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Title: Phase I/II Trial of HPV Vaccine PRGN-2009 Alone or in Combination with Anti-PD-L1/TGF-β trap (M7824) in Subjects with HPV Associated Cancers

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Drug Name:	PRGN-2009	M7824
IND Number:	19628	19628
Sponsor:	CCR, NCI	CCR, NCI
Manufacturer:	Precigen, Inc.	EMD Serono, Inc.
Supplier	Precigen, Inc.	EMD Serono, Inc.

Commercial Agents: None

PRÉCIS

Background

- Metastatic HPV associated malignancies (cervical, anal, oropharyngeal cancers, etc.) are often incurable and poorly palliated by standard therapies.
- HPV-positive (p16+) oropharyngeal cancers are the most common HPV-associated malignancy in the United States and are increasing in incidence.
- Stage II and III HPV-positive oropharyngeal cancer is primarily treated with definitive therapy.
- Although the prognosis for stage I HPV+ oropharyngeal cancer 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.
- Attempts to de-intensify treatment of HPV-positive oropharyngeal cancer by replacing high-dose cisplatin with cetuximab concurrent with radiotherapy have failed.
- Induction and neoadjuvant immunotherapy are an area of active study in this type of cancer. The aims of induction immunotherapy are to induce antigen-specific immunity prior to definitive therapy and to reduce the risk of disease relapse for patients with stage II and III disease.
- Therapeutic vaccines targeting HPV alone or in combination with M7824 (dual PD-L1 and TGF beta inhibitor) have demonstrated induction of HPV antigen-specific responses and tumor growth inhibition in multiple pre-clinical models of HPV-positive malignancy.
- In clinical studies done in the CCR, M7824 as monotherapy has produced a notable objective response rate (35-40%) for metastatic HPV + cancers including Oropharyngeal Squamous Cell Carcinoma (OPSCC) and preclinical studies support the addition of an investigational HPV vaccine with therapeutic intent (PRGN-2009, a gorilla adenoviral based vaccine) to further increase anti-tumor efficacy.

Objectives:

Phase I in participants with recurrent/metastatic HPV positive cancer:

- Primary objective: To determine the safety and recommended phase II dose (RP2D) of PRGN-2009 (HPV vaccine) alone or in combination with M7824 administered at RP2D of 1200 mg q2w.

Phase II in participants with newly diagnosed stage I (T1,T2 N1)/II/III p16-positive oropharyngeal cancer and patients with newly diagnosed operable stage II/III/IVA/IVB HPV+ sinonasal squamous cell cancer:

- Primary objective: To determine if HPV vaccine alone (Arm 2A) is able to result in a ≥ 2-fold increase in CD3+ tumor infiltrating T cells post treatment compared with pre-treatment in p16-positive oropharyngeal cancer.

Eligibility:

Phase I:

- Men or women of age ≥ 18 years old.

- Subjects with cytologically or histologically confirmed locally advanced not amenable to potentially curative local therapies or metastatic HPV associated malignancies:
    - Cervical cancers;
    - p16+ Oropharyngeal cancers;
    - Anal cancers;
    - Vulvar, vaginal, penile, and squamous cell rectal cancers
    - Other locally advanced or metastatic solid tumors (e.g., lung, esophagus) that are known HPV+.
- Prior first line systemic therapy is required.

Phase II:

- Men or women of age ≥ 18 years old.
- Subjects with newly diagnosed stage I (T1,T2 N1), II or III p16-positive oropharyngeal squamous cell carcinoma (OPSCC) or stage II/III/IVA/IVB HPV-SNSCC planned for definitive therapy.

Design:

Phase I: Recurrent/metastatic HPV associated cancer:

- A 3+3 dose escalation design will be used which will evaluate PRGN-2009 (HPV vaccine) at two dose levels ($1x10^{11}$ and $5x10^{11}$ viral particle (VP) units) given as monotherapy followed by a third dose level evaluating the RP2D dose of PRGN-2009 in combination with 1200 mg (RP2D) of M7824. In addition, the combination of PRGN-2009 at RP2D with 1200 mg of M7824 will be expanded to a total of 10 evaluable participants to gauge the preliminary efficacy of the combination of PRGN-2009 and M7824 in participants with advanced disease.
- There will be a 4-week DLT evaluation period for each dose level.
- It is expected that up to 22 participants may enroll.

Phase II:

- Newly diagnosed p16-positive oropharyngeal cancer:
    - Evaluation of HPV vaccine alone (Arm 2A: Stage I (T1,T2 N1)/II/III) as neoadjuvant/ induction therapy before definitive standard of care therapy.
    - Participants will receive neoadjuvant/ induction immunotherapy at NIH Clinical Center and then be referred back to their home institution for definitive standard of care therapy.
    - It is expected that up to 20 participants may enroll.
- Newly diagnosed stage II/III/IVA/IVB HPV-SNSCC:
    - Enrollment and treatment will occur similarly as participants with p16+ oropharyngeal cancer for exploratory correlates to advise possible future trials. Up to 2 participants may enroll in this group.

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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 Objectives

Phase I in participants with recurrent/metastatic HPV positive cancer:

- Primary objective: To determine the safety and recommended phase II dose (RP2D) of PRGN-2009 (HPV vaccine) alone or in combination with M7824 administered at RP2D of 1200 mg q2w.

Phase II in participants with newly diagnosed stage I (T1,2 N1)/II/III p16-positive oropharyngeal cancer (OPSCC) cancer:

- Primary objective: To determine if HPV vaccine alone (Arm 2A) is able to result in a ≥ 2-fold increase in CD3+ tumor infiltrating T cells post treatment compared with pre-treatment in p16-positive oropharyngeal cancer.

1.1.2 Secondary Objectives

Phase I in participants with recurrent/metastatic HPV positive cancer:

- To assess overall response rate (ORR) according to RECIST 1.1.
- To assess progression-free survival time (PFS).
- To assess overall survival (OS).
- To assess duration of response.
- To assess ratio of participants that are hospitalized because of adverse events attributed to disease progression.

Phase II in participants with newly diagnosed stage I (T1,2 N1)/II/III p16-positive oropharyngeal cancer (OPSCC) cancer:

- To determine if the use of PRGN-2009 alone results in significantly prolonged survival as compared to the expected 80% three-year historical survival for this population.
- To determine the 3-year overall and relapse-free survival rate for PRGN-2009 alone (Arm 2A) as neoadjuvant/ induction therapy before definitive standard of care therapy for this population.
- To assess the safety of the recommended phase II dose (RP2D) of PRGN-2009 (HPV vaccine) alone in this participant population.

1.1.3 Exploratory Objectives

Phase I and Phase II

- To conduct exploratory immunologic studies to understand and improve the administered treatment, including:
    - To examine the pharmacokinetics of bintrafusp alfa while on treatment.
    - To determine the ORR per RECIST v1.1 of combination of PRGN-2009 and M7824 in participants with advanced HPV associated malignancies

- Precigen, Inc. may analyze samples to be used for additional exploratory analyses, to further investigate:
    - HPV 16/18 -specific immune responses, activation status of the immune system, anti-tumor specific immune responses induced by the exposure to PRGN-2009.

Phase II only

- To determine the rate of objective response following neoadjuvant/induction immunotherapy alone per RECIST 1.1.
- To assess changes in salivary HPV DNA from pre-treatment and compared to during neoadjuvant/induction therapy with PRGN-2009 (HPV vaccine) alone in participants with HPV-OPC.
- To determine the feasibility and safety of neoadjuvant treatment therapy with PRGN-2009 (HPV vaccine) alone in participants with HPV-SNSCC.
- To assess the differences in CD3+ tumor infiltrating lymphocytes following neoadjuvant therapy with PRGN-2009 (HPV vaccine) alone in participants with HPV-SNSCC.
- To determine the objective response rate following neoadjuvant therapy with PRGN-2009 (HPV vaccine) alone in participants with HPV-SNSCC.

1.2 Background and Rationale

1.2.1 HPV Associated Malignancies

In the United States, there are more than 30,000 cases of HPV associated cancer annually (1) (**Table 1**). Metastatic HPV associated malignancies (cervical, anal, oropharyngeal cancers etc.) are often incurable and poorly palliated by standard therapies. Responses to chemotherapy are variable but generally short-lived with median PFS around 3 to 7 months (2-5). In a Gynecologic Oncology Group randomized trial comparing four cisplatin-based doublets as first line therapy for metastatic cervical cancer the response rates were 22-29% and median PFS was 4 to 6 months with median OS 10 to 13 months (6). The addition of bevacizumab to combination chemotherapy has been reported to increase OS by 3.7 months, but virtually all patients die of their disease within 2 years (7). Randomized trials of second line therapy are lacking but response rates for single agents are generally reported to be less than 20% (8). Early evidence suggests that immune checkpoint therapy also has a low response rate in this disease with a phase 1b trial (KEYNOTE 028) showing a 12.5% response rate (3/24 patients) and a phase II study showing a response rate of 13.3% (13/98 patients) to pembrolizumab in patients with recurrent or metastatic cervical cancer (9).

Table 1: Estimated annual incidence of HPV associated cancers in the US

Site	Incidence of HPV associated cancers	Cases attributed to HPV (%)
Oropharyngeal	15,738	11,000 (70.1%)
Cervix	11,771	10,700 (90.6%)
Vulvar	3554	2400 (68.8%)

Site	Incidence of HPV associated cancers	Cases attributed to HPV (%)
Vaginal	802	600 (75%)
Penis	1168	700 (63.3%)
Rectal (squamous cell)	750	700 (91.1%)
Anus	5,010	4,600 (91.1%)
Total	38,793	30,700 (79.1%)

For metastatic oropharyngeal cancer, the best estimates of the chemotherapy responsiveness are inferred from looking at the oropharyngeal site in subset analyses from clinical trials for head and neck cancers. In a pivotal clinical trial that established platinum, 5-fluorouracil (5-FU), plus cetuximab as first line therapy in head and neck cancer, patients with oropharyngeal tumors experienced PFS of 4 to 6 months and OS of 8 to 11 months (3). Immune checkpoint therapy has become the standard second line therapy for metastatic oropharyngeal cancer but response rates are still low with this therapy. As an example, the phase 1b trial (KEYNOTE-012) of pembrolizumab which led to its FDA approval for recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) as second line therapy had a 17.7% response rate (34/192 patients). Response rates were slightly better in HPV positive HNSCC (21.9%) (10), but still occurred in only a minority of the patients. More recently pembrolizumab had been FDA approved as first line therapy for metastatic PD-L1 expressing HNSCC but the response rate for pembrolizumab alone in this setting is still only 16.9%.

In regard to metastatic anal cancer, only a handful of randomized trials have been performed in the last 30 years. In the metastatic setting, most of the evidence is limited to small phase II trials, retrospective series, and case reports. A recent retrospective series looking at 77 patients, 44 (55%) of whom received 5-FU in combination with cisplatin, 24 (31%) of whom received carboplatin + paclitaxel, and 11 (14%) of whom received another regimen showed a median PFS of 7 months and median OS of 22 months (5). As with HNSCC, recent early phase trials evaluating immune checkpoint therapy in this disease have shown that responses here too are limited to around 20% of patients treated. A recent phase II trial of nivolumab for metastatic squamous cell anal cancer showed a 21% response rate (7/33 patients) and a recent phase IB trial (KEYNOTE-028) of pembrolizumab showed a 20% response rate (5/25 patients) (11). In a recently published phase II trial, Massarelli et al. evaluated the combination of a therapeutic HPV vaccine and Nivolumab (12). In contrast to previous single therapy immune checkpoint inhibitor trials, they found an overall response rate of 33% showing the potential benefit of Immuno-Oncology (I/O) combination therapy for these diseases.

Human papilloma virus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) now represents the most common HPV-associated malignancy in the United States and the incidence is rising (13, 14). Over 80% of OPSCC is HPV-positive, and nearly all HPV-positive OPSCC are caused by HPV 16 or 18. Definitive standard-of-care treatment for patients with stage II/III HPV-positive oropharyngeal head and neck squamous cell carcinoma most commonly includes multi-

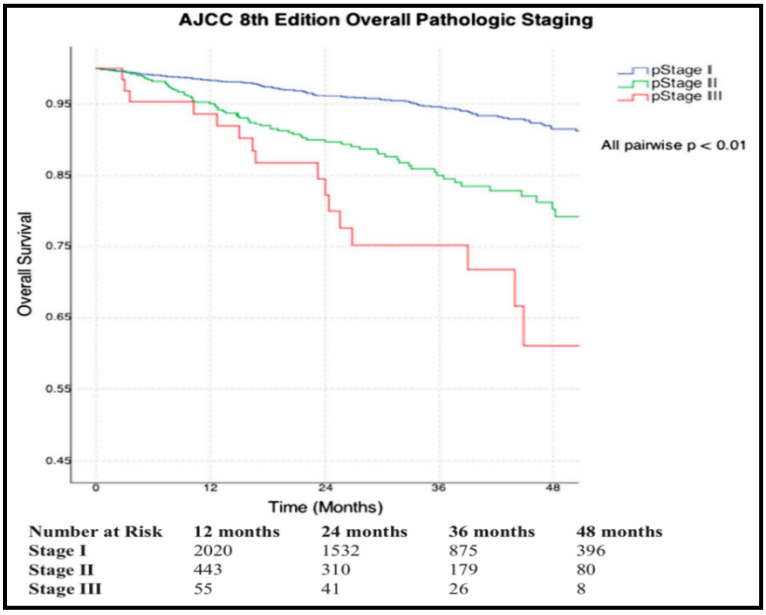
modality treatment consisting of concurrent chemotherapy and radiation (CRT) (15). Surgery is rarely utilized due to the morbidity associated with resection of oropharyngeal tissues. Despite maximum treatment, 20% of patients with stage II and 35% of patients with stage III disease will die within 4 years of diagnosis (**Figure 1**)(16). In patients cured of their disease, treatment often results in dysfunctional swallowing, taste, and speech as well as in extensive tissue fibrosis and pain (17). Patients with HPV-positive OPSCC 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 (18). Attempts to de-intensify treatment of HPV-positive OPSCC by replacing cisplatin chemotherapy with the EGFR monoclonal antibody (mAb) cetuximab have failed (15). Stage I (AJCC 8th edition) includes patients with T0-2 and N0-1 disease. Treatment within Stage I is not uniform. Recommended treatment for T1-2 N0 disease is definitive radiotherapy or surgical resection with or without ipsilateral/bilateral neck lymph node dissection and risk-stratified adjuvant treatment. Recommended treatment for T1-2 N1 with 1 lymph node (ipsilateral) not larger than 3 cm is definitive chemoradiotherapy or radiotherapy or surgical resection with or without ipsilateral/bilateral neck lymph node dissection and risk-stratified adjuvant treatment. Recommended treatment for T1-2 N1 with 1 lymph node (ipsilateral) larger than 3 cm or 2 or more ipsilateral lymph nodes none larger than 6 cm follows the paradigm of Stage II disease, with definitive chemoradiotherapy or resection plus adjuvant treatment. Consequently, while the prognosis for Stage I patients is better than Stage II and III, there is a need for de-intensification of treatment in Stage I T1-2 N1 disease.

In this clinical trial, we are studying immunotherapy consisting of PRGN-2009, an investigational HPV vaccine with therapeutic intent with or without M7824, a dual PD-L1 and TGF beta inhibitor, first as a phase I study to assess safety and tolerability, then as a phase II induction “window of opportunity” study with treatment before and after definitive therapy for patients with newly diagnosed stage I (T1,2 N1)/II/III HPV-positive OPSCC.

1.2.2 Sinonasal Squamous Cell Cancer

Sinonasal Squamous Cell Cancer (SNSCC) comprises 3-5% of all head and neck squamous cell cancers. Approximately 50% arise in the nasal cavity and the rest in the paranasal sinuses, and are usually not accompanied by nodal metastases at diagnosis (~10-20%). Emerging evidence supports a potential role for HPV in a subset of SNSCC, with studies reporting ~30% of SNSCC having HPV present, more often so in the nonkeratinizing SCC subtype, with HPV-16 the most common genotype, and less commonly genotypes HPV-18, 31 and 33. Contrary to HPV-OPC it has been reported that is more common in patients who are smoking or were smokers. Importantly, similarly to HPV-OPC, HPV positivity appears to be associated with improved prognosis, with a 5-year OS for HPV-SNSCC ranging 68.1-80.0% vs. 31-51.5% in HPV negative SNSCC. Treatment for newly diagnosed tumors consists of surgical resection with postoperative radiotherapy, but in the case of advanced stage tumors treatment is not well defined, and induction approaches are promising. For incurable tumors treatment follows the paradigm of HNSCC. In this trial we are planning to test an induction strategy in a small number of newly diagnosed HPV-SNSCC patients, following the design utilized for the HPV-OPC patient cohort.

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



1.2.3 T cell dysfunction in HPV-positive OPSCC

HPV-positive OPSCCs are infiltrated by exhausted and functionally suppressed HPV-specific T cells (19-21). 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 (22).

1.2.4 M7824

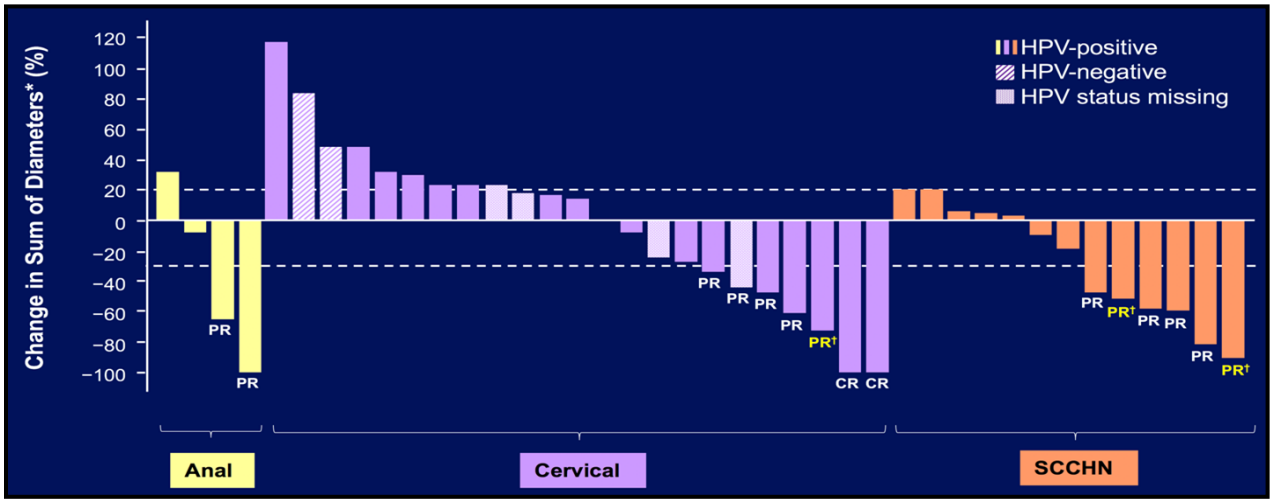
M7824 is a novel bifunctional fusion protein composed of a fully human IgG1 mAb against PD-L1 fused to the soluble extracellular domain of human transforming growth factor-beta (TGFβ)-receptor II which functions as a TGFβ "trap". Functionally, M7824 both blocks PD-L1 and neutralizes TGFβ within the tumor microenvironment (TME). Expression of PD-1/PD-L1 pathway components is present in approximately two-thirds of HPV-positive OPSCCs (23, 24). Immune checkpoint blockade of the PD-1/PD-L1 pathway with pembrolizumab is currently FDA-approved for the first-line treatment of recurrent/metastatic HPV-positive or -negative head and neck SCC in patients with a combined positive score (CPS) of >1 (24, 25) (NCT02358031). TGFβ is a major driver of local suppression of both T cell and NK cell function and is overexpressed in HPV-associated malignancies (26, 27). Through dual PD-L1 blockade and neutralization of TGFβ, M7824 induced superior tumor growth inhibition in multiple pre-clinical, syngeneic models of cancer (28, 29).

1.2.5 Clinical Experience with M7824 in HPV associated malignancies

The first in human dose escalation study of M7824 in patients with advanced solid tumors was conducted in the CCR (29). This work demonstrated on-target PD-L1 saturation and reduction of

circulating TGFβ, early evidence of clinical efficacy including in a number of patients with HPV associated malignancies, and an acceptable safety profile. Continuation of this work in patients

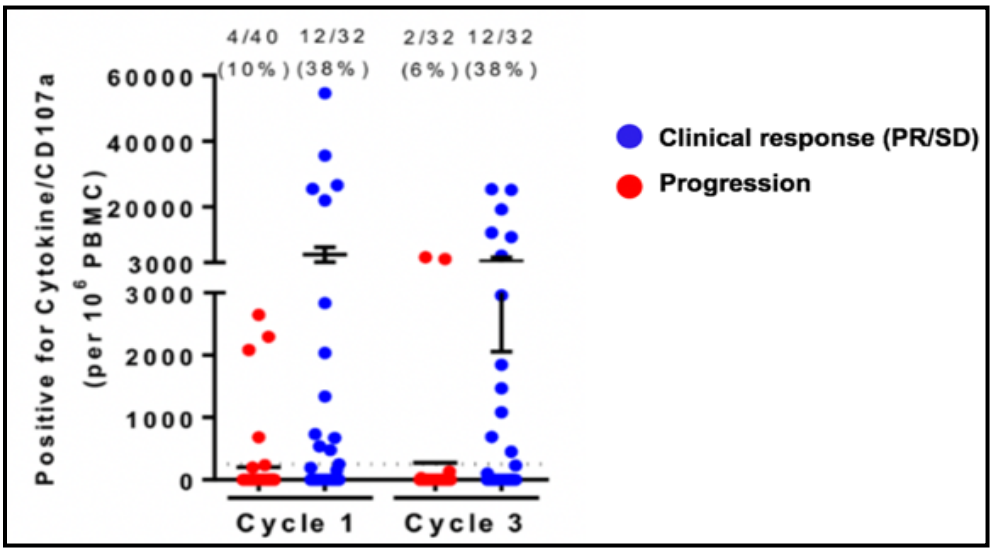
Figure 2: Waterfall plot of confirmed best overall responses in patients with immune checkpoint inhibitor-naïve, recurrent/metastatic HPV-positive OPSCC (orange).



with metastatic HPV-associated cancers which was presented at AACR 2019 revealed a 35% response rate in 43 patients including a 43% response rate in 14 patients with p16+ OPSCC (**Figure 2**). These response rates are notably higher than the 12-24% response rate seen with blockade of PD-1 or PD-L1 alone for HPV-associated malignancies (e.g., cervical, anal, P16+ oropharyngeal) in the literature (25, 30-35). In addition, the median survival of the 43 enrolled patients with HPV-associated malignancies was 16.2 months and the median survival of the 36 enrolled patients with HPV positive malignancies was not reached after 16.8 months of follow-up. It should be noted that both of these median survivals are substantially longer than the median survival reported for similar populations receiving PD-1 or PD-L1 inhibitors alone (7.5-11.5 months) in the literature (25, 32, 33, 35-37) .

Adverse events for M7824 were similar to those experienced with pembrolizumab or nivolumab, with the addition of the development of low-grade mucosal bleeding and keratoacanthomas (21%) known to be associated with systemic TGFβ blockade. Treatment with M7824 induced the expansion of peripheral blood HPV 16 antigen-specific T cells to a greater degree in patients with a best overall response of CR, PR or SD compared to patients that had a best overall response of PD (**Figure 3**). These data suggested that M7824 could reverse suppression of HPV antigen-specific immunity in patients harboring HPV-positive malignancies refractory to standard treatments.

Figure 3: PBMC from patients treated with M7824 were assayed for HPV 16 antigen-specific cytokine production or CD107a positivity



1.2.6 HPV 16/18 gorilla adenovirus 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 (38). 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 (39). 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 Precigen, Inc., 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 (40) .

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}; (41)). 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 Precigen's 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 CTL specific peptides from E6 (HPV-16/-18), E7 (HPV-16/-18), and E5 (HPV16) and 3 unique agonist peptides (40) for this multi-epitope antigen design. 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 it 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 at Advanced Bioscience Laboratories (ABL) utilizing a standardized Adenoviral vector manufacturing process that was developed at Precigen and transferred to ABL. The production cell line is Precigen's 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.

Figure 4: 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.

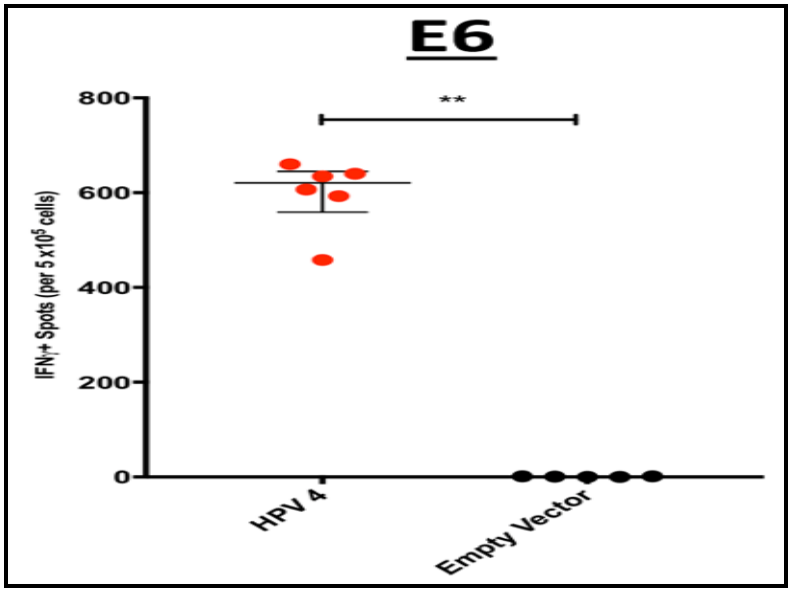
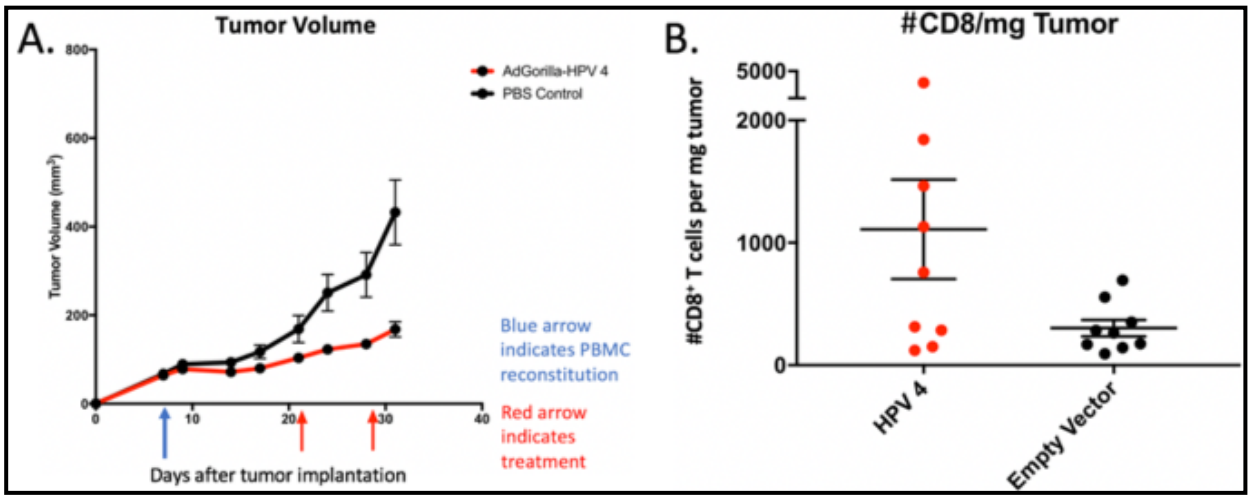


Figure 5: (A) NSG B2M-/- mice bearing SiHa tumors were immune humanized with HLA matched PBMC and mice were treated with PRGN-2009. (B) Following the second vaccine administration, SiHa tumors were assessed for CD8+ T cell infiltration by flow cytometry.



PBS vs Ad-HPV: P<0.0001 Empty vector vs HPV: P = 0.09

Pre-clinically, 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 4**). Additionally, mice bearing HPV 16-positive SiHa cervical cancers were immune humanized with HLA-matched human PBMC and treated with PRGN-2009 (**Figure 5**). Treatment

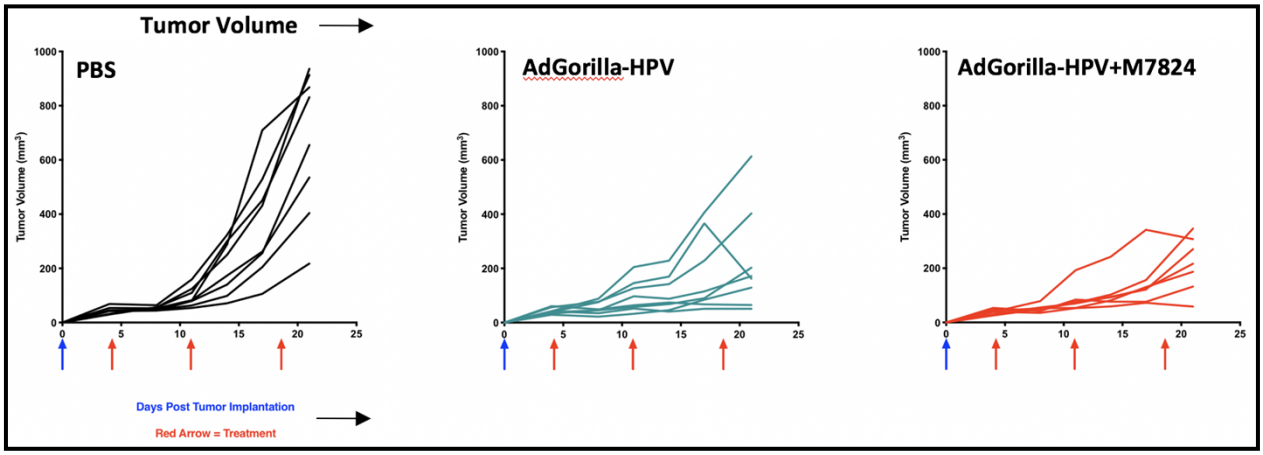
resulted in tumor growth inhibition and increased tumor infiltration of CD8+ T cells. These data demonstrated that PRGN-2009 is capable of inducing HPV antigen-specific immune responses and anti-tumor immunity sufficient to inhibit tumor growth.

1.2.7 Rationale for combination of PRGN-2009 and M7824

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 immune checkpoint 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 (12). 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 (42, 43). In addition, there is an ongoing clinical trial at CCR evaluating an adenoviral vaccine targeting CEA (ETBX-011) in combination with Avelumab and standard of care chemotherapy (FOLFOX) for patients with metastatic colorectal cancer (NCT03050814). To date 23 patients have been enrolled on this study of which 15 patients have received the combination of all study agents including the adenoviral vaccine and Avelumab. One patient had grade 2 arthritis which was managed with a steroid taper. Otherwise no notable immune related toxicities have been observed in the 15 patients receiving vaccine and Avelumab.

The specific combination of PRGN-2009 and M7824 was tested in wild-type C57BL/6 mice bearing TC-1 tumors (**Figure 6**). The addition of M7824 further enhanced tumor growth inhibition observed with PRGN-2009 alone. Cumulatively, these results indicate that the addition of M7824 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 6: C57BL/6 mice bearing TC-1 tumors were treated with PRGN-2009 alone or in combination with M7824 and assessed for primary tumor growth.



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

1.2.8 Phase I study for participants with advanced HPV associated malignancies

To first assess the safety and feasibility of treatment with PRGN-2009 alone or in combination with M7824, we plan to implement a sequential phase I study of dose-escalating PRGN-2009 (two dose levels) alone or in combination with a fixed-dose of M7824. Participants with

recurrent/metastatic HPV-positive malignancy will receive two dose levels ($1x10^{11}$ and $5x10^{11}$ VP) of PRGN-2009, followed by an evaluation of the RP2D dose of PRGN-2009 in combination with 1200 mg (RP2D) of M7824.until progression or unacceptable toxicity. This design will allow the determination of safety and tolerability of PRGN-2009 alone and in combination with M7824 and allow each participant the opportunity to benefit from combination treatment. The phase I portion of this study will allow immune checkpoint naïve or refractory participants.

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

Once the safety and tolerability of PRGN-2009 alone or in combination with M7824 is established in the phase I portion of the study, we plan to implement a two arm induction, Phase II window-of-opportunity study of PRGN-2009 (Arm 2A) or PRGN-2009 plus M7824 (Arm 2B) in participants with newly diagnosed, previously untreated stage I (T1,2 N1)/II/III p16-positive OPSCC who plan to receive definitive therapy. Induction or neoadjuvant immunotherapy for newly diagnosed, OPSCC is an area of active study in an attempt to decrease locoregional failure and the development of distant metastasis after completion of definitive treatment. Participants will receive PRGN-2009 or PRGN-2009 plus M7824 before therapy. Upon completion of the neoadjuvant or induction immunotherapy treatment participants will be referred back to their referring providers for standard of care definitive therapy.

Participants diagnosed with a new stage II/III p16-positive OPSCC with planned definitive therapy typically wait 4-6 weeks for initiation of treatment due to radiation mapping and simulation and scheduling for chemotherapy (44). During this "window of opportunity" participants receive no treatment for their cancer. The phase II portion of this study would take advantage of this opportunity and offer the participant the chance to benefit from immunotherapy.

Note: In September of 2021 we received a report by EMD Serono, 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 following review of submitted data it was decided to allow Phase I to proceed and Phase II Arm 2A (vaccine only) but to close Arm 2B (vaccine plus bintrafusp alfa) before enrollment started. The Phase II portion of this study will only consist of only Arm 2A PRGN-2009 alone. Studies that were to be conducted under Arm 2B will be removed from the rest of the protocol; however, will remain here (and in Section **2.3.2**) for background and informational purposes.

1.2.10 Rationale summary

- Clinical outcomes for recurrent/metastatic HPV-positive malignancy and newly diagnosed stage II/III p16-positive OPSCC are poor, and while they are better for I (T1,2 N1) p16-positive OPSCC de-intensification is needed to reduce long-term toxicity.
- Participants with advanced p16-positive OPSCC have tumors infiltrated with exhausted and dysfunctional HPV antigen-specific T cells.
- Treatment with a vaccine targeting HPV oncogenes to induce antigen-specific immunity (PRGN-2009) combined with immune checkpoint blockade to fully activate new and existing antigen-specific T cells (M7824) is a rational combination treatment strategy.
- Investigators at the NIH Clinical Center have significant clinical experience with M7824.

- 1200 mg flat dose of M7824 has been chosen based on a phase I dose escalation trial of M7824 in solid tumors done at the NCI and PK/ PD data from that trial (NCT02517398).
- DL1 and DL2 of PRGN-2009 has been chosen based on the dose used for other adenovirus vaccines.
- The phase I portion of the study will offer participants with immune checkpoint refractory recurrent/metastatic HPV-positive malignancy a chance to benefit from combination PRGN-2009 and M7824 treatment.
- The phase II portion of the study will offer participants the opportunity to benefit from immunotherapy during a period of time when they are normally scheduling and awaiting definitive therapy for their newly diagnosed HPV-positive OPSCC.
- 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.

1.2.11 Rationale and background for performing immune assays

Multiple immune assays have been developed in the LTIB to better define the mechanism(s) involved in the use of specific novel agents, as monotherapy or in combination therapies, both for preclinical and clinical studies. In addition to analyses of biopsies, analyses of the peripheral immunome can provide valuable information where multiple samples can be analyzed over the course of a given therapy vs. pre-therapy. The LTIB has now developed and employed (45-48) a flow cytometry–based assay that can analyze 123 immune cell subsets in human PBMC from one vial of processed PBMC (approximately 10^7 cells). This assay will be used in this clinical trial to detect multiple (n=32) subsets of CD4+ T cells, CD8+ T cells (n=29), Tregs (n=7), B cells (n=5), NK (n=20), NKT (n=4), DC (n=10), and MDSC (n=16) to better understand the role of each agent. We also plan to evaluate changes in TCR clonal diversity both in biopsies and the periphery. One example of where these assays have been employed involves an ongoing first-in-human trial of the NHS-IL12 tumor-targeting immunocytokine (NCT01417546). TCR diversity increased 6-14–fold in biopsies of patients with a high or intermediate IFN-γ response but were unchanged or decreased with a low IFN-γ response. These findings also correlated with TIL in biopsies. We are also currently employing NanoString analyses to identify a gene signature in biopsies and PBMC, pre- and post-treatment.

The infiltration of p16-positive HNSCC with tumor infiltrating lymphocytes (TIL) tends to be greater than that observed in p16-negative HNSCC (49), possibly related to the presence of strong viral antigens. Supporting that these TIL are active and possess anti-tumor function, greater numbers of TIL present within p16-postive HNSCCs is positively correlated with increased survival after standard anti-cancer treatment and associated with an increased type I immune profile within the tumor (20). A meta-analysis of all published papers exploring TIL infiltration into p16-positive HNSCC demonstrated a strong, significant, positive correlation (50). Thus, changes in TIL infiltration into p16-positive HNSCCs after immunotherapy will be used for the primary outcome of the phase II portion of this study.

In addition, the GMB will conduct multiplexed, multispectral imaging of FFPE tissue to evaluate multiple immune parameters within the TME before and after treatment in patients with available tissue.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Subjects with cytologically or histologically confirmed locally advanced not amenable to potentially curative local therapies or metastatic HPV associated malignancies (Phase I only):

- Cervical cancers;
- p16+ Oropharyngeal cancers;
- Anal cancers;
- Vulvar, vaginal, penile, and squamous cell rectal cancers;
- Other locally advanced or metastatic solid tumors (e.g., lung, esophagus) that are known HPV+.

2.1.1.2 Subjects with cytologically or histologically confirmed newly diagnosed stage I (T1,2 N1), II or III p16-positive oropharyngeal squamous cell carcinoma planned for definitive therapy or with newly diagnosed stage II or III or IVA or IVB HPV-positive sinonasal squamous carcinoma (HPV-SNSCC) eligible for primary surgery (Phase II only).

2.1.1.3 Subjects must have measurable disease, per RECIST 1.1. See Section **6.3** for the evaluation of measurable disease (Phase 1 only).

2.1.1.4 Phase I only: Participants must have received one prior line of systemic chemotherapy in the recurrent/metastatic setting as well as checkpoint blockade therapy in tumors with FDA approval (head and neck squamous cell cancer and PDL1+ cervical cancer). Exceptions to this include participants not eligible to receive standard therapy.

2.1.1.5 Men or Women; Age ≥18 years.

2.1.1.6 ECOG performance status ≤2 (see **APPENDIX A: Performance Status Criteria**).

2.1.1.7 Adequate hematologic function at screening, as follows:

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

2.1.1.8 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 institutional ULN (GFR can also be used in place of creatinine or CrCl);
- 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).

2.1.1.9 The effects of the immunotherapies (PRGN-2009 vaccine and M7824) on the developing human fetus are unknown. For this reason and because M7824 and PRGN-2009 used in this trial are possibly teratogenic, women of child-bearing potential and men must agree to use highly effective contraception (hormonal or barrier method of birth control;

abstinence) prior to study entry and up to 2 months following the last dose of M7824 study treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.10 Participants serologically positive for HIV, Hep B, Hep C are eligible as long as the viral loads are undetectable by quantitative PCR. HIV positive participants must have CD4 count ≥ 200 cells per cubic millimeter 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.

2.1.2 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 28 days prior to the first drug administration except if the investigator has assessed that all residual treatment-related toxicities have resolved or are minimal and feel the participant is otherwise suitable for enrollment. 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 Known active brain or central nervous system metastasis (less than a month out from definitive radiotherapy or surgery), seizures requiring anticonvulsant treatment (<3 months) or clinically significant cerebrovascular accident (<3 months). In order to be eligible participants must have repeated CNS imaging at least a month after definitive treatment showing stable CNS disease. Participants with evidence of intratumoral or peritumoral hemorrhage on baseline imaging are also excluded unless the hemorrhage is grade ≤ 1 and has been shown to be stable on two consecutive imaging scans.

2.1.2.4 Pregnant women are excluded from this study because M7824 and PRGN-2009 vaccine have not been tested in pregnant women and there is potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with these immunotherapies, breastfeeding should be discontinued if the mother is treated on this protocol.

2.1.2.5 Only for Phase I, Arm 1B: Active autoimmune disease that might deteriorate when receiving an immunostimulatory agent with exception of:

- Diabetes type I, eczema, vitiligo, alopecia, psoriasis, hypo- or hyperthyroid disease or other mild autoimmune disorders not requiring immunosuppressive treatment;
- Administration of steroids for other conditions through a route known to result in a minimal systemic exposure (topical, intranasal, intro-ocular, or inhalation) is acceptable;
- Subjects on systemic intravenous or oral corticosteroid therapy with the exception of physiologic doses of corticosteroids (≤ the equivalent of prednisone 10 mg/day) or other immunosuppressors such as azathioprine or cyclosporin A are excluded on the basis of potential immune suppression. For these subjects these excluded treatments must be discontinued at least 1 weeks prior to enrollment for recent short course use (≤ 14 days) or discontinued at least 4 weeks prior to enrollment for long term use (>

14 days). In addition, the use of corticosteroids as premedication for contrast-enhanced studies is allowed prior to enrollment and on study.

2.1.2.6 Only for Phase I: Subjects with a history of serious intercurrent chronic or acute illness, such as cardiac or pulmonary disease, hepatic disease, bleeding diathesis or recent (within 3 months) clinically significant bleeding events, known left ventricular ejection fraction <50% (confirmation of EF > 50% is not required for eligibility), history of myocarditis, or recent myocardial infarction (within 6 months), or other illness considered by the Investigator as high risk for M7824 drug treatment.

2.1.2.7 Only for Phase I: Subjects refusing to accept blood products as medically indicated.

2.1.2.8 History of second malignancy within 3 years of enrollment except for the following: adequately treated localized skin cancer, cervical carcinoma in situ, superficial bladder cancer, other localized malignancy which has been adequately treated or malignancy which does not require active systemic treatment (e.g., low risk CLL). For participants enrolled on the phase I portion of the protocol a second HPV driven malignancy is allowed.

2.1.2.9 Only for Phase I, Arm 1B: Subjects with a known severe hypersensitivity reaction to monoclonal antibodies or its excipients (grade >/= 3 NCI-CTCAE v5) will be evaluated by the allergy/immunology team prior to enrollment.

2.1.2.10 Prior allogenic tissue/solid organ transplant.

2.1.2.11 For participants who may receive M7824: previous life-threatening side effects resulting from prior checkpoint inhibitor therapy.

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

2.1.2.13 Participants unable to provide informed consent.

2.1.2.14 Participants whose inclusion in the trial would in the judgement of the PI lead to time from diagnosis to initiation of curative treatment of >70 days (Arm 2A only).

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

This study will be listed on www.clinicaltrials.gov. This study may be advertised at national conferences including AACR and ASCO and may also be shared with the HPV and Anal Cancer Foundation who may advertise it further. Recruitment flyers may be used to advertise this study in the future after IRB approval.

Participants for the Phase I portion of the study will be recruited from the current participant population at NIH. participants with HPV related cancers are currently being treated on another trial through LTIB, CCR, NIH, where 2-3 participants per month are being screening and enrolled. Some of these participants come from established community networks, some of these participants are self-referrals, and some of these participants are referred by another group (Dr. Norberg's group, Experimental Transplantation and Immunology Branch, CCR, NIH) who also have a large referral base for HPV related cancers. Participants on the phase II portion of the study will be recruited from locoregional hospital including the Johns Hopkin's community network of which Clint Allen, MD, an AI on this protocol, is also a part.

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 study consent or the consent for study # 01C0129 (provided the procedures are permitted on that study) on which screening activities will be performed within 28 days prior to enrollment unless otherwise indicated.

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.

- Complete medical history and physical examination (including height, weight, vital signs, and ECOG performance status).
- Imaging for Phase I:
    - CT of chest, abdomen and pelvis or MRI, if clinically indicated.
    - A brain CT scan in participants with known CNS disease as described in Section **2.1.2.3**/ MRI scan if clinically indicated.
- Imaging for Phase II:
    - CT of the neck and chest[+], or MRI, if clinically indicated, for participants with oropharyngeal cancer.
    - CT of skull, neck and chest[+], or MRI, if clinically indicated, for participants with sinonasal SCC.

  [+]Imaging of chest and neck ± skull is preferred but not required if a PET/CT is available from within the screening timeframe.
- For skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion.
- EKG
- Clinical laboratory tests (within 16 days prior to enrollment):
    - Acute care panel (Na+, K+, Cl-, total CO2, creatinine, glucose, blood urea nitrogen).
    - Hepatic panel (AST/GOT, ALT/GPT, total bilirubin, direct bilirubin).

- Mineral panel (albumin, calcium total, magnesium total, phosphorus).
- Urine or serum pregnancy test (β-HCG) for females of childbearing-potential and women < 12 months since the onset of menopause (within 5 days prior to enrollment).
- Hematology: complete blood count (CBC) with differential.
- Urinalysis.

- HBV, HCV, HIV testing including viral load for HCV, HBV and HIV if clinically indicated (within 3 months prior to enrollment). CD4 testing may also be required for HIV+ participants.
- Participants with a history of ≥ grade 3 hypersensitivity reaction to monoclonal antibodies or M7824 will be evaluated by immunologist prior to enrollment.

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 Failure

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, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a transient lab abnormality may be rescreened.

2.3.2 Treatment Assignment Procedures

2.3.2.1 Cohorts

Number	Name	Description
1	Cohort 1	Subjects with advanced or metastatic HPV associated malignancies (Phase I)
2	Cohort 2	Subjects with newly diagnosed p16+ oropharyngeal cancer (Phase II): Arm 2A: Stage I (T1,T2 N1)/II/III Arm 2B: Stage II/III Note: Closed, Will Not Enroll
3	Cohort 3	Subjects with newly diagnosed stage II/III/IVA/IVB HPV+ sinonasal squamous cell cancer (Phase II)

2.3.2.2 Arms

Number	Name	Description
1	Arm 1A	HPV vaccine at $1x10^{11}$ Viral Particles (VP) (DL1) and at $5x10^{11}$ VP (DL2) Note: Closed, Enrollment Completed
2	Arm 1B	HPV vaccine at RP2D plus M7824 at 1200 mg
3	Arm 2A	HPV vaccine at RP2D given as neoadjuvant or induction therapy
4	Arm 2B	HPV vaccine at RP2D plus M7824 at 1200 mg given as neoadjuvant or induction therapy Note: Closed, Will Not Enroll

2.3.2.3 Arm Assignment

Participants in Cohort 1 will be sequentially assigned to Arm 1A, to determine RP2D of PRGN-2009, and then Arm 1B, evaluating the combination of RP2D of PRGN plus M7824. Participants will be enrolled to these arms based upon a 3+3 dose escalation schema. In addition, Arm 1B will be expanded to a total of 10 evaluable participants to gauge the preliminary efficacy of the combination of PRGN-2009 and M7824 in participants with advanced disease.

Once the RP2D of HPV vaccine has been determined, p16+ oropharyngeal cancer participants in Cohort 2 and HPV-SNSCC participants in Cohort 3 will be assigned to Arm 2A (20 evaluable participants for Cohort 2, up to 2 for Cohort 3).

2.4 BASELINE EVALUATION

All subjects are required to complete baseline evaluations within two weeks (four weeks for Phase II imaging) prior to the first dose of the study drug (any screening evaluation done within this time period can also serve for the baseline evaluation unless otherwise indicated):

- Physical exam including weight, ECOG performance status and vital signs.
- Skin assessment.
- Medical history (Concomitant Medications and Baseline Signs and Symptoms evaluation).
- Imaging for Phase I*:
  - CT of chest, abdomen and pelvis or MRI, if clinically indicated.
  - A brain CT scan in participants with known CNS disease as described in Section **2.1.2.3**/ MRI scan if clinically indicated.
- Imaging for Phase II*:

- CT of the neck[†] and chest[+], or MRI, if clinically indicated, for participants with oropharyngeal cancer.
- CT of skull[†], neck[†] and chest[+], or MRI, if clinically indicated, for participants with sinonasal SCC.

[*]Imaging for baseline should be within 2 weeks (for Phase I) or 4 weeks (for Phase II) prior to the first dose of study drug and is not required if available within the baseline timeframe. Imaging performed at outside facilities within the timeframe may also be used for baseline.

[+]Imaging of chest is not required if a PET/CT is available within the baseline timeframe.

[†]The CT component of a PET/CT obtained within the baseline timeframe may be used as CT of the neck ± skull per PI discretion.

- Urine or serum pregnancy test (β-HCG) for females of childbearing-potential and women < 12 months since the onset of menopause (within 5 days prior to study therapy).
- Acute care panel (Na+, K+, Cl-, total CO2, creatinine, glucose, blood urea nitrogen).
- Hepatic panel (AST/GOT, ALT/GPT, total bilirubin, direct bilirubin).
- Mineral panel (albumin, calcium total, magnesium total, phosphorus).
- Thyroid tests (TSH and reflex free T3 & T4), lipase, amylase, C-Reactive Protein (CRP).
- Hematology: CBC with differential.
- Coagulation panel: PT, INR, and PTT.
- HLA typing (any time prior to study treatment initiation - may be deferred if it has been previously performed at the NIH). (For Phase II only)
- Urinalysis.
- For correlative studies: Blood and tissue sample will be collected. Please refer to Section **5.1** for details regarding samples to be collected and refer to the **Study Calendar** for collection timepoints.
- Creatinine phosphokinase (CPK).

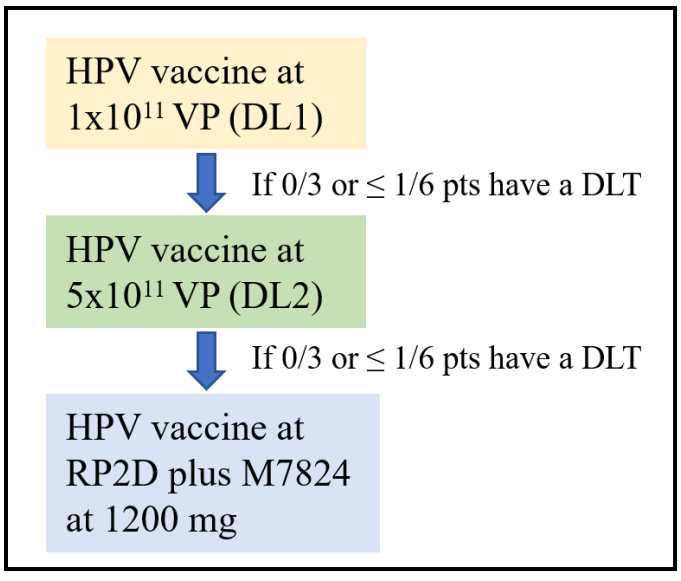
3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

Phase I: Recurrent/metastatic HPV associated cancer

- A 3+3 dose escalation design will be used which will evaluate PRGN-2009 (HPV vaccine) at two dose levels ($1x10^{11}$ and $5x10^{11}$ VP) given as monotherapy followed by an evaluation of the RP2D dose of PRGN-2009 in combination with 1200 mg (RP2D) of M7824.
- Dose escalation will follow the rules as outlined:
  - After completion of enrollment on DL1 of Arm 1A, enrollment to DL2 of Arm 1A will proceed if 0 out of 3 or 1 out of 6 participants in DL1 of Arm 1A experience a DLT.
  - After completion of enrollment on DL2 of Arm 1A, enrollment to Arm 1B will proceed if 0 out of 3 or 1 out of 6 participants in DL2 of Arm 1A experience a DLT.
  - If 2 participants in a given dose level of Arm 1A or Arm 1B experience a DLT, accrual to that arm will be halted.
- There will be a 4-week DLT evaluation period for each dose level.
- There will be a 1-week delay between the first three participants treated on a given dose level.
- All other participants will be enrolled a minimum of six days apart on the phase I portion of the protocol except between the last participant in one dose level and the first participant in the next dose level in which case the DLT period of 4 weeks will be the minimum wait time.
- In addition, Arm 1B will be expanded to a total of 10 evaluable participants to gauge the preliminary efficacy of the combination of PRGN-2009 and M7824 in participants with advanced disease.

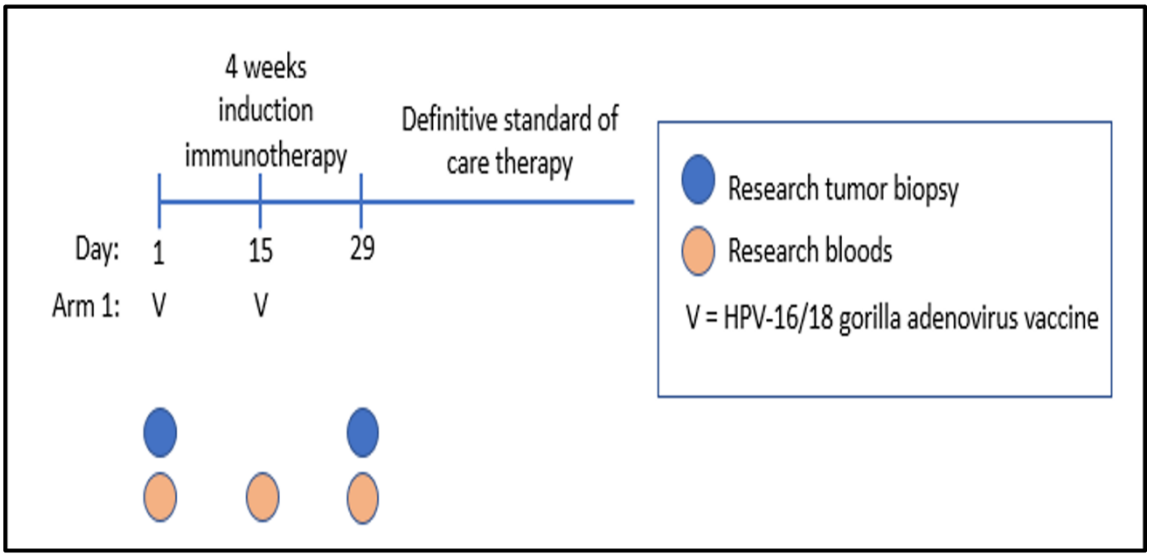
Schema for Phase I



Phase II: Newly diagnosed stage I (T1,T2 N1)/II/III HPV-positive oropharyngeal cancer and newly diagnosed stage II/III/IVA/IVB HPV-positive sinonasal squamous carcinoma:

- Evaluation of HPV vaccine alone (Arm 2A) as neoadjuvant or induction therapy in HPV-positive oropharyngeal cancer (20 participants), and HPV-SNSCC participants (2 participants).
- After completion of induction immunotherapy participants will be referred back to their home institution for definitive standard of care therapy. At outside institutions, standard of care treatment will be performed and chosen solely by outside providers. NCI investigators will have no role in treatments received at outside institutions.

Schema for Phase II



Although positive HPV testing will not be required for the phase I or II portion of the protocol prior to enrolling, HPV testing will be offered as an exploratory endpoint and participants testing negative for HPV after enrolling or whose HPV status cannot be confirmed may be replaced with other participants for the primary efficacy analysis. Participants testing negative for HPV after enrolling or whose HPV status cannot be confirmed may continue to receive treatment on study.

3.1.1 Dose Limiting Toxicity (for first 3-6 participants enrolled on Arms 1A and 1B; phase I part of the study):

Dose-limiting toxicity (DLT) will be defined as any one of the following adverse events, possibly attributable to study drugs, that occur within 28 days of the HPV vaccine monotherapy or 28 days of starting the HPV vaccine + M7824 combination therapy:

- Any Grade 3 or higher bleeding episode requiring blood transfusion(s).
- Any Grade 4 or higher adverse drug reactions (ADRs) as defined by CTCAE v5.0 and assessed as possibly related to any agent by the Investigator, except for laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 7 days without medical intervention.
- Any Grade 3 ADRs possibly attributed to any agent except for any of the following:

- Grade 3 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.
- Grade 3 Hgb decrease (< 8.0 g/dL) that is clinically manageable with blood transfusions or erythroid growth factor use does not require treatment discontinuation.
- Grade 3 laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 7 days without medical intervention.
- Keratoacanthoma and squamous cell carcinoma of the skin.
- Any endocrinopathy that can be medically managed with hormone replacement
- Any grade 3 adverse drug reaction which can be medically managed with minimal risk to the participants (e.g., placement of a pleural catheter for recurrent inflammatory pleural effusions) and resolves to at least grade one or baseline grade within 72 hours.

Where clinically appropriate subjects enrolled on Arm 1 will receive one year of treatment during which subjects will be followed with surveillance scans using CT/MRI (as appropriate) every 6-12 weeks. On a case by case basis treatment beyond a year is allowed per investigator discretion. Subjects with evidence of disease progression after completing a year of treatment will be allowed retreatment. Patients might be taken off treatment for disease progression prior to completing one year of treatment, but where clinically appropriate, treatment beyond radiographic progression is allowed if in the opinion of the investigator the subject is benefiting from treatment. Subjects will be taken off treatment if unacceptable toxicity occurs and is attributed to all therapeutic agents. If a single agent is tolerated that agent may be continued.

3.2 DRUG ADMINISTRATION

General Rule

For participants enrolled on the phase I portion of the protocol a window of +/- 14 days for a scheduled treatment is allowed in the event of scheduling issues (i.e., holiday, bad weather or other scheduling issues). For participants enrolled on the phase II portion of the protocol a window of +/- 7 days for a scheduled treatment is allowed in the event of scheduling issues (i.e., holiday, bad weather or other scheduling issues). The minimum time between administrations of a similar agent is 11 days. Where feasible PRGN-2009 should be administered first, followed by M7824 (when given).

3.2.1 PRGN-2009

On the phase I portion of the protocol PRGN-2009 will be administered on D1, D15, D29 followed by booster vaccines every 4 weeks for up to a year. The dose level given as booster will be the same dose participants will be receiving for D1, D15 and D29. On the phase II portion of the protocol PRGN-2009 will be administered on just D1 and D15.

3.2.1.1 Refer to Sections **14.1.4** Pharmacy Preparation and **14.1.5** Vaccine Preparation

3.2.1.2 Administration Procedures

- Injection Volume – The injection volume for both doses is 1.0 ml.

- Injection Route – The route of injection is by subcutaneous injection using a 1.0 ml syringe.

Follow your standard institutional clinical procedures for preparation of the subject's injection site, subcutaneous injection and post-injection bandaging of the injection site. PRGN-2009 is a non-replicating, non-integrating adenoviral vector. Since the parental adenovirus is classified as biosafety level 2 (BSL2), PRGN-2009 should be handled as BSL2 material for disposal of all materials that were used in the dose administration. Spills of PRGN-2009 should be decontaminated using a solution of 10% bleach.

3.2.2 M7824

Subjects enrolled to Arm 1B will receive M7824 via IV infusion over 1 hour (-10 minutes / +20 minutes, that is, over 50 to 80 minutes) once every 2 weeks for up to a year (Arm 1B). M7824 will be administered as a "flat" dose of 1,200 mg independent of body weight. M7824 is administered as an intravenous infusion with a mandatory 0.2 micron in-line filter. Infusion of M7824 can be administered through a PIV or CVAD or port with titanium as the main material.

Current experience revealed that IRRs to M7824 occur seldomly and are generally mild to moderate in severity. Therefore, administration of a premedication is generally not required.

In order to mitigate potential infusion-related reactions, premedication with an antihistamine and with acetaminophen (for example, 25-50 mg diphenhydramine and 500-650 mg acetaminophen) within approximately 30 to 60 minutes prior to dosing of M7824 is optional and at the discretion of the Investigator.

Management of symptoms should follow the guidelines shown in **Table 2**.

3.2.2.1 Immediate Hypersensitivity Reaction

Hypersensitivity reactions may require immediate intensive care. M7824 should be administered 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. Steroids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.

A complete guideline for the emergency treatment of anaphylactic reactions according to the Working Group of the Resuscitation Council United Kingdom and can be found at https://www.resus.org.uk/pages/reaction.pdf.

3.2.2.2 Flu-Like Symptoms

Treatment is based on clinical assessment and at the discretion of the Investigator. For prophylaxis of flu like symptoms, a nonsteroidal anti-inflammatory drug (NSAID), e.g., ibuprofen 400 mg or comparable NSAID dose, may be administered 2 hours before and 8 hours after the start of each IV infusion.

3.3 DOSE MODIFICATIONS

3.3.1 Discontinuation

Phase 1 and Phase 2:

Treatment with individual agents (PRGN-2009 alone or in combination with M7824) will be discontinued in participants experiencing specific adverse events; subjects will however still remain on study for follow up of survival. Treatment discontinuation will occur in any participant experiencing a DLT-defining toxicity and in the case of:

- Any Grade 4 or higher adverse drug reactions (ADRs) as defined by CTCAE v5.0 and assessed as possibly related to that 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 possibly attributed to that agent **except** for any of the following:
  - Grade 3 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.
  - Grade 3 Hgb decrease (< 8.0 g/dL) that is clinically manageable with blood transfusions or erythroid growth factor use.
  - Grade 3 laboratory values that are asymptomatic or resolve to Grade ≤ 1 or baseline grade within 7 days with medical management.
  - Keratoacanthoma and squamous cell carcinoma of the skin.
  - 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.

3.3.2 Dose Delay

Individual agents should be withheld for any Grade 2 or 3 ADR possibly attributed to that agent 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. Should a clinically relevant grade 2 or 3 ADR persist for more than 4 weeks, consideration should be given to discontinuing treatment with that individual agent at the discretion of the investigator.

For non-medical logistical reasons, unrelated acute illnesses, or palliative radiation, scheduled assessments and dosing can be delayed up to 2 months. Where at all possible, dosing should be restarted to keep in line with the original treatment schedule.

3.3.3 Study Accrual Halting Rules

Accrual will be halted if:

- Phase 1 and Phase 2:
  - There is an occurrence of any death (other than death related to progressive disease or accidental death) that occurs within 30 days of administration of study agents (PRGN-2009 and M7824) for both Phase 1 and Phase 2.
- Phase 2 only:
  - There is a delay in initiation of definitive standard of care therapy of 4 weeks or more beyond the planned start date due to treatment-related toxicities from HPV vaccine in two participants.

- There is occurrence of treatment-related ADRs from HPV vaccine of either Grade 4 (except for laboratory values that are asymptomatic or resolve to Grade ≤1 or baseline within 7 days) or of Grade 3 not resolving to Grade ≤1 or baseline within 7 days in two participants.

The IRB and the sponsor will be notified if one of the study accrual halting rules has been reached.

The accrual halt will be lifted once the data has been reviewed and there has been a reassessment of available safety information prior to reopening the study to accrual and allowing the study to proceed.

3.3.4 Dose Modification

During DLT period dose modifications are not allowed.

HPV vaccine: Vaccine doses will not be modified for an individual participant but doses may be skipped per investigator discretion. Once a dose is given to a participant that will always be the dose of vaccine they receive during this trial.

M7824: Dose reductions are not allowed but doses may be skipped per investigator discretion.

3.3.5 Toxicity Management

Table 2 Treatment Modification Guidance for Symptoms of Infusion-Related Reactions including Immediate Hypersensitivity

Infusion-Related Reactions (IRR) are an important risk for M7824.

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.

NCI-CTCAE Grade	Treatment Modification
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 for the next scheduled visit. • If not improving, consider administration of glucocorticoids and stop the infusion for that day. • 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.
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.

NCI-CTCAE Grade	Treatment Modification
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.	
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.	

Table 3 Immune-related adverse events (irAEs)

Immune-related AEs are specific to immunotherapies and vary by organ system.

Following immune-related AEs are important identified risks for M7824:

- Immune-related pneumonitis
- Immune-related hepatitis
- Immune-related colitis
- Immune-related nephritis and renal dysfunction
- Immune-related endocrinopathies
- (thyroid disorders, adrenal insufficiency, type 1 diabetes mellitus, pituitary disorders)
- Immune related rash
- Other immune-related events (myositis, myocarditis, encephalitis)

Following immune-related AEs are important potential risks for M7824:

- Guillain-Barré syndrome
- Uveitis
- Pancreatitis
- Myasthenia gravis/myasthenic syndrome

Recommended guidance and management for specific irAEs are provided in the current NCCN (guideline available at http://www.nccn.org).

Requirements in addition to NCCN guidelines:

- Permanent treatment discontinuation is required in case of immune-related Grade 4 rash/inflammatory dermatitis, nephritis, autoimmune hemolytic anemia, hemolytic uremic syndrome, aplastic anemia, immune thrombocytopenia, acquired thrombotic

thrombocytopenic purpura inflammatory arthritis, myositis and polymyalgia-like syndrome. • For Grade 4 immune-related lymphopenia, permanent treatment discontinuation will be required, if lymphopenia is considered immune-related in nature, no clear alternative explanation exists for the event, and it does not resolve within 14 days. Permanent treatment discontinuation is not required when the AE is manifested by a single laboratory value out of normal range without any clinical correlates. In this case, treatment should be held until the etiology is determined. If the event is not considered immune-related and resolves to Grade ≤ 1, restarting treatment may be considered. • For Grade 1 immune-related pneumonitis: continue treatment. If clinically indicated, monitor participants weekly or more frequently as needed with history, physical examination and pulse oximetry. If symptoms appear and/or changes in the physical exam are noted, treat as Grade 2. • For myositis: in case of management with rituximab, treatment should be discontinued. • For Grade 3 or 4 endocrinopathies: withhold until clinically stable or permanently discontinue depending on severity. • For hepatitis with no tumor involvement of the liver: withhold if total bilirubin increases to more than 1.5 and up to 3 times ULN, permanently discontinue if more than 3 times ULN. • Hepatitis with tumor involvement of the liver: permanently discontinue if total bilirubin increases to more than 3 times ULN.

Table 4 Management of M7824 mediated Skin Reactions

Skin reactions are considered important identified risk for M7824.
• Hyperkeratosis • Keratoacanthoma • Cutaneous squamous cell carcinoma (cSCC) • Basal cell carcinoma • Actinic keratosis
Management
• Discontinuation or termination not required in most cases. Continuation of treatment should be evaluated by the Investigator. • Emollients may be used • Develop diagnostic and treatment plan in collaboration with Investigator and dermatologist • Treatment follow-up will depend on number and localization of lesions.   o Single lesion: full excision may be recommended

○ Multiple lesion or location not suitable for full excision: Mohrs surgery, cryotherapy or other standard treatment options depending on pathology. Retinoids may be used after discussion with Investigator. • Close clinical follow-up for re-evaluation, resolution and potential recurrence should be implemented • In general, treatment of skin lesions should be based on local guidelines/standard of care.
Additional consideration: Keratoacanthoma lesions may resolve spontaneously without surgical intervention within weeks after discontinuing M7824. Consult with Medical Monitor as needed for management of skin lesions.

Table 5 Management of Anemia

• Anemia is considered an important identified risk for M7824. • All relevant hematological testing for treatment-related anemias should be done prior to a blood transfusion, if clinically feasible
Basic Anemia Evaluation
• CBC with emphasis on red cell indices • If indicated and at clinical discretion, the following should be considered: ○ Iron studies ○ Serum Folate and Vit B12 values ○ Coagulation factors ○ Fecal occult blood ○ Urinalysis ○ Hormone panel: TSH, Erythropoietin ○ Peripheral blood smear
Further Recommendation Based on Suspected Etiology (in Addition to Basic Anemia Testing)
• Suspected Hemolysis ○ bilirubin, LDH, Coombs test, haptoglobin • Suspected bleeding: ○ Consider imaging/interventional radiology consultation as indicated ○ Consider imaging and/or endoscopy as clinically indicated • Suspected aplastic anemia: ○ Hematology consultation ○ Consider bone marrow aspiration/morphologic evaluation

Additional consideration: In general, blood transfusions and erythroid growth factors are permitted as clinically indicated.

Table 6 Management of Bleeding Adverse Events

Bleeding Adverse Events	
• Bleeding adverse events are considered important identified risk for M7824. • In general, mild and moderate mucosal bleedings resolve without discontinuation of treatment. • These events may include, but are not limited to the following: o Epistaxis o Hemoptysis o Gingival bleeding o Hematuria	
Non-tumor Bleeding	
Grading	Management
Grade 2	• If resolves to Grade ≤ 1 by the day before the next infusion, study intervention may be continued • If not resolved to Grade ≤ 1 by the day before the next infusion, but is manageable and /or not clinically relevant, assessment if clinically reasonable to administer the following infusion will be per PI discretion.
Grade 3	• Permanently discontinue treatment unless an alternative explanation can be identified (such as concomitant use of antithrombotic agents, traumatic events, etc.) • In case of alternative explanations, hold study treatment until the event recovers to Grade ≤ 1
Grade 4	• Treatment must be permanently discontinued if no alternative explanation is identified.
Tumor Bleeding	
Grade ≥ 2	• Study treatment must be held till the event recovers to Grade ≤ 1

	• Permanently discontinue treatment if the Investigator considers the participant to be at risk for additional severe bleeding.

Table 7 Impaired Wound Healing

• Impaired wound healing is considered important potential risk for M7824 • Elective surgery on study will be allowed per PI discretion • It is recommended to hold study intervention for approximately 4 weeks post major surgery for observation. Post-operative wound healing should be closely monitored

3.4 STUDY CALENDAR

3.4.1 Study Calendar for Phase I

Procedure	**Screening[1]**	**Baseline / Day 1[2]**	**Odd numbered Weeks (W1, W3, W5, W7 onwards)[3]**	**EOT[4]**	**Safety follow up[5]**	**Long Term follow up[6]**
Treatment[3]		X	X			
NIH Advance Directives Form [7]		X				
Medical History [17]	X					
Height	X					
Physical exam, weight, vital signs, ECOG [16]	X	X	X	X	X	
Skin assessment [18]		X	X	X	X	
HIV, HCV, HepB	X					
EKG	X			X	X	
CBC with differential	X	X	X	X	X	
Biochemical profile [8]	X	X	X	X	X	
Tumor Markers [9]		X	X	X		
CD4 [10]	X					
TSH with reflex Free T3 and T4, lipase, amylase, CRP		X	X[11]			
Urinalysis	X					
Urine or serum pregnancy testing in women of childbearing potential	X	X	X			
PT, INR, PTT		X				
Tumor evaluation (CT Scan / MRI)	X	X	X[12]			X
Brain CT/MRI [13]	X					
Concomitant Medications		X	X	X	X	
Adverse events		X	X	X	X	
Biopsy for immune analysis [14]		X	X			
Research Blood [15]		X	X	X		
Telephone Follow Up					X	X

1. Screening evaluations performed within 28 days prior to the first drug administration, unless specified in Section **2.2.**

2. Baseline evaluations performed within 2 weeks prior to first drug administration.

Note: Baseline Labs will not be redrawn if done already as screening within the 2 weeks prior to drug administration.

3. Treatment:

- Trial treatment will be given with either HPV vaccine monotherapy (Arm 1A) or the combination of HPV vaccine + M7824 (Arm 1B) for a year.
- HPV vaccine will be given on D1, D15 and D29 followed by every 4 weeks thereafter and M7824 will be given every 2 weeks.
- Both of these agents may be discontinued early for unacceptable toxicity or disease progression. Treatment beyond a year may be given per PI discretion.
- Administration of study agents and indicated evaluations can be done up to 14 days earlier or delayed up to 14 days due to holidays, inclement weather, conflicts, or similar reasons.

The timing of subsequent administrations is adjusted to maintain a minimum of 11 days between treatments of similar agents.

4. EOT – End of treatment visit: Where feasible, on the day of or within 7 days of the decision to discontinue treatment prematurely before completion of one year of treatment.

5. Approximately 28 days (+/- 7 days) after last treatment. If subjects are not willing to come to NIH for FU visit, they will be contacted by phone to assess adverse events and for such participants no labs will be needed at FU.

6**.** Participants who have come off treatment for disease progression will be followed by phone annually for survival. Participants who have not progressed on treatment will continue to be followed and scanned per investigator discretion until progression. Initial and follow up courses of treatment may extend beyond a year per investigator discretion.

7. As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advanced directive form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

8. Biochemical profile: Acute care, hepatic and mineral panel as described in Sections **2.2** and **2.4**.

9. Evaluate CEA, CA19-9, CA125 and CA15-3 at baseline and follow elevated values thereafter (for participants enrolled on the phase I portion of the protocol).

10. In HIV positive participants.

11. Every 8 weeks for participants.

12. Imaging will be performed every 8 weeks. In the event of a PR or CR tumor imaging assessments may be performed every 3 months (+/- 2 weeks) at the discretion of the investigator. Tumor assessment should be continued beyond end of treatment in participants who have not experienced PD until they experience PD in order to assess PFS. MRI (with gadolinium) will be used when CT scan is not an option to follow the disease. Bone scans and other imaging assessments may also be performed as clinically indicated.

13. In participants with known CNS disease as described in Section **2.1.2.3;** MRIs may be performed as clinically indicated in this population.

14. Where feasible, optional biopsies may be done at baseline and at week 9 and will likely be CT guided. If a recent (≤ 6 months preferred) outside biopsy has been performed that specimen may be used in lieu of a baseline biopsy for both phase I and II participants.

15. Where feasible, research blood (see Section **5.1**) will be collected at the following time points:

- PKs and ADAs – performed only on Arm 1B (bintrafusp alfa plus vaccine)
    - Samples for PK analysis will be taken prior to infusion and following infusion on W1 and W5 and prior to infusion on W3 and W11 or with end of treatment labs if patients comes off treatment prior to any of those timepoints; and
    - Samples for ADA analysis will be taken prior to infusion on W1, W5, and W11 or with end of treatment labs if participants come off treatment prior to any of those timepoints.
- Samples for AVA analysis will be taken prior to infusion on W1, W3, W5, W11, and restaging visits or with end of treatment labs if participants come off treatment prior to any of those timepoints.
- All other blood samples
    - Baseline, Week 3, and Week 5; restaging visits per PI discretion.
- Note: Samples may be sent to Precigen, Inc for additional analyses (see Section **5.1.9**).

16. Vital signs at screening should include pulse oximetry on room air.

17. Medical history will include monitoring for signs of bleeding.

18. Skin assessment must be performed at baseline and at least every 6 weeks, starting on week 5 during treatment and at the end of treatment or at the 28 (±7 days) day safety follow-up (if not performed in the previous 6 weeks).

3.4.2 Study Calendar for Phase II

Procedure	**Screening[1]**	**Baseline[2]**	**Week 1**	**Week 3**	**Week 4-5[18]**	**EOT[4]**	**Safety follow up[5]**	**Long Term follow up[6]**
Treatment [3]		X	X	X				
NIH Advance Directives Form [7]		X						
Medical History [14]	X							
Height	X							
Physical exam, weight, vital signs, ECOG [13]	X	X	X	X	X	X	X	
Skin assessment [16]		X	X	X	X	X	X	
HIV, HCV, HepB	X							
EKG	X					X	X	
CBC with differential	X	X	X	X	X	X	X	

Procedure	Screening[1]	Baseline[2]	Week 1	Week 3	Week 4-5[18]	EOT[4]	Safety follow up[5]	Long Term follow up[6]
Biochemical profile [8]	X	X	X	X	X	X	X	
CD4 [9]	X							
TSH with reflex Free T3 and T4, lipase, amylase, CRP		X	X	X	X			
Urinalysis	X							
Urine or serum pregnancy testing in women of childbearing potential	X	X	X	X				
HLA typing [17]		X						
PT, INR, PTT		X						
Tumor evaluation (CT Scan / MRI)[10]	X	X			X			
Concomitant Medications		X	X	X	X	X	X	
Adverse events		X	X	X	X	X	X	
Biopsy for immune analysis [11]		X			X			
Research Blood [12]		X	X	X	X	X		
Saliva sample [15]		X	X	X	X	X	X	
Taken off treatment for definitive treatment [19]					X			
Telephone/email Follow Up							X	X

1. Screening evaluations performed within 28 days prior to the first drug administration, unless specified in Section **2.2.**

2. Baseline evaluations performed within 2 weeks prior to first drug administration.

Note: Baseline Labs will not be redrawn if done already as screening within the 2 weeks prior to drug administration.

Note: Imaging within 4 weeks prior to drug administration.

3. Therapeutic agents administered will be as outlined in Section **3.1**:

- Participants will be planned to receive two doses of treatment (HPV vaccine alone in Arm 2A) before definitive standard of care therapy.
- Administration of study agents and indicated evaluations can be done up to 3 days earlier or delayed up to 3 days due to holidays, inclement weather, conflicts, or similar reasons.

The timing of subsequent administrations is adjusted to maintain a minimum of 11 days between treatments of similar agents.

4. EOT – End of treatment visit: Where feasible, on the day of or within 7 days of the decision to discontinue treatment prematurely before completion of one year of treatment prematurely or one month of neoadjuvant or induction treatment. Note: Does not need to be completed if drug is withheld after these timepoints and such participants will be considered off-treatment.

5. Approximately 28 days (+/- 7 days) after last treatment. If subjects are not willing to come to NIH for FU visit, they will be contacted by phone to assess adverse events and for such participants no labs will be needed at FU.

6**.** Participants who have completed neoadjuvant or induction immunotherapy on the phase II portion of the protocol will be followed by phone or email for recurrence, adverse events and survival starting 2 months (+/- 2 weeks) after safety visit and then annually (+/- 1 month) until the patient is 5 years out from surgery or completion of chemoradiotherapy.

7. As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advanced directive form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended but is not required.

8. Biochemical profile: Acute care, hepatic and mineral panel as described in Sections **2.2** and **2.4**.

9. In HIV positive participants.

10. Imaging (CT of neck and chest/ MRI, if indicated, for oropharyngeal cancer participants; CT of skull, neck and chest/ MRI, if indicated, for sinonasal SCC; no CT chest required if PET/CT available) at baseline/screening, and at Week 4-5 (CT of neck/ MRI, if indicated, for oropharyngeal cancer participants; CT of skull, neck/ MRI, if indicated, for sinonasal SCC). MRI (with gadolinium) will be used when CT scan is not an option to follow the disease. Bone scans and other imaging assessments may also be performed as clinically indicated. Post-treatment imaging is to be strongly pursued but is not required and may be waived at the discretion of the PI.

11. Where feasible, biopsies will be done at baseline as well as at week 4-5. Biopsies will be done by ENT under direct visualization. Where feasible at least 2 cores will be obtained. If a recent (≤ 6 months preferred) outside biopsy has been performed that specimen may be used in lieu of a baseline biopsy. For patients that have surgery as definitive treatment, Week 4-5 biopsy may be omitted: in this case tumor biospecimens will be obtained from the institution where the patient underwent surgery after the patient signs that institution's release document.

12. Where feasible, research blood (see Section **5.1**) will be collected at baseline, week 3 and week 45 with the following exceptions:

- Samples for AVA analysis will be taken prior to infusion on W1, W3, with research labs on 4-5 or with end of treatment labs if participants come off treatment prior to any of those timepoints.
- Note: Samples may be sent to Precigen, Inc for additional analyses (see Section **5.1.9**).

13. Vital signs at screening should include pulse oximetry on room air.

14. Medical history will include monitoring for signs of bleeding.

15. Only for p16+ oropharyngeal cancer patients: Collection of saliva by mouth rinse and gargle with 15-20 mL 0.9% NaCl for 30 seconds and spitting into the collection tube. Where possible, saliva samples will be analyzed for HPV DNA detection and quantification.

16. Skin assessment must be performed at baseline and at least every 6 weeks starting on week 5 during treatment and at the end of treatment or at the 28 (±7 days) day safety follow-up (if not performed in the previous 6 weeks).

17. HLA A, B, C and DR, DQ and NGS A, B, C and DR, DQ, at any time prior to treatment start. Patients who have had HLA typing previously performed at NIH do not require retesting.

18. Preferably scheduled for Week 5, with Week 4 an option to not interfere with activities required for arrangements for definitive treatment. An earlier timepoint may also be used to avoid interfering with definitive treatment, but should be at least a day after the second vaccination.

19. Upon completion of induction or neoadjuvant immunotherapy participants on the phase II portion of the protocol will be taken off treatment and referred back to their home institutions for definitive standard of care therapy and follow up will be performed per superscript # 6 above.

3.5 COST AND COMPENSATION

3.5.1 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 if they are not covered by an insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.5.2 Compensation

Subjects will not receive compensation for participation in this study.

3.5.3 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.

3.6 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.

3.6.1 Criteria for Removal from Protocol Therapy

- Completion of protocol therapy.
- Clinical or radiographic progression of disease except when the investigator feels the subject is still benefiting from treatment. (It is generally preferable for participants to remain on treatment past initial radiographic progression in case there is pseudo - progression, except when the investigator feels that the clinical picture warrants changing therapy at initial progression).
- Unacceptable toxicity possibly attributed to all active therapies (see Section **3.3**).
- Participant requests to be withdrawn from active therapy.
- Start of another systemic anticancer treatment or participation in another investigational therapeutic trial (except for standard of care therapy for phase II participants). For participants enrolled in the phase I portion of the protocol, focal palliative radiotherapy, ablation, or surgery to a site of disease will not necessitate removal from protocol therapy.
- Investigator discretion.
- Positive pregnancy test.
- Intercurrent illness that prevents further administration of treatment, in the judgement of the investigator.

3.6.2 Off-Study Criteria

- Failure to maintain eligibility criteria prior to starting treatment.
- Screen failure.
- PI decision to end the study.
- Participant requests to be withdrawn from study.
- Participant lost to follow-up.
- Investigator discretion.
- Death.
- Completion of 5-year follow up period. (Only for Phase II)

3.6.3 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 subject welfare or alleviate symptoms and will not interfere with the trial medication may be given at the Investigator's discretion.

Palliative radiotherapy delivered in a normal organ-sparing technique may be administered during the trial. The assessment of PD will not be based on the necessity for palliative radiotherapy.

4.2 PROHIBITED MEDICATIONS

The following treatments should not be administered during the trial:

- Other immunotherapies or immunosuppressive drugs (for example, chemotherapy or systemic corticosteroids except for prophylaxis or treatment of allergic reactions, endocrine replacement therapy at low dose 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). Steroids with no or minimal systemic effect (topical, inhalation) are allowed.

- Prophylactic use of corticosteroids for infusion related reactions. Corticosteroid administration prior to CT scans in participants with intravenous contrast allergy is allowed.
- 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).
- Systemic anticancer treatment (except standard of care therapy for phase II participants).

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Test/assay	Volume (approx.) per Timepoint	Type of tube [a]	Collection point	Location of specimen analysis
Standard and 123 immune cell subsets by FACS	60-80 mL blood for PBMCs	Sodium heparin (green top) tubes	See **Study Calendar**	LTIB [Storage at Clinical Services Program (CSP)]
Functional Analysis of immune cell subsets by FACS				LTIB (Storage at CSP)
Antigen Specific Immune Response by cytokine staining assay				LTIB (Storage at CSP)
T cell clonality by immuno-seq platform				LTIB and NCI Frederick Genomic Core Facility (Storage at CSP)
RNA expression level of 770 genes				LTIB and NCI Frederick Genomic Core Facility
Soluble Factors (to include sCD27 and sCD40 ligand) by ELISA	8 mL blood for serum	SST		LTIB (Storage at CSP)
M7824 Pharmacokinetics	4 mL blood for serum	SST		EMD Serono (Storage at BPC)
ADA by ELISA	4 mL blood for serum	SST		EMD Serono (Storage at BPC)
AVA by ELISA	1 mL blood for serum	SST		Precigen (Storage at BPC)
Immune Markers by IHC	Tumor samples	N/A		GMB TIME LAB (Storage at LP, CSP)
Single-cell Proteomic analysis	Tumor samples	N/A		Precigen (Storage at LP, CSP)

Test/assay	Volume (approx.) per Timepoint	Type of tube [a]	Collection point	Location of specimen analysis
Immune transcriptomic analysis	Tumor samples	N/A		Precigen (Storage at LP, CSP)
RNA expression level of 770 genes	Tumor samples	N/A		LTIB and NCI Frederick Genomic Core Facility (Storage at LP, CSP)
HPV status by PCR of DNA	Tumor samples	N/A		Dr. Norberg's Team (Storage at LP, CSP)
Circulating free tumor DNA (cftDNA) by PCR system	Plasma	EDTA (lavender top)		LTIB and NCI Frederick Genomic Core Facility (Storage at CSP)
T cell clonality by immunoseq platform	Tumor samples	N/A		LTIB and NCI Frederick Genomic Core Facility (Storage at LP, CSP)
Salivary HPV DNA	15-20 mL 0.9% NaCl rinse	50 mL conical tube		LTIB and NCI Frederick Genomic Core Facility (Storage at CSP)
[a] Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.				

Research blood samples will be sent either to the Clinical Services Program – Leidos Biomedical Research, Inc. (CSP) (Section **5.2.1**) or the Blood Processing Core (BPC) (Section **5.2.2**) for barcoding, initial processing and storage. Tissue will be sent to the Laboratory of Pathology as described in Section **5.2.3**. Additional tissue for research may also be stored in CSP (Section **5.2.1**). From these facilities, coded, linked samples will be sent to the designated labs for analysis upon request.

5.1.1 Immune Phenotyping

Exploratory immunologic studies will be conducted to evaluate the study drug's effect on the immune response before and after treatment, to gain insight into potential biomarkers, and help improve the administered therapy. Blood will be collected as per the **Study Calendar**. The following immune assays may be performed at the Laboratory of Tumor Immunology and Biology (LTIB) at the NCI's Center for Cancer Research (CCR) in select participants where adequate samples are available:

1. PBMCs may be analyzed for changes in 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 123 immune cell subsets, using multi-color flow cytometry.

2. PBMCs from selected subjects 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.

5.1.2 Soluble Factors

Samples for soluble factor analysis will be collected as per the **Study Calendar**.

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).

5.1.3 AVA

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

5.1.4 M7824 Pharmacokinetics

PK measurements of M7824 will be taken in Arm 1B only to collect data which will provide insight into population PKs of M7824 in participants receiving these novel combinations.

The schedule is in the **Study Calendar**. Serum M7824 PK measurements will be done at EMD Serono.

5.1.5 ADA

Anti-Drug Antibody (ADA) development is an accepted mechanism of loss of efficacy of administered human monoclonal antibodies. Measuring titers will ensure that lack of efficacy of M7824 is not due to ADA development.

The schedule is in the Study Calendar – samples will be collected in Arm 1B only. The investigation will be done by EMD Serono using ELISA.

5.1.6 Tumor Tissue Analyses for Immune Markers

Analyses may be performed in pre-treatment vs. post-treatment (at Week 4-5 for phase II participants) tumor tissue. Where available, archival tumor samples may be requested for pre-treatment analysis (preferably tissue samples from the last 6 months). For participants with lesions amenable to biopsy, two biopsies may be performed at baseline and at Week 4-5 (phase II participants) or Week 9 (phase I participants). Tumor samples will be sent to the Laboratory of Pathology for disease evaluation; remaining samples will be used for research. Tissue samples for research may also be stored in CSP as described in Section **5.2.1**.

Where available, study of immune infiltration as well as PD-L1 status within the tumor microenvironment pre vs. post treatment by IHC and/or multiplex immunofluorescence may be performed by the GMB TIME Laboratory.

5.1.7 Single-cell proteomic analysis

Where available, tumor tissue single-cell proteomic analysis of immune and signaling pathways may be performed with the Isoplexis Single Cell Functional proteomic platform by Precigen.

5.1.8 Immune transcriptomic analysis

Where available, tumor tissue immune transcriptomic analysis may be performed with the Nanostring platform by Precigen.

5.1.9 Additional Biomarker Studies

De-identified, coded blood (PBMC, plasma, and/or serum) or tissue samples may be sent to Precigen Inc., the manufacturer of PRGN-2009, 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, 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 Precigen 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.

5.1.10 Assessment of HPV status

In participants with available tumor tissue (either archival or by optional biopsy), HPV testing will be performed using the Roche Cobas or Becton Dickinson HPV PCR based DNA assay, if no prior HPV testing of the tumor has been performed. This will be done through collaboration with Dr. Norberg's Team.

5.1.11 Circulating tumor DNA

If sufficient plasma is available, select participant samples may be analyzed for circulating tumor DNA (LTIB and NCI Frederick Genomic Core Facility). Plasma DNA will be isolated with an automated purification system. The circulating tumor/HPV DNA will be quantified with a digital droplet PCR system from Bio-Rad to obtain precise quantification. This is just quantification by PCR. There is no DNA sequencing.

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

Where possible, RNA expression and T-cell receptor clonality analysis will be done on the peripheral blood as well as archived tumor tissue or optional biopsies 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 Nanostring platform and T-cell receptor clonality using the ImmunoSeq platform (LTIB and NCI Frederick Genomic Core Facility).

NCI Fredrick Genomic Core Facility:
Leidos Biomedical Research, Inc:
ATRF, Rm C3016
8560 Progress Drive
Frederick, MD 21701
Ph. 301-846-7677

5.1.13 Salivary HPV DNA

Participants in Phase 2 Cohort 2 with p16-positive oropharyngeal cancer will have collection of saliva by mouth rinse and gargle with 15-20 mL 0.9% NaCl for 30 seconds and spitting into the collection tube (timepoints per **Study Calendar**). Where possible, saliva samples will be analyzed for HPV DNA detection and quantification (LTIB and NCI Frederick Genomic Core Facility). Salivary HPV DNA will be quantified with digital droplet PCR; no sequencing is involved.

5.2 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.

5.2.1 Sample Management and Storage at Clinical Services Program – Leidos Biomedical Research, Inc. (CSP)

Clinical Services Program - Leidos Biomedical Research, Inc.
Attn: Theresa Burks
1050 Boyles Street
Bldg. 496/Room 121
Frederick, MD 21702

On days samples are drawn, Jen Bangh at CSP (part of NCI Frederick Central Repositories) should be notified (phone: [301] 846-5893; fax: [301] 846-6222). She 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 training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is 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 NCI Frederick Central Repository in Frederick, Maryland.

NCI Frederick 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. 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.

It is the intent and purpose of the subcontractor to accept only coded, linked 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.

5.2.2 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.2.2.1 BPC contact information

Please email NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.2.2.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to participant information are required to complete the Human Subjects Research course.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Participant demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.2.3 Sample Storage

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80 C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.2.3 Procedures for Storage of Tissue Specimens in the 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.

5.2.4 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 withdraws consent the participant's 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.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR 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. For participants enrolled on the phase II portion of the protocol adverse events which occur while participant is being treated with standard chemoradiation and which are not attributed to immunotherapy need not be captured.

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.

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

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center).
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through*:*

- An NIH-funded or approved public repository: clinicaltrials.gov
- BTRIS (automatic for activities in the Clinical Center).
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

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

6.3.1 Antitumor Response

Tumor assessments may include the following evaluations: physical examination (with photograph and measurement of skin lesions, as applicable); cross-sectional imaging using computed tomography (CT) or magnetic resonance imaging (MRI) scan of the chest, abdomen, and pelvis (pelvis scan is optional unless known pelvic disease is present at baseline); nuclear bone scan for subjects with known/suspected bone lesions; and CT or MRI scan of the brain (only as clinically warranted based on symptoms/findings). The preferred method of disease assessment is CT with contrast. If CT with contrast is contraindicated, CT of the chest without contrast and MRI scan of the abdomen/pelvis is preferred.

At baseline, tumor lesions will be selected and categorized as target or non-target lesions. Target lesions include those lesions that can be accurately measured in at least 1 dimension as ≥ 20 mm with conventional techniques or ≥ 10 mm with CT scan. Malignant lymph nodes with a short axis diameter ≥ 15 mm can be considered target lesions. Up to a maximum of 2 target lesions per organ and 5 target lesions in total will be identified at baseline. These lesions should be representative of

all involved organs and selected based on their size (those with the longest diameter) and their suitability for accurate repeated measurements. A sum of the longest lesion diameter (LLD) for all target lesions will be calculated and reported as the baseline sum LLD. For malignant lymph nodes identified as target lesions, the short axis diameter will be used in the sum of LLD calculation. All other lesions (or sites of disease) should be identified as non-target lesions (including bone lesions).

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 at baseline should be used throughout the course of the study unless subject safety necessitates a change (e.g., allergic reaction to contrast media).

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

6.3.2 Disease Parameters

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

- 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/pulmonitis, 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.

6.3.3 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.

6.3.4 Response Criteria

All the scans performed at Baseline and other imaging performed as clinically required (other supportive imaging) need to be repeated at subsequent visits. In general, lesions detected at Baseline need to be followed using the same imaging methodology and preferably the same imaging equipment at subsequent tumor evaluation visits.

Brain CT / MRI scan should be performed, if clinically indicated by development of new specific symptoms or on the discretion of the Principal Investigator. 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 sequential 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**.

The foreseen treatment duration is until disease progression verified by a scan subsequent to the initial documentation of PD, unacceptable toxicity, or any criterion for withdrawal from the trial occurs (see Section **6.3**). Before stopping the treatment, progressive disease should be confirmed by imaging 4 to 6 weeks (preferably 6 weeks, but not later) after progression has been diagnosed according to RECIST 1.1 (51). If progression is based on the occurrence of a new lesion in an area not scanned at Baseline, a further on-study scan 6 weeks later should be considered before performing the 28-Day Safety Follow-up visit. Treatment may be continued despite progression according to RECIST 1.1 at any time if:

- There are no new or concerning symptoms.
- There is no decrease in ECOG PS.
- The Investigator does not consider it necessary to administer a salvage therapy.

The treatment should be stopped immediately, if the subject does not tolerate M7824 or if therapeutic failure occurs, which requires urgent treatment with an additional drug or results in clinically significant progression / deterioration.

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.

Any CR or PR should be confirmed according to RECIST 1.1. In the case of a PR or CR, a confirmatory CT or MRI scan should be done no sooner than 4 weeks (preferably at the scheduled 6-week interval).

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

As an exploratory endpoint antitumor activity will also be evaluated according to iRECIST (52).

Using iRECIST criteria the following will be incorporated into assessment:

1. An increase in the sum of target lesions of more than 20%, unequivocal increase in non-target lesions or new lesions result in iUPD (unconfirmed progressive disease); iUPD can be assigned multiple times as long as iCPD (confirmed progressive disease) is not confirmed at the next assessment.

2. Progression is confirmed in the target lesion category if the next imaging assessment after iUPD (4–8 weeks later) confirms a further increase in sum of measures of target disease from iUPD, with an increase of at least 5 mm. Progression is confirmed in the non-target lesion category if subsequent imaging, done 4–8 weeks after iUPD, shows a further unequivocal increase in non-target lesions. Progression is confirmed in the new lesions category if at next assessment additional new lesions appear or an increase in size of previously seen new lesions is seen (≥5 mm for sum of new lesion target).

3. However, the criteria for iCPD (after iUPD) are not considered to have been met if complete response, partial response, or stable disease criteria (compared with baseline and as defined by RECIST 1.1) are met at the next assessment after iUPD. The status is reset (unlike RECIST 1.1, in which any progression precludes later complete response, partial response, or stable disease). iCR, iPR, or iSD should then be assigned; and if no change is detected, then the timepoint response is iUPD.

6.3.5 Response Criteria by RECIST 1.1

6.3.5.1 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.

6.3.5.2 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).

6.3.5.3 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.			

6.3.5.4 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.

6.3.5.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.3.6 Immune Response Evaluation

6.3.6.1 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 (Opal™ method). This method explained below uses tissue sparingly and ensures simultaneous staining of different markers in the same tissue plain. 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 (GMB TIME Laboratory), for presence of tumor and tissue integrity.

6.3.6.2 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 Leica Biosystems' BOND RX. First, sections will be subjected to deparaffinization and antigen retrieval (HIER). 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 (GMB TIME Laboratory) 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.

The Opal method is based on PerkinElmer's TSA® (Tyramide Signal Amplification) Plus reagents and offers many advantages compared to conventional indirect immunofluorescence labeling, including brighter signals for faster scanning, balanced signals for higher level multiplexing, signals that do not photobleach, and freedom from species-based interference. The Opal method is explained in more details in the attachment (Opal_Assay_Development_Guide_Aug2017).

6.3.6.3 Digital Imaging Protocol: Once all antigens of interest are stained, 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) by research pathologist (GMB TIME Laboratory). The scans generated at high resolution are called multispectral images (MSIs) and are available in .im3 format. These can only be analyzed using inform software (available in GMB TIME Laboratory). The software allows for spectral unmixing based on a spectral library (built based on single reference stains) and artificial intelligence based unmixing algorithm. 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.

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 NCICCRQA@mail.nih.gov 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 the 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 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 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 existing hospitalization;
    - A hospitalization/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, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for participant convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. 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.

8.1.6 Adverse Events of Special Interest (AESI)

Adverse events of special interest (AESIs) are serious or nonserious AEs that are of clinical interest and should be closely followed.

AESIs include following:

- Infusion-related reactions including immediate hypersensitivity.
- Immune-related adverse events.
- TGFβ inhibition mediated skin reactions.
- Anemia.
- Bleeding AEs.

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 administration must be reported to the sponsor with the exception of any listed in Section **8.4**.

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.**

Hospitalizations related to adverse events attributed to disease progression will not need to be reported expeditiously as these events will be captured as a separate study endpoint.

All SAE reporting must include the elements described in Section **8.1.6**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov 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.

In addition, the Sponsor is responsible for reporting adverse events to the FDA in compliance with 21 CFR 312.32. The Sponsor must report all serious, unexpected and related or possibly related adverse events within 15 calendar days after the study team receives knowledge of the event, and all life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent within 7 calendar days after the study team receives knowledge of the event.

8.4 Waiver of Expedited Reporting to CCR

As death/hospitalization due to disease progression is/are part of the study objectives, and captured as an endpoint in this study, they 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**

As M7824 is known to result in immune related toxicities as well as an increased risk of mucosal bleeding as documented in the informed consent and protocol and shared with the FDA, only those SAEs that are unexpected will be reported to the Sponsor in an expedited manner.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of participants treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at OSROSafety@mail.nih.gov.

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: OSROSafety@mail.nih.gov 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

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 two months after the last dose of M7824.

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 2 months after the last dose 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. 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.

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
- that 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 before the study starts is 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 take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO 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 STATISTICAL HYPOTHESIS

10.1.1 Primary Endpoints

10.1.1.1 Phase I:

The primary endpoint of the phase I portion of the study is to determine the recommended phase II dose (RP2D) of PRGN-2009 (HPV vaccine) alone and in combination with M7824.

10.1.1.2 Phase II:

The primary endpoint of the phase II portion of the study is to determine if HPV vaccine alone (Arm 2A) is able to result in a ≥ 2-fold increase in CD3+ tumor infiltrating T cells post treatment compared with pre-treatment in participants with p16+ oropharyngeal cancer.

10.1.2 Secondary Endpoints

10.1.2.1 Phase I:

The secondary endpoint of the phase I portion of the study are to determine overall response rate (ORR), progression-free survival time (PFS), overall survival (OS), ratio of participants that are hospitalized because of adverse events attributed to disease progression, and duration of response of the combination therapy in participants with advanced disease.

10.1.2.2 Phase II:

The secondary endpoints of the phase II portion of the study are:

- To determine if the use of HPV vaccine alone results in potentially prolonged survival as compared to the expected 80% three-year historical survival for this population.
- To determine the 3-year overall and relapse-free survival rate for HPV vaccine alone (Arm 2A) in combination with standard of care chemoradiation in participants with p16+ oropharyngeal cancer.

10.1.3 Exploratory Endpoints

The exploratory endpoints of the Phase II portion of the study are:

- To determine if the use of HPV vaccine alone results in potentially prolonged survival as compared to the expected 80% three-year historical survival for this population.
- To determine the 3-year overall and relapse-free survival rate for HPV vaccine alone (Arm 2A) in combination with standard of care chemoradiation in HPV-SNSCC participants.

10.2 SAMPLE SIZE DETERMINATION

The phase I portion of the trial will be conducted using 3+3 dose escalation design evaluating the safety of PRGN-2009 at two dose levels as monotherapy and at the RP2D of PRGN-2009 in combination with a fixed dose of M7824. Using this design up to 22 participants may be enrolled on the phase I portion of the study.

In arm 2A, phase II, 20 participants with p16+ oropharyngeal cancer 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 in arm 2A, 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.

Expecting that up to 22 evaluable participants will enroll on the phase I portion of the study, that 20 evaluable p16+ oropharyngeal cancer participants will enroll on phase II 2A, and that up to 2 evaluable HPV-SNSCC participants may enroll on phase II 2A, a total of up to 44 evaluable participants will be planned to enroll on this protocol. To allow for a few inevaluable participants the accrual ceiling for this protocol will be set at 50 participants.

10.3 POPULATIONS FOR ANALYSES

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

For phase II, only HPV-OPC participants will be evaluable for primary and secondary objective endpoints. HPV-SNSCC participants will be evaluable only for exploratory objectives.

For phase II, participants who receive at least two doses of therapy and have an adequate pretreatment and posttreatment biopsy will be evaluable for immune infiltration assessments. For phase II, participants who receive at least two doses of immunotherapy and go on to receive standard definitive therapy will be evaluable for relapse free survival and overall survival assessments.

For phase I and II participants, only those participants who have measurable disease present at baseline and have had their disease re-evaluated will be considered evaluable for response. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

The phase I portion of the study will be conducted as a safety assessment using a 3+3 dose escalation design. In addition, Arm 1B will be expanded to a total of 10 evaluable participants to gauge the preliminary efficacy of the combination of PRGN-2009 and M7824 in participants with advanced disease. The phase II portion of the study will be conducted to evaluate for a potentially significant increase in CD3+ tumor infiltrating lymphocytes with immunotherapy induction.

10.4.2 Analysis of the Primary Endpoints

Regarding the phase I portion of the study, participants will be evaluated with respect to the grades and types of toxicities obtained. The results will be presented descriptively and tabled if appropriate. Regarding the phase II portion of the study the rate of doubling of baseline CD3+ tumor infiltrating lymphocytes will be provided as well as the 95% confidence interval.

10.4.3 Analysis of the Secondary Endpoints

For the phase I portion of the study data will be obtained on ORR, duration of response, PFS, OS, and ratio of participants that are hospitalized because of adverse events attributed to disease progression.

10.4.4 Duration of 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 PD is objectively documented and is evaluated using the Kaplan-Meier method.

10.4.4.1 Progression-Free Survival and Relapse-Free survival

PFS and RFS will be evaluated using Kaplan-Meier methods. PFS will be defined as the time from the date of first treatment to the date of disease progression or death (any cause) whichever occurs first. 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.

10.4.4.2 Objective Response

Objective response is a complete or partial radiographic response as defined by RECIST 1.1 (Section **6.3.5**).

10.4.4.3 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.

10.4.5 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 within dose cohorts and 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.

10.4.6 Baseline Descriptive Statistics

Baseline Characteristics will be described.

10.4.7 Planned Interim Analyses

Interim assessment of safety will be made based on DLT assessments according to the 3+3 dose escalation design in the phase I portion of the study and the first six participants enrolled on the phase II portion of the study.

10.4.8 Tabulation of Individual Participant Data

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

10.4.9 Exploratory Analyses (Immune Responses)

Where feasible, exploratory immune analyses will be conducted to evaluate anti-tumor immune responses. Immune response will be assessed among all subjects treated in each cohort. The

magnitude of immune responses will be described. A subject will be considered evaluable for immune response if they receive at least one dose of treatment. The percentage of subjects with a positive immune response will be evaluated by cohort. For flow cytometry analyses on PBMC samples, Student T tests or Kruskal-Wallis and Wilcoxon rank sum tests as appropriate will be performed on percentages of TNF-α and/or IFN-γ expressing cells among the different cohorts to determine any significant differences in cell populations. For antigen specific T cell responses, a positive immune response is defined by CMI reactivity in *ex vivo* stimulation using a flow cytometric readout (cytokine production or CD107 expression). Antigen-specific peptide challenge assays require a readout of >250 reactive T-cells/million cells above the background (53).

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

A CRADA (02666) is in place with EMD Serono for the supply of M7824.

A CRADA (03209) is in place with Precigen for the supply of PRGN-2009.

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.

Patients who do not accept blood transfusions will be excluded. As there is a risk of severe bleeding with this study drug, participants must be willing to receive blood transfusions if medically necessary for their own safety. Participants must be able to receive blood transfusions in order to minimize the risks of receiving M7824.

In addition, these patients could compromise the scientific validity of the study. For example, death from blood loss could make it difficult to assess other aspects of the investigational immunotherapy's safety—a primary scientific goal in this early-phase immunotherapy trial, which are carried out in small numbers of participants.

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 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.4.2**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR as needed.

Please see Section **12.5.1** for consent procedure.

12.4 RISK/BENEFIT ASSESSMENT

12.4.1 Known Potential Risks

Some of the procedures performed on this study are not known to be associated with risk. These include urine tests and EKGs. Below are a list of procedures and study interventions that are associated with risk.

12.4.1.1 Risks Associated with Study Drugs

Potential adverse reactions attributable to the administration of the study drug utilized in this trial are discussed in Section **14**. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. Participants will be examined and evaluated prior to enrollment. All evaluations to monitor the treatment of participants will be recorded in the medical record. If participants suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Institutes of Health, Bethesda, Maryland.

Participants may be harmed from being in this study by toxicity due to the drug or combination of drugs given during this study. M7824 is similar to immune check point inhibitors. There are preliminary data to suggest that not all patients benefit from immune check point inhibitors nor M7824. Additionally, there are preliminary data to suggest that an unexpectedly rapid progression of disease occurs in some patients receiving immunotherapy such as immune checkpoint inhibitors.

12.4.1.2 Risk of Biopsies

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

12.4.1.3 Risks of Exposure to Ionizing Radiation

12.4.1.3.1 Phase I

This research study involves the possibility of 8 CT CAP scans, 2 brain CT scan, and 2 CT guided biopsies collected for research purposes for Phase I participants.

The amount of radiation exposure is equal to approximately 10.6 rem. This level of exposure results in an increased risk of cancer.

12.4.1.3.2 Phase II

This research study involves the possibility of 3 CT neck and chest scans for research purposes for Phase II participants with oropharyngeal cancer and 3 CT neck, chest, and skull scans for research purposes for Phase II participants with SNSCC.

The amount of radiation exposure is equal to approximately 0.99 rem and 1.29 rem for Phase II participants with oropharyngeal cancer and SNSCC, respectively. This level of exposure could result in an increased risk of cancer.

12.4.1.4 CT Scan Risk

CT scans create low levels of radiation, which has a small potential to cause cancer and other defects. However, the risk associated with scan is small.

12.4.1.5 Risks Due to Contrast Agents for CT

Contrast agents can cause allergic reactions and kidney damage. Allergic reactions can include mild itching associated with hives but can also result in a serious life-threatening emergency from difficulty breathing. If this occurs, it is treatable.

12.4.1.6 Risk of MRI

People are at risk for injury from the MRI magnet if they have some kinds of metal in their body. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss.

There are no known long-term risks of MRI scans.

12.4.1.7 Risk of Gadolinium Enhanced MRI

The gadolinium infusion may cause mild symptoms such as coldness in the arm during the injection, a metallic taste, headache, and nausea. There are risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling.

Procedure-related risks from MRI and gadolinium enhanced MRI will be explained fully during informed consent.

12.4.1.8 Risks of Clinical Endoscopy and Nasopharyngo-laryngoscopy

In this procedure, a physician gently places a small, thin, flexible endoscope with a camera through participant's nose to view their upper airway anatomy above the vocal cords. This procedure takes only a few minutes, is painless and does not require any sedation. It is a normal part of an ENT exam. Risks of clinic endoscopy include epistaxis or vasovagal syncope, each of which occur in <1% of all participants.

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. Risks of operative biopsies include post-op bleeding (<1% of all participants), dental damage from laryngoscopy instrumentation (<1% of all participants) and temporary tongue paresthesia/dysgeusia (<10% of all participants) that resolves within a few days.

12.4.1.9 Risk from 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.

12.4.1.10 Research Blood Collection Risks

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.

12.4.1.11 Risks of 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.

12.4.1.12 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document and this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4.2 Known Potential Benefits

The potential benefit to a participant that goes onto study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival.

12.4.3 Assessment of Potential Risks and Benefits

Locally advanced, metastatic or refractory/recurrent HPV associated malignancies (cervical, anal, oropharyngeal cancers, etc.) need improved therapy options. Preclinical studies suggest that the use of a combination of multiple immunotherapy agents may improve anti-tumor efficacy. Therefore, PRGN-2009 alone or in combination with M7824 may improve clinical outcome of participants with HPV associated malignancies. 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 alone or in combination with M7824 for this group of participants outweigh the risks associated with the proposed entry into this protocol with PRGN-2009 alone or in combination with M7824.

12.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) 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 NIH 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 subject 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.

As there is an optional biopsy for research in this protocol, the participant/consent designee will be asked to sign a separate consent at the time of the procedure. If the participant/consent designee refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.5**.

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, funding agency, 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, 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 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 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 sites 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)

14.1.1 Source / Acquisition and Accountability

PRGN-2009 will be provided by Precigen through a CRADA with NCI to Pharmacy. All study interventions will be given at 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 has not been tested in humans. 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 provides 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.

PRGN-2009 is a non-replicating, non-integrating adenoviral vector. Since the parental adenovirus is classified as biosafety level 2 (BSL2), PRGN-2009 should be handled as BSL2 material for disposal of all materials that were used in the dose preparation. Avoid generation of aerosols. Spills of PRGN-2009 should be decontaminated using a solution of 10% bleach.

This is a first in human study with PRGN-2009. Possible side effects may include injection side reaction, fever, flu like symptoms (e.g., fatigue, headache, muscle aches), poor appetite, increase in blood sugar, and skin rash.

Table 8: Summary of Treatment-Emergent Adverse Events in Phase 1/2 Study in Patients with HPV+ Malignancies (NCT04432597) (Reference: Table 7 in PRGN-2009 IB Version 3)

	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
All TEAEs	79	3/3 (100%)	20	3/3 (100%)	132	11/11 (100%)	88	22/22 (100%)
Grade 1	46	3/3 (100%)	11	3/3 (100%)	90	10/11 (90.9%)	60	20/22 (90.9%)
Grade 2	22	3/3 (100%)	9	3/3 (100%)	35	8/11 (72.7%)	26	15/22 (68.2%)
Grade 3	11	2/3 (66.7%)	0	0	4	3/11 (27.3%)	2	2/22 (9.1%)
Grade 4	0	0	0	0	2	2/11 (18.2%)	0	0
Grade 5	0	0	0	0	1	1/11 (9.1%)	0	0
PRGN-2009 treatment-related TEAE	32	3/3 (100%)	4	2/3 (66.7%)	45	10/11 (90.9%)	53	20/22 (90.9%)
PRGN-2009 treatment-related AE ≥Grade 3	0	0	0	0	0	0	0	0
All SAEs	7	2/3 (66.7%)	1	1/3 (33.3%)	6	3/11 (27.3%)	0	0
Treatment related SAEs	0	0	0	0	0	0	0	0
DLT	0	0	0	0	0	0	0	0

DLT = dose-limiting toxicity; SAE = serious adverse event; TEAE = treatment-emergent adverse event

Table 9: Adverse Events Related to PRGN-2009 Treatment by System-Organ Class in Phase 1/2 Study in Patients with HPV+ Malignancies (NCT04432597) (Reference: Table 8 in PRGN-2009 IB Version 3)

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

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
Investigations								
Alkaline phosphatase increased	0	0	1	1/3 (33.3%)	1	1/11 (9.1%)	0	0
Aspartate aminotransferase increased	0	0	0	0	2	2/11 (18.2%)	0	0
Creatinine increased	1	1/3 (33.3%)	1	1/3 (33.3%)	3	2/11 (18.2%)	1	1/22 (4.5%)
Lymphocyte count decreased	0	0	0	0	2	2/11 (18.2%)	2	2/22 (9.1%)
Metabolism and nutrition disorders								
Hyperglycemia	0	0	0	0	3	2/11 (18.2%)	0	0
Hyponatremia	0	0	0	0	2	2/11 (18.2%)	1	1/22 (4.5%)
Nervous system disorders								
Dysgeusia	0	0	0	0	1	1/11 (9.1%)	1	1/22 (4.5%)
Headache	1	1/3 (33.3%)	0	0	3	3/11 (27.3)	5	5/22 (22.7%)

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
Psychiatric disorders								
Anxiety	0	0	0	0	1	1/11 (9.1%)	1	1/22 (4.5%)
Renal and urinary disorders								
Hemoglobinuria	1	1/3 (33.3%)	0	0	2	2/11 (18.2%)	0	0
Proteinuria	3	1/3 (33.3%)	1	1/3 (33.3%)	0	0	0	0
Respiratory, thoracic and mediastinal disorders								
Epistaxis	0	0	0	0	3	3/11 (27.3%)	0	0
Sore throat	0	0	1	1/3 (33.3%)	0	0	1	1/22 (4.5%)
Skin and subcutaneous tissue disorders								
Dry skin	1	1/3 (33.3%)	0	0	2	2/11 (18.2%)	0	0
Rash maculo-papular	1	1/3 (33.3%)	0	0	7	4/11 (36.4%)	0	0

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 ABL) – 4.0 ml/vial (frozen); and
- Sterile empty glass vials (manufactured by ALK) for preparing dilutions (if required).

14.1.3.1 Stability and Storage

PRGN-2009 (manufactured by ABL) 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 5×10^{11} PU/ml. Therefore, each vial contains approximately 5×10^{11} PU.

14.1.3.2 Diluent

Final formulation buffer (FFB; manufactured by ABL) will be used as the diluent for PRGN-2009 and will be supplied by Precigen. Diluent 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. Each vial contains 4.0 ml (extractable volume) of PRGN-2009. NOTE: no other diluents other than FFB should be used with PRGN-2009.

Two different doses of PRGN-2009 will be evaluated during the phase I dose escalation phase of the trial:

- Initial dose: 1×10^{11} PU – A single vial of PRGN-2009 and a single vial of Diluent will be used for dilution and administration of the initial dose of 1×10^{11} PU. See instructions below for preparation of the initial dose.
- Maximum dose: 5×10^{11} PU – A single vial will be used for administration of the maximum dose of 5×10^{11} PU. No pooling of vials will be required. A Diluent vial will not be needed for the 5×10^{11} PU dose.

14.1.4 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.

14.1.5 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% 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 (Precigen) 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.

Two different doses of PRGN-2009 will be evaluated during the dose escalation phase of the trial:

- Preparation of the 1 x 10^{11} PU dose - A single vial of PRGN-2009 will be thawed and diluted 1:5 using the Diluent provided by Precigen, prior to administration to a subject as follows. After thawing, 0.4 ml of PRGN-2009 should be aseptically withdrawn from the vial and transferred into an empty sterile glass vial (provided by Precigen) using an appropriately sized sterile syringe. It is permissible to push air into the vial before withdrawing the specified volumes, as long as aerosols are not created. Next, 1.6 ml of thawed Diluent will be aseptically withdrawn from the Diluent vial using an appropriately sized sterile syringe and this volume will be transferred into the glass vial into which the 0.4 ml of PRGN-2009 has been transferred in the previous step. The contents should be gently swirled to thoroughly mix (do not vigorously shake). A volume of 1.0 ml of the diluted PRGN-2009 will then be aseptically withdrawn using an appropriately sized sterile syringe and administered for the initial dose of 1 x 10^{11} PU. The remaining contents of the PRGN-2009 vial, the remaining contents of the Diluent vial, the glass dilution vial, and used syringes should be discarded as biohazardous waste. The syringe containing PRGN-2009 can be stored at ambient temperature after preparation. The total time for exposure of PRGN-2009 to ambient temperature after thawing the vial is 2 hours, including dilution, syringe filling and administration. Do not store prepared PRGN-2009 syringe on wet ice or in a refrigerator. Also, do not share vials of PRGN-2009 or Diluent between subjects.

- 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.

14.1.6 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 SriSai Biopharmaceutical Solutions, Frederick, MD.

- 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 Precigen 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.

14.1.7 Incompatibilities

Not available.

14.2 M7824 (IND #19628)

14.2.1 Source / Acquisition and Accountability

M7824 is manufactured and supplied for the trial by EMD Serono Research and Development Institute to Pharmacy. All study interventions will be given at 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.

14.2.2 Toxicity

The immunoglobulin portion of M7824 molecule is identical to avelumab (Bavencio). Respective warnings and precautions for grade 2 or higher immune-mediated pneumonitis, immune-mediated colitis, immune-mediated endocrinopathies, immune-mediated hepatitis) and infusion reactions are included in the prescribing for Bavencio (bavencio.com). Participants will be pre-medicated to prophylax against infusions reactions. The following additionally significant immune-mediated adverse reactions have occurred in less than 1% of 1738 participants treated with BAVENCIO: myocarditis with fatal cases, myositis, psoriasis, arthritis, exfoliative dermatitis, erythema multiforme, pemphigoid, hypopituitarism, uveitis, Guillain-Barré syndrome, and systemic inflammatory response. The above irAEs are all considered an anticipated risk of treatment with M7824 and thus will not be considered DLTs.

In a phase 1, open-label 3+3 dose-escalation study of M7824 in 16 participants, 3 participants experienced grade 3 drug-related adverse events including skin infection secondary to grade 2 bullous pemphigoid, lipase increased, and colitis with associated anemia. There were no grade 4 – 5 treatment related adverse events. Please see table below for details.

Treatment-related adverse events

	3 mg/kg (n = 3)		*10 mg/kg (n = 3)*		*20 mg/kg (n = 7)*		*Total (n = 16)*	
	Any Grade	Grade 3	Any Grade	Grade 3	Any Grade	Grade 3	Any Grade	Grade 3
Participants with any event**	**2 (66.7)**	**1 (33.3)**	**1 (33.3)**	**0 (0.0)**	**4 (57.1)**	**2 (28.6)**	**7 (43.8)**	**3 (18.8)**
Anemia					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Bullous pemphigoid	1 (33.3)						1 (6.3)	
Colitis					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Dermatitis acneiform			1 (33.3)				1 (6.3)	
Dyspnea exertional***					1 (14.3)		1 (6.3)	
Hyperthyroidism					1 (14.3)		1 (6.3)	
Hypophosphatemia					1 (14.3)		1 (6.3)	
Hypothyroidism			1 (33.3)		1 (14.3)		2 (12.5)	
Infusion-related reaction					1 (14.3)		1 (6.3)	
Keratoacanthoma					1 (14.3)		1 (6.3)	
Lipase increase					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Nausea	1 (33.3)						1 (6.3)	
Pruritus	1 (33.3)						1 (6.3)	
Rash maculo-papular	1 (33.3)		1 (33.3)				2 (12.5)	
Skin infection	1 (33.3)	1 (33.3)					1 (6.3)	1 (6.3)
Vomiting	1 (33.3)						1 (6.3)	

**There were no treatment-related AEs in the 3 participants treated with 1 mg/kg M7824.

***The differential for this dyspnea was pneumonitis vs. lymphangitic spread of disease (disease progression).

As of August 2017, > 500 participants have been treated with M7824 across multiple solid tumor expansion cohorts. The safety profile is consistent with other monotherapy checkpoint inhibitors, with the exception of keratoacanthomas and cutaneous squamous cell carcinomas which have occurred in approximately 3-5% of participants and are well managed with surgical excision. These lesions have not been a criterion for treatment discontinuation, but thus far have all spontaneously regressed following treatment discontinuation.

In addition, after discussion among NCI investigators on multiple protocols using M7824 bleeding events ranging from low grade gingival bleeding and epistaxis to more serious hemoptysis, GI bleeding and hematuria have been observed. Some of these events can be attributed to bleeding events related to cancer directly. However, there remains the possibility that M7824 may increase the overall risk of bleeding in ways that may not be directly related to direct tumor bleeding. It is hypothesized that this possible increased bleeding risk may be due to TGF beta inhibition which has an effect on angiogenesis. Accordingly, participants will be notified of the same possible risk in the informed consent document for this study (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool).

Potential risks include anemia, alterations in wound, healing or repair of tissue damage, and embryofetal toxicity.

In addition, at least 2 instances of nodular regenerative hyperplasia have been observed with the use of this agent.

14.2.3 Formulation and Preparation

M7824 is provided as a sterile liquid formulation and packaged at a 10 mg/mL concentration in USP/ Ph Eur type I 50R vials that are filled with drug product solution to allow an extractable volume of 60 mL (600 mg/60 mL). The vials are closed with rubber stoppers in serum format complying with USP and Ph Eur with an aluminum crimp seal closure. Each single-use vial contains 600mg of M7824, formulated as 10mg/mL of active, 6% (w/v) Trehalose, 40 mM NaCl, 5 mM Methionine, 0.05% (w/v) Tween 20, 10 mM LHistidine at pH 5.5.

The liquid formulation is diluted directly with 0.9% sodium chloride solution for injection. The estimated volumes of delivery are anticipated to be no more than 250mL. The verified concentration range in the infusion solution is 0.16 mg/mL to 9.6 mg/mL.

14.2.4 Stability and Storage

M7824 must be stored at 2℃ to 8℃ until use. Product stored at room temperature for extended periods of time might be subject to degradation. M7824 must not be frozen. Rough shaking of the reconstituted solution must be avoided.

The chemical and physical in-use stability for the infusion solution of M7824 in 0.9% sodium chloride for injection has been demonstrated for a total of 72 hours at room temperature; however, from a microbiological point of view, the diluted solution should be used immediately and is not intended to be stored unless dilution has taken place in controlled and validated aseptic conditions.

No other drugs should be added to the infusion containers containing M7824. See Manual of Preparation of approved ancillary supplies.

14.2.5 Administration Procedures

See Section **3.2.2**.

14.2.6 Incompatibilities

Not available.

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16 APPENDIX A: PERFORMANCE STATUS CRITERIA

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