	3/22 (13.6%)
Oral hemorrhage	0	0	0	0	2	2/11 (18.2%)	0	0
Vomiting	1	1/3 (33.3%)	3	2/3 (66.7%)	0	0	0	0

System Organ Class Preferred Term	Phase 1						Phase 2	
	PRGN-2009, 1 x 10^{11} PU (n=3)		PRGN-2009, 5 x 10^{11} PU (n=3)		PRGN-2009, 5 x 10^{11} PU + M7824, 1200 mg (n=11)		PRGN-2009, 5 x 10^{11} PU (n=22)	
	# of events	# of patients	# of events	# of patients	# of events	# of patients	# of events	# of patients
Blood and lymphatic system disorders								
Anemia	10	2/3 (66.7%)	1	1/3 (33.3%)	5	4/11 (36.4%)	2	2/22 (9.1%)
Ear and labyrinth disorders								
Ear pain	0	0	0	0	0	0	2	2/22 (9.1%)
Gastrointestinal disorders								
Abdominal pain	1	1/3 (33.3%)	1	1/3 (33.3%)	3	3/11 (27.3%)	0	0
Constipation	1	1/3 (33.3%)	2	2/3 (66.7%)	1	1/11 (9.1%)	1	1/22 (4.5%)
Diarrhea	1	1/3 (33.3%)	0	0	2	2/11 (18.2%)	0	0
Dry mouth	0	0	0	0	2	2/11 (18.2%)	0	0
Duodenal hemorrhage	0	0	0	0	2	2/11 (18.2%)	0	0
Dyspepsia	0	0	0	0	0	0	1	1/22 (4.5%)
Mucositis oral	0	0	0	0	2	2/11 (18.2%)	1	1/22 (4.5%)
Nausea	2	2/3 (66.7%)	2	1/3 (33.3%)	3	2/11 (18.2%)	3	3/22 (13.6%)
Oral hemorrhage	0	0	0	0	2	2/11 (18.2%)	0	0
Vomiting	1	1/3 (33.3%)	3	2/3 (66.7%)	0	0	0	0

System Organ Class Preferred Term	Phase 1						Phase 2	
	PRGN-2009, 1 x 10^{11} PU (n=3)		PRGN-2009, 5 x 10^{11} PU (n=3)		PRGN-2009, 5 x 10^{11} PU + M7824, 1200 mg (n=11)		PRGN-2009, 5 x 10^{11} PU (n=22)	
	# of events	# of patients	# of events	# of patients	# of events	# of patients	# of events	# of patients
General disorders and administration site conditions								
Fatigue	6	2/3 (66.7%)	0	0	7	4/11 (36.4%)	8	5/22 (22.7%)
Fever	0	0	0	0	2	2/11 (18.2%)	0	0
Flu-like symptoms	4	2/3 (66.7%)	2	1/3 (33.3%)	15	6/11 (54.5%)	19	17/22 (77.3%)
Injection site reaction	21	3/3 (100%)	2	1/3 (33.3%)	16	9/11 (81.8%)	22	16/22 (72.7%)
Pain	1	1/3 (33.3%)	1	1/3 (33.3%)	1	1/11 (9.1%)	8	7/22 (31.8%)
Injury, poisoning and procedural complications								
Fall	1	1/3 (33.3%)	0	0	2	2/11 (18.2%)	0	0
Fracture	0	0	0	0	2	2/11 (18.2%)	0	0

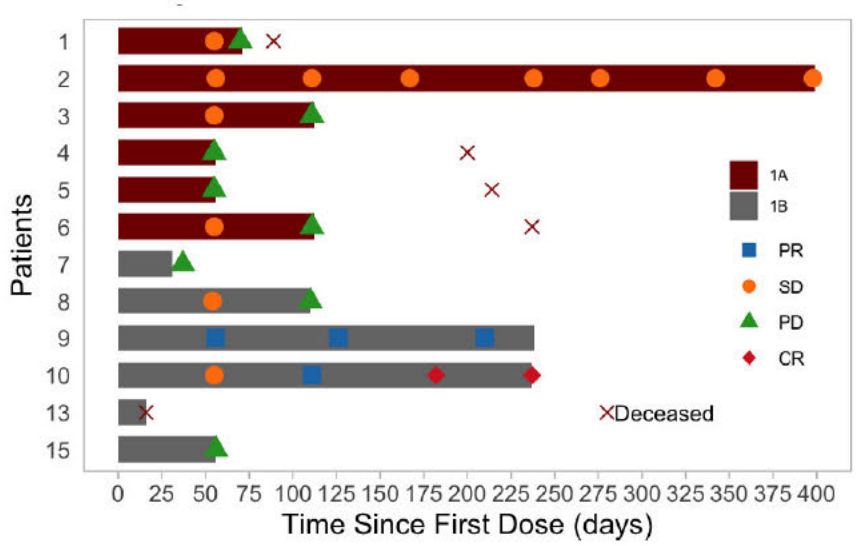
In a preliminary analysis of immune responses, most patients developed T-cell responses against HPV 16 and 18, and the cascade antigens brachyury and MUC1, and in some patients, the HPV-16 and HPV-18 specific T cell responses were augmented after `repeated vaccination.

Table 2: T-cell responses in Phase I clinical trial of PRGN-2009 alone or with M7824 (NCT04432597); Arm 1A PRGN-2009 monotherapy; Arm 1B combination PRGN-2009 with M7824

T-cell responses Target	**Arm 1A** n/T (%)	**Arm 1B** n/T (%)
HPV-16	4/6 (67)	4/5 (80)
HPV-18	5/6 (83)	4/5 (80)
Brachyury	6/6 (100)	2/3 (67)
MUC1	3/3 (100)	0/2 (0)
T: number of patients tested		

At the time of presentation, in the PRGN-2009 vaccine monotherapy arm one patient had stable disease going on for more than a year, and in the combination arm of PRGN-2009 with M7824 2 of 5 evaluable patients had confirmed ongoing responses, one with partial response (PR), and one with complete response (CR) (**Figure 5**).

Figure 5: Time course of study treatment and response in Phase I clinical trial of PRGN-2009 alone or with M7824 (NCT04432597); Arm 1A PRGN-2009 monotherapy, completed (6 participants); Arm 1B combination of PRGN-2009 with M7824 (6 participants).



1.2.6.2 Phase II window of opportunity study for participants with newly diagnosed stage I (T1,2 N1)/II/III p16-positive OPC

The Phase II part of the ongoing study is a window-of-opportunity study of neoadjuvant/induction immunotherapy in patients with newly diagnosed early-stage (node-positive Stage I; Stages II and

III) p16^{+} OPC, eligible for definitive chemoradiotherapy/surgery with curative intent. Initially this Phase consisted of two Arms. Participants would receive PRGN-2009 (Arm 2A) or PRGN-2009 plus bintrafusp alfa (Arm 2B). before definitive therapy. Upon completion of the neoadjuvant or induction immunotherapy treatment, participants would be referred back to their referring providers for definitive therapy.

Enrollment to Arm 2A started after completion of Arm 1A, and enrollment is ongoing, approaching completion. There have been no delays in initiation of definitive treatment beyond the planned start date due to treatment-related toxicities, and the only treatment-related adverse events that have been noted are Grade 1 and 2 injection site reactions and flu-like syndrome.

In September of 2021 we received a report by [redacted], the manufacturer of M7824, that three randomized clinical trials (two in non-small cell lung cancer and one in biliary tract cancer) had been terminated due to futility and the suggestion of increased toxicity in some subjects, or increased progression in a subset of subjects. This was reported to the Sponsor and the IRB and it was decided to close Arm 2B before enrollment to that arm started. Arm 2B will be replaced by the present trial, which will be conducted as an independent single-arm trial to test the combination of PRGN-2009 with pembrolizumab as neoadjuvant/induction immunotherapy in patients with newly diagnosed early-stage p16-positive OPC eligible for definitive treatment with curative intent. Therefore, the present study (neoadjuvant/induction PRGN-2009 plus pembrolizumab) will be compared with the currently enrolling study of neoadjuvant/induction PRGN-2009.

1.2.7 Pembrolizumab

Tumor-infiltrating lymphocytes (TILs) in cancer tissue have been shown to correlate with favorable prognosis in various malignancies.[23-32] In particular, the presence of CD8^{+} T cells and the ratio of CD8^{+} effector T cells/FoxP3^{+} regulatory T cells seems to correlate with improved prognosis and long-term survival in many solid tumors. The PD-1 receptor-ligand interaction is a utilized by tumors to evade immunosurveillance. [33] PD-1 (encoded by the gene *Pdcd1*) is expressed on the cell surface of activated T cells and down-modulates unwanted or excessive immune responses, including autoimmune reactions. It is an immunoglobulin (Ig) superfamily member related to CD28 and CTLA-4 and it negatively regulates antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4^{+} and CD8^{+} T-cells, B cells, T regs and Natural Killer (NK) cells. The ligands for PD-1, PD-L1 and PD-L2, are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. Both PD-L1 and PD-L2 are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Healthy tissues express little (if any) PD-L1, but a variety of cancers were demonstrated to express abundant levels and it has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma. [31, 34-36] This suggested that the PD- 1/PD-L1 pathway plays a critical role in tumor immune evasion and led to its consideration as an attractive target for therapeutic intervention.

Pembrolizumab is a high affinity, highly selective, humanized immunoglobulin G4 kappa (IgG4κ) mAb that binds to the PD-1 receptor, thus blocking its interaction with PD-L1 and PD-L2.

1.2.7.1 Pre-clinical and clinical data

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD1/PD-L1 interaction enhances infiltration of tumor-specific $CD8^+$ T-cells and leads ultimately to tumor rejection, either as a monotherapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a monotherapy in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, and colorectal carcinoma. Blockade of the PD-1 pathway effectively promoted $CD8^+$ T-cell infiltration into the tumor and the presence of IFN-gamma, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo. [37]

Pembrolizumab has demonstrated robust antitumor activity and a favorable safety profile in patients with multiple solid tumor types and is currently approved in more than 80 countries for the treatment of one or more advanced malignancies. Blockade of the immune checkpoint PD-1/PD-L1 pathway with pembrolizumab is currently FDA-approved as monotherapy for the first-line treatment of recurrent/metastatic HNSCC in patients with a PD-L1 combined positive score (CPS) of >1 or combined with platinum and 5-FU for PD-L1 negative tumors.

1.2.8 Rationale for Selection of Primary Endpoint

Primary endpoint for this study is if PRGN-2009 plus pembrolizumab can result in a ≥2-fold increase in $CD3^+$ tumor infiltrating T lymphocytes post treatment compared with pre-treatment.

This endpoint was chosen for the ongoing trial NCT04432597as an endpoint of biological activity: TILs in cancer tissue have been shown to correlate with favorable prognosis in various malignancies. [23-32] In addition, TIL infiltration is associated with better prognosis in patients with OPC [38] and in HPV-related OPC the intratumoral HPV16-specific cells T cells are associated with improved survival. [9] Following neoadjuvant checkpoint blockade, increases in CD4 cell populations have also been reported. [39] While CD8 TIL targeting tumor antigens in these cancers may be dysfunctional and exhausted, but they are not terminally exhausted, and function can be rescued with systemic immunotherapy (unpublished data). The choice of a 2-fold increase as a threshold for a categorical TIL response was considered to be a meaningful increase and allow for a statistical treatment simpler than that of treating the increase in TIL as a continuous variable. The increase in the ratio of post-treatment to pre-treatment $CD8^+$ TIL density (favorable prognostic biomarker in OPC) in surgical specimens by 0.5 (doubling) was used as a primary endpoint in the study by Ferrarotto et al (neoadjuvant immunotherapy in OPC, any HPV status), and it was reported that it positively associates with tumor pathologic response (a secondary endpoint) in OPC patients receiving neoadjuvant PD-1 with or without CTLA-4 blockade [13]. Relapse-free survival (RFS) was not preferred as a primary endpoint for this study because of constraints regarding sample size and trial duration, given that $p16^+$ OPC has comparatively better outcomes than $p16^-$ OPC and other HNSCC sites. Therefore, RFS is included as a secondary endpoint.

1.2.9 Summary

- Clinical outcomes for newly diagnosed Stage I (T1,2 N1)/ II/ III p16-positive OPC are better than for p16-negative OPC but need improvement and de-intensification to reduce long-term toxicity.

- Participants with advanced p16-positive OPC have tumors infiltrated with exhausted and dysfunctional HPV antigen-specific T cells.
- Treatment with a vaccine targeting HPV oncogene antigens to induce antigen-specific immunity (PRGN-2009) combined with immune checkpoint blockade to fully activate new and existing antigen-specific T cells (pembrolizumab) is a rational combination treatment strategy.
- Pembrolizumab is FDA-approved for first-line treatment in recurrent/metastatic HNSCC. Its use in neoadjuvant treatment has been shown to be safe.
- PRGN-2009 has shown excellent safety profile and immunogenicity in a Phase I trial either alone or in combination with anti-PD-L1 (M7824). Its use as neoadjuvant/induction monotherapy has been shown to be safe in an ongoing trial.
- The study will offer participants the opportunity to benefit from immunotherapy during a period when they are scheduling and awaiting definitive therapy for their newly diagnosed HPV-positive OPC.
- Pre-treatment and post-treatment tumor biopsies before definitive therapy can provide tissue for studies aimed at interrogating the effects of single agent or combination immuno-oncologic treatments on the tumor microenvironment and correlating those effects with clinical activity.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Subjects must have cytologically or histologically confirmed newly diagnosed stage I (T1,2 N1), II or III p16-positive oropharyngeal squamous cell carcinoma (SCC) planned for definitive therapy (surgery or chemoradiotherapy).

2.1.1.2 Subjects must have measurable disease, per RECIST 1.1. See Section **6.3**.

2.1.1.3 Age ≥18 years.

2.1.1.4 Eastern Cooperative Oncology Group [ECOG] performance status ≤ 2 (see Appendix A: Performance Status Criteria).

2.1.1.5 Adequate hematologic function at screening, as follows:

- Absolute neutrophil count (ANC) ≥1 x 10^9/L;
- Hemoglobin (Hgb) ≥ 9 g/dL;
- Platelets ≥ 75,000/microliter.

2.1.1.6 Adequate renal and hepatic function at screening, as follows:

- Serum creatinine ≤ 1.5 x upper limit of normal (ULN) OR measured or calculated creatinine clearance ≥ 40 mL/min for participant with creatinine levels > 1.5 x ULN (glomerular filtration rate [GFR] can also be used in place of creatinine or CrCl);
- Total bilirubin ≤ 1.5 x ULN OR in subjects with Gilbert's syndrome, a total bilirubin ≤ 3.0 x ULN;
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 x ULN, unless liver metastases are present, then values must be ≤ 3 x ULN.

2.1.1.7 Participants serologically positive for HIV, Hepatitis B, or Hepatitis C are eligible if the viral loads are undetectable by quantitative PCR. Note: HIV positive participants must have CD4 count ≥ 200 cells/mm^3 at enrollment, be on stable antiretroviral therapy for at least 4 weeks and have no reported opportunistic infections or Castleman's disease within 12 months prior to enrollment.

2.1.1.8 Women of child-bearing potential (WOCBP) must agree to use effective contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and for at least 4 months following the last dose of pembrolizumab.

2.1.1.9 Participants must be willing to undergo two research biopsies on this study.

2.1.1.10 Ability of participant to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Participants with prior investigational drug, live vaccine, chemotherapy, immunotherapy, or any prior radiotherapy (except for palliative bone directed therapy) within the past 4 weeks prior to the first drug administration. Participants may continue adjuvant hormonal therapy in the setting of a definitively treated cancer (e.g., breast).

2.1.2.2 Major surgery within 28 days prior to the first drug administration (minimally invasive procedures such as diagnostic biopsies are permitted).

2.1.2.3 Pregnant individuals as evaluated by a positive serum or urine β-hCG at screening

2.1.2.4 Active autoimmune disease that might deteriorate when receiving an immunostimulatory agent with the **exception** of:

- Diabetes type I, eczema, vitiligo, alopecia, psoriasis, hypo- or hyperthyroidism or other mild autoimmune disorders not requiring immunosuppressive treatment.
- Administration of glucocorticoids through a route known to result in a minimal systemic exposure (topical, intranasal, intraocular, or inhalation).

2.1.2.5 Systemic (intravenous or oral) glucocorticoid (except for physiologic doses of corticosteroids, i.e., ≤ the equivalent of prednisone 10 mg/day) or other immunosuppressors such as azathioprine or cyclosporin A, are excluded because of potential immune suppression. These treatments must be discontinued at least 1 week

prior to enrollment for recent short course use (≤ 14 days). Glucocorticoids as premedication for contrast-enhanced studies is allowed prior to enrollment and on study.

2.1.2.6 Participants with a prior or concurrent malignancy whose natural history or treatment that has potential to interfere with the safety or efficacy assessment of the regimen.

2.1.2.7 Prior allogenic tissue/solid organ transplant.

2.1.2.8 Participants with pulse oximetry < 92% on room air at screening.

2.1.2.9 Uncontrolled intercurrent illness that would limit compliance with study requirements suggested by medical history, physical examination or standard clinical assessments such as imaging and laboratory studies.

2.1.3 Recruitment Strategies

The protocol may be abstracted into a plain language announcements (see below). Information to be abstracted includes: study title; purpose of the study; protocol summary; basic eligibility criteria; study site location(s); and how to contact the site for further information. Should we add additional descriptive information about the study to these announcements, we will seek additional IRB approval.

Recruitment strategies that be used for this study *may* include:

1. The study may be publicized on
    a. NIH official websites; (e.g., www.clinicaltrials.gov., NCI cancer clinical trial listing (PDQ), CCR)
    b. NIH moderated social medical platforms [e.*g.,* Facebook, Twitter, Instagram]. These sites comply with government terms of service per the NIH Social Media Guidelines
    c. Scientific conferences, such as AACR (https://www.aacr.org/) and ASCO (www.asco.org) who may advertise it further
    d. Research Match (https://www.researchmatch.org/about/)
    e. Newsletters
2. Television advertisements/radio advertisements
    a. Clinical Center TV (CCTV) – Clinical Center Television - CCTV | NIH Clinical Center - America's Research Hospital TVs placed all around the NIH Clinical Center will rotate through various messages including one for this study.
3. Newspaper advertisements
4. Videos
5. Publicly posted recruitment flyers and posters
6. Brochures
7. Clinical Center Office of Patient Recruitment Services (OPR)
8. Recruitment Letters
    a. Doctor to patient informational letter: distribution of letters to private health care physicians who will then share the letters with potential participants. This information will be made available in electronic and/or a hard copy as needed.

(Note: if material is not to be shared with participants, these are not submitted to the IRB).

b. Investigator to potential participant letters.

Prior to distribution of any individual potential participant-facing recruitment materials, such materials will be submitted for IRB review, as appropriate.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images.
- Review of existing photographs or videos.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for this study.

Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

See also the Study Calendar, Section **3.5**.

2.2.2.1 Performed at any time prior to treatment initiation

- Pathology confirmation of p16-positive OPC: pathology reports by an accredited laboratory will be accepted as evidence of histologic confirmation. If pathology report or archival tumor specimen is unavailable for confirmation of diagnosis, then fresh tumor biopsy must be performed.

  Note: If fresh biopsy is done for confirmation of diagnosis, sufficient tissue for baseline research biopsy may be obtained at the same time; refer to Section **5.1.2**.

2.2.2.2 Performed within 28 days prior to treatment initiation

- Clinical examination
  - Medical history: complete medical history with comprehensive physical examination (H&P) including vital signs, height, and weight, including prior cancer therapies, surgical history.
  - Performance status evaluation (see **Appendix A: Performance Status Criteria**)
  - Oxygen saturation test
  - Immunologist evaluation for participants with a history of ≥ grade 3 hypersensitivity reaction to pembrolizumab or monoclonal antibodies.
- Clinical laboratory data

- Complete blood count (CBC) with differential and platelet count, creatinine, eGFR AST, ALT, total bilirubin
- Coagulation panel (PT and/or INR, PTT)
- HBV, HCV, HIV testing (including viral load testing where indicated)
- CD4 testing for HIV^+ participants

- Radiographic studies
  - Tumor imaging or assessment
    - Computed tomography (CT) of neck (or MRI if CT scan cannot be performed on a participant or is insufficient)
    - CT of the chest or fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET/CT).

- Performed within 5 days prior to treatment initiation
  - Urine or serum pregnancy test (β-HCG) in WOCBP*

  ***WOCBP**: any woman who has experienced menarche and has not had hysterectomy or bilateral oophorectomy or is not postmenopausal (amenorrheic 12 months or more following cessation of exogenous hormonal treatments; if <50 years old need follicle stimulating hormone FSH in the post-menopausal range).

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a transient underlying condition not related to the condition under study may be rescreened once after the underlying condition has resolved.

2.3.2 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Cohort 1	Subjects with newly diagnosed p16+ OPC: Stage I (T1, T2 N1)/II/III

Arms

Number	Name	Description
1	Arm 1	PRGN $5x10^{11}$ Viral Particles (VP) SC plus pembrolizumab 200mg IV as induction/neoadjuvant therapy

Arm Assignment

Participants in Cohort 1 will be directly assigned to Arm 1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase II, open label, single-arm evaluation of PRGN-2009 plus pembrolizumab as induction or neoadjuvant therapy in newly diagnosed stage I (T1, T2 N1)/II/III HPV-OPC.

PRGN-2009 will be administered on an outpatient setting on day 1 and day 15, for a total of two administrations. A window of ±3 days for a scheduled treatment is allowed in the event of scheduling issues (i.e., holiday, inclement weather, or other scheduling issues).

Pembrolizumab will be administered in a single dose concurrently with the first vaccine dose (see **Figure 6**).

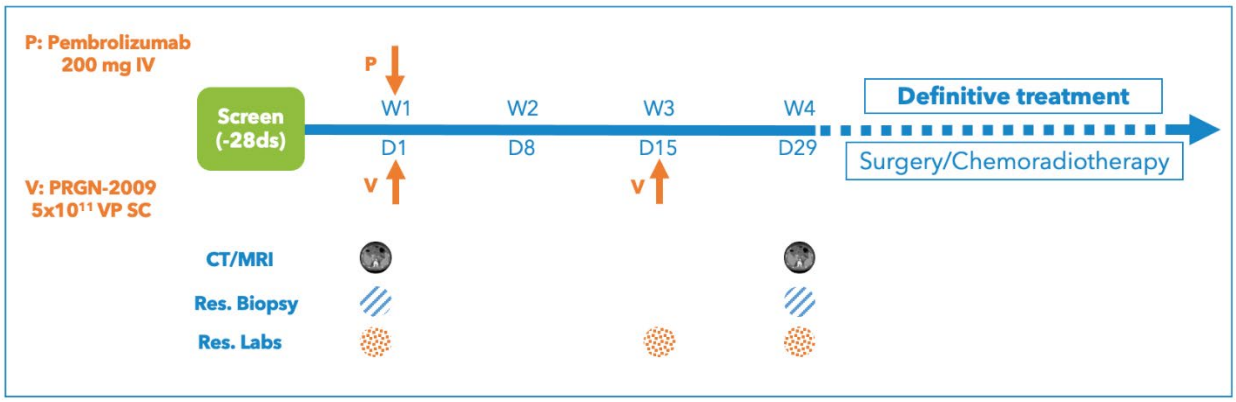
Where feasible, PRGN-2009 should be administered first, followed by pembrolizumab.

The first 6 participants will be evaluated in an early safety assessment: before enrolling the 7th participant, a period of 28 days from starting PRGN-2009 plus pembrolizumab combination therapy must pass without intolerable toxicity (per Section **3.3.2**) in the first 6 participants.

Participants will be followed by phone or email for recurrence and survival at 3 and 6 months from the first study drug administration and then annually until they are 5 years out from surgery or completion of chemo/radiotherapy (see Section **3.4.1**).

After completion of induction immunotherapy, participants will be referred back to their home institution for definitive standard of care therapy. The standard of care treatment will be performed and chosen solely by the providers, and not driven by this study or by NCI investigators for participants continuing treatment at outside institutions.

Figure 6: Trial Schema



Positive HPV testing will not be required for prior to enrolling, but HPV testing will be performed as an exploratory endpoint and participants testing negative for HPV after enrolling or whose HPV status cannot be confirmed will be replaced with other participants for the primary endpoint analysis. Participants testing negative for HPV after enrolling or whose HPV status cannot be confirmed may continue to receive treatment on study.

3.2 Drug Administration

3.2.1 PRGN-2009

PRGN-2009 will be administered as a SC injection using a 1.0 ml syringe at a dose of $5x10^{11}$ VP. The injection volume for each dose is 1.0 ml.

For additional details, refer to Sections **14.1.3** and **14.1.4**.

3.2.2 Pembrolizumab

Pembrolizumab 200 mg (standard approved dose) will be administered as an IV infusion over 30 minutes (-5 minutes and +10 minutes, i.e., between 25 and 40 minutes). Pembrolizumab will be administered on an outpatient basis, in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures – e.g., glucocorticoids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.

Current experience is that infusion-related reactions (IRR) occur infrequently, and are mild to moderate, so administration of premedication is not required. Only if medically warranted in response to an IRR will the investigator modify the infusion parameters (e.g., decrease the rate of infusion, interrupt the infusion).

Management of symptoms should follow the guidelines shown in **Table 3**.

3.3 Dose Modifications

3.3.1 PRGN-2009

Dose reductions are not allowed. The second/last dose may be skipped if participants develop a significant reaction to the first dose or other clinical indication that requires definitive treatment to be initiated prior to administration of 2nd dose.

3.3.2 Pembrolizumab

Dose reductions are not applicable (i.e., administered one time).

3.3.1 Dose Delay

PRGN-2009 should be withheld for any Grade 2 or 3 adverse drug reactions (ADR) attributed to PRGN-2009 until resolution to Grade ≤ 1, unless the ADR in the opinion of the investigator is not clinically relevant or can be medically managed with minimal risk to the participant.

Pembrolizumab may be given up to 3 days post PRGN-2009 in the case of an immediate reaction to PRGN-2009.

3.3.2 Treatment Discontinuation

Treatment will be discontinued in participants experiencing specific adverse events; subjects will however remain on study for follow up of survival.

Treatment discontinuation will occur in any participant experiencing any of the following, when attributable to study drugs, occurring within 28 days of starting the PRGN-2009 plus pembrolizumab combination therapy:

- Any Grade 4 ADRs as defined by CTCAE v5.0 and assessed as related to any agent by the Investigator, **except** for any of the following:
    - Laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 7 days without medical intervention.
    - Any endocrinopathy that can be medically managed with hormone replacement.
- Any Grade 3 ADRs attributed to any agent **except** for any of the following:
    - Flu-like symptoms or fever, as well as associated symptoms of fatigue, headaches, nausea, emesis which can be controlled with conservative medical management.
    - Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor.
    - Laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 7 days without medical intervention.
    - Any endocrinopathy that can be medically managed with hormone replacement.
    - Any other grade 3 adverse drug reaction that resolves to Grade ≤ 1 or baseline grade within 72 hours.

3.3.3 Study Accrual Halting Rules

For safety reasons, accrual will be temporarily halted until an expedited safety report has been evaluated by the investigators and IND Sponsor if:

- There is an occurrence of a Grade 5 toxicity possibly attributable to the study treatment within 30 days of administration of study agents (PRGN-2009 and pembrolizumab).
- There is occurrence of treatment-related ADRs of either Grade 4 toxicity (except for laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline within 7 days) or of Grade 3 toxicity not resolving to Grade ≤ 1 or baseline within 7 days, in two participants.

- There is delay in initiation of definitive therapy of ≥ 4 weeks beyond the planned start date due to treatment-related toxicities from the study treatment in two participants.

3.3.4 Toxicity Management

3.3.4.1 Infusion Reactions

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in **Table 3**.

Table 3: Pembrolizumab Infusion Reaction Treatment Guidelines

NCI-CTCAE Grade	Treatment Modification
Grade 1 - mild Mild transient reaction; in general, infusion interruption not indicated; intervention not indicated	• Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • Hold infusion if deemed necessary by the investigator.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, nonsteroidal anti-inflammatory drugs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	• Stop the infusion of the study intervention. • Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • If symptoms resolve quickly, resume infusion at 50% of original rate with close monitoring of any worsening signs and symptoms, otherwise dosing held until resolution of symptoms with mandated premedication • If not improving, consider administration of glucocorticoids. • If the participant has a second IRR Grade ≥ 2 on the slower infusion rate despite premedication, the infusion should be stopped, and the investigator may consider withdrawal of this participant from the study.

NCI-CTCAE Grade	Treatment Modification
Grade 3 or Grade 4 – severe or life-threatening o Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. o Grade 4: Life-threatening consequences; urgent intervention indicated.	• Stop the infusion of study intervention immediately and disconnect infusion tubing from the participant with additional appropriate medical measures and closely monitor until deemed medically stable by the attending Investigator. Hospitalization and/or close monitoring is recommended • Administration of glucocorticoids may be required • For Grade 3 or 4 IRRs, permanent discontinuation of study intervention is mandated.
Once the infusion is interrupted or rate reduced to 50% of previous infusion rate, it must remain decreased for all subsequent infusions. *For all types and grades of infusion reactions, details about drug physical constitution, method of preparation, and infusion must be recorded.* *Participants should be instructed to report any delayed reaction immediately.* *Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration.*	

3.3.4.2 Immune-related Adverse Events (irAEs)

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These irAEs may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, administer corticosteroids per management guidelines. Toxicity management guidelines for irAEs associated with pembrolizumab are published and should be consulted for management in conjunction with the clinical judgement of the investigator [40]. NCCN, ASCO, and ESMO are all nationally recognized guidelines for the management of irAEs and can be found at the links below:

NCCN: https://jnccn.org/view/journals/jnccn/18/3/article-p230.xml

ASCO: https://ascopubs.org/doi/10.1200/jco.2017.77.6385

ESMO: https://www.annalsofoncology.org/article/S0923-7534(19)42153-4/pdf

3.4 On Study Assessments/Evaluations

3.4.1 Timing of Procedures

The following describes all tests and procedures to be conducted during treatment. Refer to Study Calendar (Section **3.5**) for timing and applicable windows.

For each time period, consider the following order of assessments:

- **Screening**: Refer to Section **2.3**. Screening procedures are required to be done within 28 days unless otherwise indicated.
- **Baseline**: With the exception of the imaging assessments that are required to be done within 4 weeks prior to treatment initiation, baseline evaluations should be performed within 2 weeks of first study drug administration. Any screening evaluation performed within the appropriate timeframe can also serve for the baseline evaluation.
- **Follow-up dosing:** The results of all assessments must be available and reviewed up to 3 days prior to initiation of the study drug administration. The therapy should be delayed if criteria described in Dose Modifications, Section **3.3** are met.
- **Study Drug Administration:**
  - **PRGN-2009**: Two fixed doses of PRGN-2009 will be given approximately 2 weeks apart.
  - **Pembrolizumab** will be given at a fixed dose concurrently with the first PRGN-2009 dose.
- **Completion Visit**: It is preferred that this visit occur on Day 29 (-7). However, so as not to interfere with activities required for arrangements for definitive treatment, this visit may occur as soon as the day after the 2nd/last PRGN-2009 administration.
- **Unscheduled Visits:** In the event of an unscheduled/unplanned visit (e.g., additional clinical assessment(s) due to toxicity), the investigator should use best clinical judgement as to the necessary assessments. In the event that the decision is made to continue treatment, all tests/assessments as required by the next visit on the Study Calendar (Section **3.5**) should still be conducted (or repeated) within the applicable windows. If a decision is made to discontinue treatment, the participant should have completion visit, with tests/assessments completed (or repeated) within the applicable windows.
- **Safety Follow-up Visit:** The Safety Follow- up (FU) visit should be conducted 28 days (+ 7 days) after last treatment. Subjects will be contacted by phone to assess adverse events. See also Section **6.1**.
- **Post-Therapy Follow-up:** Participants who have completed neoadjuvant or induction immunotherapy will be followed by phone or email for recurrence, and survival at 3 months (± 2 weeks) and at 6 months (± 2 weeks) from the date of first study drug administration and then annually (± 1 month) until the participant is 5 years out from surgery or completion of chemo/radiotherapy.
- **Telehealth/Remote Visits:** For any timepoint where an in-person visit is not required, participants will be contacted remotely. In these instances, participants will be contacted by phone, email or other NIH approved remote platform used in compliance with local policy, including HRPP Policy 303.

3.4.2 Description of Procedures

The following is a description of all procedures:

- Symptom assessment: verbal review of participant's current symptoms pertaining to eligibility on study.

- Medical history: verbal review of participant's current symptoms, ongoing medical conditions pertaining to eligibility on study and involvement during study.
- Physical exam: standard assessments including review of organ systems, weight, and vital signs, (i.e., temperature, pulse, pulse oximetry on room air, respirations, blood pressure). After initiation of study drugs, symptom-directed physical examinations will be performed as clinically indicated in the investigator's judgment.
- Performance status (ECOG): an assessment of activities of daily living; see **APPENDIX A: Performance Status Criteria**. Performed in order to assess eligibility at screening and to assess clinical status at subsequent timepoints.
- Clinical laboratory tests: the following comprises the required tests/analytes to assess eligibility at the time of screening and/or the safety of the study drugs at subsequent timepoints unless otherwise noted. Additional laboratories may be ordered as part of panels (e.g., acute care panel, comprehensive chemistry, etc.). Given that the methodologies utilized are similar across all laboratories, no significant variability is expected and there is no anticipation that study data will be affected.
    - CBC with differential
    - Serum Chemistries:
        - Acute care panel [Sodium (Na), Potassium (K), Chloride (Cl) Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen]
        - Hepatic panel (ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, alkaline phosphatase)
        - Mineral Panel (Albumin, Calcium, Magnesium, Phosphorus)
    - Thyroid tests: Thyroid stimulating hormone (TSH), Free T4
    - C-reactive protein (CRP)
    - HLA typing: HLA A, B, C and DR, DQ and NGS A, B, C and DR, DQ, at any time prior to treatment start. Participants who have had HLA typing previously performed at NIH do not require retesting.
    - Coagulation panel: PT/INR and aPTT
    - Pregnancy test: Urine or serum β-HCG for WOCBP assessed beyond screening to determine treatment continuation
    - Urinalysis
- Imaging assessments
    - Performed for eligibility determination/baseline
        - CT of neck (or MRI if CT scan cannot be used or is insufficient) and chest, or ^{18}FDG-PET/CT. Imaging performed at outside facilities within the timeframe may also be used.
    - Performed for response assessment:

  - CT of neck (or MRI, if CT scan cannot be used or is insufficient). Post-treatment imaging is to be strongly pursued but is not required and may be waived at the discretion of the PI.

- Adverse events and concomitant medication review: Adverse events (assessed using the NCI CTCAE v5.0) and concomitant medication will be continuously monitored throughout the study until approximately 28 (± 7 days) after last treatment. Adverse events that occur beyond 28 days after the last administration will be recorded per Section **6.1**.
- Correlative studies: Refer to Section **5**.

3.5 STUDY CALENDAR

Procedure	Screening	Baseline	Treatment (week)		Follow-Up		
			W1	W3	Completion Visit	Safety Follow-up Visit	Post-Therapy Follow-up
Window(s):			*NA*	*±3 days*	*-7 days or at least the day after the second vaccination*	*28±7 days*	*at 3 and 6 months (± 2 weeks) from the date of first study drug administration and then annually (± 1 month) through year 5 post surgery/other therapy*
	Section 2.3	*Section 3.4.1*			*Section 3.4.1*		
Confirmation of diagnosis	X						
Medical history	X						
Height	X						
Physical exam, weight, vital signs	X	X	X	X	X	X	
O_2 saturation test	X						
ECOG PS	X	X	X	X	X	X	
Immunologist evaluation (if indicated)	X						
Clinical Laboratories							
CBC with differential and platelet count, creatinine, eGFR AST, ALT, total bilirubin	X						
CBC with differential		X	X	X	X	X	
Acute care, hepatic, and mineral panels		X	X	X	X	X	
PT, INR, PTT	X						
HIV, HCV, HepB and viral load if positive	X						
CD4 (HIV^+ only)	X						
Serum or Urine β-hCG	X	X	X	X			
TSH and Free T4, CRP		X	X	X	X		
HLA typing		X					
Urinalysis		X					

Procedure	Screening	Baseline	Treatment (week)		Follow-Up		
			W1	W3	Completion Visit	Safety Follow-up Visit	Post-Therapy Follow-up
Window(s):			*NA*	*±3 days*	*-7 days or at least the day after the second vaccination*	*28±7 days*	*at 3 and 6 months (± 2 weeks) from the date of first study drug administration and then annually (± 1 month) through year 5 post surgery/other therapy*
	Section 2.3	*Section 3.4.1*			*Section 3.4.1*		
Radiology/ Other Assessments							
EKG		X			X	X	
Imaging (CT or PET/CT scan; MRI, if CT scan cannot be used or is insufficient)	X (neck and chest CT or PET/CT)	X (neck and chest CT or PET/CT)			X (neck CT only)		
Current/Concomitant medications	X	X	X	X	X	X	
Symptoms/ Adverse events			X	X	X	X	
Survival follow-up (telephone/email)							X
Study Drug							
PRGN-2009			X	X			
Pembrolizumab			X				
Correlative Studies		See Section 5.1.3 for details					
Biopsy		X			X		
Saliva sample		X	X	X	X	X	
Research blood		X	X	X	X		

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs.

3.6.2 Compensation

Participants will not be compensated on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Regardless of reason for removal from study therapy, participants will be asked to have a 28 day follow up safety visit. Participants who are unable or unwilling to return for this visit will be asked to review any safety concerns by phone within this time period.

3.7.1 Criteria for Removal from Protocol Therapy

- Completion of protocol therapy.
- Unacceptable adverse event(s) (see Section **3.3**)
- Participant requests to be withdrawn from study therapy.
- Start of another systemic anticancer treatment or participation in another investigational therapeutic trial.
- Intercurrent illness that prevents further administration of treatment, in the judgement of the investigator.
- Requirement for use of prohibited therapies as listed in Section **4.2**.
- Investigator discretion
- Positive pregnancy test
- Study is cancelled for any reason.

3.7.2 Off-Study Criteria

- Completion of 5-year follow up period.
- PI decision to end the study.
- Participant requests to be withdrawn from study.
- Lost to follow-up.
- Death
- Screen failure or failure to maintain eligibility prior to initiation of treatment.

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 4 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the next 2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS / MEASURES

4.1 PERMITTED MEDICATIONS

Any medications (other than those excluded by the clinical trial protocol) that are considered necessary to protect participant welfare or alleviate symptoms and will not interfere with the trial medication may be given at the investigator's discretion.

4.2 PROHIBITED MEDICATIONS

The following treatments should **not** be administered until after the last study drug administration:

- Glucocorticoids
    - Systemically administered, except for prophylaxis or treatment of allergic reactions, replacement therapy at physiologic doses (e.g., prednisone ≤ 10 mg daily or equivalent), for the treatment of irAEs, or for short courses (≤ 14 days) as appropriate medical therapy for unrelated medical conditions (e.g., asthma).
    - Prophylactic use of glucocorticoids for infusion related reactions.
    - Glucocorticoids administration prior to CT scans in participants with intravenous contrast allergy is allowed.
    - Glucocorticoids without significant systemic effect (topical, inhalation) are allowed.
- Other immunotherapies or immunosuppressive drugs (e.g., methotrexate).
- Systemic anticancer treatment: allowed after completion of the study treatment, per primary team administering definitive treatment.
- Any live vaccine therapies for the prevention of infectious disease. Administration of inactivated vaccines is allowed (for example, inactivated influenza vaccines or locally approved COVID vaccines).

- Radiotherapy

5 CORRELATIVE STUDIES FOR RESEARCH

Blood, saliva, and tumor samples will be collected and processed according to validated standard operating procedures (SOPs) to maintain sample quality and participant confidentiality. All biospecimens will be collected during visits to NIH. Samples will be distributed to the appropriate laboratory for each future assay performed. Please refer to Section 5.1 for sample collection and timepoints.

5.1 BIOSPECIMEN COLLECTION

5.1.1 Blood Samples

Blood samples will be collected pre- and post-treatment. Samples may be initially processed and stored prior to shipment and analysis.

5.1.2 Tumor Tissue

Biopsies will be performed pre- and post-treatment. To avoid interfering with the definitive treatment, the post-treatment biopsy, may be collected starting on the day after the second vaccination. The biopsies will be performed by NCI ENT/Surgery consultants under direct visualization via endoscopy and nasopharyngo-laryngoscopy per routine standard of care, with local anesthesia or under general anesthesia, as clinically indicated. If a biopsy is done for clinical reason, samples may be collected for research. Where feasible, at least 2 cores will be obtained. No more than 2 research biopsies will be performed on this protocol for research purpose only. Consent for the biopsy procedure will be obtained by the interventionalist at the time of the procedure per local policy.

For participants that have surgery as definitive treatment, the post-treatment biopsy may be omitted: in this case tumor biospecimens will be obtained from the institution where the participant underwent surgery.

5.1.3 Biospecimen Collection Timepoints

A description of correlative studies including a brief statement of rationale and processing information is made below.

<table>
<tr><th>Test/assay</th><th>Sample type</th><th>Type of tube and volume[a]</th><th>Collection point(s)</th><th>Storage Lab/Analysis Lab</th></tr>
<tr><td>Immune cell subsets/Functional analysis (FACS)</td><td rowspan="4">Peripheral Blood Mononuclear Cells (PBMCs)</td><td rowspan="4">6-8 10 mL Sodium Heparin (green top) tubes</td><td rowspan="4">Baseline, W1, W3, Completion Visit [b]</td><td rowspan="2">Clinical Services Program (CSP)/Center for Immuno-Oncology (CIO)</td></tr>
<tr><td>Antigen Specific Immune Response (Cytokine staining assay)</td></tr>
<tr><td>T-cell clonality (Immunoseq platform)</td><td rowspan="2">CSP/CIO and NCI [redacted] Genomic Core Facility</td></tr>
<tr><td>RNA expression (Nanostring platform/RNA-Seq)</td></tr>
</table>

Test/assay	Sample type	Type of tube and volume[a]	Collection point(s)	Storage Lab/Analysis Lab
Soluble factor levels (ELISA)	Blood	1 x 8 mL SST		CSP/CIO
AVA (ELISA)				CSP/[redacted]
Circulating free HPV 16/18 DNA (cfDNA) (PCR)	Plasma	2 x 10 mL EDTA (lavender top)		CSP/CIO and NCI [redacted] Genomic Core Facility
Immune markers (IHC/multiplex IF)	Tumor tissue Fresh biopsies (3-4 cores)	N/A	Baseline, Completion Visit [c]	[redacted] Lab/CIO
Transcriptomic analysis (RNA-Seq)				
HPV status (PCR)				[redacted] and NCI Genomic Core Facility
Genomic analysis (exome sequencing)				
T cell clonality (Immunoseq platform)				
Additional biomarker studies	Blood and tumor tissue		Baseline, W1, W3, Completion Visit [b]	[redacted]
Salivary HPV DNA (digital droplet PCR)	Saliva	15-20 mL 50 mL conical tube	Baseline, W1, W3, Completion Visit [b], Safety FU	CSP/[redacted] Facility
Salivary soluble factors	Saliva	5 mL saliva in conical tubes	Baseline, W1, W3, Completion Visit [b], Safety FU	CSP/CIO lab

a. *Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.*
b. *On completion visit if participants come off treatment prior to any of those timepoints*
c. *An earlier timepoint may also be used to avoid interfering with definitive treatment but should be at least a day after the second vaccination. Post treatment biopsy can be omitted if participants have surgery as definitive treatment and can be obtained from the institution where the participant underwent surgery.*

5.2 PLANNED ANALYSES

It may be noted that these are the planned analyses and specific analytes proposed. For the exploratory correlates, adjustments may be made based on knowledge and available assays at the time of analysis as long as the intent of the original and approved objectives are observed.

5.2.1 Immune Phenotyping

Exploratory immunologic studies will be conducted to evaluate the study drug's effect on the immune system before and after treatment. The following immune assays may be performed at the where adequate samples are available:

1. PBMCs may be analyzed for changes in standard immune cell types (CD4 and CD8 T cells, NK cells, Tregs, MDSCs, and dendritic cells) as well as immune cell subsets, using multi-color flow cytometry.
2. PBMCs may be analyzed for function of specific immune cell subsets, including CD4 and CD8 T cells, NK cells, Tregs, and MDSCs using flow-based assays.
3. PBMCs may be analyzed for tumor antigen-specific immune responses using an intracellular cytokine staining assay. PBMCs will be stimulated in vitro with overlapping 15-mer peptide pools encoding the tumor-associated antigens HPV 16 E6 and E7 and HPV18 E6 and E7; control peptide pools will involve the use of human leukocyte antigen peptide as a negative control and CEFT peptide mix as a positive control. CEFT is a mixture of peptides of CMV, Epstein-Barr virus, influenza, and tetanus toxin. Post-stimulation analyses of CD4 and CD8 T cells will involve the production of IFN-γ, IL-2, TNF, and the degranulation marker CD107a. If sufficient PBMCs are available, assays may also be performed for the development of T cells to other tumor-associated antigens.

5.2.2 Soluble Factors

Sera may be analyzed pre- and post-therapy for the following soluble factors: sCD27, sCD40 ligand using commercial ELISA kits.

Sera may be analyzed for changes in cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers using ELISA or multiplexed assays (e.g., Mesoscale, Luminex, cytokine bead array).

Samples will be sent to the NCI Center for Immuno-Oncology (CIO) for analysis. Please contact [redacted] (CIO, CCR, NCI), [redacted] at [redacted].

5.2.3 Anti-vector antibodies

Anti-vector antibodies (AVA) are one mechanism of neutralization and inefficacy of viral vectors. Longitudinal detection and titer measurement by ELISA of AVA to the vaccine adenoviral vector will assist in characterizing cases of loss of efficacy.

Samples will be shipped to Precigen for analysis.



5.2.4 Circulating free tumor DNA

If sufficient plasma is available, select participant samples may be analyzed for circulating free tumor DNA (cfDNA). The circulating tumor/HPV DNA will be quantified with a digital droplet PCR system from Bio-Rad to obtain precise quantification. There is no DNA sequencing.

5.2.5 Immune Markers Analysis

Where available, study of immune infiltration as well as PD-L1 status within the tumor microenvironment by IHC and/or multiplex immunofluorescence (IF) may be performed.

5.2.5.1 Tumor Biopsies

Biopsies obtained will be stained for multiple immune cell markers (CD4, CD8, CD3, PD-1, PD-L1) as well as tumor cell markers (pancytokeratin) using an automated multiplex immunofluorescence (MxIF) staining protocol. An average of 5 consecutive biopsy recuts (4-5-micron thick formalin fixed paraffin embedded tissue sections (FFPE) mounted on 25 x 75mm, charged glass slides) or unstained slides (US) per each biopsy time point is needed for this analysis. Hematoxylin and Eosin (H&E) staining (done on last or deepest recut level) will be examined by a research pathologist (CIO), for presence of tumor and tissue integrity.

5.2.5.2 Staining Protocol

Formalin-fixed paraffin-embedded (FFPE) tumor sections (US) will be immuno-stained using Opal Multiplex Automation IHC Detection Kits on the BOND RX from L[redacted]. Sections will be subjected to deparaffinization and antigen retrieval. Subsequent Opal staining of each antigen occurs as follows: slides will be blocked with blocking buffer (Akoya/Leica) for 10 min then incubated with primary antibodies at optimized concentrations (CIO) followed by Opal HRP polymer (or secondary antibody) and one of the Opal fluorophores. Individual antibody complexes will be stripped after each round of antigen detection. After the final stripping step, DAPI (4',6-diamidino-2- phenylindole) counterstain will be applied and slides will be removed from the BOND RX for cover slipping.

5.2.5.3 Digital Imaging Protocol

A multiplex scanning protocol will be created on Vectra Polaris scanner (Akoya/Perkinelmer), to allow for whole slide imaging at 10-20X. This is followed by higher resolution scan of regions of interest (ROIs) picked (5 on average) or whole slide analysis (if feasible) (CIO). The scans generated at high resolution (multispectral images -MSIs) will be analyzed (CIO) using that allows for spectral unmixing. Individual ROIs will be segmented based on nuclear counterstain DAPI, and cells will be counted based on expression of various markers (CD3, CD4, CD8) in a binary fashion allowing for objective measurements of density, absolute counts, and percentages of each cell type. Averages of representative ROIs or whole areas of interest (if feasible) for each time point will be generated for statistical analysis.

5.2.6 Assessment of HPV status

HPV testing for ascertainment of HPV positivity and HPV subtype will be performed using HPV DNA assay.

5.2.7 Genomic Studies

Samples will be sent to [redacted]

[redacted]

5.2.7.1 Immune transcriptomic analysis

Where available, tumor tissue immune transcriptomic analysis to study the effect of the treatment on immune gene expression may be performed with the Nanostring platform/RNA-Seq.

5.2.7.2 Genomic analysis

Where possible, genomic analysis (DNA sequencing, exome) will be performed on tumor tissue to study the mutation profile of the tumor.

5.2.7.3 Management of Results

Participants will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of secondary findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Unless requested otherwise, participants may be contacted at this time with a request to provide a sample to be sent to a CLIA certified laboratory. (Note: Commercial labs will generally be utilized for this purpose.) If the research findings are verified in the CLIA certified lab, the subject participant will be offered the opportunity to schedule a visit, in person (at our expense) or via telemedicine on an NIH approved platform to have genetic education and counseling to explain this result. If the participant does not want to come to NIH, or participate in a telemedicine visit, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that secondary findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Participants will be requested to maintain up to date contact information to enable future contact.

5.2.7.4 RNA sequencing and T-cell Receptor Clonality Analysis of Blood and Tumor Tissue

Where possible, RNA sequencing and T-cell receptor clonality analysis will be done on the PBMCs and tumor tissue to help further evaluate changes in immune response and RNA expression levels with treatment as well as to determine tumor and infiltrating lymphocyte characteristics which may be predictive of response to treatment. In addition, these analyses will also be used to gauge resistance mechanisms and additional targets for future therapy. Coded, linked samples may be analyzed for RNA expression levels using the RNA Sequencing/Nanostring platform and T-cell receptor clonality using the ImmunoSeq platform.

5.2.7.5 Salivary soluble factors

Saliva will be collected in 5 mL saliva in conical tubes, prior to the collection for salivary HPV DNA, and will be stored/transported at 4 ^{O}C to [redacted] for aliquoting. There it will be mixed by inversion and vortexing for several seconds, aliquoted and frozen 1 mL/vial, then stored at -80^{o}C until assay.

5.2.7.6 Salivary HPV DNA

Saliva will be collected by mouth rinse and gargle with 15-20 mL 0.9% NaCl for 30 seconds and spitting into the collection tube. Samples will be stored/transported at 4 ^{O}C to [redacted]k. Where possible, saliva samples will be analyzed for HPV DNA detection and quantification with digital droplet PCR; no sequencing is involved.

5.2.8 Additional Biomarker Studies

PBMC, plasma, and/or serum or tissue samples may be sent to [redacted]. (see Section 5.2.3) to further investigate HPV 16/18 -specific immune responses, activation status of the immune system (e.g., leukocyte subsets, exhaustion, cytokines profile), antigen cascade, anti-tumor specific immune responses induced by the exposure to PRGN-2009, proteomic analysis, and potential predictive/prognostic biomarker candidates related to the drug and/or cancer (e.g., specificity of tumor infiltrating cells), and results of these analyses will be shared with the NCI. These analyses are exploratory and dependent on the quality and availability of sufficient materials. The panel of biomarkers might be adjusted based on results from ongoing research; therefore, any remaining samples can be stored at [redacted] and can be used for future exploratory research on the drug and/or disease-related aspects. Only remaining samples will be used to this purpose, no additional samples will be collected.

5.3 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside the National Institutes for Health (NIH) without appropriate approvals and/or agreements, if required.

5.3.1 Sample Management and Storage at Clinical Services Program – [redacted]

On the days that samples are collected, please notify the courier staff at CSP [redacted] They will arrange same-day courier delivery of the specimens.

All data associated with the participant samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the [redacted] for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80ºC freezer or vapor phase liquid nitrogen. These freezers are located at [redacted]

[redacted] Central Repositories (managed under a subcontract) store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited-access facilities with sufficient security, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance (FWA). The

subcontractor's role limited to clinical research databases and repositories containing participant specimens. The subcontractor does not conduct or have any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects. The subcontractor's IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

It is the intent and purpose of the subcontractor to accept only de-identified samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input, and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.3.2 [redacted] Laboratory

Tissue samples will be stored and processed by the NIDCD [redacted] Lab.

[redacted]

Notify [redacted] of all incoming specimens; [redacted], email: [redacted]

Backup contact: [redacted], Cell: [redacted], email: [redacted].

Samples will be stored in freezers, liquid nitrogen containers or tissue cartridge shelves in the NCI Core laboratory, located in [redacted] on the NIH campus, secured by locked doors.

Samples and associated data will be stored permanently unless the participant withdraws consent.

5.3.3 Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissues are stored for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the participant was enrolled.

5.3.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value. If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if so requested). The PI will report any loss or unanticipated destruction of samples per Section **7.2.1**. If the participants withdraw consent, the participants' data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Following completion of the planned analyses, samples will remain in storage as detailed above. Tissue specimens and derived tissue lysates, RNA and DNA collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study that are not expressly stated in the present protocol. However, this research may only be done if the proposed research has undergone prospective IRB review and approval, including consent/reconsent if required.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The Principal Investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study treatment, study day 1, through 28 days after the subject received the last product administration. After 28 days, only adverse events which are serious and related to the study investigational agent need to be recorded.

SAE assessment after the 28 days will be documented by a licensed physician listed on the 1572 in the source documents.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study;
- Is associated with clinical signs or symptoms;
- Requires treatment or any other therapeutic intervention;
- Is associated with death or another serious adverse event, including hospitalization;
- Is judged by the Investigator to be of significant clinical impact; and/or
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.

The PI (or designee) evaluation of each AE not captured in the clinical database determining that it meets the criteria above will be documented in the source documents. Note: the investigator performing the assessment must be a clinician licensed to diagnose and listed on the 1572.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers no sooner than three years after the completion of the primary endpoint by contacting the Principal Investigator.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the exploratory endpoint antitumor activity will be evaluated with target and/or non-target lesions according to RECIST Version 1.1.

Acceptable methods for disease assessment include CT (including CT obtained as part of PET/CT), and MRI; CT with contrast preferred.

All post-baseline response assessments should follow the same lesions identified at baseline. The same mode of assessment (e.g., CT) used to identify/evaluate lesions prior to treatment should be used throughout the course of the study unless subject safety necessitates a change (e.g., allergic reaction to contrast media).

6.3.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥ 10 mm
    - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥ 10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

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

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

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

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and

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

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

6.3.2 Methods for Evaluation of Measurable Disease

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

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

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

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT 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. MRI is also acceptable in certain situations (e.g., for body scans).

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

6.3.3 Response Criteria

For each subject, the Investigator will designate 1 or more of the following measures of tumor status to follow for determining response: CT or MRI images of primary and / or metastatic tumor masses, physical examination findings, and the results of other assessments. All available images collected during the trial period will be considered. The most appropriate measures to evaluate the tumor status of a subject should be used. The measure(s) to be chosen for evaluation during the trial have to correspond to the measures used to document the progressive tumor status that qualifies the subject for enrollment. The tumor response assessment will be assessed and listed according to the Study Calendar (see Section **3.5**).

Tumor responses to treatment will be assigned based on the evaluation of the response of target, non-target, and new lesions according to RECIST 1.1 (all measurements should be recorded in metric notation).

To assess objective response, the tumor burden at baseline will be estimated and used for comparison with subsequent measurements. At baseline, tumor lesions will be categorized in target and non-target lesions according to RECIST 1.1.

Results for these evaluations will be recorded with as much specificity as possible so that pre-and post-treatment results will provide the best opportunity for evaluating tumor response.

The Investigator may perform scans in addition to a scheduled trial scan for medical reasons or if the Investigator suspects PD.

6.3.4 Response Criteria by RECIST 1.1

6.3.4.1 Evaluation of Target Lesions

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

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

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

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

6.3.4.2 Evaluation of Non-Target Lesions

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

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

6.3.4.3 Evaluation of Best Overall Response

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

For Participants with Measurable Disease (i.e., Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥6 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

6.3.4.4 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. 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 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP Office of Compliance and Training / IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

Note: Only IND Safety Reports that meet the definition of an **unanticipated problem** will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI Clinical Director Reporting

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to [redacted] within one business day of learning of the death.

7.4 Institutional Biosafety Committee (IBC) Reporting Criteria

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of PRGN-2009 as soon as possible but in no event later than 7 calendar days of initial receipt of the information.

Serious adverse events that are unexpected and associated with the use of PRGN-2009, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the NCI Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.3 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial;

- clinical site;
- the Principal Investigator;
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons;
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed; and
- if the trial has been completed, a brief description of any study results.

7.4.4 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system;
- a summary of all serious adverse events submitted during the past year;
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications;
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (approximately weekly) when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the NCI Principal Investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The Principal Investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The Principal Investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL / SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death;
- A life-threatening adverse event (see Section **8.1.3**);
- Inpatient hospitalization or prolongation of hospitalization
    - An inpatient admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, will not be considered SAEs.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for participant or subject convenience) will not be considered SAEs.
    - Emergency room visits and stays in observation units that do not result in inpatient admission will not be considered SAEs. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate 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.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death (21CFR312.32).

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site Principal Investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site Principal Investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site Principal Investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded from the time of first investigational product Study Day 1, through 28 days after the subject received the last product administration must be reported to the sponsor with the exception of any listed in Section **8.4**. After 28 days, only adverse events which are serious and related to the study investigational agent need to be reported.

8.3 Reporting of Serious Adverse Events

Any AE that meets a protocol-defined serious criterion or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in Section **8.4.**

All SAE reporting must include the elements described in Section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: [redacted] and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at:
https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

As overall survival and progression free survival are part of the study objectives, and therefore death due to disease progression is captured as an endpoint in this study, it will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to Section **8.3**.

The PI will submit a summary table of all Grade 3-5 AEs, whether or not considered related to the product, every 6 months. The report will include a line listing of all events including the PID, date of onset of the event, date of resolution of the event, date of dosing for each study intervention in relationship to the event, attribution of the event, grade of the event, CTCAE term of the event and the System Organ Class of the event. In addition, a listing of all adverse events including the number of participants experiencing each adverse event, cumulative and per grade, and per causality assessment will be included. The report will indicate the date of the data cut off, total number of participants treated to date, and dictionary for any coded field. The report is cumulative and new information for the last 6 month period should be highlighted. Reports will be submitted to the Center for Cancer Research (CCR) at [redacted]

The Sponsor might request case summaries for those events if, upon review, the Sponsor determines that an aggregate safety report is required (21CFR312.32(c)(1)(iv)).

8.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

Reporting will be per the collaborative agreement.

8.6 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: [redacted] and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal Exposure

Female participants should refrain from becoming pregnant during the study and for 4 months after the last dose of pembrolizumab or 2 months after the last dose of PRGN-2009.

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of pembrolizumab or 2 months after the last dose of PRGN-2009.

Pregnancy of the participant's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 4 months after the last dose of pembrolizumab or 2 months after the last dose of PRGN-2009 should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance with 21CFR 312.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

8.9 SAFETY OVERSIGHT COMMITTEES

8.9.1 Safety Monitoring Committee (SMC)

The sponsor SMC is an independent group of at least 3 experts that monitors participant safety and advises The Sponsor. SMC members will be separate and independent of study staff participating in this trial and should not have scientific, financial, or other conflicts of interest related to this trial. The SMC will consist of members with appropriate expertise to contribute to the interpretation of data from this trial. A quorum will consist of a simple majority.

The SMC will review cumulative safety data from this trial at least annually.

The SMC will have a final review meeting at the end of the study.

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review applicable data, including, but not limited to, enrollment, demographics, dosing data, clinical

laboratory data, and safety data, at scheduled timepoints during this trial as defined in the SMC charter. The SMC will review blinded aggregate data in the open session of the SMC meetings.

Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by the Sponsor. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study product administration, and to continue, modify, or terminate this trial.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine if the use of PRGN-2009 with pembrolizumab in participants with p16-positive OPC can result in a ≥ 2-fold increase in $CD3^+$ tumor infiltrating T cells post treatment compared with pre-treatment	$CD3^+$ tumor infiltrating lymphocytes will be assessed by multiplex immunofluorescence from the surgical/ biopsy tissue collected at week 4-5 and compared with pre-treatment	T-cell infiltration is a marker of immune response. Success of regimens will be determined by these endpoints
Secondary		
To determine if the use of PRGN-2009 plus pembrolizumab results in significantly prolonged survival as compared to the expected 80% three-year historical survival in p16-positive OPC participants	Assess the anti-tumor activity of PRGN-2009 plus pembrolizumab at 3 and 6 months from the date of first study drug administration	Standard endpoint for clinical trials. Success of regimens will be determined by these endpoints
To determine the 3-year OS and RFS rate for PRGN-2009 plus pembrolizumab as neoadjuvant/induction therapy before definitive standard of care therapy in p16-positive OPC participants	Assess OS and RFS once a year for 5 years	
To assess the safety of PRGN-2009 plus pembrolizumab in p16-positive OPC participants	Incidence of treatment-related serious adverse events at baseline, W1, W3, on day 29, and 28 days after last treatment as defined by Common Terminology Criteria for Adverse Events (CTCAE) v5.0	Safety evaluation is a standard endpoint for clinical trials
To determine if the rate of ≥2-fold increase in tumor infiltrating lymphocytes by immunohistology following PRGN-2009 in combination with pembrolizumab is significantly more than the rate with PRGN-2009 alone (data from ongoing study NCT04432597)	Compare the induction of immune response of PRGN-2009 plus pembrolizumab with PRGN-2009 at completion visit compared with pre-treatment with endpoint the ≥2-fold increase in tumor infiltrating lymphocytes	T-cell infiltration is a marker of immune response. Success of regimens will be determined by these endpoints.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Exploratory		
To determine ORR per RECIST v1.1 in subjects receiving PRGN-2009 plus pembrolizumab	Response rate assessed by review of imaging at completion visit, EOT, safety FU, at 2 months after safety FU, and annually through year 5 post surgery/other therapy	Standard endpoint for phase 2 cancer clinical trials used to assess clinical efficacy of treatment.
Standard immune cell types (CD4 and CD8 T cells, NK cells, Tregs, MDSCs, and dendritic cells) as well as immune cell subsets	Evaluate the study drug's effect on the immune system at baseline, W1, W3, and completion visit.	Standard endpoints for correlative studies. These endpoints will explore changes within timepoints as stipulated within the protocol to understand the effect of PRGN-2009 plus pembrolizumab in p16-positive OPC participants
Function of specific immune cell subsets (CD4 and CD8 T cells, NK cells, Tregs, and MDSCs)	Evaluate the study drug's effect on the function of the immune system at baseline, W1, W3, and completion visit.	
Immune responses to HPV antigens	To assess the immune responses to HPV antigens with treatment at baseline, W1, W3, and completion visit.	
T-cell clonality	To evaluate changes in immune response with treatment as well as to determine tumor and infiltrating lymphocyte characteristics which may be predictive of response to treatment at baseline, W1, W3, and completion visit.	
RNA expression	To evaluate changes in RNA expression levels with treatment at baseline, W1, W3, and completion visit.	
Exome sequencing	To detect the mutation profile of the tumor at baseline and completion visit.	
Soluble factors, cytokines, chemokines, antibodies, tumor-associated antigens, and/or other markers	To evaluate changes in cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers at baseline, W1, W3, and completion visit.	
Anti-vector antibodies (AVA)	To characterize cases of loss of vaccine efficacy at baseline, W1, W3, and completion visit.	

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Circulating free HPV 16/18 DNA	To evaluate changes in the levels of circulating free HPV DNA at baseline, W1, W3, and completion visit.	
Immune markers	To evaluate immune infiltration as well as PD-L1 status within the tumor microenvironment pre vs. post treatment at baseline and completion visit.	
HPV status to assess HPV positivity and subtype	HPV testing for ascertainment of HPV positivity and HPV subtype at baseline and completion visit	
To assess changes in salivary HPV DNA and salivary soluble factors	To assess changes in salivary HPV DNA and salivary soluble factors at baseline, W1, W3, completion visit, and safety FU	
To assess HPV 16/18-specific immune responses, activation status of the immune system, and anti-tumor specific immune responses induced by the exposure to PRGN-2009 with biomarker studies	Biomarker studies with remaining samples performed at baseline, W1, W3, and completion visit when possible.	

10.2 Statistical Hypotheses

We hypothesize that PRGN-2009 plus pembrolizumab can result in a $\geq$ 2-fold increase in $CD3^+$ tumor infiltrating T cells post treatment compared with pre-treatment in p16-positive OPC.

10.3 Sample Size Determination

A total of 20 participants with $p16^+$ OPC will be enrolled based on the following reasoning. Assume that a success for any individual participant will be defined as that participant experiencing a two-fold increase in $CD3^+$ tumor infiltrating T cells post treatment compared with pre-treatment. With 20 participants, there would be 80.4% power to detect a difference between 50% and 80% of participants experiencing a success, with an exact binomial test using a one-sided 0.05 significance level. As an illustration, with 20 participants, if 15 participants were able to have a two-fold increase, the lower two-sided 90% confidence bound on 15/20 is 54.4% and the upper two-sided 90% confidence bound is 89.6%. Thus, 15/20 participants would demonstrate the intended magnitude of effect. If 14/20 had this outcome, the lower 90% two-sided bound is 49.2% and the upper two-sided 90% bound is 86.0%, essentially demonstrating nearly the same result.

To determine if the use of the PRGN-2009 plus pembrolizumab is associated with significantly prolonged survival as compared to the historically expected 80% 3-year overall survival with this population, using the method of Brookmeyer and Crowley, with 20 participants, there is 82% power to demonstrate that the therapy improves upon 80% three-year survival and is consistent

with 95% survival, with a one-sided alpha=0.10. In practice, a confidence interval will be constructed for the Kaplan-Meier curve.

A total of up to 20 evaluable participants will be enrolled on this protocol. To allow for a few inevaluable participants and screen failures the accrual ceiling for this protocol will be set at 29 participants. It is anticipated that it will take approximately 12 months to enroll 20 evaluable participants.

To determine if the combination of PRGN-2009 and pembrolizumab is able to have a greater fraction of participants who have a 2-fold increase over PRGN-2009 alone, a Fisher's exact test with a one-sided 0.10 significance level would have 88.8% power to detect the difference between 45% and 85% when there are 20 subjects in each group. Data for PRGN-2009 alone will come from an ongoing study (NCT04432597).

10.4 POPULATIONS FOR ANALYSES

All participants who receive any investigational treatment will be evaluable for safety and toxicity evaluations.

Participants who receive two doses of PRGN-2009 and the single dose of pembrolizumab and have an adequate pre-treatment and post-treatment biopsy will be evaluable for immune infiltration assessments.

Participants who receive two doses of PRGN-2009 and the single dose of pembrolizumab and go on to receive standard definitive therapy will be evaluable for relapse free survival and overall survival assessments.

10.5 STATISTICAL ANALYSES

10.5.1 General Approach

The study will be conducted primarily to evaluate whether there is a potentially significant increase in $CD3^+$ tumor infiltrating lymphocytes with immunotherapy induction.

10.5.2 Analysis of the Primary Endpoints

The fraction of doubling of baseline $CD3^+$ tumor infiltrating lymphocytes will be provided as well as the 95% confidence interval

10.5.3 Analysis of the Secondary Endpoints

A Kaplan-Meier curve as well as the 95% confidence interval will be provided for survival and relapse-free survival

10.5.3.1 Relapse-Free survival

Relapse-free survival (RFS) will be evaluated using Kaplan-Meier methods. RFS will be defined as the time from completion of standard chemoradiation to the date of disease recurrence or death (any cause) whichever occurs first. Subjects who do not have disease progression, disease recurrence or have not died at the end of follow up will be censored at the last known date the subject was progression or relapse free.

10.5.3.2 Objective Response

Objective response is a complete or partial radiographic response as defined by RECIST 1.1 (Section **6.3.4**).

10.5.3.3 Overall Survival

OS will be evaluated using Kaplan-Meier methods. OS will be defined as the time from the date of first treatment to the date of death (any cause). Subjects who are alive at the end of follow up will be censored at the last known date alive.

10.5.4 Safety Analyses

Safety endpoints will be analyzed as summary statistics during treatment and/or as change scores from baseline assessments. AEs will be coded as defined in the Medical Dictionary for Regulatory Activities (MedDRA). All AEs will be recorded and tabulated following each treatment. AEs will be recorded by severity, frequency, and relationship to the study intervention and will be presented by System Organ Class (SOC) designations and preferred term groupings. Information on each AE will include start date, stop date, severity, relationship, expectedness, outcome, and duration. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be presented either in a Table or a Listing.

In addition, overall safety will be assessed by descriptive analyses using tabulated frequencies of AEs by grade using CTCAE Version 5 for the overall study population in terms of treatment-emergent AEs, SAEs, and clinically significant changes in safety laboratory tests, physical examinations, ECGs, and vital signs.

10.5.5 Baseline Descriptive Statistics

Baseline characteristics will be described.

10.5.6 Tabulation of Individual Participant Data

Individual responses in a cohort may be depicted using a waterfall plot or spider diagram.

10.5.7 Exploratory Analyses (Immune Responses)

It is expected that each of the exploratory objectives will be evaluated using descriptive statistical methods. Any exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

This study will receive the PRGN-2009 under a CRADA with [redacted] (CRADA Number: 03209).

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. Efforts will be made to extend accrual to a representative population. Due to impaired cellular immunity which may affect the efficacy of treatment, participants with poorly controlled HIV as well as patents with detectable viral loads of hepatitis B and C will be excluded. Pregnant participants are not permitted, and contraception will be required because the teratogenic effects

of PRGN-2009 vaccine with pembrolizumab are unknown. Furthermore, because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the of the breastfeeding participant with pembrolizumab, breastfeeding should be discontinued if the mother is treated on this protocol. These potential risks may also apply to other agents used in this study.

12.2 PARTICIPATION OF CHILDREN

Individuals under the age of 18 will not be eligible to participate in this study because of unknown toxicities in pediatric patients.

12.3 RISK/BENEFIT ASSESSMENT

12.3.1 Known Potential Risks

12.3.1.1 Study Drugs

Potential adverse reactions attributable to the administration of the study drugs utilized in this trial are discussed in Section **14**.

Participants may be harmed from being in this study by toxicity due to the drug or combination of drugs given during this study.

12.3.1.2 Saliva collection

Saliva collection is not known to be associated with risk. There may be some discomfort from the oral rinse and gargle with saline.

12.3.1.3 Blood collection

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop. Up to 130 mL (~9 tablespoons) of blood will be collected at any visit but no more than 634 ml (~43 tablespoons) in an 8 week period.

12.3.1.4 Urine collection

There are no risks associated with urine collection.

12.3.1.5 EKG

Some skin irritation can occur where the EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

12.3.1.6 Biopsies

All care will be taken to minimize risks that may be incurred by tumor sampling via endoscopy and nasopharyngo-laryngoscopy. Risks may include major bleeding (<1% of all participants); nosebleed; drop in heart rate; dental damage (<1% of all participants); numbness of the tongue/ (paresthesia) or altered taste (dysgeusia) (<10% of all participants).

12.3.1.7 Local anesthesia

Biopsies may be done under local anesthesia. There may also be an allergic reaction to the local anesthetic used to control pain while obtaining the biopsy. Potential side effects of local anesthesia include drowsiness, headaches, blurred vision, twitching muscles or shivering, continuing numbness, weakness or pins and needles sensation.

12.3.1.8 General anesthesia

If participant's tumors are not accessible in the clinic or a biopsy in the clinic is deemed unsafe by the study doctor, biopsies may be performed in the operating room under general anesthesia. Side effects of general anesthesia may include temporary confusion, difficulty passing urine, bruising or soreness from the IV drip, nausea and vomiting, shivering and feeling cold, and sore throat.

12.3.1.9 MR Imaging

The risks of MR imaging are relatively small. Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage. MRIs include the additional risk of damage to hearing.

12.3.1.10 Imaging

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

12.3.1.11 Delaying potentially curative treatment

Side effects of this investigational treatment may cause delay in scheduled curative therapy. A prior study has shown that people with oropharyngeal cancer who received treatment more than 10 weeks after their initial diagnosis did significantly worse than those who received treatment sooner.

12.3.1.12 Radiation exposure

On this study, participants will receive up 3 neck CTs, 2 chest CT and 2 PET/CT. The total radiation dose for research purposes will be approximately 3.23 rem.

12.3.2 Non-physical Risks of Genetic Research

12.3.2.1 Risk of receiving unwanted information

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Participants will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with participants, family members or health care providers.

1.1.1.1.1 Risk related to possibility that information may be released

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the participants, family members or health care providers, this risk will be included in the informed consent document.

1.1.1.1.2 Risk to Family or Relatives

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems.

12.3.3 Known Potential Benefits

The potential benefit to a participant that goes onto study is an increase in the immune response against the tumor which may be accompanied by reduction in the bulk of their tumor and which may or may not have favorable impact on symptoms and/or survival.

12.3.4 Assessment of Potential Risks and Benefits

The risks associated with participation and exposure to potential adverse events are noted in Section **14**. Locally advanced HPV-associated OPC needs improved therapy options. Preclinical studies suggest that the use of a combination of immunotherapy agents may improve anti-tumor efficacy. Therefore, PRGN-2009 in combination with pembrolizumab may improve clinical outcome of participants with HPV-associated OPC. A number of clinically appropriate strategies to minimize risk to participants have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefits of PRGN-2009 in combination with pembrolizumab for this group of participants outweigh the risks associated with the proposed entry into this protocol.

12.4 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other site approved remote platforms used in compliance with local policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent

remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and IRB and, as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have

a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute (NCI) has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 PRGN-2009 (IND # 19628)

Please refer to the investigational brochure (IB) for complete product information.

14.1.1 Source / Acquisition and Accountability

PRGN-2009 will be provided by [redacted] through a CRADA with NCI. All study interventions will be given at the NIH Clinical Center and documented in the electronic medical record. The investigator or designee (e.g., pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the manufacturer and the site.

14.1.2 Toxicity

PRGN-2009 antigen was successfully evaluated in three different types of *in vitro* analyses designed to identify potential oncogenicity of novel DNA in mammalian cells. The HPV PRGN-2009 antigen did not induce an oncogenic profile in the host cells in any assay, whereas cells transformed with wild type HPV oncogene versions unambiguously displayed morphological and phenotypic changes consistent with oncogenesis, both qualitatively and quantitatively. This data provided confidence that the HPV PRGN-2009 molecular vaccine candidate is safe for use in mammalian cells and will not induce aberrant growth consistent with carcinogenesis.

The Phase I study of PRGN-2009 in advanced HPV-associated solid tumors, has been ongoing in the Clinical Center: Arm 1A, PRGN-2009 vaccine monotherapy dose escalation study was completed, having enrolled 6 participants, with no DLTs, and observed AEs as described in Section **1.2.6.1** and **Table 1**.

14.1.3 Formulation and Preparation

Precigen will provide the following materials:

- The concentration of the PRGN-2009 in supplied vials is 5 x 10E11 PU/ml;
- PRGN-2009 vials (manufactured by ABL) – 1.0 ml/vial (extractable volume; frozen);
- Final formulation buffer (FFB) Diluent vials (manufactured by [redacted]) – 4.0 ml/vial (frozen); and
- Sterile empty glass vials (manufactured by [redacted]) for preparing dilutions (if required).

14.1.3.1 Stability and Storage

PRGN-2009 (manufactured by [redacted]) is provided as a sterile, frozen, injectable liquid that has been aseptically filled into crystal zenith (CZ) vials fitted with a butyl rubber stopper and an aluminum flip-cap seal. PRGN-2009 must be stored in an ultracold freezer in the temperature range of -60 to -90°C. Each vial contains 1.0 ml (extractable volume) of PRGN-2009 at a target concentration of 5 x 10^{11} PU/ml. Therefore, each vial contains approximately 5 x 10^{11} PU.

14.1.3.2 Diluent

FFB (manufactured by [redacted]) will be used as the diluent for PRGN-2009 and will be supplied by [redacted]. Diluent is provided as a sterile, frozen, injectable liquid that has been aseptically filled into CZ vials fitted with a butyl rubber stopper and an aluminum flip-cap seal. Each vial contains 4.0 ml (extractable volume) of PRGN-2009. NOTE: no other diluents other than FFB should be used with PRGN-2009.

- A single vial will be used for administration of the maximum dose of 5 x 10^{11} PU. No pooling of vials will be required. A Diluent vial will not be needed for the 5 x 10^{11} PU dose.

14.1.3.3 Pharmacy Preparation

Follow institutional procedures for handling of a BSL-2 organism. Since the PRGN-2009, is provided with a butyl rubber stopper with aluminum flip-tear seal, these materials do not required handling within a biological safety cabinet. Care should be taken to avoid creation of aerosols during syringe withdrawals through the butyl rubber stopper. If institutional policies dictate, the steps involving a syringe transfer should be conducted within a biological safety cabinet. Spills of PRGN-2009 should be decontaminated using a solution of 10% bleach. All materials should be disposed of according to institutional procedures for a BSL-2 organism.

14.1.3.4 Vaccine Preparation

Thawing – For each subject, a single vial of PRGN-2009 should be removed from the ultracold freezer and rapidly thawed in a 37°C water bath until the contents are just thawed (avoid prolonged exposure of the thawed vial to the 37°C water bath). Once thawed, do not refreeze. After thawing, vials should be wiped down with 70% isopropyl alcohol (IPA). Thawed vials of PRGN-2009 can be stored for up to 2 hours at ambient temperature prior to administration. Do not store thawed vials on wet ice or in contact with a cold pack or inside a refrigerator.

Dilution of PRGN-2009 and storage of the diluted product at ambient temperature is addressed in the pharmacy manual, which should be referenced if dilution is required.

Visually inspect the vialed products before use. If the appearance does not match the appearance listed below, contact the manufacturer [redacted]) and quarantine the vial(s) until further guidance is provided. Do not use the product if they appear to be damaged in any way.

- PRGN-2009 should appear to be a clear to slightly opalescent, colorless liquid and should be free of visible particulates.
- FFB should appear to be a clear, colorless liquid and should be free of visible particulates.

Preparation of the 5 x 10^{11} PU dose - The top dose of 5 x 10^{11} PU will be obtained by aseptically withdrawing 1.0 ml of PRGN-2009 from a single thawed vial using an appropriately sized sterile syringe. One ml of the undiluted PRGN-2009 will be administered for the top dose of 5 x 10^{11} PU. The empty PRGN-2009 vial should be discarded as biohazardous waste. The total time for exposure of PRGN-2009 to ambient temperature after thawing the vial is 2 hours, including syringe filling and administration. Do not store on wet ice or in a refrigerator.

14.1.4 Stability and Storage

PRGN-2009 and diluent are provided in cardboard cartons containing 10 vials of frozen liquid. Each 10-pack carton is provided within a sealed, labeled Mylar pouch. The sealed pouch is shipped inside a cardboard box that contains dry ice so that the contents will remain frozen during shipment. This Mylar pouch protects the vials from direct exposure to dry ice vapors. PRGN-2009 and Diluent will be shipped to the clinical site from [redacted]

After receipt, the package should be opened and the contents inspected to confirm that a quantity of dry ice remains and that the Mylar pouch is intact. (Report to [redacted] any issues observed when receiving and opening a package). The Mylar-enclosed carton of 10 vials should be immediately transferred to an ultracold freezer and stored in the range of -60 to -90°C. This freezer should be appropriately monitored for temperature excursions. Any excursions that occur outside the recommended storage temperature range for PRGN-2009 should be documented and reported to the Sponsor.

The carton can remain within the Mylar pouch until a vial of PRGN-2009 is required for administration to a subject. After the Mylar pouch is opened, the carton should be quickly returned to the ultracold freezer to avoid exposure of PRGN-2009 to ambient temperature. After the pouch is opened it can be disposed of and the cardboard carton can be stored directly within the ultracold freezer. Do not expose unprotected vials (without Mylar pouch) directly to dry ice vapors after opening and removing the pouch.

14.1.5 Incompatibilities

Not available.

14.2 PEMBROLIZUMAB

Please refer to the FDA-approved package insert for complete product information.

14.2.1 Source

Pembrolizumab will be obtained from commercial sources and dispensed by the NIH Pharmacy.

14.2.2 Administration Procedures

See Section 3.2.2.

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16 LIST OF ABBREVIATIONS

Abbreviation	Term
AACR	American Association of cancer Research
[redacted]	[redacted]
ACAT	Ability to Consent Assessment Team
ADR	Adverse drug reaction
AE	Adverse Event/Adverse Experience
AI	Associate Investigator
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
AVA	Anti-vector antibodies
BSL	Biosafety level
BTRIS	Biomedical Translational Research Information System
CAP	College of American Pathologists
CBC	Complete blood count
CCR	Center for Cancer Research
cfDNA	Circulating free DNA
CFR	Code of Federal Regulations
CIO	Center for Immuno-Oncology
CLIA	Clinical Laboratory Improvement Amendments
CLL	Chronic lymphocytic leukemia
CMP	Clinical Monitoring Plan
COV	Close-out Visit
CPD	Confirmed progressive disease
CPS	Combined positive score
CR	Complete Response
CRIS	Clinical Research Information Service
CRP	C-reactive protein
CRT	Chemoradiotherapy
CSP	Clinical Services Program
CRADA	Cooperative Research and Development Agreement
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTL	Cytotoxic T lymphocyte
CZ	Crystal zenith
DNA	Deoxyribonucleic acid
DS	Drug Substance
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EF	Ejection fraction

Abbreviation	Term
EGFR	Epithelial Growth Factor Receptor
EKG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ENT	Ear, nose, and throat
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FFB	Final formulation buffer
FFPE	Formalin fixed paraffin embedded
FU	Follow up
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
HCG	Human chorionic gonadotropin
H&E	Hematoxylin and Eosin
Hep B/HBV	Hepatitis B virus
Hep C/HCV	Hepatitis C virus
HHS	Health and Human Services
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
HRPP	Human Research Protection Program
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
IC	Inhibitory concentration
ICB	Immune checkpoint blockade
ICH	International Council on Harmonisation
IFA	Immunofluorescence Assay
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IgG4κ	Immunoglobulin G4 kappa
IHC	Immunohistochemistry
IL	Interleukin
IND	Investigational New Drug
INR	International normalized ratio
IPA	Isopropyl alcohol
irAE	Immune-related adverse event
IRB	Institutional Review Board

Abbreviation	Term
IRBO	Institutional Review Board Office
IRR	Infusion-related reaction
ITIM	Immunoreceptor tyrosine-based inhibition motif
ITSM	Immunoreceptor tyrosine-based switch motif
IV	Intravenous
JCAHO	Joint Commission on Accreditation of Healthcare Organizations
LAR	Legally Authorized Representative
LLD	Longest lesion diameter
LP	Laboratory of Pathology
LTIB	Laboratory of Tumor Immunology and Biology
mAb	Monoclonal antibody
MCV	Mean corpuscular volume
MDSC	Myeloid-derived suppressor cells
MedDRA	Medical Dictionary for Regulatory Activities
MEL	Melanoma
MOI	Multiplicity of infection
MOP	Manual of Operational Procedures
MRI	Magnetic Resonance Imaging
MVB	Master Virus Bank
MxIF	Multiplex immunofluorescence
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCT	National Clinical Trial (number)
NIDCD	National Institute on Deafness and Other Communication Disorders
NIH	National Institutes of Health
NK	Natural killer
OHSRP	Office for Human Subjects Research Protections
OPC	Oropharyngeal cancer
ORR	Overall response rate
OS	Overall survival
OSRO	Office of Sponsor and Regulatory Oversight
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD	Progressive Disease
PD-1	Programmed cell death 1
PD-L1/2	Programmed cell death ligand 1/2
PDTS	Protocol Deviation Tracking System
PET	Positron Emission Tomography
PFS	Progression-free survival
PI	Principal Investigator
PR	Partial Response
PS	Performance Status

Abbreviation	Term
PT	Prothrombin time
PTT	Partial thromboplastin time
QA	Quality Assurance
QC	Quality Control
RBC	Red blood cell
rCR	Revised Common Rule
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	Relapse-free survival
RNA	Ribonucleic Acid
RP2D	Recommended Phase II dose
RDW	Red cell distribution width
SAE	Serious Adverse Event/Serious Adverse Experience
SAV	Site Assessment Visit
SC	Subcutaneously
SCC	Squamous cell carcinoma
SIV	Site Initiation Visit
SD	Stable Disease
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedure
SROS	Sponsor and Regulatory Oversight Support
TNF	Tumor necrosis factor
Tregs	Regulatory T cells
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
UPD	Unconfirmed progressive disease
US	United States
VP	Viral particles
WBC	White blood cells
WOCBP	Woman of childbearing-potential
WT	Wild type

17 APPENDICES

17.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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