Abbreviated Title: Combination Immunotherapy

NIH Protocol #: 18C0005

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NCT #: NCT03315871

IBC #: RD-17-V111-09

Title: Phase II Trial of Combination Immunotherapy in Biochemically Recurrent Prostate Cancer

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[redacted]

Drug Name	PROSTVAC-V	PROSTVAC-F	CV301	MSB0011359C (M7824)
IND Number	[redacted]	[redacted]	[redacted]	[redacted]
Sponsor	Center for Cancer Research, NCI	Center for Cancer Research, NCI	Center for Cancer Research, NCI	Center for Cancer Research, NCI
Manufacturer/ Supplier	[redacted]	[redacted]	[redacted]	[redacted]

PRÉCIS

Background:

- Androgen deprivation therapy (ADT) and surveillance are treatment options for prostate cancer patients with biochemical progression after localized therapy (i.e., biochemically recurrent [BCR] prostate cancer). The primary goal in these patients is to prevent morbidity from their cancer that results from disease progression and metastatic disease on conventional imaging.
- ADT can lower the PSA in these patients, but because of its substantial side effect profile and ambiguous long-term impact, it is generally deferred by most patients until there is a rapid escalation in their PSA.
- Immunotherapy presents an alternative option for these patients that is especially attractive because it is not associated with substantial toxicity. Also, since immunotherapy can have lasting effects after treatment due to a sustained activated immune response, patients will not be required to take these treatments indefinitely to potentially benefit clinically.
- Current and previous clinical trials have demonstrated that single agent immunotherapy can impact PSA in patients in this population.
- The focus of this study is to determine if combination immunotherapy with immune-cell mobilizing vaccines can initiate an immune response in the first 4 months that is then augmented by an immune checkpoint inhibitor in the following 3 months.
- In addition to PSA responses (the primary metric in this population), safety, changes in immune responses, and PSA kinetics will also be evaluated.

Objectives:

Primary Objectives:

- Safety Lead-In: To evaluate the safety and tolerability of combination immunotherapy in participants with castration-resistant prostate cancer
- Biochemical Recurrence: To determine if the combination immunotherapy can induce a 30% decline in PSA in 28% of participants with biochemically recurrent prostate cancer.

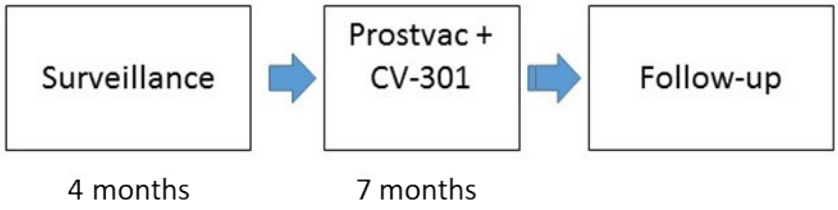
Eligibility Criteria (for biochemical recurrence):

- Histologically confirmed adenocarcinoma of the prostate
- Participants with negative CT Scan and Tc-99m Bone Scan
- Participants with a PSA over 0.8 ng/ml for participants following radical prostatectomy or for participants following definitive radiation therapy: a rise in PSA of ≥ 2 ng/mL above the nadir
- Participants with a PSA doubling time of 5-15 months
- No history of active autoimmune disease or history of organ compromising autoimmune disease
- ECOG 0-1
- Safety lead-in cohort will evaluate 6 participants with castration resistant prostate cancer

Design:

- Three-arm, non-randomized study
- Accrual goal is a total of 37 evaluable participants (6 in an initial safety cohort, 6 participants who received M7824 as part of the initial investigation and 25 homogenously treated participants) to evaluate response
- Participants from an on-going study (NCT02649439) with nearly identical eligibility can serve as a contemporary control for secondary endpoints
- Following the safety lead-in, all participants will be enrolled and may undergo a surveillance period during which up to 4 consecutive monthly PSA values will be captured by the labs.
- After surveillance period, if applicable, participants will be treated with 2 vaccines concurrently, Prostvac and CV301, during months 1-4. For months 5-7, MSB0011359C [an anti-PD-L1 antibody (avelumab) with TGFβ-Trap molecule] will be added to the regimen.
    - Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3)
- Participants will be monitored for on-treatment and post-treatment PSA, immune and imaging responses.

SCHEMA



Prostvac: Q2 weeks for 1 month, then monthly for 6 additional months (7 months total)

CV301: Q2 weeks for 1 month, then monthly for 6 additional months (7 months total)

MSB0011359C: Given q 2 weeks for 3 months at phase II dose (flat dose of 1200 mg) starting after 4 months of vaccines. Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3). This change is reflected in the new cohort 2 schema above.

- With Amendment v05/10/2023, participants that have at least 3 PSA measurements in the surveillance period, with at least one of them sufficient to provide the doubling time from baseline, will not undergo any further PSA measurements in the surveillance period.

Phase 1 Lead-in: 6 participants treated with Prostvac+CV-301 and anti-PDL1/TRAP*

- *Phase 1 dosing will be given concurrently in castration-resistant prostate cancer participants until disease progression or toxicity limits dosing
- Participants in the Phase 1 Lead-in will not undergo the surveillance period

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference 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

- Safety Lead-In: To evaluate the safety and tolerability of combination immunotherapy in participants with castration-resistant prostate cancer
- Biochemical Recurrence: Determine if the combination immunotherapy can induce a 30% decline in PSA in 28% of participants with biochemically recurrent prostate cancer.

1.1.2 Secondary Objectives

- Assess the safety of the combination immunotherapy regimen
- Changes in PSA kinetics after treatment with the combination immunotherapy, relative to baseline PSA kinetics

1.1.3 Exploratory Objective

- Immune changes after treatment with 2 vaccines for 4 months
- Correlate changes in PET images to changes in PSA over time
- Evaluate changes in the microbiome of the gastrointestinal tract and correlate to changes in treatment, PSA and immune cell subpopulations
- Evaluate the decline in PSA in participants who received vaccine as well as MSB0011359B prior to its being discontinued.

1.2 BACKGROUND AND RATIONALE

1.2.1 Rationale for conducting the study in biochemically recurrent prostate cancer patients

Although surgery or radiation can cure the majority of newly diagnosed patients, 20-40% are likely to have recurrent disease manifested as a rising PSA despite negative conventional imaging studies (CT and technetium-99m (Tc-99m) Bone Scan) used to detect metastasis.[1, 2] This stage of disease is often referred to as biochemically recurrent prostate cancer.

Despite the fact that nearly 90,000 men who are diagnosed each year with prostate cancer will ultimately develop biochemically recurrent prostate cancer there is no clear standard of care for these patients.[3] Treatments range from active surveillance to intermittent or continuous androgen deprivation therapy (ADT) and in some cases salvage radiation or surgery. Ideal effective non-hormonal treatment strategies in this population would avoid or delay the onset of metastatic castration resistant prostate cancer (mCRPC). There are two significant obstacles to developing therapies in this asymptomatic population; (1) therapies need to have minimal toxicity (2) conventional clinical endpoints (metastatic progression and overall survival) can take 5-10 years.

1.2.2 Therapeutic Cancer Vaccines in Prostate Cancer

The goal of therapeutic cancer vaccines is to generate a targeted immune response leading to immune-mediated anti-tumor activity. Sipuleucel-T is a therapeutic cancer vaccine generated from peripheral blood mononuclear cells. This vaccine is generated after a patient's peripheral immune cells are collected via leukapheresis, transported to a regional processing center where they are exposed in vitro to a PAP/GM-CSF fusion protein. At the end of this process, the activated cellular product is re-infused into the patient. A full course of therapy repeats this process 3 times every 2 weeks for 1 month.[4, 5] A phase III trial (n = 512) demonstrated an overall survival benefit for the vaccine (25.8 months vs. 21.7 months; P = 0.032).[4] Based on these overall survival findings, the FDA approved sipuleucel-T for the treatment of asymptomatic or minimally symptomatic mCRPC, making it the first FDA-approved therapeutic cancer vaccine for the treatment of any malignancy.

1.2.3 Prostvac

Prostvac [redacted] offers an alternative strategy to sipuleucel-T.[6, 7] [redacted] To target prostate-specific antigen (PSA), Prostvac vaccine employs genetically altered poxviruses to deliver targeting information to immune cells and generate an immune response. Administered subcutaneously, the poxviruses deliver the transgenes for the tumor associated antigens (PSA) to antigen presenting cells through cellular infection. Once these pox viruses are within the cellular cytoplasm, the transgenes are processed. The end result is an antigen presenting cell expressing a PSA peptide within the major histocompatibility complex, resulting in PSA-specific cytolytic T lymphocytes activation.[8, 9] (**Figure 1**) This approach does not require expensive, labor-intensive *ex vivo* preparation of patients' peripheral blood. Prostvac is thus potentially more logistically and financially feasible over the long-term than sipuleucel-T.[10]

Prostvac has been investigated in 2 phase II trials in mCRPC, both of which administered the vaccine at monthly intervals until disease progression. An industry-sponsored, placebo-controlled, multicenter trial in 125 mCRPC patients randomized them 2:1 in favor of Prostvac; the placebo was an empty poxviral vector containing no transgenes. As was seen in the sipuleucel-T studies, patients receiving vaccine showed no change in short term disease progression, yet had an overall survival benefit (25.1 months with Prostvac vs. 16.6 months with placebo; P = 0.0061).[11] (**Figure 2**) A second phase II study of Prostvac in 32 mCRPC patients at the NCI demonstrated that the vaccine was able to generate a T-cell specific immune response.[12] Based on the findings in these trials, a phase III trial of Prostvac in mCRPC has completed accrual with approximately 1300 patients (NCT01322490).

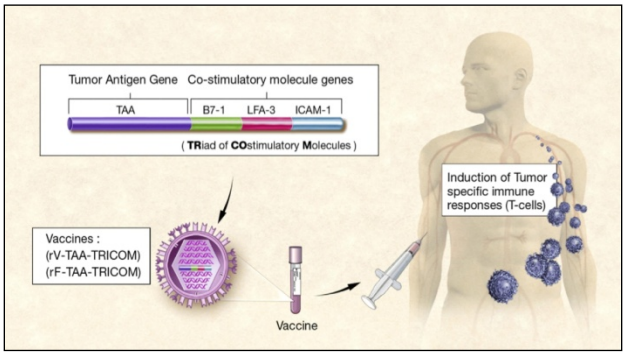


Figure 1:Poxviral vaccine strategy: Modified poxvirus contains transgenes for the tumor-associated antigen PSA and 3 T-cell costimulatory molecules [8, 9].

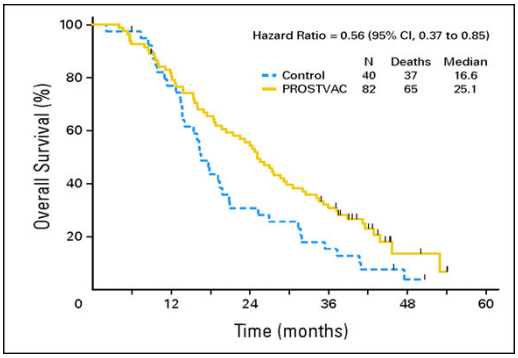


Figure 2:Prostvac improved survival in mCRPC patients in a randomized multi-center phase II trial. [11]

Prostvac is very well-tolerated, with common side effects of grade 1 injection-site reactions or flu-like symptoms.[11, 12] A favorable side effect profile and the potential ability to induce a sustained antitumor immune response, clinical trials are ongoing evaluating strategies to use Prostvac in earlier disease patients. A previous study of Prostvac in biochemically recurrent prostate cancer suggested that this treatment could slow/improve PSA doubling in 25 patients from a median of 5.3 months to 7.7 months.[13] A randomized prospective study is currently evaluating this hypothesis with the same treatment dosing and schedule as the previous study (NCT02649439). The treatment dosing and schedule for this combination immunotherapy study is the same as the previous studies.

1.2.4 CV301

Both CEA and MUC-1 have been reported as relevant immunologic targets in prostate cancer. CEA has been reported as elevated in the circulation of nearly 50% of 141 patients evaluated with advanced disease.[14] A separate study suggested that more than 60 percent of primary tumors have been found to express MUC1 and over 90% of metastatic lesions in lymph nodes have also been found to express MUC1.[15] Furthermore, more aggressive disease (higher Gleason Score) has also been associated with greater MUC1 expression.[15] Immunologic studies here at the NCI have demonstrated that despite initial targeting of PSA by pox virus-based vaccines, increased immunologic targeting of MUC1 and CEA have occurred.[16] Across several other trials, broad immunologic targeting of multiple antigens after immunotherapy has been associated with better clinical outcomes.[17]

Earlier efforts to generate efficient cancer immunotherapy were directed towards the development of PANVAC, a prime and boost approach based on the administration of a replicating-competent Vaccinia-based delivery vector followed by a Fowlpox vector. Both vectors encoded the transgenes for B7.1, ICAM-1 and LFA-3, together with the CEA and MUC-1. In order to establish its therapeutic potential, several preclinical studies were performed, showing that PANVAC activates antigen-specific human T cells *in vitro* [18],elicits antibody and cellular immune responses *in vivo,* and shows significant antitumor efficacy against tumors expressing either human CEA or MUC-1 in mice, resulting in programmed death ligand 1 (PD-L1) up-regulation within the tumor microenvironment. Subsequently, the clinical development program continued with the design of several clinical trials to further confirm the efficacy and safety of this intervention. Overall, the results of Phase I and II clinical trials in different cancer populations employing PANVAC monotherapy or in combination with GM-CSF or docetaxel demonstrate a good safety profile accompanied by some hints of clinical benefits.[19, 20]

A second-generation cancer immunotherapy strategy was developed, giving rise to CV301 involving a modified vaccinia. CV301 is a recombinant non-replicating poxvirus-based immunotherapy encoding the same 5 transgenes used in PANVAC (B7.1, ICAM-1, LFA-3, CEA and MUC-1) and thus designed to activate an antigen-specific response. Therefore, tumors expressing these antigens, including prostate cancer, qualify as a potential indication for CV301.

Given the potential risks associated with the replicating capacity of the vaccinia-based PANVAC-V construct, the next generation cancer immunotherapeutic candidate MVA-BN-CV301 was generated [redacted] It constitutes a recombinant non-replicating poxvirus- based immunotherapy derived from the MVA-BN construct. This novel viral vector was developed from the highly attenuated vaccinia virus known as MVA, which after more than 500 serial passages in chicken embryo fibroblasts lost approximately 15% of the original vaccinia genome. Furthermore, MVA-BN differs from other MVA strains in its extensive plaque purification and propagation in serum-free conditions, and has already undergone extensive investigation as a new smallpox vaccine. Within this comprehensive clinical development program, MVA-BN has been administered to more than 7,700 subjects without major safety concerns, hence exhibiting a robust safety profile. Accordingly, besides decreasing the risk of cardiac adverse events associated with earlier generation vaccinia-based strategies, the improved MVA-BN enables previously excluded segments of the general population, such as immunocompromised individuals and persons diagnosed with atopic dermatitis, to receive the potential benefits offered by this novel approach. Therefore, it embodies a highly novel approach and offers a reliable technological platform that enables the safe delivery of different transgenes to produce a tailored immune response against specific antigens.

The new CV301 strategy encodes the same 5 transgenes expressed by the previous PANVAC vector: CEA, MUC-1, B7.1, ICAM-1 and LFA-3. However, these inserts encode each of the transgenes with amino acid sequences that are slightly different from the original vectors. In fact, MUC-1 has been modified with additional agonistic epitopes. Consequently, the CV301 construct is expected to promote an antigen-specific targeted immune response for tumors expressing these antigens. In addition, a change in biosafety also characterizes the new CV301 construct. Thus, whereas vaccinia possesses a BSL-2, MVA has been categorized as BSL1 in Europe. As well, similarly to the earlier PANVAC concept, CV301 also encompasses a heterologous prime-boost regimen employing an MVA-BN-CV301 priming dose followed by a Fowlpox-based FPV-CV301 boosting dose, thus replacing the former PANVAC-V and PANVAC-F components. Given the

existing correspondences between the CV301 and PANVAC vectors, the clinical development program designed for CV301 will rely on the extensive nonclinical and clinical data currently available from PANVAC.

CV-301 Phase I Experience

A phase I study was just completed at the NCI with no dose limiting toxicities. (unpublished) As expected, CV-301 was well tolerated in all 12 patients with no DLT noted. The only attributable toxicities were grade 1 and 2 and mostly injections site reactions and flu-like symptoms, with other rare toxicities such as headache and nausea (also grade 2 or less) also reported. There were no grade 3 or greater toxicities reported in the study.

1.2.5 PD-L1 TGFβ Trap (MSB0011359C)

MSB0011359C is a bifunctional fusion protein that combines an anti-programmed death ligand 1

(PD-L1) antibody and the soluble extracellular domain of transforming growth factor beta (TGFβ) receptor type II as a TGFβ neutralizing "trap," into a single molecule. This anti-PD-L1 / TGFβ-Trap molecule is designed to target 2 major mechanisms of immunosuppression in the tumor microenvironment. The anti-PD-L1 moiety of MSB0011359C is identical to avelumab, except for three amino acid substitutions in the heavy chain constant regions, which result in a different human IgG1 allotype, and one amino acid substitution in the heavy chain for antibody stability. Avelumab (proposed international non-proprietary name for MSB0010718C) is currently in Phase II / III clinical development [redacted].

The PD-1/PD-L1 axis is an important mechanism for tumor immune evasion.[21] Effector T cells chronically sensing antigen take on an exhausted phenotype marked by PD-1 expression, a state under which tumor cells engage by upregulating PD-L1. Blocking the axis restores the effector function in these T cells. Additionally, in the tumor microenvironment, myeloid cells, macrophages, parenchymal cells, and T cells upregulate PD-L1. TGFβ has growth inhibitory effects on normal epithelial cells, functioning as a regulator of epithelial cell homeostasis, and it acts as a tumor suppressor during early carcinogenesis. As tumors progress toward malignancy, the growth inhibitory effects of TGFβ on the tumor are lost via mutation in one or more of the TGFβ pathway signaling components or through oncogenic reprogramming.[22] Upon loss of sensitivity to TGFβ inhibition, the tumor continues to produce high levels of TGFβ, which then serve to promote tumor growth.[22] The TGFβ cytokine is overexpressed in various cancer types with correlation to tumor stage.[22, 23] Many types of cells in the tumor microenvironment produce TGFβ, including the tumor cells themselves, immature myeloid cells, regulatory T cells, and stromal fibroblasts; these cells collectively generate a large reservoir of TGFβ in the extracellular matrix. TGFβ signaling contributes to tumor progression by promoting metastasis, stimulating angiogenesis, and suppressing innate and adaptive antitumor immunity.[22] As a broadly immunosuppressive factor, TGFβ directly down regulates the effector function of activated cytotoxic T cells and natural killer (NK) cells and potently induces the differentiation of naïve CD4+ T cells to the immunosuppressive regulatory T cells (Treg) phenotype.[23] In addition, TGFβ polarizes macrophages and neutrophils to a wound-healing phenotype that is associated with production of immunosuppressive cytokines.[24] As a therapeutic strategy, neutralization of TGFβ activity has the potential to control tumor growth by restoring effective antitumor immunity, blocking metastasis, and inhibiting angiogenesis. In prostate cancer, TGFβ has been associated in poor outcomes or disease recurrence in early stage patients, perhaps suggesting that it may be a therapeutic target ([25],[26]).

MSB0011359C also binds TGFβ (preferentially 1 and 3 isoforms), an inhibitory cytokine produced in the tumor microenvironment by cells including apoptotic neutrophils, myeloid-derived suppressor cells, T cells, and tumor.[21, 27] Inhibition of TGFβ by soluble TGFβRII reduced malignant mesothelioma tumors in a manner that was associated with an increase in CD8+ T cell antitumor effects.[28] The absence of TGFβ1 produced by activated CD4+ T cells and Treg cells has been shown to inhibit tumor growth, and protect mice from spontaneous cancer.[29] Thus, TGFβ appears to be important for tumor immune evasion.

Combining these pathways, PD-1 / PD-L1 and TGFβ, is attractive as an antitumor approach. A recent report found that blockade of TGFβ signaling in T cells or deletion of TGFβ1 from T cells in a mouse model led to diminished PD-1 expression in tumor-infiltrating CD8+ T cells.[29] Concomitant PD-1 and TGFβ blockade can restore pro-inflammatory cytokines.[30] In a murine model of hepatocellular carcinoma, TGFβ appeared to increase the expression of PD-L1 in dendritic cells, which in turn promoted T-cell apoptosis and increased percentage of CD25+, Foxp3+ T regulatory cells.[31] Higher levels of circulating myeloid-derived suppressor cells (MDSCs), a significant source of TGFβ, are associated with failure to respond to anti-PD-1 therapy.[32]

NCT02517398 is a phase 1, open label, 3+3 dose-escalation study and this preliminary data was presented at AACR in April, 2017. Eligible patients received MSB0011359C at 1, 3, 10, or 20 mg/kg Q2W until confirmed progressive disease, unacceptable toxicity, or trial withdrawal; treatment beyond progression is generally allowable. The primary objective is to determine the safety and maximum tolerated dose of MSB001359C; secondary objectives include pharmacokinetics (PK), immunogenicity, and best overall response per RECIST v1.1. As of Oct 3, 2016, 16 heavily pretreated pts with ECOG performance status 0-1 have received MSB0011359C. Our PK data show a dose-linear increase in exposure; furthermore, at the examined dose levels, MSB0011359C saturates peripheral PD-L1 and sequesters any released plasma TGF-β1, -β2, and -β3 throughout the dosing period. Grade 3 drug-related treatment-emergent adverse events (TEAEs) occurred in 3 patients (skin infection secondary to grade 2 bullous pemphigoid [BP], lipase increased, anemia, and colitis); there were no grade 4-5 drug-related TEAEs. BP and colitis responded well to steroids. Colitis and its secondary events of anemia and rectal hemorrhage (in a previously radiated area) were considered dose-limiting in 1 patient. (See full table of AEs in table below). There was preliminary evidence of efficacy across all dose levels, including 1 ongoing confirmed complete response (cervical cancer), 1 durable partial response (pancreatic cancer), a 25% reduction in the sum of diameters of target lesions after only 2 doses of MSB001359C (cervical cancer), and 2 cases of prolonged stable disease (pancreatic cancer; carcinoid). Updated data will be presented. Preliminary data from this phase 1 dose-escalation study suggest that MSB0011359C has a manageable safety profile in patients with heavily pretreated advanced solid tumors. Early evidence of clinical efficacy warrants further study. The recommended phase 2 dose is a flat dose of 1200 mg every 2 weeks.

	n = 16 cohort		**n = 3 backfill data**	
Treatment Related Adverse Events (Gulley JL et al., ASCO 2017)	Any Grade	Grade 3	Any Grade	Grade 3

	n = 16 cohort		**n = 3 backfill data**	
Patients with any event, n (%)	**7 (43.8)**	**3 (18.8)**	**2 (66.7)**	
Anemia	1 (6.3)	1 (6.3)		
Bullous pemphigoid	1 (6.3)			
Colitis	1 (6.3)	1 (6.3)		
Dermatitis acneiform	1 (6.3)			
Dyspnea exertional	1 (6.3)			
Hyperthyroidism	1 (6.3)		1 (33.3)	
Hypophosphatemia	1 (6.3)			
Hypothyroidism	2 (12.5)		1 (33.3)	
Infusion-related reaction	1 (6.3)			
Keratoacanthoma	1 (6.3)		1 (33.3)	
Lipase increase	1 (6.3)	1 (6.3)		
Nausea	1 (6.3)			
Peripheral motor neuropathy			1 (33.3)	
Pruritus	1 (6.3)			
Rash maculopapular	2 (12.5)		1 (33.3)	
Skin infection	1 (6.3)	1 (6.3)		
Vomiting	1 (6.3)			

As of 24 August 2018, clinical activity of MSB0011395C has been observed in the dose escalation cohorts of EMR200647-001. For the 40 participants analyzed at the 1200mg level, Overall Response Rate (ORR) assessed by the Investigator was 25%, with median progression-free survival (PFS) of 2.7 months and overall survival of 17.1 months (data cutoff 15 October 2019).

1.2.6 Rationale to Combine Vaccines

Previous studies have demonstrated that in vivo broadening of the immune response through targeting of additional antigens after immunotherapy can lead to better outcomes. This process is called antigen spreading or antigen cascade.[33] Although this has been seen and reported previously with single vaccine administration, it is possible that *in vivo* broadening of the immune response could be augmented by the use of multiple vaccines.

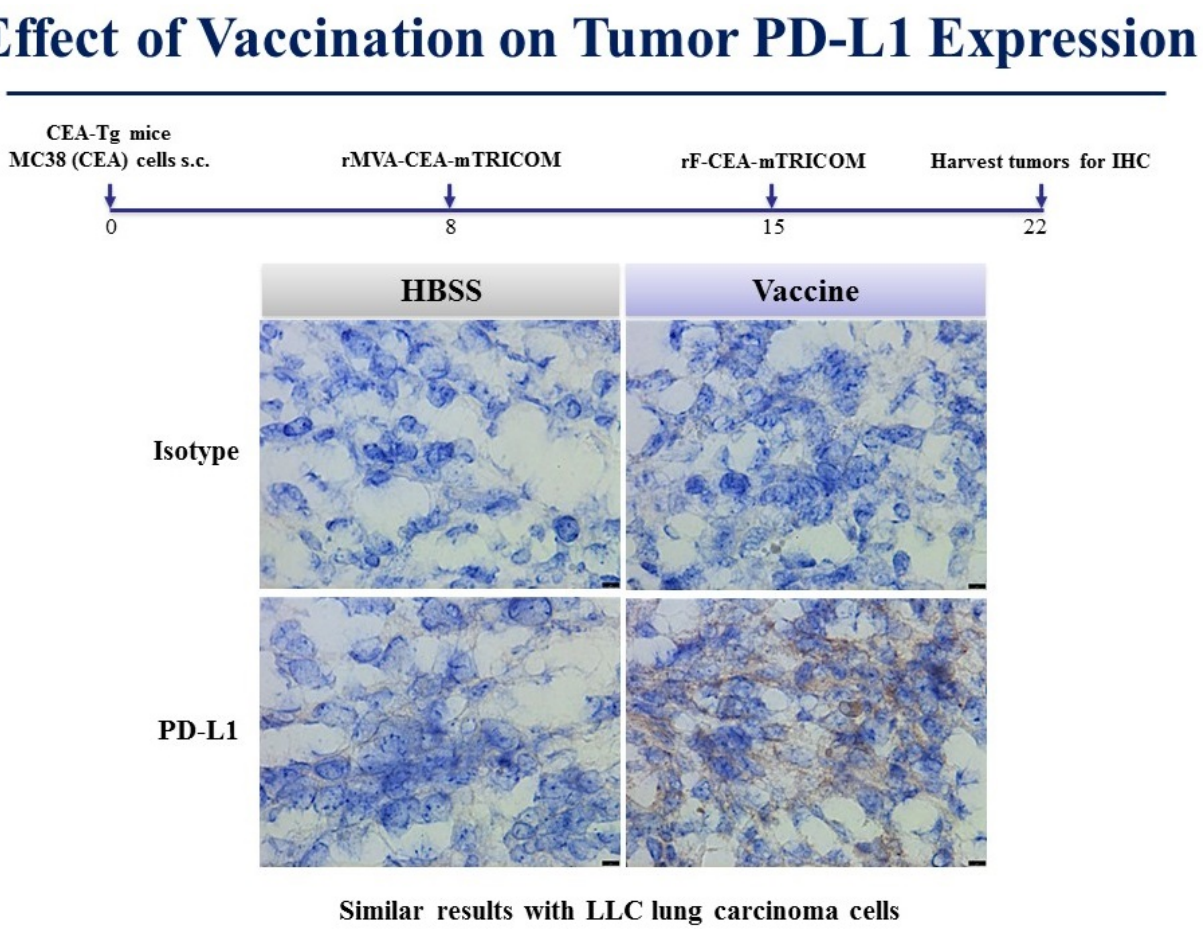
Preclinical models support this hypothesis using two vaccines in a female C57BL/6 (H-2b) mouse model.[34] These studies demonstrated that concurrent administration of two vaccines induced a more diverse T-cell population that lead to enhanced antitumor efficacy. These studies provide the rationale for clinical studies investigating concurrent administration of vaccine platforms to enhance the antigen-specific immune response. This strategy has not been tested in prostate cancer to this point and this study will be the first to explore this strategy and its impact immunologically and on PSA in biochemical recurrence. Given that both vaccines are well tolerated with negligible side effect profiles, there are limited concerns about toxicity from this combination.

1.2.7 Rationale to Combine Immune Checkpoint Inhibitors with Vaccines

To this point, immune checkpoint inhibitors have failed to demonstrate clear single agent efficacy in prostate cancer. Two studies with ipilimumab failed to demonstrate an overall survival in phase 3 studies.[35, 36] Anti-PD1 and anti-PDL1 have demonstrated only limited benefits. The study that has generated the most interest to this point involves pembrolizumab in mCRPC.[37] That study has reported a response rate of about 15-20% in the first 30 patients, typical of findings across multiple cancer subtypes. In order to enhance response to anti-PD1/anti-PDL1 inhibition, combinations with other therapies that can mobilize immune cells to the tumor microenvironment have been proposed.[38] In this manner, immune cells in the tumor microenvironment could be enabled by PD1/PDL1 inhibition.

This is likely the underlying mechanism for the approved ipilimumab /nivolumab combination in metastatic melanoma.[39] Anti-CTLA-4 (ipilimumab) is able to activate immune cells in the periphery and within tumor, leading to enhanced outcomes in combination with anti-PD1 (nivolumab), improving progression free survival (PFS) in metastatic melanoma relative to nivolumab alone (11.5 vs. 6.9 months; $p<.001$) and ipilimumab alone (2.9 months, $p<0.001$). Interestingly a subgroup analysis from this study indicated that patients with PDL-1 positive tumors had no benefit from the combination vs. nivolumab alone (PFS of 12.4 vs. 12.4 months).[39] This data would suggest that for patients lacking immune cells in the tumor microenvironment (devoid of PD-L1 upregulation) benefited from ipilimumab induced immune mobilization from the periphery.[38] The toxicity of ipilimumab in prostate cancer patients limits its use in biochemical recurrence, but it is possible that vaccine combinations could accomplish the same results.

Indeed, preclinical models from the LTIB have suggested that vaccine-based therapies can lead to upregulation of PDL1 in the tumor microenvironment (unpublished). As demonstrated below, in murine model with CEA transgenic mice, a CEA vaccine led to upregulation of PDL1 expression. Mechanistically, the CEA vaccine likely increased immune cells in the tumor microenvironment after immune activation ultimately leading to increased PDL1 expression in the tumor.



Preclinical investigations with a therapeutic cancer vaccine, TEGVAX, help mechanistically illustrate this hypothesis. TEGVAX has demonstrated the ability to increase interferon-gamma production in multiple murine models.[40] One series of experiments showed that PDL1 upregulation on tumors was interferon-gamma dependent. Another set of experiments demonstrated synergistic anti-tumor activity when TEGVAX was combined with anti-PD1 antibody. This benefit, however, was mitigated when an interferon-gamma blocking antibody was added to the model, demonstrating its integral role.

The sequence of the anti-PDL1 therapy in this study will allow for priming of the immune system and the perhaps "inflame" the tumor microenvironment prior to potentiation of a clinical response with the anti-PDL1 therapy. Although this treatment will only be given for 4 months, it should provide sufficient time for proof-of-concept for the efficacy of this therapy.

1.2.8 Safety

Safety is a paramount concern in this population of patients who may live another decade. Vaccines have been frequently studied in this population of biochemical recurrence with minimal toxicities of injection site reactions and transient flu like symptoms. We do not expect combination vaccine therapy to induce more sustained toxicities. This is an extrapolation based on data where vaccines have been associated with vivo expansion, resulting in the targeting of multiple antigens (antigen spread/antigen cascade) and such patients often have better clinical outcomes and are not associated with greater toxicity.[16] [41] The addition of an immune checkpoint inhibitor does add

potential for greater toxicities but these toxicities should be manageable as demonstrated in other clinical trials using checkpoint inhibitors as monotherapy. 18 patients with mCRPC were treated with avelumab (the anti-PDL1 antibody that is the backbone of MSB0011359C) and the toxicities are reported in **Table 1**. Immune-mediate adverse events are the greatest concern for these patients, and patients will have detailed informed consent discussions on this manner.

	Reported Toxicities in mCRPC patients treated with Avelumab (n=18)			
	Maximum grade, (%)			
	1	2	3	4
All	13 (72%)	8 (44%)	2 (11%)	0
Fatigue	5 (28%)	0	0	0
Joint pain	1 (5%)	0	0	0
Infusion reaction	0	4 (22%)	0	0
Rash	2 (11%)	0	0	0
Hypothyroidism	1 (5%)	3 (17%)	0	0
Elevated liver enzymes	0	2 (11%)	0	0
Amylase/Lipase elevation	**0**	**1 (5%)**	**2 (11%)**	**0**

Table 1: Reported toxicities in mCRPC patients treated with avelumab (the anti-PDL1 antibody backbone of MSB0011359C).

Nonetheless, out of an abundance of caution the three agents will be used together in a 6 patient phase I lead in cohort in advanced (castration resistant prostate cancer). Once all 6 patients have completed a 6-week toxicity assessment period the toxicity data will be shared with IRB before moving for with the planned study in biochemical recurrence.

Please note: As of December 21, 2018, the 6 patients in the lead in cohort have completed the 6-week toxicity assessment period and no SAEs were seen. Per the protocol design patients will now be enrolled to the BCR cohort. The toxicity assessment of patients in the lead-in cohort is described in section **1.2.13**.

1.2.9 Evaluating the Immunologic Response

Our group in the LTIB has previously evaluated immunologic parameters in clinical trials with Prostvac among several immunotherapy studies.[12, 42] While these findings are not surrogate markers of response, they have improved our knowledge of a vaccine-generated immune response and provided a better understanding of what factors are potentially important in mounting a sufficient anti-tumor immune response that could be associated with improved clinical outcomes. These and other data may allow optimization of vaccines in subsequent clinical trials. Few groups are as well positioned and have the experience as the LTIB to conduct this rigorous immune testing from clinical samples of patients treated with Prostvac.

Antigen-Specific Immune Responses. While many clinical trials have been done in biochemically recurrent prostate cancer, there is little reported on the impact of those therapies on the immunologic response that is near the depth and scope that is proposed in this trial. The proposed immunologic studies will be done on all patients by the Laboratory of Tumor Immunology and Biology at the NCI Clinical Center under the direction of Dr. Jeffrey Schlom.

Unlike previous studies involving Prostvac, which evaluated immune responses to a single 9 amino acid section of the 244 amino acid PSA protein, the immune analysis in this trial will evaluate overlapping 15 amino acid sequences of the entire PSA protein. (**Figure 3**). This more extensive analysis will provide a more thorough understanding of the immune response initiated by Prostvac and a greater opportunity for clinical correlations. This type of analysis has not been previously prospectively performed in biochemically recurrent prostate cancer patients. Advantages of this approach include the ability to assess both CD4 and CD8 T cells, identification of multifunctional T cells (those producing ≥2 cytokines), and identification of T lymphocytes with lytic potential (CD107a expression) (**Table 2**).

The rationale for evaluating intracellular cytokine staining is that it is one method to demonstrate to what extent the immune system has been specifically activated by an immunotherapy (vaccine) to target specific tumor antigens.

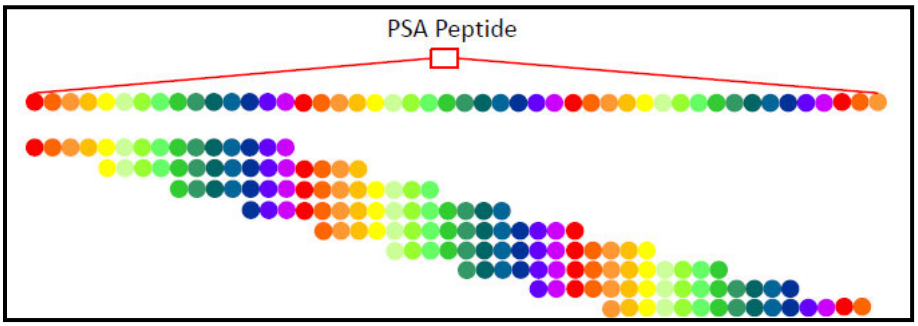


Figure 3: Overlapping PSA Peptide Assessment. This innovative approach to evaluating immune response allows for assessment of immune response to the entire PSA protein using overlapping 15 amino acid sequences. (Previous techniques have focused on assessing just one of these peptides).

		Immune Responses to PSA							
		CD4				CD8			
	PT	CD107a	IFNg	IL2	TNF	CD107a	IFNg	IL2	TNF
Cohort 1 – No Vaccine	11								
	13								
	20								
	22					1427			274
	25								630
	3								
	5								
	10								
Cohort 2 with Prostvac	2		786		374	5269	453		323
	8		345			633			
	12								
	18				402				
	21								
	24					1242			
	14	821							
	16								
	26	815							
	27								

Table 2: Preliminary Data from a trial of Prostvac in mCRPC. This table shows how this multi peptide approach can be used in this proposal. In this previous (unpublished) trial, 2 cohorts of patients were evaluated using the proposed methods. (Responses are listed as absolute # of CD4 or CD8 T Lymphocytes Producing Cytokine or Positive for CD107a per $1x10^6$ cells plated.) This readout shows the breadth of this analysis as both CD4 and CD8 tell cells are evaluated using multiple parameters (cytokine production signifying activation – IFN-γ, IL-2 and TNF-α) and lytic potential as measured by CD107a expression. When only one peptide was used to analyze the immune responses, no immune responders were identified (unpublished).

Briefly, the methodology takes cryopreserved peripheral blood mononuclear cells (PBMCs) from patients before therapy and at the time of restaging. They are thawed and rested overnight at 37°C, 5% CO_2 in complete media (IMDM supplemented with 10% Human AB, 2mM glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin). The next day (Day 0), PBMCs are seeded in 12 well plates ($2.5 x10^6$ in 1 mL), and stimulated with peptide mixes (0.1ug/mL per peptide); cultures were supplemented on days 3 and 5 with cytokines (IL7 and IL15, 10 ng/mL, PeproTech, Rocky Hill, NJ) and fresh media, and on day 7 are rested (with removal of cytokine and peptide). On day 11, $1x10^6$ cells were restimulated for 24 hours in 96 well plates with peptide mixes in the presence of anti-CD107a-APC (clone H4A3, BD Biosciences); brefeldin A (1ul/mL) and monensin (0.7ul/mL) (BD Biosciences) are added to cultures 2 hours after the start of the restimulation and incubated for the final 22 hours. PBMCs are then stained with anti-CD4-PerCP-Cy5.5 (clone OKT4, Biolegend), anti-CD8-AF700 (clone OKT8, Ebioscience), and anti-TNF-PE (clone MAb11), anti-IFNγ-PE-Cy7 (clone 4SB3), and anti-IL-2-BV521 (clone 5344.111) (BD Biosciences). At least $3x10^5$ events in the live gate are acquired with a BD LSR-II flow cytometer equipped with a UV, violet, blue, and red laser. FCS files are analyzed with FlowJo V.9.7 for Macintosh (TreeStar, Ashland, OR). Fluorescence minus one (FMO) controls are used for gaiting, and non-viable cells were excluded. The absolute number of $CD4^+$ or $CD8^+$ lymphocytes

producing cytokine or positive for CD107a is calculated per $1x10^6$ cells plated at the start of the IVS. The background signal (obtained with the HLA peptide pool), and values obtained prior to therapy were subtracted from those obtained post-therapy. Values ≥250 are scored as positive for TAA-specific immune response following therapy (**Figure 4**).

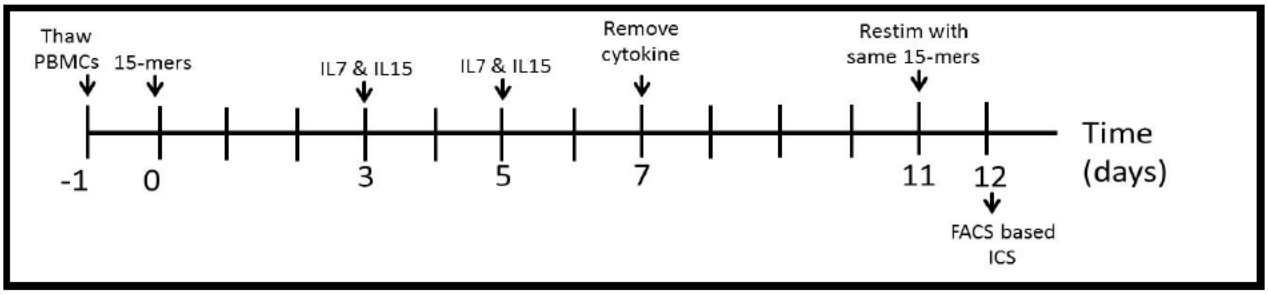


Figure 4: Methodology of Assessing PSA-specific Immune Responses

Flow Cytometry Analysis of Immune subsets (Biomarker Development)

As part of the extensive immune interrogation, peripheral immune cells will be evaluated by 30 markers to assess 127 immune subsets (**Table 3**). This methodology has been previously described. [43] This added analysis will provide preliminary data to develop a Peripheral Immunoscore based on the frequency of specific pre-determined immune cell subsets in the blood of patients prior to therapy. The score is calculated from the frequency of certain immune cell subsets prior to vaccine treatment. The panel of markers will also be used to measure immune response to the vaccine. The ultimate goal is to use this Peripheral Immunoscore as a biomarker that could serve as an intermediate marker of immune response in future studies. Data from this study would be used to establish the relevant markers and can be confirmed in future studies. The first step has been performed in a previous study with another pox viral vaccine in breast cancer (**Figure 5**).

Table 3: Analysis of Peripheral Immune Cells

1. **CD4**: Helper T lymphocytes (32 subsets)
2. **CD8**: Cytotoxic T lymphocytes (29 subsets)
   - **Markers of PD-1 pathway and T cell activation (in CD4 and CD8):**
     - **EOMES:** activation
     - **TCR-αβ:** activation
     - **Tbet:** activation
     - **BATF:** activation/exhaustion
   - **Maturation status of T lymphocytes (in CD4 and CD8)**:
     - **Naïve**: CD45RA$^+$ CCR7$^+$
     - **Effector Memory**: CD45RA$^-$ CCR7$^-$
     - **Terminal (EMRA)**: CD45RA$^+$ CCR7$^-$
   - **T lymphocyte markers (in CD4 and CD8)**:
     - **CTLA-4:** inhibition
     - **PD-1:** activation/inhibition
     - **PD-L1:** activation/cross-inhibition
     - **TIM-3:** inhibition
     - **ICOS**: activation (only on CD4)
3. **Tregs**: Regulatory T lymphocytes (CD4$^+$ CD25$^+$ FoxP3$^+$ CD127$^-$) (7 subsets)
   - **CD45RA**: Tregs highly expandable *in vitro*
   - **CTLA-4**: Treg suppression
   - **CD49d:** "contaminating" effector lymphocytes (non-Tregs)
   - **ICOS**: Treg suppression
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
4. **B lymphocytes**: CD19$^+$ (5 subsets)
   - **CTLA-4:** inhibition
   - **TIM-3:** inhibition
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
5. **NK**: Natural killer cells (CD56$^+$ CD3$^-$) (20 subsets)
   - **CD16$^+$ CD56^{br}**: Functional intermediate, lytic and cytokine production
   - **CD16$^+$ CD56^{dim}**: Mature NK, cytokine production
   - **CD16$^-$ CD56^{br}:** Immature, abundant in human placenta
   - **CD16$^-$ CD56^{dim}**: non-lytic, non-cytokine production
   - **TIM-3:** activation
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
6. **NK-T**: CD56$^+$ CD3$^+$ (4 subsets)
   - **TIM-3:** activation
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
7. **cDCs (Conventional DCs):** CD3$^-$CD56$^-$CD1c$^+$CD303$^-$ (5 subsets)
8. **pDCs (plasmacytoid DCs):** CD3$^-$CD56$^-$CD1c$^-$CD303$^+$ (5 subsets)
   - **Markers of DC activation**
     - **CD83:** activation
     - **TIM-3:** inhibition
     - **PD-1:** activation/inhibition
     - **PD-L1:** cross-inhibition
9. **MDSCs**: Myeloid-derived suppressor cells (CD11b$^+$ HLA-DR^{low-} CD33$^+$) (20 subsets)
   - **CD14**: Common Myeloid Marker (high in monocytes, dim in granulocytes)
   - **CD15**: Granulocyte marker
   - **CD16**: most immature monocytic MDSCs
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition

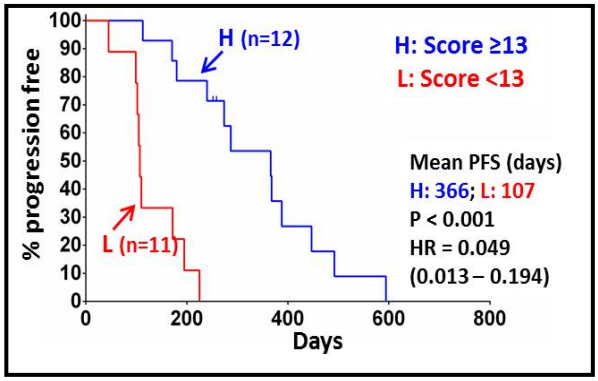
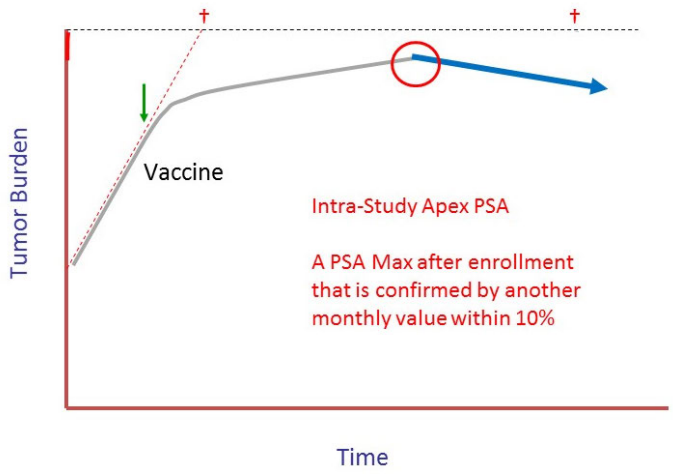


Figure 5: Preliminary Data of Immunoscore Associations with Clinical Outcomes. This data (unpublished) from a recently completed breast cancer study involving a therapeutic cancer vaccine highlights the potential of Immunoscore. In this study, patients with peripheral immunoscore (H, more than 13 parameters positive, measured prior to therapy) had a significant increase in progression free survival. This difference was only observed in the vaccine arm and not in the standard of care alone arm. A separate assessment is required in prostate cancer and will be done in this study. If similar findings are seen, the Immunoscore could be prospectively evaluated in future trials using the data from this study to establish which immune subsets are most relevant.

1.2.10 Rational for Determining PSA Response

It has been noted previously that immunotherapy may have delayed clinical impact and preliminary data with prostvac alone in this population supports that hypothesis. This data comes from the ongoing NCI study: Prostvac in Patients with Biochemically Recurrent Prostate Cancer (NCT02649439). Of the first 20 patients enrolled, 18 are evaluable for PSA responses >9 months after enrollment (2 patients withdrew from the study and are not included). In a preliminary analysis, those patients that ultimately experienced a PSA decline had an intra study apex PSA determined. The intra study apex PSA is defined as the maximum on-study PSA that can be corroborated by another on-study PSA value within 10%. PSA values beyond 10% may represent lab error and therefore are not used to determine the intra-study apex (see **Figure 6** below).

Figure 6:



Of those 18 evaluable patients, 5 (27.8%) have had confirmed (at least 2 consecutive) PSA declines from an intra-study apex PSA with 3 declines being confirmed at ≥30% (see **Figure 7**). Two additional patients have unconfirmed declines, but are still being followed for confirmation. It is of note because of the 10 patients on the study thus far who had monthly PSAs evaluated as part of a protocol mandated surveillance cohort, no patients had any similar confirmed declines >10%. These findings suggest that late effects of the vaccine may impact PSA in this population.

PSA declines similar to this have not been reported previously in this population with immunotherapy, but this approach (using the intra-study apex PSA) may be a reasonable way to assess clinical impact of immunotherapy in this setting. Correlative immune data could also support biomarker development in this population and have broader applications to other types of cancer. This proposed trial of combination immunotherapy in biochemically recurrent prostate cancer represents a reasonable follow-up to the current study (expected to complete accrual at the NCI by Quarter 3, 2017).

Figure 7:

Declines from Intra-Study Apex
in 7 of first 18 Evaluable Patients

Patient #	% Decline from Intra-Study Apex
1	**99.9%**
7	**30.0%**
12	12.8% (unconfirmed)
14	13.6%
15	**48.8%**
18	19.4% (unconfirmed)
19	15.4%

10 Patients on Surveillance for 6 months – no patients had similar confirmed declines

1.2.11 Rationale for Research PET Scans

When logistically feasible, PET imaging will be done on patients in the BCR cohort as part of this study to evaluate for possible changes in PET images over time as they relate to PSA. In addition, such imaging could help identify patient who may have responses (e.g., patients with negative scans on other imaging, less tumor/less bone-based disease).

1.2.12 ^{18}F-DCFPyL-PET/CT

PET imaging based on prostate specific membrane antigen (PSMA), including use of the radiotracer DCFPyL (Pylarify; manufacturer: Lantheus), which binds to PSMA, has emerged as a sensitive modality to detect localized and metastatic prostate cancer. ^{18}F-DCFPyL is a second generation, high affinity agent that has shown outstanding efficacy in detecting prostate cancer, that received FDA approval in May 2021.

^{18}F-DCFPyL appears to be a sensitive and specific modality for staging prostate cancer and molecular analyses are underway as correlates to previous trials to identify which patients are most likely to be exceptional responders. Alternatively, early ^{18}F-DCFPyL PET/CT scan (e.g., after ~2 months) may predict which patients go on to have exceptional responses. Thus, there is clinical benefit that could be derived from the ability of ^{18}F-DCFPyL PET/CT imaging to monitor response to therapy.

1.2.13 Toxicity Assessment of Patients in Lead-In Cohort

As planned, 6 patients were enrolled in the lead in cohort. As of December 21, 2018, the first three patients were removed from treatment for Progressive Disease. All six patients have completed the 6-week toxicity assessment period and no SAEs were seen. Additionally, none of the toxicities listed under the early stopping rules in section 8.4.1 have occurred on this trial. Of note, an asymptomatic increased Lipase - Grade 3, was captured on subject #1; treatment was not held as per protocol section 3.3.3. The AE resolved on its own without clinical symptoms. In addition, patient 6 had a brief episode of hematochezia (but no associated diarrhea). Out of an abundance of caution he was evaluated with a colonoscopy that demonstrated internal hemorrhoids and diverticulitis but no findings consistent with autoimmune colitis. The patient then continued all therapies. In addition, there have been no unexpected AEs to this point. At this

point, there is too little experience to make an estimation of clinical benefit, but there have been no unexpected safety signals therefore, we will initiate enrollment of BCR cohort per protocol design.

1.2.14 Rationale for removing MSB0011359C (M7824)

We have decided to remove Bintrafusp Alfa from this study based on a global assessment of the risks and benefits of this agent in this population of cancer patients who have an expected median survival of approximately 10 years. Through the first six patients treated with this agent in the biochemical recurrence (main cohort), we saw one case of colitis and one case of myocarditis. The latter required extensive steroid dosing to resolve and the patient continues to have residual side effects from the high doses of steroids. Furthermore, through the first 6 patients in biochemical recurrence cohort and the first 6 patients of castration resistant prostate cancer, there was no clear clinical impact of the Bintrafusp Alfa in any of the patients (no radiographic changes or PSA declines). The use of Bintrafusp Alfa was reviewed by the Clinical Immunotherapy Team here at the NCI in a specific toxicity meeting in May 2021.

Treatment regimen without an immune checkpoint inhibitor was considered to be optimal for patients of castration resistant prostate cancer. Given the longevity of this population, the favorable response without immune check inhibitor and the life expectancy of this population assessing a risk/benefit analysis, it was decided that Bintrafusp Alfa will be removed.

The rationale leading to this decision was discussed and approved [redacted].

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria for All Subjects

2.1.1.1 Histopathological documentation of prostate cancer confirmed in either the Laboratory of Pathology at the National Institutes of Health (NIH) Clinical Center, or Walter Reed National Military Medical Center prior to enrollment. If no pathologic specimen is available, participants may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.

2.1.1.2 Recovery to baseline from acute toxicity related to prior therapy, including surgery and radiation (28 days removed from last systemic therapy, 14 days removed from last radiation therapy).

- Hepatic function eligibility parameters (within 16 days before starting therapy):
    - Bilirubin ≤ ULN (OR in participants with Gilbert's syndrome, a total bilirubin ≤ 3.0), AST and ALT ≤ 1.5 times upper limit of normal.

2.1.1.3 Adequate renal function defined by an estimated creatinine clearance > 50 mL/min according to the Cockcroft-Gault formula or by measure of creatinine clearance from 24 hour urine collection.

2.1.1.4 No other active malignancies within the past 36 months (with the exception of nonmelanoma skin cancers or carcinoma in situ of the bladder) or life-threatening illnesses.

2.1.1.5 Willing to travel to the NIH for follow-up visits.

2.1.1.6 18 years of age or older.

2.1.1.7 Able to understand and sign informed consent.

2.1.1.8 The effects Prostvac and CV301 on the developing human fetus are unknown. For this reason, men must agree to use highly effective contraception (that is, methods with a failure rate of less than 1% per year) prior to study entry, for the duration of study therapy and at least four months after the last treatment administration. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.2 Additional Inclusion Criteria Specific to Safety Lead-In Cohort

- Castrate testosterone level (<50ng/dl or 1.7nmol /L)
- Progressive disease at study entry defined as one or more of the following criteria occurring in the setting of castrate levels of testosterone:
    i. Radiographic progression defined as **any new** or enlarging bone lesions or growing lymph node disease, consistent with prostate cancer

    OR

    ii. PSA progression defined by sequence of rising values separated by >1 week (2 separate increasing values over a minimum of 2ng/ml (PCWG2 PSA eligibility criteria). If participants had been on flutamide, PSA progression is documented 4 weeks or more after withdrawal. For participants on bicalutamide or nilutamide disease progression is documented 6 or more weeks after withdrawal.
- Participants must agree to continuation of androgen deprivation therapy (ADT) with a gonadotropin-releasing hormone agonist/antagonist or bilateral orchiectomy
- ECOG performance status of 0–2 (Karnofsky >80%, see **APPENDIX A**).
- Hematological eligibility parameters (within 16 days before starting therapy; see **APPENDIX D**):
    - Granulocyte count ≥ 1000/mm3
    - Platelet count ≥ 100 000/mm3
    - Hgb ≥ 9 g/dL
    - PT ≤ 1.5 x ULN
    - aPTT ≤ 1.5 x ULN

2.1.3 Additional Inclusion Criteria Specific to Biochemical Recurrence Cohort

2.1.3.1 Biochemical progression defined as follows:

- For participants following definitive radiation therapy: a rise in PSA of ≥ 2 ng/mL above the nadir (per RTOG-ASTRO consensus criteria)
- For participants following radical prostatectomy: rising PSA after surgical procedure (participants must have a PSA ≥ 0.8 ng/mL)

2.1.3.2 Participants must have a rising PSA as confirmed by 3 values a minimum of 1 week apart over at least a 1 month period of time.

2.1.3.3 Participants must have a PSA doubling time of 5-15 months.

2.1.3.4 ECOG performance status of 0–1 (Karnofsky ≥ 80%, see **APPENDIX A**).

2.1.3.5 Negative CT scan/MRI and bone scan for metastatic prostate cancer.

2.1.3.6 Baseline testosterone ≥ 100 ng/dl.

2.1.3.7 PSA ≤ 30 ng/mL.

2.1.3.8 Hematological eligibility parameters (within 16 days before starting therapy; see **APPENDIX D**):

- Granulocyte count ≥ 1000/mm^3
- Platelet count ≥ 100 000/mm^3
- Hgb ≥ 10 g/dL

2.1.4 Exclusion Criteria

2.1.4.1 Immunocompromised status due to:

- Human immunodeficiency virus (HIV) positivity.
- Active autoimmune diseases such as Addison's disease, Hashimoto's thyroiditis, systemic lupus erythematosus, Sjogren syndrome, scleroderma, myasthenia gravis, Goodpasture syndrome or active Grave's disease.
  - Participants with a history of autoimmunity that has not required systemic immunosuppressive therapy or does not threaten vital organ function including CNS, heart, lungs, kidneys, skin, and GI tract will be allowed.
  - Participants with diabetes type I, vitiligo, or alopecia are allowed.
- Other immunodeficiency diseases
- Splenectomy

2.1.4.2 Receipt of any organ transplantation, including allogeneic stem-cell transplantation, but with the exception of transplants that do not require immunosuppression (e.g., corneal transplant, hair transplant).

2.1.4.3 Chronic administration (defined as daily or every other day for continued use > 14 days) of systemic corticosteroids within 28 days before the first planned dose of

investigational therapy. Use of corticosteroids with minimal systemic absorption (e.g., inhaled steroids, nasal sprays, and topical agents) is allowed.

2.1.4.4 Serious intercurrent medical illness that, in the judgment of the investigator, would interfere with participant's ability to carry out the treatment program.

2.1.4.5 Other medications used for urinary symptoms including 5-alpha reductase inhibitors (finasteride and dutasteride) and alternative medications known to alter PSA (e.g., phytoestrogens and saw palmetto).

2.1.4.6 History of prior chemotherapy (chemotherapy allowed for lead-in cohort in castration resistant disease.)

2.1.4.7 History of prior immunotherapy within the last 3 years (immunotherapy allowed for lead-in cohort in castration resistant disease.)

2.1.4.8 Receipt of an investigational agent within 28 days (or 56 days for an antibody-based therapy) before the first planned dose of study drugs.

2.1.4.9 Major surgery within 4 weeks prior to enrollment

2.1.4.10 History of allergic reactions attributed to compounds of similar chemical or biologic composition to poxviral vaccines (e.g., vaccinia vaccine)

2.1.4.11 Previous serious adverse reactions to smallpox vaccination

2.1.4.12 History of allergic reactions attributed to monoclonal antibodies (grade ≥ 3)

2.1.4.13 Known allergy to eggs, egg products, aminoglycoside antibiotics (e.g., gentamicin or tobramycin).

2.1.4.14 History of atopic dermatitis or active skin condition (acute, chronic, exfoliative) that disrupts the epidermis

2.1.4.15 Unable to avoid close contact or household contact with the following high-risk individuals for three weeks after the Day 1 vaccination: (a) children ≤ 3 years of age, (b) pregnant or nursing women, (c) individuals with prior or concurrent extensive eczema or other eczemoid skin disorders, or (d) immunocompromised individuals, such as those with HIV.

2.1.4.16 Participants who test positive for HBV or HCV

2.1.4.17 Clinically significant cardiovascular/cerebrovascular disease as follows: cerebral vascular accident/stroke (< 6 months prior to the first planned dose of study drugs), myocardial infarction (< 6 months prior to the first planned dose of study drugs), unstable angina, congestive heart failure (New York Heart Association Classification

Class ≥ II), serious cardiac arrhythmia, or uncontrolled hypertension (SBP>170/ DBP>105).

2.1.4.18 Participants who have received a red cell transfusion within 2 weeks prior to enrollment.

2.1.4.19 Participants unwilling to accept blood products as medically indicated.

2.1.4.20 Individual tumor lesion(s) in the liver or chest which are 10 cm or larger.

2.1.5 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 available websites (www.clinicaltrials.gov, https://www.cancer.gov/about-cancer/treatment/clinical-trials/search) and participants will be recruited from the current patient population at NIH.

2.2 SCREENING/ELIGIBILITY EVALUATION

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

A. Pathological confirmation of diagnosis by either the Laboratory of Pathology at the NIH or Walter Reed National Military Medical Center may be obtained any time prior to enrollment (if specimen is available). If no pathologic specimen is available, participants may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.

B. The following parameters will be obtained within 8 weeks prior to start of enrollment:

1. HIV test (anti-HIV 1/2 antibody)
2. Hepatitis B and C (HBs AG screening and anti-HCV antibody)
3. Tc-99 whole-body scintigraphy
4. CT (or MRI may be substituted at investigator's discretion) of chest, abdomen and pelvis

C. The following parameters will be obtained within 16 days prior to start of enrollment:

1. History and physical examination with ECOG
2. Serum PSA
3. Complete blood count plus differential, and platelet count
4. Hepatic Panel (Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin)
5. Acute Care Panel (Sodium [NA], Potassium [K], Chloride [CL], Total CO2 [Bicarbonate], Creatinine, Glucose, Urea Nitrogen, eGFR)
6. Testosterone level
7. 24-hour urine collection if creatinine clearance to be confirmed by this method

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 Treatment Assignment Procedures (for registration purposes only)

Cohorts

Number	Name	Description
1	*Lead-In Cohort* ***(closed December 2018)***	*Participants with advanced castration resistant prostate cancer (first six participants)*
2	*Biochemical Recurrence Cohort*	*Participants with biochemically recurrent prostate cancer*

Arms

Number	Name	Description
1	*Combination therapy* ***(closed December 2018)***	*Prostvac + CV301+ MSB0011359C*
2	*Combination therapy + surveillance* ***(closed)***	*Surveillance followed by Prostvac + CV301 then Prostvac + CV301 + MSB0011359C*
3	*Combination vaccine therapy+/- surveillance*	*Surveillance as needed followed by Prostvac + CV301*

Arm Assignment

Participants in cohort 1 will be directly assigned to Arm 1 of the study. This cohort has been closed as of December 2018.

Participants in cohort 2 will be directly assigned to Arm 2, or Arm 3 of the study based upon time of enrollment. Participants enrolling after approval of amendment dated 7/29/2021 are assigned to Arm 3.

Please note: As of December 21, 2018, the 6 participants in the lead in cohort have completed the 6-week toxicity assessment period and no SAEs were seen. Per the protocol design participants will now be enrolled to the BCR cohort. The toxicity assessment of participants in the lead-in cohort is described in section **1.2.13**.

2.4 BASELINE EVALUATION

A. The following parameters will be obtained **within 8 weeks** prior to enrollment:
   1. Baseline electrocardiogram (EKG) on all participants, and appropriate cardiologic evaluation, as clinically indicated, to provide baseline function and identify any participants who should be monitored closely for cardiac risks associated with vaccinia vaccination.

B. The following parameters will be obtained **within 16 days** prior to start of treatment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe):

1. Clinical evaluation
   - History and physical examination

- ECOG performance status (see **APPENDIX A**)
- Height, weight

2. Laboratory studies
   - Serum PSA
   - Serum testosterone level
   - CBC/differential, with platelet count
   - Serum chemistries (Na^+, K^+, Cl^-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
   - Serum amylase and lipase
   - Serum thyroid stimulating hormone (TSH)
   - Lymphocyte phenotyping CD3/CD4/CD8
   - Urinalysis in participants unless not feasible (i.e.. participant has incontinence)
   - Prostate acid phosphatase (PAP)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a three-arm, open-label trial of combination immunotherapy in participants with biochemical recurrent prostate cancer. 37 evaluable participants will be enrolled (after the Safety Lead-In cohort with 6 participants (arm 1), 6 participants who received M7824 as part of the initial investigation for PSA response (arm 2), and 25 homogenously treated participants (arm 3) with an accrual ceiling of 40 participants.

A 6-participant phase I lead in cohort in advanced castration resistant prostate cancer participants will be conducted to determine safety. Dose levels for the lead in cohort are equivalent to the biochemical recurrence cohort. Participants will not have an observation period for the safety lead-in; dosing will begin after eligibility is confirmed (see Study Calendar in Section **18.2**). Once all 6 participants have completed a 6-week toxicity assessment period the toxicity data will be shared with IRB before moving forward with the planned study in biochemical recurrence. Participants may remain on treatment with vaccines in the lead in cohort as long as the participant is willing and their disease remains stable on follow-up imaging. As of December 21, 2018, the 6 participants in the lead in cohort have completed the 6-week toxicity assessment period and no SAEs were seen; therefore this cohort is closed.

Participants enrolled in Cohort 2 will undergo surveillance with four consecutive monthly PSA checks followed by Prostvac and CV301 during months 1-4 then Prostvac and CV301 during months 5-7.

- Existing participants with biochemical recurrent prostate cancer undergoing surveillance and PSA monitoring at the NIH may be enrolled at any point during the surveillance period provided the PSA monitoring is consistent with PSA monitoring in the Study Calendar (**APPENDIX C**) and the participant meets all other eligibility criteria. For example, if an eligible participant has 3 monthly PSA values, he may be enrolled onto the study, obtain one additional PSA value at a one month interval to complete the surveillance period, then

begin C1D1 with vaccine therapy. In this case, the 3 PSA values obtained prior to enrollment will be evaluated as if they were collected during the surveillance period while enrolled and will factor into the determination of the intra-study apex value (See Section **6.3.2**) highest PSA that is within 10% of another peak PSA for the participant on-study. Any other peak PSA values that are beyond 10% of other PSA values on the study will be determined as potential lab variability and excluded from response assessment.

- With Amendment v05/10/2023, participants that have at least 3 PSA measurements in the surveillance period, with at least one of them sufficient to provide the doubling time from baseline, will not undergo any further PSA measurements in the surveillance period.

Prostvac will be administered nearly identical to the Phase III dosing with Prostvac-V given day 1 (week 1) followed by Prostvac-F on weeks 3 and 5 then monthly for an additional 5 months. Therefore, Prostvac-V is given one time (priming vaccine) followed by 7 doses of Prostvac-F (booster vaccine). The administration schema for CV301 is representative of the Phase I dose escalation trial. MVA-BN-CV301 (priming vaccine) is administered on day 1 (week 1) and week 3 followed by FPV-CV301 (booster vaccine) administered on week 5 then monthly for an additional 5 months.

MSB0011359C (anti-PDL1 TGFβ Trap) will be administered at the recommended phase II dose of 1200mg by IV infusion every 2 weeks for 3 months (6 doses) starting after 4 months of concurrent Prostvac and CV301.

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3). This cohort will now receive Prostvac and CV301 during months 1-7 following surveillance.

3.1.1 Safety Assessment

For safety purposes, a minimum of 6 weeks must elapse between C1D1 of the third participant enrolled and C1D1 of the fourth participant enrolled which will allow for an adequate toxicity evaluation period of the combination therapies (i.e., 3 participants will have received combination vaccine and anti-PDL1 TGFβ Trap before any additional participants will be treated with that combination).

3.2 DRUG ADMINISTRATION

3.2.1 Prostvac

- The Prostvac vaccine regimen consists of Prostvac-V (priming vaccine) and Prostvac-F (booster vaccine).
- Prostvac will be administered nearly identical to the Phase III regimen with Prostvac-V ($2x10^8$ infectious units per 0.5 mL) given day 1 (week 1) followed by Prostvac-F ($1x10^9$ infectious units per 0.5 mL) on weeks 3 and 5 then monthly for an additional 5 months.
- Prostvac is administered subcutaneously preferably in the upper thigh. Alternate subcutaneous sites may be used with approval of the principal investigator.
- Prostvac should be handled according to the guidelines outlined by each department. Study staff administering the vaccine or assessing the vaccine site should wear personal protection consistent with the standard of practice outlined by each department or unit. Participants receiving Prostvac-V should be isolated just prior to the administration of

vaccine and can be removed from isolation after the vaccine is administered and an appropriate sterile dry dressing is secured over the injection site.

- Participants should be provided with the Vaccinia-Prostvac Participant Instruction Sheet at the time of or prior to administration of Prostvac-V (**APPENDIX B**).

3.2.2 CV301

- The CV301 vaccine regimen consists of MVA-BN-CV301 (priming vaccine) and FPV-CV301 (booster vaccine).
- MVA-BN-CV301 is administered as four subcutaneous injections (4 x 10^8 infectious units/0.5 mL each) on C1D1 and C1D15 followed by FPV-CV301 (1 x 10^9 infectious units/ 0.5mL) administered on C2D1 then monthly for an additional 5 months.
    - MVA-BN-CV301 is administered as one injection in each of the following locations: upper left arm, upper right arm, upper left thigh and upper right thigh (i.e., four injection sites per dose). Alternate subcutaneous sites may be used with approval of the principal investigator.
    - FPV-CV301 is administered as a subcutaneous injection preferably in the upper arm (i.e., one injection site per dose). An alternate subcutaneous site may be used with approval of the principal investigator.

3.2.3 MSB0011359C

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3).

- MSB0011359C (anti-PDL1 TGFβ Trap) will be administered at a dose of 1200mg by IV infusion over 60 minutes every 2 weeks for 3 months (6 doses) starting after 4 months of concurrent Prostvac and CV301. Use of an in-line 0.2 or 0.22 micron filter during infusion administration is required.
- Current experience revealed that infusion related reactions (IRRs) to MSB0011359C seldom occur and are generally mild to moderate in severity. Therefore, administration of a premedication is generally not required.
    - If an Investigator deems it necessary to administer a premedication to a particular participant, an antihistamine (e.g., diphenhydramine 25-50mg and acetaminophen 650mg intravenously or equivalent oral dose) is recommended approximately 30 - 60 minutes prior to the MSB0011359C infusion. If Grade ≥ 2 infusion reactions are seen during the first two infusions, premedication should not be stopped. Premedication regimens may be adjusted based on local site procedures or participant-specific factors. Steroids as premedication are not permitted.
- Infusion-related reactions to MSB0011359C
    - Participants experiencing a grade 4 infusion related reaction will have the infusion stopped immediately and will not be rechallenged.

- Participants experiencing a ≥ grade 2 infusion related reaction will have the infusion duration extended to 120 minutes for remainder of that infusion and all subsequent infusions.

- MSB0011359C 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.

3.2.4 Administering multiple agents

- When CV301 and Prostvac are administered concurrently, it is preferable that CV301 be administered prior to Prostvac. When CV301 and Prostvac are administered in the same appendage, the vaccines must be administered a minimum of 2 inches apart. The sterile dry dressing to cover the Prostvac-V site should NOT overlap or cover the CV301 injection site.

- When CV301, Prostvac, and MSB001359C are administered concurrently, the following order of administration is preferred: CV301 followed by Prostvac followed by the MSB0011359C infusion. Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3).

3.3 TREATMENT MODIFICATIONS

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3).

3.3.1 Dose modifications

Dose reductions or dose escalations are not permitted for Prostvac, CV301 or MSB0011359C.

3.3.2 Dosing delays

To account for scheduling or logistical issues, the scheduled vaccine may be given ± 3 days from the scheduled administration date in month one of vaccine combination. Once participants are beyond C2D1, there will be a ±7 day window for scheduling to allow for logistical and travel constraints. If the vaccines or anti-PDL1 TGFβ Trap are not given within this window, it will be considered a missed dose and the next dose will be given at the planned administration date. If one agent is delayed, all agents given concurrently should also be delayed so the agents are administered on the same day. However, if a dose of one agent is missed for a toxicity unequivocally attributed to that agent, the other agents may be administered as scheduled. If for any reason, the immunotherapy regimen is held for >56 days, the participant will not receive further treatment and will be removed from the protocol.

The immunotherapy regimen must be delayed for the following drug-related AEs:

- Any grade ≥ 2 drug-related AE with the following exceptions
  - Grade 2 drug-related fatigue, dermatologic AEs, and asymptomatic laboratory abnormalities do not require a treatment delay

- Grade 2 electrolyte abnormalities that can be managed with replacement therapy do not require a treatment delay
- Grade 2 hypothyroidism that can be managed with replacement therapy does not require a treatment delay
- Grade 2 lymphopenia or leukopenia does not require a dose delay.
- Asymptomatic ALT/AST or amylase/lipase elevation not associated with clinical manifestations does not require a dose delay. Increased monitoring may be considered.

- Any grade 3 drug-related AE with the following exceptions
  - Grade 3 lymphopenia or leukopenia does not require a dose delay
  - Grade 3 asymptomatic electrolyte abnormalities that can be managed with replacement therapy do not require a treatment delay
  - Asymptomatic grade 3 amylase or lipase elevation not associated with clinical manifestations of pancreatitis does not require a dose delay. Increased monitoring is required.

Criteria for resuming the immunotherapy regimen:

- Participants with a required delay in treatment may resume the immunotherapy regimen when the drug-related AEs resolve to grade ≤ 1 or baseline with the following exceptions
  - Participants who develop grade 3 injection site reactions will have their vaccine(s) held until injection site reaction resolves to grade ≤ 2.
  - Participants with a grade 3 dermatologic AE may resume when their skin toxicity resolves to grade ≤ 2.

3.3.2.1 MSB0011359C

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3).

3.4 PROTOCOL EVALUATION

NOTE: Also see **APPENDIX C**.

- All participants who are deemed eligible and who sign an informed consent will be enrolled in this trial.
- A complete history and physical examination, including ECOG performance status, will be done within 16 days before enrollment.
- Laboratory Studies (See also **APPENDIX C**)
  - Serum PSA
  - CBC/differential with platelet count
  - Serum testosterone level
  - Serum chemistries (Na^+, K^+, Cl^-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
  - Serum amylase and lipase
  - Serum thyroid stimulating hormone (TSH)

- HIV/Hepatitis B/Hepatitis C tests (within 8 weeks prior to start of enrollment)
- Lymphocyte phenotyping CD3/CD4/CD8
- PAP (prostatic acid phosphatase)
- Urinalysis unless not feasible (i.e., participant has incontinence)

3.4.1 Radiographic assessment

Participants will undergo scheduled radiographic assessment for metastatic disease at screening, prior to vaccine therapy and, at the end of study therapy, then every 6 months thereafter as long as participants remain in follow-up. After year 1, the PI has the discretion to perform scans q6 months or as clinically indicated. Radiologic studies consisting of bone scan, CT scan of chest, and CT scan of abdomen/pelvis will be performed at baseline. MRI may be substituted for CT scan at the discretion of the investigator. The window for the scans is ± 21 days,

For participants in the BCR cohort, NaF PET scans will be obtained when feasible at any point during the up to 4-month surveillance period and at the post treatment follow up visit. If a NaF PET scan was done prior to consent within 8 weeks, it will not need to be repeated during the surveillance period.

3.4.1.1 Imaging with ^{18}F-DCFPyL (Pylarify)

When feasible, participants in the BCR cohort will undergo two ^{18}F-DCFPyL PET/CT scans: one in the first two months during surveillance and within 8 weeks after completing treatment. All PET data sets will be reviewed for quality and completeness. Participants will be instructed to maintain good hydration for the 24 hours prior to the ^{18}F-DCFPyL PET/CT (recommended 1-2 liters of fluid, unless medically contraindicated). There are no dietary or food restrictions.

Participants will report to the appropriate imaging center on the day of their ^{18}F-DCFPyL PET/CT imaging session and peripheral venous access will be obtained.

A static whole-body PET/CT will be performed 1 hour (± 15 minutes) post injection of ^{18}F-DCFPyL. Only a single injection of ^{18}F-DCFPyL is required.

3.4.2 Assessment for immunotherapy administration

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3).

Dosing and administration of CV-301, Prostvac, and MSB0011359C will be performed in the Clinical Center Oncology Day Hospital or on a 3rd floor oncology inpatient unit. Participants will be monitored with vital signs (blood pressure, heart rate, respiratory rate, temperature) prior to and within 1 hour after the initial vaccine treatment. On subsequent vaccine visits, participants will have vital signs checked prior to vaccine administration and then be monitored for 30 minutes after vaccine treatments (post-vaccine vital signs will not be required except for the first vaccination). When all three agents (CV-301, Prostvac, MSB011359C) are administered concurrently, vital signs will be monitored prior to vaccine administration and within 1 hour after the completion of the MSB011359C infusion. Documentation of any participant reported symptoms occurring between dosing will be included in the assessment. Laboratory assessments will be conducted as per **APPENDIX C**.

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

Participants will not be compensated on 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

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 28 days (± 7 days) following the last dose of study therapy.

If a participant experiences a toxicity that can unequivocally be attributed to one agent, he may continue on the remaining investigational agents at the discretion of the principal investigator.

3.6.1 Criteria for removal from protocol therapy

Participants will be removed from treatment for the following:

- Clinical or radiographic progression of disease as defined in Section **6.3.1**. (Clinical progression includes the initiation of ADT in participants with biochemical recurrent prostate cancer.)
- AE-related treatment delays lasting > 56 days, as defined in Section **3.3.2**
- Any Grade 4 toxicity that is possibly, probably or definitely related to the protocol treatment will require a participant to be off-treatment
- If at any time the constraints of this protocol are detrimental to the participant's health, the participant may be removed from protocol therapy and reasons for withdrawal will be documented
- Requirement for androgen deprivation therapy
- Completion of protocol therapy

3.6.2 Off-Study Criteria

- For lead-in cohort, subjects will be taken off study at the completion of safety follow-up visit/period.
- Participant requests to be taken off study. Reasons for withdrawal will be documented.
- Requirement for androgen deprivation therapy

- Noncompliance with protocol guidelines (participant removed at discretion of Principal Investigator)
- Investigator decision to end the study
- Death

3.7 FOLLOW-UP EVALUATIONS

After subjects have stopped taking the study medication for any of the reasons listed in Section **3.6.1**, they will be seen at the study site, if logistically feasible, for a safety visit approximately 28 days (+/- 14 days) after drug discontinuation. For the biochemical recurrence cohort, follow up will continue monthly for the first five months. For those participants who remain on study 5 months beyond their last treatment follow-up visits could be conducted at 4-8 week intervals.

The following assessments will be performed at the follow up safety visit:

- History and Physical Examination
- Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
- Serum amylase/lipase
- Serum TSH
- CBC/differential with platelet count
- Serum PSA level
- Serum PAP
- Adverse event reporting
- NaF PET scan (for Participants in the BCR cohort)

After the safety visit, if there are no unresolved grade 3 or higher AEs, we may, when feasible contact the participant annually to find out how they are doing and to determine survival status. If there are unresolved grade 3 – 4 AEs and participants cannot return for logistical reasons or refuse follow-up at NIH, participants may be followed by their local physician. In the latter case, we will obtain the physician's medical notes and we will assess the status of the participant's adverse events based on the physician's reports.

Any scans performed outside of the study site will also be obtained when possible.

4 CONCOMITANT MEDICATIONS

4.1 EXCLUDED MEDICATIONS

- Concurrent hormonal therapy, anticancer treatment with chemotherapy, radiation therapy, major surgical procedures for prostate cancer, anti-cancer radionuclides and non-protocol-related immunotherapy will not be permitted.
- Systemic corticosteroids (daily or every other day for continued use > 14 days) will not be permitted. Participants requiring systemic steroids for treatment of immune-related AEs may remain on-study at the discretion of the investigator. Corticosteroids with minimal

systemic absorption (e.g., topical and inhaled steroids) are allowed. Ophthalmologic steroids will not be permitted within 3 days prior to and within one month after receiving the Prostvac-V injection.

- Vaccination with live vaccines other than the investigational agents is prohibited (e.g., Zostavax). Administration of inactivated vaccines is allowed (e.g., inactivated influenza vaccine). Locally approved COVID vaccines are permitted.

4.2 SUPPORTIVE CARE

4.2.1 General supportive care

For the administration of Prostvac and CV301 , antiemetics, stool softeners and antidiarrheal agents may be administered as required but are not anticipated to be needed and should not be used prophylactically on the first cycle. The selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens will not include steroids.

Other supportive care with blood components, analgesics, general medical therapy, etc., will be delivered as required. Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm3. If invasive procedures are planned or the participant develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of > 50,000/mm3.

Any evidence of disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) including thrombocytopenia, hemolytic anemia, renal failure, fever or neurologic changes should be thoroughly evaluated and closely monitored and supported as clinically indicated.

4.2.2 Antibiotics

Antibiotics will be delivered as required. Co-administration of protocol treatments can be done at the discretion of the investigator. Prophylactic antibiotics (e.g., prevention of urinary tract infections) may be allowed at the discretion of the investigator.

4.2.3 Infusion-Related Reactions

See section **3.2.3**.

4.2.4 Immune-Related Adverse Events (irAEs)

Immune-related AEs (irAEs) require early recognition and prompt intervention. If an irAE is suspected, all investigational agents must be held. See management algorithms in **Error! Reference source not found.**.

4.3 TREATMENT OF VACCINIA VACCINATION COMPLICATION

4.3.1 Vaccinia Immune Globulin:

First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in participants that are systemically ill and whose

condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG.

VIG is contraindicated, however, for the treatment of isolated vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. Upon receipt of a call from a participant or upon direct observation of a participant or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible:

1) to initiate review of the clinical case,
2) to seek consultation on the appropriateness of VIG therapy,
3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and
4) to determine how to access and have the appropriate doses of VIG delivered.

Early institution of VIG therapy is advised following recognition of clinical symptoms compatible with some vaccinia complications (eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of inadvertent inoculation). The effectiveness of VIG therapy appears to be time dependent. VIG has not proven to be of benefit in the treatment of post-vaccinial encephalitis, and is contraindicated for treatment of isolated vaccinial keratitis due to the increased risk of corneal scarring.

An intravenous formulation of VIG is available through the CDC, which has a lower level of aggregated protein, allowing it to be used by either the IM or IV route. This formulation will most likely be preferred for administration and investigators will be instructed by the CDC regarding appropriate dosing and method of administration based on formulation and availability. There is no guarantee that VIG will successfully treat complications. At present, there are no other anti-viral therapies of proven benefit for the treatment of vaccinia-related complications.

4.3.2 Cidofovir (Vistide®, Gilead Sciences):

Cidofovir is an FDA-approved antiviral drug for the treatment of CMV retinitis among participants with AIDS. Cell-based in vitro studies and animal model studies have demonstrated antiviral activity of this agent against certain orthopoxviruses. Currently, efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, "VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be released by the CDC after all inventories of VIG have been exhausted, after a participant fails to improve with VIG treatment, or as a last effort for a participant who is otherwise near death." [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia

Immune Globulin and Cidofovir. Last updated February 11, 2003. Available at:

https://www.cdc.gov/smallpox/clinicians/vaccine-medical-management6.html].

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

5.1.1 Immunologic Parameters

- Antibodies to PSA, vaccinia, fowlpox may be tested
- Leukocyte CD3, CD4, CD8 subsets; CD4:CD8 ratio will be drawn according to the Research Bloods schedule in the study calendar (see **APPENDIX C**) while the participant remains on trial.
- The results of the HIV antibody need to be available before treatment to determine eligibility.
- Additional studies will include but are not limited to quantitative and qualitative assessments of regulatory T-cells, Natural Killers cells, Myeloid Derived Suppressor Cells, anti-glycan antibodies and Naïve T-cell/new thymic emigrants.
- Assessment of levels of cytokines.
- Immunologic studies will be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined not to be treatment-related.

5.1.2 Summary of Sample Collection

Sample	**Collection Details***	**Collection point**	**Location of specimen analysis**
Blood sample for PBMC: Immune Monitoring	6 x 10 mL green top sodium heparin tubes (also listed in **APPENDIX D**)	See study calendar in **APPENDIX C**	Clinical Services Program NCI Frederick Research and Development Center
Blood sample for serum: Immune Monitoring	2 x 8 mL SST tubes (also listed in **APPENDIX D**)	See study calendar in **APPENDIX C**	Clinical Services Program NCI Frederick Research and Development Center
Stool Samples	Stool Collection Tubes	See study calendar in **APPENDIX C**	Figg Lab

*Tubes/media may be adjusted at the time of collection based upon materials available. The amount of blood that may be drawn from adult participants for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

NOTE: Portions of all samples may be banked for future research analyses; prospective consent will be obtained during the informed consent process.

5.1.3 Immunologic Assays

5.1.3.1 CD4 T Cell Proliferation Assay

It is planned that all participants will undergo exploratory analysis of the ability to detect CD4-positive responses using a whole-protein PSA assay, as well as a peptide mix with 63 different 15-mer peptides by ELISPOT and/or ELISA.

5.1.3.2 Sera Antibody Analysis

Serum will be stored at -80 degrees Celsius and there will be planned analysis for generation of antibodies to PSA, BCG, PAP, PSMA, PSCA, and/or MUC-1.

5.1.3.3 Flow cytometry analysis of thymic emigrant

To determine recent thymic emigrants, flow cytometry analysis will be performed on peripheral blood mononuclear cells. Cells will be resuspended in staining buffer (phosphate-buffered saline containing 3% fetal bovine serum) and stained for 30 minutes at 4°C with the combination of following antibodies: APC-H7-conjugated anti-CD4, PE-CY7-conjugated anti-CD3; FITC-conjugated CD45RA, PE-conjugated CD31, PerCP-CY5.5-conjugated Ki-67, AF-700-conjugated-CD197, V450-conjugated CD8, APC-conjugated CD103, V500-conjugated CD27 all purchased from BD Pharmingen, San Diego, CA). After that, FoxP3 intra-cellular staining will be performed on the cells stained with anti-CD4 and anti-CD25. They will be fixed and permeabilyzed using a fix/perm kit (eBioscience, San Diego, CA) according to the manufacturer's manual, and will be labeled with FITC-conjugated anti-Foxp3 antibody (236A/E7 clone) or its isotype control antibody (eBioscience). Flow cytometry will be performed on a Becton Dickinson LSRII (BD Biosciences) device.

5.1.3.4 Natural Killer (NK) Cells

The number and phenotype of NK cells will be determined by phenotypic analysis of PMBCs stained for CD56, CD3, CD8, and CD16 by flow cytometry.

5.1.3.5 Immune Subsets

Subsets of immune cells will also be followed in response to treatment.

5.1.3.6 Regulatory T Cells

Regulatory T cells have been shown to inhibit the activation and function of T cells that participate in antigen-specific immune responses. Higher levels of regulatory T-cells have been reported in the peripheral blood mononuclear cells of participants with several types of tumors. The number and phenotype of regulatory T-cells in peripheral blood mononuclear cells from participants in this study will be determined by 7-color flow cytometry analysis. Cells will be resuspended in staining buffer (phosphate-buffered saline containing 3% fetal bovine serum) and stained for CD4, CD25, CD127, FoxP3, CTLA-4, CD45RA, and CD8. The ratios between regulatory T-cells and CD4 effector cells and the ratios between regulatory T-cells and CD8 Effector cells will also be analyzed.

5.1.4 Stool (optional)

Participants will be asked to provide stool in order to evaluate changes in microbiome of gastrointestinal tract and correlate to changes in treatment, PSA and immune cell subpopulations.

5.1.5 Additional Assays

Blood samples may be used for additional research studies, which may include phenotypic and functional analysis of immune-cell subsets and analyses for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor associated antigens, and/or other markers

All samples will be labeled with the following identifier system.

- Participant's enrollment #
- Trial number
- Participant's initials

  Example: 01-ABC

These labels are used only to send the samples from the NIH Clinical Center to the NCI Frederick Central Repository. The NCI Repository will process all samples, appropriately discard the label on the blood tube, and then store the samples with unique identifiers, to which only NCI study personnel will have the code to link to participant specific clinical information. Samples will be tracked according to Section **5.3**.

5.2 COLLECTION OF SPECIMENS

5.2.1 Blood Samples

6 (10ML) Green Top Sodium Heparin Tubes for PBMC; 2 (8ML) SST Tubes for serum will be collected at the timepoints indicated in **APPENDIX C**.

5.2.2 Handling and Processing of Specimens

The samples will be processed/stored through:

Clinical Services Program
NCI Frederick Cancer Research and Development Center
[redacted]

On days samples are drawn, [redacted] at CSP should be notified [redacted] She will arrange same-day courier delivery of the specimens.

5.2.3 Stool (optional)

Stool specimens will be collected from willing participants during the surveillance period, after 3-4 months on treatment and after completion of 7 months of treatment. In some instances, subjects may be asked to ship collected stool specimens to the research team at NIH. In those instances, participants will be given instructions for home self-sampling of stool (**APPENDIX F** and **APPENDIX G**) as well as instructions to ship the specimen (**APPENDIX H**). Samples will be stored for future studies, including whole genome shotgun sequencing of host and/or microbial DNA.

Participants will receive a cooler for transporting to clinic and/ or shipping materials which include: one brown top container and one purple top container for specimen collection, a white cardboard box with shipping materials, two biohazard plastic bags and a return label. Participants will be instructed to write the date and time of the collection on both tubes. Participants will be

instructed to freeze the specimens for at least 4 hours prior to transporting/ shipping and to ship the samples back as soon as they can after freezing so that the research staff can process the samples. The sample will be shipped to the Blood Processing Core (BPC; Figg lab) prior to transfer of coded samples for analysis to:

[redacted]

5.2.4 Imaging Evaluations

An exploratory objective has been added to evaluate possible changes in PET images over time as they relate to PSA (i.e., NaF PET) (Section **3.4.1**). The procedures for performing the NaF PET will follow clinical policies, no special procedures apply to this additional assessment for research purposes.

5.2.5 Questionnaires (OPTIONAL)

5.2.5.1 Evaluating Quality of Life in the BCR Population

Little has been formally evaluated about the impact of PSA kinetics on participants with BCR. Beginning with amendment A, participants enrolled in the BCR cohort will be asked to complete a prostate cancer specific QOL survey with 18 questions (MAX-PC) as well as the FACT-P survey at the following time points: Baseline, start of vaccines, at month 4 and month 7 and approximately every 3 months thereafter.

Each survey will take approximately 5 minutes to complete and they will be administered in English. The surveys being used have been published and vetted as appropriate QOL tools [44-46].

5.3 SAMPLE STORAGE, TRACKING, AND DISPOSITION

5.3.1 Blood Samples/Immunologic Assays

Samples will be ordered in CRIS and tracked through Clinical Trial Data management system. Should a CRIS screen not be available, CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required. Any transfer of materials to other NIH or non-NIH investigators will occur following NIH Intramural Research Program guidelines. If the subject 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.

All data associated with the participant samples is protected by using a secure database. All samples drawn at the NIH Clinical Center will be transported to the NCI Frederick Central Repository by couriers.

Samples will be tracked and managed by Central Repository database. All samples will be stored in either a -20˚C or -80˚C freezer. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

A subcontractor manages the NCI Frederick Central Repositories under subcontract to Leidos Biomedical Research, Inc. NCI Frederick Central Repositories 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.

The subcontractor's role is limited to clinical research databases and repositories containing participant specimens. It does not conduct or have any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects.

The NCI Frederick Central Repository will accept only coded, linked samples and sample information. Protected information will not be 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 withdraw request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No participant-specific information is encoded in this ID.

Investigators are granted view, input, and withdraw 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.

Samples will be used for research analysis, including immunologic monitoring as outlined in Section **5.1**. All specimens for analysis will be requested from Leidos Biomedical, Inc. and will be delivered by Leidos Biomedical, Inc. couriers to the Laboratory of Tumor Immunology and Biology.

5.3.2 Stool Samples (Blood Processing Core/Figg Laboratory)

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.

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 participant 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 participant 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.).

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.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

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), and reported as such to the IRB. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

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.

5.3.3 Protocol Completion/Sample Destruction

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples, provided they have an IRB-approved protocol and participant consent.

Samples and associated data will be stored permanently unless the participant withdraws consent. If the participant 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. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Description of the scope of genetic/genomic analysis

Genomic sequence characterization is increasingly important in understanding tumor biology, host/microbial interactions, and skin conditions. The current study proposes to perform DNA sequencing on biologic samples, including stool samples – although the likelihood of incidental human genomic findings in stool sampling is expected to be very low/rare, the possibility for this is included in an abundance of caution.

The technology platforms used to interrogate genomic structure and function are dynamic and the specific methodology, e.g. DNA/RNA sequencing, DNA copy number analysis, DNA

methylation analysis, employed will be determined at the time that samples are ready for analysis.

5.4.1 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Confidentiality for genetic samples will be maintained as described (Section **5.3**). In addition, a Certificate of Confidentiality has been obtained for this study.

5.4.2 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. Subjects will be contacted at this time with a request to provide a sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

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

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

- Eligible participants must be confirmed and checklist completed. Consent form must be signed prior to registration with Central Registration Information Services.
- Data will be secured in a 21 CR-Part 11-compliant data capture system provided by the NCI CCR. Data will be collected using protocol-specific case report forms, and verified for accuracy and completeness. Hard copies of data will be stored in locked secured areas and data will be entered onto a secured electronic data base. The following protocol-specific study forms will be complete and stored: eligibility checklist (developed by Central Registration Office, CRO). A copy of all serious AE forms will be kept in the research record.
- Treatment is given according to protocol (dated notes about doses given, complications, and clinical outcomes).
- Toxicity is assessed according to protocol (laboratory report slips, etc.)
- Response is assessed according to protocol (X-ray, scan, lab reports, and date noted on clinical assessment, as appropriate).
- Drug Accountability Records are kept for each participant.

The PI will be responsible for overseeing entry of data into a 21 CR-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 intervention, Study Day 1, through 28 days after last dose of study treatment.

An abnormal laboratory value will be considered an AE 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
- 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).
- Coded, linked or identified 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. Insert name or names:__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

Response assessments will be performed per the schedules in the study calendars in **APPENDIX C**.

6.3.1 Disease Progression

- PSA will not be used to measure progression of disease. Participants will be offered androgen deprivation therapy (ADT) as clinically indicated. Starting ADT will be done at the discretion of the investigator, although this will result in treatment termination and removal from the clinical trial. Participants may go on ADT at any time, although this may result in removal from the trial. PSA will be used to calculate PSA kinetics/tumor growth kinetics.
- Development of a new bone lesion on bone scan.
- Development of a soft tissue mass, identified on CT scan or physical exam, consistent with metastatic prostate cancer. If identified on physical exam, the lesion may be biopsied to confirm the presence of prostate cancer.
- Development of urethral, ureteral, or spinal cord obstruction secondary to tumor.
- Development of cytologically positive pleural effusion or lymphangitic spread in the lungs.
- Symptoms which in the opinion of the investigator are consistent with clinical progression.

6.3.2 Intra-study PSA response

Participants will be monitored for PSA kinetics once enrolled. Participants who have an intra study decline in PSA will be evaluated for PSA response. Such responses would be defined by an intra-study apex PSA, which will be established by determining the maximum PSA value that can be validated by another intra-study PSA value within 10%. (The goal here is to not capture lab variations which may be seen as isolated PSA spikes (i.e. greater than 10%) in the context of participant follow-up. Responders will then be evaluated using confirmed PSA declines (at least 2 consecutive values after the intra-study apex) of at least 30%. Participants with intra-study PSA declines greater than 50% will also be identified.

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 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING/DATA AND SAFETY MONITORIING 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 **s**hould 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 PROSTVAC Vaccine 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 PROSTVAC Vaccine, but are not fatal or life-threatening, much 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 information described below shall be submitted. 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.

7.4.2.1 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,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 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
- 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 Principal Investigator, lead associate investigator and the research nurse will meet weekly at each clinic to review all adverse events for each subject in this trial. Unexpected adverse events and/or serious adverse events will be reported to the NIH Intramural Institutional Review Board (IRB) and sponsor/FDA as outlined above. If trends are noted and/or risks warrant it, accrual will be interrupted, dose levels expanded and/or the protocol and/or consent will be modified accordingly.

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 **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 patient or research subject 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 4.0.

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

Adverse events of special interest (AESIs) are serious or nonserious AEs that are of clinical interest and should be closely followed.
AESIs include the 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 protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

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

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.

8.4 Waiver Of Expedited Reporting To CCR

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

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

All events listed below must be reported in the defined timelines to OSROSafety@mail.nih.gov. OSRO Safety will send all reports to the manufacturers as described below.

8.5.1 Reporting to [redacted] Research and Development Institute, Inc.

- Adverse events (AEs) which are considered Suspected, Unexpected Serious Adverse Events (SUSARs) will be reported as soon as they have been reported to the FDA in the same format (MedWatch 3500A or Council for International Organizations of Medical Services [CIOMS report].
- A copy of the FDA IND annual report or any other safety reports requested by the FDA.

Adverse event reports will be sent to [redacted] electronically at the following addresses (include as applicable-protocol number, subject number, site number/PI name and SAE/Onset date):

[redacted]

2. [redacted]

8.5.2 Reporting to [redacted].

The sponsor should also submit all safety reports that are sent to the FDA using the Medwatch 3500 Form (or equivalent) to:

[redacted]

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 Paternal Exposure

Male participants should refrain from fathering a child or donating sperm during the study and for four months after the last dose of the investigational immunotherapy agent. 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 four 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,
- the quality and integrity of study data and data collection methods are maintained.

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

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

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

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

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR

Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STUDY OBJECTIVES

10.1.1 Primary

The primary objective is to determine if the combination immunotherapy can result in a confirmed 30% decline in PSA in a modest fraction of participants enrolled

10.1.2 Secondary

The secondary objectives are to:

- Assess the safety of the combination immunotherapy regimen
- Test for changes in PSA kinetics after treatment with the combination immunotherapy, relative to baseline PSA kinetics

10.2 SAMPLE SIZE DETERMINATION

The trial will begin with a 6-participant safety lead-in. Following determination of safety, the primary objective of this study is to determine if the use of two vaccines combined with an immune checkpoint inhibitor can be associated with a modestly high fraction of participants who can experience at least a 30% decline from their maximum PSA anytime during treatment to the minimum value during treatment.

Safety data for the 6-participant lead-in will be shared with IRB before accrual commences in the biochemical recurrence cohort. Beyond safety, this cohort will just be evaluated in a descriptive manner.

Because participants may be receiving the proposed treatment and be followed for PSA changes over an extended period, and the evaluation of the endpoint may take place at any point during this potentially long period, the trial will use a single stage design for evaluating efficacy. With 25 evaluable participants who do not receive M7824, there would be 82% power to rule out 15% and be consistent with 35% who may be able to experience a 30% decline in PSA, using an exact binomial test with a 0.10 one-sided significance level. As an illustration, if 25 evaluable participants are enrolled, 7 of 25 (28.0%) who experience at least a 30% PSA change from the maximum level would have an associated one-sided lower 90% confidence interval bound of 16.3%, and simultaneously an associated one-sided upper 90% confidence interval bound of 42.6%. These demonstrate that 7/25 would be a desirable result based on the parameters selected.

With the amendment v7/29/2021, the initial 6 participants who received M7824 will be evaluated separately in exploratory fashion and will not be included among the 25 main participants.

In addition, because a trial of participants with similar eligibility (16-C-0035) may result in approximately 36 participants who are undergoing surveillance for 6 months, this other trial may serve as a control for a secondary evaluation. The changes in PSA on this trial during the initial 6 months can be compared to the changes in PSA during the initial 6 months on the surveillance arm of the control trial as a secondary evaluation. With 25 evaluable participants on this trial and 36 evaluable participants on the other trial, there would be 81% power to identify a difference between 35% of participants on this trial with a 30% PSA maximum change and 10% of participants on the

control trial with a 30% PSA maximum change with a 0.10 one-sided significance level Fisher's exact test.

It is anticipated that 1-2 participants per month can be enrolled onto this trial; thus, it expected that accrual of 37 evaluable participants (6 for the lead-in, 6 who received M7824 as part of the initial investigation for PSA response and 25 homogeneously treated participants to evaluate PSA response) can be completed within 1 to 2 years. To allow for a small number of inevaluable participants, the accrual ceiling will be set at 40 participants.

10.3 POPULATIONS FOR ANALYSIS

Modified intention to treat: all participants who receive at least one dose of the two vaccines and the immune checkpoint inhibitor will be included in the statistical analyses performed. The lead-in cohort will be evaluated separately in a descriptive fashion and for safety primarily.

10.4 STATISTICAL ANALYSES

10.4.1 General approach

Following a safety determination in 6 participants meeting separate eligibility requirements, the PSA decline measured from the maximal value obtained while on study to the minimum value obtained while on study will be obtained for each patient, and will be considered a success if the maximal PSA decline is 30% or more. The fraction of evaluable participants who experience at least a 30% decline will be determined. Quantitative parameters will be tested for changes over time using paired tests.

10.4.2 Analysis of the primary efficacy endpoints

The fraction of evaluable participants who experience at least a 30% decline from the maximum to the minimum PSA value while on study will be determined. A two-tailed 80% and 95% confidence interval will be formed around the fraction of participants with 30% decline to interpret the fraction who experience a decline of that magnitude. This primary analysis will not include the 6 patients who received M7824 before it was removed from future use in this study.

10.4.3 Analysis of the secondary efficacy endpoints

A 6-participant phase I lead in cohort in advanced (castration resistant prostate cancer) participants will be conducted to determine safety. Once all 6 participants have completed a 6-week toxicity assessment period the toxicity data will be shared with IRB. If the IRB approves accrual to evaluate PSA response, then the trial will move forward with the planned study in biochemical recurrence. The participants in the safety cohort will only be analyzed for safety and toxicity, and not PSA changes.

Safety of the combination regimen will be assessed by reporting the grade of adverse events noted in each participant, and reporting the fraction with grade 3 and grade 4 adverse events.

To test for changes in PSA kinetics after treatment with the combination immunotherapy relative to baseline PSA kinetics, the changes in PSA over defined intervals (e.g., 1 to 3 months or similar) after administering the vaccines as well as after administering the checkpoint inhibitor will be determined by calculating the slope of the PSA change over time. Then, the slopes obtained after administering the vaccines alone or in combination with the checkpoint inhibitor will be tested vs. the slopes obtained prior to administering the vaccines using a two-tailed 0.05 significance level paired t-test or Wilcoxon signed rank test (if the paired differences are not normally distributed).

In addition, because a trial of participants with similar eligibility (16-C-0035) may result in approximately 36 participants who are undergoing surveillance for 6 months, this other trial may serve as a control for a secondary evaluation. The changes in PSA on this trial during the initial 6 months can be compared to the changes in PSA during the initial 6 months on the surveillance arm of the control trial as a secondary evaluation. The fractions of participants who experience a 30% PSA maximum change on the present study as well as the approximately 36 participants on 16-C-0035 who underwent surveillance will be compared using a one-sided significance level Fisher's exact test.

Please note: As of December 21, 2018, the 6 participants in the lead in cohort have completed the 6-week toxicity assessment period and no SAEs were seen. Per the protocol design participants will now be enrolled to the BCR cohort. The toxicity assessment of participants in the lead-in cohort is described in section **1.2.13.**

10.4.4 Safety Analyses

Safety of the combination regimen will be assessed by reporting the grade of adverse events noted in each participant, and reporting the fraction with grade 3 and grade 4 adverse events.

10.4.5 Baseline Descriptive Statistics

Demographic and clinical characteristics of all participants will be reported.

10.4.6 Planned interim analyses

None will be performed.

10.4.7 Subgroup analyses

None will be performed, except to consider exploratory evaluations based on the use of M7824

10.4.8 Tabulation of individual participant data

None will be provided.

10.4.9 Exploratory analyses

The study will aim to evaluate immune changes after treatment with 2 vaccines for 4 months.

Many immune parameters (See Section **5.1**) will be obtained at baseline and at several time points beginning at the time the two vaccines are first administered. The changes in the immune parameters after treatment with 2 vaccines for 4 months will be determined and tested for statistical significance using a two-tailed 0.05 significance level paired t-test or Wilcoxon signed rank test (if the paired differences are not normally distributed). Since there are potentially a moderately large number of these tests, they will be reported as being of secondary intent and presented without adjustment for multiple comparisons but in the context of the number of such tests performed.

Willing participants will continue to be followed after cycle 7 until progression or ADT is clinically indicated.

Patients in cohort 2 who received M7824 as part of their therapy will have their PSA response evaluated in a descriptive fashion, and may be compared informally to those from the remaining patients who do not receive M7824.

10.4.10 Halting Rules

If either of the below occurs, the Principal Investigator will halt accrual to the trial and will discuss with the NIH Intramural Institutional Review Board (IRB) whether any changes need to be made to the protocol.

- One occurrence of grade 5 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.
- Two occurrences of grade 4 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.
- More than two episodes of grade 3 colitis

This will apply to both cohorts-the phase I lead-in cohort and the main cohort of biochemical recurrence.

11 COLLABORATIVE AGREEMENTS

11.1 COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

This study is conducted under a Collaborative Research and Development Agreement (CRADA) [redacted]

12 HUMAN SUBJECT PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

12.1.1 Selection Based on Gender, Ethnicity, and Race

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. Women are not eligible for this study as this disease occurs only in men.

12.1.2 Justification for Exclusions

Due to impaired cellular immunity with the concomitant increased risk of serious side effects from vaccinations with infectious agents, the Centers for Disease Control and Prevention recommends that HIV infected participants be excluded, in addition, participants with chronic hepatitis infection, including B and C, because of potential immune impairment.

12.2 PARTICIPATION OF CHILDREN

Men under the age of 18 will not be eligible for participation in this study based on the fact that participants under 18 are unlikely to have this disease and there are unknown toxicities in pediatric participants.

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**), all subjects 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 **12.6.1** for consent procedure.

12.4 EVALUATION OF BENEFITS/RISKS/DISCOMFORTS

There is no standard therapy for participants with prostate cancer and rising PSA following local definitive therapy. Potential risks of Prostvac, CV-301 and MSB0011359C in this patient population include the range of side effects outlined in Section **14** and Section **1.2**. Prostvac has been well tolerated in previous large trials. CV-301 and MSB001359C have demonstrated acceptable adverse events in phase I dose escalation trials.

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3). Prostvac has been well tolerated in previous large trials. CV-301 has demonstrated acceptable adverse events in phase 1 dose escalation trials.

12.4.1 Risks associated with blood sampling

Side effects of blood draws include pain and bruising in the area where the needle is inserted, lightheadedness, and rarely, fainting. Participants in the CRPC lead-in cohort will have approximately the following amounts of blood collected for routine laboratory testing: 2 ½ tablespoons during screening, 3 tablespoons at the start of each cycle, day 15 of cycles 1 – 3, end of treatment and safety visit. Participants in the biochemical recurrence portion will have approximately the following amounts of blood collected for routine laboratory testing: 2.5 tablespoons during screening, 3 tablespoons during baseline, day 1 of each cycle, on day 15 of cycles 1, 5, 6, and 7, end of treatment and during follow up. We will collect approximately 5 tablespoons of blood for research at baseline, and the start of cycle 1, cycle 2, cycle 5, end of treatment and during follow up. Over a two-week period, approximately 6 ½ tablespoons of blood will be collected in the lead in portion and approximately 9 tablespoons of blood will be collected in the biochemical recurrence portion.

12.4.2 Risks of genetic research

The planned genetic research on biospecimens are non-physical and include the following:

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

parents/guardians choose to be contacted if a clinically actionable gene variant is discovered (Section **5.4.2**).

- Risk related to possibility that information may be released: This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies.
- Risk to family or relatives: Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems.

12.4.3 Alternative Approaches or Treatments

Participants will be advised verbally and in writing regarding the risks and benefits of this trial, treatment requirements, and alternative approaches to entering the trial. Written consents will be obtained.

12.4.4 Procedures to Eliminate or Minimize Potential Risks

This study may involve unforeseeable risks for participants, such as side effects whose exact nature and severity are unpredictable. Scrupulous care will be taken to minimize such side effects. All participants will be given blood tests, physical examinations, and scans, as described in the monitoring schedule (**APPENDIX C**), and must have a local physician to provide long-term care and monitoring for complications. No compensation is available, but any injury will be evaluated and treated in keeping with the benefits or care to which participants are entitled under applicable regulations.

12.4.5 Provisions for Monitoring Data Collection to Ensure Subject Safety

As information is gathered from this trial, clinical results will be shared with participants. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a participant's willingness to participate further, will be explained.

Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

12.5 RISKS/BENEFITS ANALYSIS

This study involves clinical research with an experimental combination immunotherapy designed to generate an immune response against prostate cancer. Participants will undergo multiple vaccinations with Prostvac and CV-301. Participants in Arm 1 and Arm 2 also received multiple infusions of MSB001359C until that drug was removed from the study as of amendment v07/29/2021 due to immune related adverse events observed with M7824 in this and other studies. Side effects of the immunotherapy are outlined elsewhere (see Section **14** and Section **1.2**). Potential risks are applicable for all participants on study, including those that may become cognitively impaired while on study. Whether the combination immunotherapy regimen will have any clinical effect is unknown; therefore, benefit cannot be promised, nor the chance of benefit accurately predicted. Potential benefits for all participants on study, including those that may

become cognitively impaired while on study, could include shrinking of tumor or lessening of symptoms, such as pain, that are caused by the cancer. Participation in this study is voluntary, and refusal will not result in penalty or loss of benefit to which the participant is otherwise entitled. We believe that genetic analyses of study samples will pose no more than minimal risk to the subjects or their family members, as the results will not be reported with any identifiable information. The codes linking the subject with samples will be maintained by the principal investigator and research coordinator in a locked electronic data base, with access only to the PI and research coordinators.

Participation may be discontinued at any time without penalty, and the participant will be encouraged to discuss any concerns or questions.

12.5.1 Risks related to Imaging

CT scans, Tc-99 scans and PET scans often use a contrast agent. There is a small risk of having a reaction to the contrast and most often include nausea, pain in the vein where the contrast is given, headache, metallic and/ or bitter taste in the mouth and a warm, flushing feeling. Rarely, some people have more severe allergic reactions to the contrast which may include skins rashes, shortness of breath, wheezing or low blood pressure.

There is a small risk of having a reaction to the contrast agent ^{18}F-DCFPyL (Pylarify) and most often include headache, metallic and/or bitter taste in the mouth, and fatigue. Participants may have discomfort from lying on a hard surface for about 2 hours during the scans, infection at the IV site, or leaking of the contrast into the skin and tissue around the IV. Rarely, some people may have an allergic reaction to the contrast.

12.5.2 Risks from Radiation Exposure

The procedures for performing the CT scans (chest/ abdomen/ pelvis), Tc-99 whole body scintigraphy scans, 18F NaF PET/CT scans, and two ^{18}F-DCFPyL PET scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive additional radiation exposure in one year from up to three (3) CT scans of the chest, abdomen, and pelvis, three (3) Tc-99 whole body scintigraphy scans and two (2) 18F NaF PET/CT scans.

The total additional radiation dose for research purposes will be approximately 7.2 rem in one year.

12.5.3 Risks from other study procedures

There are minimal risks associated with electrocardiogram as this is a relatively safe procedure.

Questionnaires may contain questions that are sensitive in nature. The participants are asked to only answer questions they are comfortable with.

12.6 CONSENT PROCESS AND DOCUMENTATION

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

investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other 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 participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at:
https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

12.6.1 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 **12.6**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, the Investigational New Drug (IND) 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(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference 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 each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site 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 RECOMBINANT FOWLPOX-PSA(L155)/TRICOM™

14.1.1 Background

Other Names: PROSTVAC-F/TRICOM™; PROSTVAC-F

Classification: Recombinant fowlpox virus vector vaccine of the genus *Avipoxvirus.*

Product Description: Recombinant Fowlpox-PSA(L155)/TRICOM™ is a recombinant fowlpox virus vector vaccine containing the genes for human PSA and three co-stimulatory molecules (designated TRICOM™): B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The PSA gene coding sequence is modified to code for a single amino acid substitution [isoleucine to leucine at amino acid position 155 of the PSA antigen (designated L155)], which is designed to enhance immunogenicity. This modification occurs in a 10-mer, HLA-A2-restricted, immunodominant epitope of the antigen [designated PSA-3 (amino acids 154-163)]. An attenuated, live, plaque-purified isolate from the POXVAC-TC strain of fowlpox virus was used as the parental virus for this recombinant vaccine. A plasmid vector containing the modified PSA gene and the genes for the three co-stimulatory molecules is used for *in vivo* recombination between the plasmid vector and parental fowlpox virus genome. The recombinant vaccine is manufactured by infection of primary chicken embryo dermal (CED) cells with the recombinant fowlpox virus. Fowlpox virus can infect mammalian cells and express

the inserted transgenes to stimulate both humoral and cellular immunity, but cannot replicate in non-avian species, making systemic infections unlikely.

14.1.2 How Supplied

Recombinant Fowlpox-PSA(L155)/TRICOM™ will be supplied by the manufacturer, Bavarian Nordic, Inc.

Recombinant Fowlpox-PSA(L155)/TRICOM™ is supplied in vials containing 0.75 mL of the vaccine at a final viral concentration titer of 2 x 10^9 infectious units/mL formulated in phosphate-buffered saline containing 10% glycerol.

14.1.3 Preparation

Thaw vials completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds prior to dose preparation. Withdraw 0.5 mL (1 x 10^9 infectious units) into a 1 mL syringe for administration by subcutaneous injection.

14.1.4 Storage

Store intact vials of Recombinant Fowlpox-PSA(L155)/TRICOM™ at –70°C or colder.

14.1.5 Stability

Shelf-life stability studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8°C. Do not re-freeze thawed vials. Vials of Recombinant Fowlpox-PSA(L155)/TRICOM™ are for single-use only and do not contain a preservative. Administer prepared doses as soon as possible following preparation (*i.e.*, within one hour). If necessary, store prepared doses at 2-8°C for up to 4 hours following preparation.

14.1.6 Route of Administration

Recombinant Fowlpox-PSA(L155)/TRICOM™ is administered by subcutaneous injection preferably in the upper thigh. Alternate subcutaneous sites may be used with approval of the principle investigator.

14.1.7 Special Handling

Fowlpox virus is classified as a Biosafety Level 1 agent. These agents are not known to cause disease in healthy human adults and are of minimal potential hazard to personnel and the environment under ordinary conditions of use. Clinicians can use techniques generally acceptable for nonpathogenic material. The recombinant vaccine is a preparation of a live virus (infectious for birds) containing DNA sequences derived from the human genome. Handle the recombinant vaccine as a hazardous biological substance and dispose of waste materials as hazardous biological waste, with incineration according to local institutional policy and according to local, state, and federal regulations. Healthcare workers handling the recombinant fowlpox vaccine should avoid direct contact with pet birds for at least 72 hours after working with the agent.

Preparation, Handling and Disposal Recommendations

1. Strictly adhere to standard microbiological practices and techniques.
2. Limit/restrict access to preparation areas while dose preparation is in progress.

3. Use appropriate infection control measures (e.g., thorough hand washing) after handling any materials.
4. Institute and follow policies for safe handling of sharps.
5. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eye wear, hair cover].
6. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
7. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
8. Place an empty biohazard sharps container lined with a leak-proof biohazard bag in or near the biosafety cabinet to dispose of all waste generated.
9. Transport the agent from the freezer to the work area in leak proof bag.
10. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
11. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
12. Place all waste into the sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
13. Incinerate waste according to institutional policy and according to local, state, and federal regulations.
14. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    - Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    - Use protective apparel, eyewear, mask, and gloves.
    - Cover spills with disposable absorbent towels.

- Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
- Dispose of all waste as biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.

15. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the site's Institutional Biosafety Committee (IBC) by the site's PI. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

For more information about biohazard risk group classification and biohazard safety levels see:

- *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). See current version at: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html*
- *Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at:*

  http://www.cdc.gov/biosafety/publications/index.htm

Participant Care Implications and Contraindications

Cover vaccination sites with a sterile dry dressing (e.g., Telfa pad). Once the injection site is healed, no further barrier is necessary. As a precaution, protect injection sites that are exhibiting evidence of weeping, oozing or ulceration with a sterile dry dressing. In these circumstances, instruct participants to avoid direct contact of the injection site with susceptible individuals (e.g.; those who may be immunocompromised by disease or therapy). Instruct participants to avoid fathering a child for at least 4 months following therapy completion with the recombinant vaccine. Instruct participants receiving fowlpox vaccines to avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site.

Due to the method of manufacturing (virus grown in primary chicken embryo dermal cells), participants with a history of allergy to eggs or egg products should not receive the vaccine.

14.2 RECOMBINANT VACCINIA-PSA(L155)/TRICOM™

14.2.1 Background

Other Names: PROSTVAC-V/TRICOM™; PROSTVAC-V

Classification: Recombinant vaccinia virus vector vaccine of the genus *Orthopoxvirus.*

Product Description: Recombinant Vaccinia-PSA(L155)/TRICOM™ is a recombinant vaccinia virus vector vaccine containing the genes for human PSA and three co-stimulatory molecules (designated TRICOM™): B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The PSA gene coding sequence is modified to code for a single amino acid substitution [isoleucine to leucine at amino acid position 155 of the PSA antigen (designated L155)], which is designed to enhance immunogenicity. This modification occurs in a 10-mer, HLA-A2-restricted, immunodominant epitope of the antigen [designated PSA-3 (amino acids 154-163)]. An attenuated, live, derivative of the Wyeth (New York City Board of Health) strain of vaccinia virus is used as the parental virus for the recombinant vaccine. A plasmid vector

containing the modified PSA gene and the genes for the three co-stimulatory molecules is used for *in vivo* recombination between the plasmid vector and parental vaccinia virus genome. The recombinant vaccine is manufactured by infection of primary chicken embryo dermal (CED) cells with the recombinant vaccinia virus. Vaccinia virus can infect mammalian cells and express the inserted transgenes, and is a potent immune stimulator, eliciting both a strong humoral and cellular immune response. Vaccinia virus is replication competent in mammalian cells, making systemic infections possible.

14.2.2 How Supplied

Recombinant Vaccinia-PSA(L155)/TRICOM™ will be supplied by the manufacturer, Bavarian Nordic, Inc.

Recombinant Vaccinia-PSA(L155)/TRICOM™ is supplied in vials containing 0.75 mL of the vaccine at a final viral concentration titer of 4 x 10^8 infectious units/mL formulated in phosphate-buffered saline containing 10% glycerol.

14.2.3 Preparation

Thaw vials completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds prior to dose preparation. Withdraw 0.5 mL (2 x 10^8 infectious units) into a 1 mL syringe for administration by subcutaneous injection.

14.2.4 Storage

Store intact vials of Recombinant Vaccinia-PSA(L155)/TRICOM™ at –70°C or colder.

14.2.5 Stability

Shelf-life studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8°C. Do not re-freeze thawed vials. Vials of Recombinant Vaccinia-PSA(L155)/TRICOM™ are for single-use only and do not contain a preservative. Administer prepared doses as soon as possible following preparation (*i.e.*, within one hour). If necessary, store prepared doses at 2-8°C for up to 4 hours following preparation.

14.2.6 Route of Administration

Recombinant Vaccinia-PSA(L155)/TRICOM™ is administered by subcutaneous injection preferably in the upper thigh. Alternate subcutaneous sites may be used with approval of the Principal Investigator.

14.2.7 Special Handling and Precautions

Vaccinia virus is classified as a Biosafety Level 2 agent. These agents are associated with human disease and are of moderate potential hazard to personnel and the environment. The recombinant vaccine is a preparation of a live virus affecting humans and contains DNA sequences derived from the human genome. Handle the recombinant vaccine as an infectious hazardous biological substance and dispose of waste materials as infectious hazardous biological waste, with incineration according to local institutional policies and according to local, state, and federal regulations.

Preparation, Handling and Disposal Recommendations

1. Prepare a biosafety manual which advises personnel of special hazards and specific instructions on practices and procedures.
2. Post warning hazard signs on access doors, identifying the agents, the biosafety level, the name and phone number of the Principal Investigator or other responsible person, and any special requirements for entry.
3. Establish policies and procedures allowing only personnel who are knowledgeable of the potential hazards and meet specific entry requirements (*e.g.*, immunization) into agent preparation or storage areas.
4. Strictly adhere to standard microbiological practices and techniques.
5. Limit/restrict access to preparation areas while dose preparation is in progress.
6. Use appropriate infection control measures (*e.g.*, thorough hand washing) after handling any materials.
7. Institute and follow policies for safe handling of sharps. Use only needle-lock syringes and needles for dose preparation. Use extreme caution to prevent autoinoculation. Do not bend, shear, or replace the needle guard from the syringe following use. Promptly place used needles and syringes in puncture-resistant containers for disposal.
8. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eyewear, hair cover].
9. Perform all procedures carefully to minimize aerosol creation.
10. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
11. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
12. Place an empty biohazard sharps container in the biosafety cabinet to dispose of all waste generated.
13. Transport the agent from the freezer to the work area in leak proof bag.
14. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
15. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.

16. Place all waste into a sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
17. Place all waste and protective apparel in a leak proof biohazard bag, and place the bag inside a biohazard sharps container for incineration according to institutional policy and according to local, state, and federal regulations.
18. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    a. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    b. Use protective apparel, eyewear, mask, and gloves.
    c. Cover spills with disposable absorbent towels.
    d. Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
    e. Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the site's Institutional Biosafety Committee (IBC) by site's PI. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

    For more information about biohazard risk group classification and biohazard safety levels:

    - *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). See current version at: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html*
    - *Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at: http://www.cdc.gov/biosafety/publications/index.htm*

Precautions for Healthcare Workers

Recombinant vaccinia virus transmission risk to exposed healthcare workers is unknown. To date, no reports of transmission to healthcare personnel from vaccine recipients have been published. If appropriate infection control precautions are observed (such as covering the vaccination site and washing hands after contact with the vaccination site or bandages), healthcare workers are probably at less risk of infection than laboratory workers because of the smaller volume and lower titers of virus in clinical specimens as compared with laboratory material. However, because of the potential risk for transmission, healthcare personnel who prepare or administer doses of recombinant vaccinia vaccine or have direct contact with contaminated dressings or other infectious material from participants in clinical studies must adhere to appropriate infection control measures and should be offered vaccination with the licensed vaccinia vaccine. Do not administer

routine, non-emergency vaccination with the licensed vaccinia vaccine to healthcare workers, if they, or for at least three weeks after vaccination, their close household contacts (close household contacts are those who share housing or have close physical contact):

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (e.g., burns, impetigo, varicella zoster, severe acne, or other open rashes or wounds), until the condition resolves.
- are pregnant or intend to become pregnant within 4 weeks of vaccination or are breast-feeding.
- are immunodeficient or immunocompromised (by disease or therapy), including HIV infection.

Additionally, do not administer routine, non-emergency vaccination with the licensed vaccinia vaccine to healthcare workers if the vaccine:

- has a moderate or severe acute illness, until the illness resolves.
- is less than 18 years of age, unless specifically indicated.
- is undergoing topical steroid therapy for inflammatory eye diseases or undergoing therapy with systemic steroids; potential immune suppression increases risk for vaccinia-related complications.
- has a history of allergy or serious reaction to prior vaccinia vaccination or any of the vaccine's components.
- As a precaution, the CDC recommends that individuals with known cardiac disease (e.g., previous MI, angina, CHF, cardiomyopathy, stroke or TIA) or who have ≥ 3 known risk factors for cardiac disease (e.g., hypertension, hypercholesterolemia, diabetes, first degree relative with onset of cardiac complications prior to age 50, smoker), not receive routine, non-emergency, prophylactic vaccination with the licensed vaccinia vaccine while a possible causal relationship between vaccination and cardiac events is being evaluated.

Avoid exposure to the recombinant vaccinia vaccine, any contaminated dressings, or other infectious materials from participants, or the participant's inoculation site if you are pregnant or breast-feeding; have a history or presence of active eczema or atopic dermatitis; have acute, chronic or exfoliative skin conditions; or, are immunocompromised. More information on vaccinia precautions for healthcare workers can be obtained from http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm#tab2 and http://www.cdc.gov/mmwr/PDF/rr/rr5207.pdf.

The CDC is the only source of the licensed vaccinia vaccine. The CDC will provide vaccinia vaccine to protect laboratory and other healthcare personnel, whose occupations place them at risk of exposure to vaccinia and other closely related orthopoxviruses, including vaccinia recombinants. The vaccine should be administered under the supervision of a physician selected by the study institution. Revaccination is recommended every 10 years. For instructions on obtaining the licensed vaccinia vaccine, contact Drug Services, National Center for Infectious Diseases, CDC at (404) 639-3670.

Recombinant Vaccinia Vaccine Participant Care Implications, Contraindications and Potential Complications

Participant Care Implications and Contraindications

Cover vaccination sites with a sterile dry dressing (*e.g*., Telfa pad). Instruct participants on proper hand-hygiene, sterile dressing care, bathing, laundering of clothing, *etc*. Treat participant bandages or dressings removed from the vaccination site as infectious waste and dispose in appropriate biohazard containers. Do not administer the recombinant vaccinia vaccine if the recipient, or for at least three weeks after vaccination, their close household contacts (close household contacts are those who share housing or have close physical contact):

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (*e.g*., burns, impetigo, varicella zoster, severe acne, contact dermatitis, psoriasis, herpes or other open rashes or wounds), until the condition resolves.
- are pregnant or intend to become pregnant (due to the potential risk of fetal vaccinia); or are breast-feeding (due to the potential risk of contact transmission and inadvertent inoculation). It is currently unknown if vaccinia virus or antibodies are excreted in breast milk. Participants (*i.e*., vaccinees) should avoid fathering a child for at least 4 months following completion of therapy with the recombinant vaccine.
- are in close contact with children less than 3 years of age (due to the potential risk of contact transmission and inadvertent inoculation).
- are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection.

Additionally, do not administer the recombinant vaccinia vaccine if the vaccinee:

- has a moderate or severe acute illness, until the illness resolves.
- is undergoing topical steroid therapy for inflammatory eye diseases, or undergoing therapy with systemic steroids; potential immune suppression increases risk for vaccinia-related complications.
- At this time, until a more definitive causal relationship is determined, it is recommended that participants with known CHF or clinically significant cardiomyopathy, not be vaccinated with recombinant vaccinia-based vaccines, due to the potential for development of myocarditis and/or pericarditis.

  Although the CDC believes that there is no evidence to conclude that the licensed vaccinia vaccine used in the Smallpox Vaccination Program causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends excluding individuals with underlying heart disease from participation in the current Smallpox Vaccination Program. Participants are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Participants will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice.

Due to the method of manufacturing (virus grown in primary chicken embryo dermal cells), do not administer the recombinant vaccinia vaccine to participants with a history of allergy to eggs or

egg products. Do not administer the recombinant vaccinia vaccine to participants with a history of allergy or serious reaction to prior vaccinia vaccination (*e.g.*, smallpox vaccination).

Potential Complications Associated with Recombinant Vaccinia Vaccination

When vaccinia vaccine is administered by scarification for vaccination against smallpox, expected local reactions in individuals that have not previously been vaccinated with vaccinia include the appearance of a red papule in 3-4 days, followed by vesiculation in 5-6 days, and then the formation of a pustule on days 8-9. A large area of erythema may surround the vesicle and pustule. A crusted scab usually forms by the second week and sloughs by the third week, leaving a well-formed scar. Maximal viral shedding occurs from days 4-14, but can continue until the scab is shed from the skin. Other normal local reactions can include development of local satellite lesions, regional lymphadenopathy that can persist for weeks following healing of the skin lesion, considerable local edema, and intense inflammation from the vaccination (*i.e.*, viral cellulites), which may be confused with bacterial cellulites. Systemic symptoms typically occur between 8-10 days post-vaccination and include fever, malaise, headache, chills, nausea, myalgia, local lymphadenopathy, soreness and intense erythema surrounding the vaccination site.

When administered by scarification in individuals that have previously been vaccinated with vaccinia, expected local reactions include the appearance of a clear cut pustule 6-8 days following vaccination or the development of an area of definite induration around a central lesion that may be an ulcer or scab 6-8 days following vaccination. The response to re-vaccination depends on the degree of residual immunity following previous vaccination. Similar systemic symptoms may occur, but typically at a lower frequency.

When recombinant vaccinia vaccines are administered by intradermal, intralesional, subcutaneous or intramuscular routes of injection, milder local reactions are expected, but similar systemic symptoms may occur.

There have been a number of complications reported in the literature associated with vaccinia vaccination for smallpox. Reported complications from vaccinia vaccine when given by scarification include: a) auto-inoculation of other sites with vaccinia, b) generalized vaccinia, c) eczema vaccinatum, d) progressive vaccinia (vaccinia necrosum), or e) post-vaccinial encephalitis. In a 1968 ten-state survey, cases of these complications per million vaccinations in adult recipients (≥ 20 years of age) of vaccinia primary vaccination and revaccination were:

	Primary Vaccination	Revaccination
auto-inoculation	606.1	25
generalized vaccinia	212.1	9.1
eczema vaccinatum	30.3	4.5
progressive vaccinia	none reported	6.8
postvaccinial encephalitis	none reported	4.5

Based on a 1968 national survey, the number of deaths in primary vaccinees was approximately 1 per million and the number of deaths in recipients of revaccination was approximately 0.25 per million. Deaths were most often the result of postvaccinial encephalitis or progressive vaccinia.

Information has been reported by the US Department of Defense (DoD) during the post-vaccination surveillance assessment of adverse events in military personnel following implementation of a Smallpox Vaccination Program from the period of December 13, 2002 through May 28, 2003.

Although not directly comparable to historical numbers, due to differences in multiple population variables, estimated cases (number of cases per million vaccinations based on vaccination of 450,293 personnel, with a median age of 26 years and 70.5% as primary vaccinees) of these same complications per million vaccinations were:

auto-inoculation	107
generalized vaccinia	80
eczema vaccinatum	none reported
progressive vaccinia	none reported
postvaccinial encephalitis	2.2

Generally, self-limited adverse reactions that can be serious, but not life-threatening include autoinoculation, erythematous and urticarial rashes, and generalized vaccinia. More serious life-threatening complications include progressive vaccinia, eczema vaccinatum, and post-vaccinial encephalitis/encephalomyelitis. The complications of vaccinia vaccination may involve a number of different reactions:

1. **Non-specific erythematous or urticarial rashes:** These rashes can appear approximately 10 days after vaccination and may sometimes be confused with generalized vaccinia, but are generally self-limiting. Patients are usually afebrile and these benign rashes usually resolve spontaneously within 2-4 days. Erythema multiforme can present as different types of lesions, including macules, papules, urticaria, and bull's eye lesions (dark papule or vesicle surrounded by a pale zone and an area of erythema). These lesions may be extremely pruritic, lasting up to four weeks. Rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome) may occur, requiring hospitalization. Vaccinia Immune Globulin (VIG) therapy is not indicated to treat these rashes. Supportive care measures are warranted since these rashes are likely manifestations of an immune response or hypersensitivity reaction to the vaccine and are not likely to contain vaccinia virus.
2. **Bacterial Infection:** Vaccination site infection, most likely due to staphylococcus and streptococcus normal skin flora, is rare. Onset is approximately 5 days post-vaccination and is more common in children. Appropriate antibiotic therapy is required.
3. **Inadvertent Inoculation**: This can occur in the vaccinee (autoinoculation) as well as in close contacts (contact transmission). Accidental infection is the most common complication of vaccinia vaccination, accounting for approximately 50% of all complications associated with vaccination and revaccination. This usually results from autoinoculation of vaccinia virus transferred from the site of the vaccination. Sites typically involved include the face, eyelids, nose, mouth, genitalia, or rectum, but can also involve the arms, legs, and trunk. Contact transmission of vaccinia, with accompanying toxicities, may occur when a recently vaccinated individual has contact with a susceptible individual. In a 1968 ten-state survey, contact transmissions were reported to occur at a rate of 27 infections per million vaccinations. The age group in which contact transmission occurred most commonly was in children ≤ 5 years. Eczema vaccinatum as a result of contact transmission may result in a more severe syndrome than that seen in vaccinees, perhaps because of multiple simultaneous inoculations. About 30% of eczema vaccinatum cases reported in the 1968 ten-state survey were a result of contact transmission. It is possible that the number of cases of contact transmission would be greater in today's population, due to a largely unvaccinated patient population against smallpox. Contact transmission

rarely results in postvaccinial encephalitis or progressive vaccinia. Most cases of inadvertent inoculation usually resolve without specific therapy and resolution of lesions follow the same course as the vaccination site in immunocompetent individuals. VIG can be used for severe cases involving extensive lesions or if comorbid conditions exist. VIG is contraindicated in the presence of isolated keratitis due to the risk of increased corneal scarring. VIG use can be considered in cases of ocular implantation, with keratitis, if vision-threatening or if other life-threatening vaccinia-related complications exist that require VIG therapy.

4. **Generalized vaccinia:** Generalized vaccinia (GV) is characterized by a disseminated maculopapular or vesicular rash of varying extent on any part of the body and typically develops 6-9 days after vaccination. The lesions follow the same course as the vaccination site lesion. The lesions are hematogenously spread and may contain vaccinia virus. In immunocompetent individuals, the rash is generally self-limiting and requires supportive care therapy. VIG treatment can be used in severe cases for patients who are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.

   The differential diagnosis of GV includes eczema vaccinatum, erythema multiforme, inadvertent inoculation at multiple sites, and uncommonly, early stages of progressive vaccinia or other vesicular diseases (*e.g.*, severe chickenpox or disseminated herpes). Several publications have investigated the reporting of GV among those individuals who received smallpox vaccinations during 2003. Out of 38,440 vaccine recipients, 29 reports of possible GV during January 2003–December 2003 were identified but only 2 reports met the case definition. More than 75% of the reports received a final diagnosis of hypersensitivity reaction or nonspecific rash after review by dermatologists or because laboratory results were negative for vaccinia and other orthopoxviruses. Of 74 cases investigated in 753,226 smallpox vaccinations administered in December 2002 to December 2004, 50 (67.6%) met the case definition of possible GV. Cases occurred more frequently in primary vaccinees (rate, 81 per 1 million vaccinees) than in those revaccinated (rate, 32 per 1 million vaccinees). However, none met the case definition of probable or confirmed GV, including 15 with virologically negative laboratory evaluations (*e.g.*, culture, polymerase chain reaction, or skin biopsy). Twenty-one reports of postscab lesions were made between January and August 2003 among 37,542 smallpox vaccinees. The lesions (scab and/or fluid) of seven patients were tested for vaccinia virus by use of polymerase chain reaction and/or immunohistochemistry; all were found to be negative. In addition, the postscab lesions of four of the patients were biopsied. The results from two of the biopsies suggested an allergic contact dermatitis, and results of one each demonstrated chronic dermatitis and squamous cell carcinoma. None of the four biopsied lesions had histologic evidence of viropathic changes and no evidence supported smallpox vaccination as a cause for any of the lesions.

5. **Eczema vaccinatum:** Eczema vaccinatum is a serious complication in persons with eczema and other types of chronic or exfoliative skin conditions. It can also occur among eczematous contacts of recently vaccinated persons. Vaccinial lesions (generalized papular, vesicular or pustular lesions) develop on areas of the skin that are, or had at one time been, eczematous. These areas become highly inflamed and lesions may spread to healthy skin. The rash is often accompanied by fever and individuals are systemically ill.

The fatality rate for untreated cases (prior to availability of VIG) has been reported from 30-40%. Following availability of VIG, mortality was reduced to approximately 7%. Early diagnosis and prompt treatment with VIG is necessary to reduce mortality.

6. **Progressive vaccinia:** Progressive vaccinia is the most serious cutaneous complication, occurring when the local vaccination lesion fails to heal and develops progressive necrosis, with destruction of large areas of skin, subcutaneous tissue, and underlying structures. Progressive lesions may spread to other skin surfaces and to bone and viscera. Progressive vaccinia is associated with a high mortality rate. This complication has been seen in patients with a compromised immune system due to a congenital deficiency, lymphoproliferative disease, immunosuppressive treatment, or HIV infection. Management should include aggressive VIG therapy.

7. **Post-Vaccinial Encephalitis/Encephalomyelitis**: Vaccinial complications affecting the CNS are unpredictable. Post-vaccinial encephalitis typically affects children < 2 years of age and is characterized by an onset of symptoms 6-10 days following vaccination, which include seizures, hemiplegia, aphasia, and transient amnesia. Histopathological changes include generalized cerebral edema, mild lymphocytic meningeal infiltration, ganglion degenerative changes and perivascular hemorrhages. Older children and adults can develop encephalitis or encephalomyelitis characterized by an onset of symptoms 11-15 days following vaccination, which include fever, vomiting, headache, malaise, and anorexia, progressing to loss of consciousness, amnesia, confusion, disorientation, restlessness, delirium, drowsiness, seizures and coma. Histopathological changes include demyelination with lymphocytic infiltration, but limited cerebral edema. Mortality rates have ranged from 15-25%, with 25% of patients who recover being left with varying degrees and types of neurological deficits. VIG has not been shown to be effective in treating CNS disease and is not recommended. Post-vaccinial encephalitis/

   encephalomyelitis are diagnoses of exclusion and are not believed to be a result of replicating vaccinia virus. Although no specific therapy exists, supportive care, anticonvulsants, and intensive care might be required. A review of vaccinia-related deaths (68) during a 9-year period (1959–1966 and 1968) revealed that among first-time vaccines, 36 (52%) patients died as a result of post-vaccinial encephalitis.

8. **Fetal Vaccinia**: Fetal vaccinia is a rare, but serious complication following vaccinia vaccination during pregnancy or shortly before conception (e.g., within four weeks). To date, fewer than 50 cases have been reported and often result in fetal or neonatal death. Efficacy of VIG therapy in a viable infant or used prophylactically in women during pregnancy is unknown. The CDC has established a National Smallpox Vaccine in Pregnancy Registry. This registry will follow women during their pregnancies and their babies, after they are born, to determine the outcome of such pregnancies. The CDC can be contacted at (404) 639-8253.

9. **Myocarditis/Pericarditis:** The CDC has recommended a temporary medical deferral to the voluntary Smallpox Vaccination Program for persons with heart disease or cardiovascular risk factors (March 25, 2003) and issued "interim supplementary information" regarding evidence that smallpox vaccination may cause myocarditis and/or pericarditis (March 31, 2003) in people recently vaccinated with the smallpox vaccine. The cardiac events reported include myocardial infarction, angina, myocarditis, pericarditis,

and myopericarditis. Although the CDC believes that there is no evidence to conclude that the licensed vaccinia vaccine causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends that individuals with underlying heart disease be excluded from participation in the current Smallpox Vaccination Program. While it is currently not possible to fully evaluate the risk of cardiac events or the risk of myocarditis, pericarditis, or myopericarditis associated with vaccinia vaccination, it is reasonable to inform patients participating in studies using recombinant vaccinia virus of these reports and provide relevant guidance for evaluating these events. Further investigation from the ongoing vaccine program may provide additional data regarding an association or lack of association with cardiovascular disease. Patients are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice. At this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare event. If not otherwise excluded, patients with known CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility.

Out of a total of 540,824 military personnel vaccinated with a New York City Board of Health strain of vaccinia from December 2002 through December 2003, 67 developed symptomatic myopericarditis. In the 61 ECGs that were reviewed, an identifiable abnormality was evident in 46 (75.4%). The most common abnormalities included ST-segment changes observed evident in 40 patients (65.6%); 5 patients (8.2%) had normal variant early repolarization, and T-wave abnormalities were noted in 11 patients (18.0%). In addition, cardiac enzymes were elevated in 60 of 61 (98.4%) patients evaluated with this assay. On follow-up of 64 patients, all patients had objective normalization of electrocardiography, echocardiography, graded exercise testing, laboratory testing, and functional status; 8 (13%) reported atypical, non-limiting persistent chest discomfort. Among 37,901 health care workers vaccinated with the identical strain, 21 myo/pericarditis cases were identified; 18 (86%) were revacinees. Twelve met criteria for either myocarditis or myopericarditis, and 9 met criteria for pericarditis only (6 suspected and 3 probable). The severity of myo/pericarditis was mild, with no fatalities, although 9 patients (43%) were hospitalized.

Treatment of Vaccinia Vaccination Complications

Vaccinia Immune Globulin (VIG): First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in patients that are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG. VIG is contraindicated, however, for the treatment of isolated vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is only available through the CDC's Strategic National Pharmaceutical Stockpile by contacting the CDC's Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100. Upon receipt of a call from a patient or upon direct

observation of a patient or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible: 1) to initiate review of the clinical case, 2) to seek consultation on the appropriateness of VIG therapy, 3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and 4) to determine how to access and have the appropriate doses of VIG delivered. Early institution of VIG therapy is advised following recognition of clinical symptoms compatible with some vaccinia complications (eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of inadvertent inoculation). The effectiveness of VIG therapy appears to be time dependent. VIG has not proven to be of benefit in the treatment of post-vaccinial encephalitis, and is contraindicated for treatment of isolated vaccinial keratitis due to the increased risk of corneal scarring. A new IV formulation of VIG that has a lower level of aggregated protein allowing it to be used by either the IM or IV route is available through the CDC. This formulation will most likely be preferred for administration and the CDC will instruct investigators regarding appropriate dosing and method of administration based on the formulation and availability. There is no guarantee that VIG will successfully treat complications. At present, there are no other anti-viral therapies of proven benefit for the treatment of vaccinia-related complications.

14.3 CV301

14.3.1 Source

CV301 (MVA-BN-CV301 and FPV-CV-301) will be supplied by the manufacturer, Bavarian Nordic.

14.3.2 Toxicity

Updated clinical data has been presented in Table 4.

Table 4. **Suspected Adverse Drug Reactions Reported by ≥1% of Subjects in the Completed MVA-BN Clinical Trials[a] (N=8992[b])**

Preferred Term (PT)	No. of reports by subjects	Frequency %
Injection site pain	7370	82.0%
Injection site erythema	5875	65.3%
Injection site swelling	4488	49.9%
Injection site induration	3988	44.4%
Injection site pruritus	3573	39.7%
Myalgia	3017	33.6%
Fatigue	2886	32.1%
Headache	2704	30.1%
Nausea	1316	14.6%
Rigors/chills	842	9.4%
Body temperature increased	269	3.0%
Pyrexia	259	2.9%
Injection site nodule	228	2.5%
Appetite disorder	218	2.4%
Arthralgia	209	2.3%
Injection site discolouration	207	2.3%
Pain in extremity	148	1.7%
Injection site haematoma	107	1.2%
Axillary pain	93	1.0%
Injection site warmth	90	1.0%

[a] POX-MVA-001, -002, -004, -005, -006, -007, -008, -009, -010, -011, -013, -023, -024, -027, -028, -029, -030, -031, -036, -037, -03X, HIV-NEF-004 and HIV-POL-002.

[b] 8 subjects exposed but not included in analysis. 7 subjects in POX-MVA-009 received Dryvax either on the same day or within 7 days after MVA-BN administration and were therefore not included to avoid a potential bias in the adverse event reporting. 1 subject in POX-MVA-029 was not vaccinated according to the randomization, therefore removed from analysis set.
Source: MVA-BN IB Ed. 25.0, Table 10

A phase I study was just completed at the NCI with no dose limiting toxicities (unpublished). As expected, CV-301 was well tolerated in all 12 patients with no DLT noted. The only attributable toxicities were grade 1 and 2, and primarily injections site reactions and flu-like symptoms, with other rare toxicities such as headache and nausea (also grade 2 or less) also reported. There were no grade 3 or greater toxicities reported in the study.

Initial safety data show that the most frequently occurring treatment-related AEs were temporary and self-limiting, grade 1 or 2 in severity and included injection site reactions (injection site erythema, pruritus, pain, induration and swelling) and general symptoms including fever/chills, flu-like symptoms, headache, fatigue/weakness, nausea/vomiting, myalgia and arthralgia. There was no occurrence of any of the pre-specified adverse events of special interest (immune-related events and cardiac events). There were no related SAEs. There were no dose limiting toxicities.

MVA-BN-CV301 (Prime Vaccine)

The most common side effects associated with MVA-BN and other MVA-BN-derived vaccines include mild to moderate flu-like symptoms such as: fever, chills, muscle or joint ache, and tiredness (fatigue). In addition, some localized reactions at the site where the vaccine is injected under skin (subcutaneously). These injection site reactions may include any or all of the following: swelling, localized pain, hardness of a small area of skin around the injection site induration), and redness.

FPV-CV301 (Boost Vaccine)

The most common side effects from the use of fowlpox vaccines are mild and may include the following: fever, tiredness (fatigue), low red blood cell count (anemia), low white blood cell count (leucopenia).

In addition, some localized reactions at the site where the vaccine is injected under skin (subcutaneously). These injection site reactions may include any or all of the following: swelling, localized pain, hardness of a small area of skin around the injection site induration), and redness.

14.3.3 Formulation and preparation

MVA-BN-CV301 and FPV-CV301 encode the human MUC-1 and the human CEA gene in combination with human TRICOM. No marker gene is present in both recombinant viruses.

MVA-BN-CV301 is a liquid-frozen, highly attenuated, live recombinant virus based on the viral vector MVA-BN. It is administered as s.c. application. Packaging and vials will be labeled according to the respective product specifications.

One MVA-BN-CV301 vaccine vial has a nominal titer of 4 x 10^8 infectious units (Inf.U) in 0.5 mL of the drug product.

FPV-CV301 is a liquid-frozen, highly attenuated, live recombinant virus. It is administered as s.c. application. The packages and vials will be labeled according to the respective product specifications.

One FPV-CV301 vaccine vial has a nominal virus titer of 1 x 10^9 Inf.U in 0.5 mL of the drug product.

MVA-BN-CV301 and FPV-CV301 are each supplied in 2 mL type I borosilicate glass vials closed with sterile bromobutyl rubber stoppers, crimped with aluminum caps and covered with polypropylene closures.

14.3.4 Stability and Storage

Supplies of both the MVA-BN-CV301 and the FPV-CV301 vaccines will be shipped temperature controlled and monitored to the clinical trial site. Once at the site, the package should be handed over to personnel in charge of vaccine preparation (e.g., the pharmacist or representative). Site personnel are responsible for proper storage of vaccine upon receipt.

Both the MVA-BN-CV301 and the FPV-CV301 vaccines must be shipped to site and stored at a temperature of -80°C. A vial must not be re-frozen once it has been thawed.

14.3.5 Administration procedures

Each dose of MVA-BN-CV301 consists of 4 injections administered subcutaneously (one in each arm and one in each leg).

Each dose of FPV-CV301 consists of 1 injection administered subcutaneously (preferably in the non-dominant arm).

14.4 MSB0011359C (M7824, BINTRAFUSP ALFA)

Effective with amendment v7/29/2021, MSB0011359C will no longer be given as part of the treatment combination for the Biochemical Recurrence cohort (Arms 2 and 3).

14.4.1 Source

MSB0011359C (M7824) will be supplied by the manufacturer, EMD Serono.

14.4.2 Toxicity

Updated clinical data has been presented in **Table 5** and **Table 6**.

Table 5. **Adverse Drug Reactions by Preferred Term in the Pooled SafetyAnalysis Set**

System Organ Class Preferred Term	Pooled Safety Analysis Set (N=765) All Grades n (%)	 Grade ≥ 3 n (%)
Blood and lymphatic system disorders		
Anaemia	222 (29.0)	132 (17.3)
Blood loss anaemia	2 (0.3)	1 (0.1)
Disseminated intravascular coagulation	2 (0.3)	2 (0.3)
Haemolytic anaemia	1 (0.1)	1 (0.1)
Increased tendency to bruise	1 (0.1)	0
Microcytic anaemia	1 (0.1)	0
Normocytic anaemia	1 (0.1)	1 (0.1)
Cardiac disorders		
Myocarditis *	1 (0.1)	1 (0.1)
Ear and labyrinth disorders		
Ear haemorrhage	2 (0.3)	0
Endocrine disorders		
Adrenal insufficiency *	14 (1.8)	6 (0.8)
Autoimmune thyroiditis *	1 (0.1)	0
Basedow's disease *	1 (0.1)	0
Hyperthyroidism *	4 (0.5)	0
Hypophysitis *	3 (0.4)	2 (0.3)
Hypopituitarism *	2 (0.3)	1 (0.1)
Hypothyroidism *	33 (4.3)	2 (0.3)
Lymphocytic hypophysitis *	1 (0.1)	0
Secondary adrenocortical insufficiency *	1 (0.1)	0
Thyroiditis *		
Eye disorders	3 (0.4)	0
Conjunctival haemorrhage	6 (0.8)	0
Retinal haemorrhage	2 (0.3)	0
Vitreous haemorrhage	1 (0.1)	1 (0.1)
Gastrointestinal disorders		
Abdominal pain	111 (14.5)	15 (2.0)
Anal haemorrhage	1 (0.1)	0
Autoimmune colitis *	2 (0.3)	2 (0.3)
Colitis *	5 (0.7)	3 (0.4)
Constipation	137 (17.9)	3 (0.4)
Diarrhoea	105 (13.7)	5 (0.7)

System Organ Class Preferred Term	Pooled Safety Analysis Set (N=765) All Grades n (%)	 Grade ≥ 3 n (%)
Diarrhoea *	8 (1.0)	5 (0.7)
Diverticulum intestinal haemorrhagic	1 (0.1)	1 (0.1)
Duodenal ulcer haemorrhage	1 (0.1)	1 (0.1)
Enterocolitis *	1 (0.1)	0
Gastric haemorrhage	2 (0.3)	1 (0.1)
Gastritis haemorrhagic	1 (0.1)	1 (0.1)
Gastroduodenal haemorrhage	1 (0.1)	1 (0.1)
Gastrointestinal haemorrhage	14 (1.8)	8 (1.0)
Gastrointestinal vascular malformation haemorrhagic	1 (0.1)	1 (0.1)
Gingival bleeding	38 (5.0)	0
Haematemesis	6 (0.8)	1 (0.1)
Haematochezia	5 (0.7)	0
Haemorrhagic ascites	1 (0.1)	0
Haemorrhoidal haemorrhage	8 (1.0)	2 (0.3)
Intra-abdominal haematoma	1 (0.1)	1 (0.1)
Intra-abdominal haemorrhage	3 (0.4)	3 (0.4)
Lower gastrointestinal haemorrhage	4 (0.5)	1 (0.1)
Melaena	12 (1.6)	2 (0.3)
Mouth haemorrhage	9 (1.2)	0
Nausea	137 (17.9)	12 (1.6)
Oesophageal haemorrhage	1 (0.1)	1 (0.1)
Oesophageal varices haemorrhage	2 (0.3)	2 (0.3)
Rectal haemorrhage	5 (0.7)	0
Small intestinal haemorrhage	1 (0.1)	0
Upper gastrointestinal haemorrhage	14 (1.8)	8 (1.0)
Vomiting	100 (13.1)	11 (1.4)
General disorders and administration site conditions		
Asthenia	126 (16.5)	15 (2.0)
Chills	3 (0.4)	0
Fatigue	160 (20.9)	24 (3.1)
Mucosal haemorrhage	2 (0.3)	0
Oedema peripheral	79 (10.3)	2 (0.3)
Pyrexia	141 (18.4)	7 (0.9)
Hepatobiliary disorders		
Hepatic function abnormal *	1 (0.1)	1 (0.1)
Hepatic haemorrhage	1 (0.1)	1 (0.1)
Hepatitis *	2 (0.3)	1 (0.1)
Immune system disorders		
Drug hypersensitivity	1 (0.1)	0
Injury, poisoning and procedural complications	1 (0.1)	0
Contusion	9 (1.2)	1 (0.1)
Extradural haematoma	1 (0.1)	1 (0.1)
Infusion related reaction	26 (3.4)	1 (0.1)
Subcutaneous haematoma	1 (0.1)	0
Subdural haematoma	1 (0.1)	1 (0.1)
Investigations		
Alanine aminotransferase increased *	6 (0.8)	4 (0.5)
Aspartate aminotransferase increased	87 (11.4)	19 (2.5)
Aspartate aminotransferase increased *	6 (0.8)	5 (0.7)

System Organ Class Preferred Term	Pooled Safety Analysis Set (N=765) All Grades n (%)	 Grade ≥ 3 n (%)
Blood creatine phosphokinase increased *	2 (0.3)	1 (0.1)
Blood thyroid stimulating hormone increased *	2 (0.3)	0
Haemoglobin decreased	4 (0.5)	3 (0.4)
Transaminases increased *	1 (0.1)	0
Metabolism and nutrition disorders		
Decreased appetite	189 (24.7)	18 (2.4)
Latent autoimmune diabetes in adults *	1 (0.1)	1 (0.1)
Type 1 diabetes mellitus *	1 (0.1)	0
Musculoskeletal and connective tissue disorders		
Back pain	1 (0.1)	0
Myositis *	2 (0.3)	2 (0.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)		
Basal cell carcinoma	6 (0.8)	1 (0.1)
Bowen's disease	2 (0.3)	2 (0.3)
Intracranial tumour haemorrhage	1 (0.1)	1 (0.1)
Keratoacanthoma	57 (7.5)	5 (0.7)
Lip squamous cell carcinoma	4 (0.5)	2 (0.3)
Skin neoplasm bleeding	1 (0.1)	0
Squamous cell carcinoma of skin	27 (3.5)	11 (1.4)
Tumour haemorrhage	13 (1.7)	10 (1.3)
Nervous system disorders		
Cerebellar haemorrhage	1 (0.1)	1 (0.1)
Cerebral haemorrhage	1 (0.1)	1 (0.1)
Haemorrhage intracranial	3 (0.4)	3 (0.4)
Haemorrhagic transformation stroke	1 (0.1)	0
Headache	86 (11.2)	5 (0.7)
Immune-mediated encephalitis *	1 (0.1)	1 (0.1)
Renal and urinary disorders		
Acute kidney injury *	3 (0.4)	3 (0.4)
Haematuria	22 (2.9)	3 (0.4)
Nephritis *	1 (0.1)	1 (0.1)
Tubulointerstitial nephritis *	1 (0.1)	1 (0.1)
Reproductive system and breast disorders		
Breast haemorrhage	1 (0.1)	0
Vaginal haemorrhage	4 (0.5)	0
Respiratory, thoracic and mediastinal disorders		
Bronchial haemorrhage	1 (0.1)	0
Cough	87 (11.4)	0
Dyspnoea	134 (17.5)	31 (4.1)
Epistaxis	81 (10.6)	3 (0.4)
Haemoptysis	41 (5.4)	4 (0.5)
Haemothorax	1 (0.1)	1 (0.1)
Interstitial lung disease *	4 (0.5)	2 (0.3)
Laryngeal haemorrhage	1 (0.1)	0

System Organ Class Preferred Term	Pooled Safety Analysis Set (N=765) All Grades n (%)	 Grade ≥ 3 n (%)
Pharyngeal haemorrhage	1 (0.1)	0
Pneumonitis *	8 (1.0)	3 (0.4)
Pulmonary haemorrhage	4 (0.5)	2 (0.3)
Respiratory tract haemorrhage	1 (0.1)	0
Skin and subcutaneous tissue disorders		
Actinic keratosis	12 (1.6)	0
Blood blister	3 (0.4)	0
Dermatitis acneiform *	14 (1.8)	2 (0.3)
Drug eruption *	1 (0.1)	0
Ecchymosis	1 (0.1)	0
Erythema *	3 (0.4)	0
Hyperkeratosis	12 (1.6)	2 (0.3)
Lichen planus *	1 (0.1)	0
Pemphigoid *	3 (0.4)	3 (0.4)
Petechiae	2 (0.3)	0
Pruritus	143 (18.7)	2 (0.3)
Pruritus *	38 (5.0)	3 (0.4)
Purpura	5 (0.7)	0
Rash *	42 (5.5)	11 (1.4)
Rash erythematous *	4 (0.5)	0
Rash macular *	8 (1.0)	2 (0.3)
Rash maculo-papular *	44 (5.8)	16 (2.1)
Rash papular *	2 (0.3)	1 (0.1)
Rash pruritic *	9 (1.2)	1 (0.1)
Skin haemorrhage	1 (0.1)	0
Toxic skin eruption *	1 (0.1)	1 (0.1)
Vascular disorders		
Flushing	2 (0.3)	0
Haematoma	6 (0.8)	0
Hypotension	5 (0.7)	0
Shock haemorrhagic	1 (0.1)	1 (0.1)

Source: ADSL 23DEC2020 8:38, ADAE 23DEC2020 9:27,OutputID:T-38-aefrq.

* Immune-related adverse reaction as identified via customized MedDRA PT query and assessed based on adetailed medical review using predefined criteria.

Table 6. **Serious Adverse Drug Reactions by Preferred Term (reported for ≥ 2 Participants per PT) Considered Expected for the Purpose of SafetyReporting to Regulatory Authorities in the European Union**

System Organ Class Preferred Term	Pooled Safety Analysis Set (N=765) Treatment-related SARs n (%)
Blood and lymphatic system disorders	
Anaemia	6 (0.8)
Endocrine disorders	

System Organ Class Preferred Term	Pooled Safety Analysis Set (N=765) Treatment-related SARs n (%)
Adrenal insufficiency	7 (0.9)
Hypophysitis	2 (0.3)
Gastrointestinal disorders	
Colitis	4 (0.5)
Autoimmune colitis	2 (0.3)
Diarrhoea	2 (0.3)
Musculoskeletal and connective tissue disorders	
Myositis	2 (0.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	
Squamous cell carcinoma of skin	19 (2.5)
Keratoacanthoma	10 (1.3)
Lip squamous cell carcinoma	3 (0.4)
Basal cell carcinoma	2 (0.3)
Bowen's disease	2 (0.3)
Renal and urinary disorders	
Acute kidney injury	2 (0.3)
Respiratory, thoracic and mediastinal disorders	
Pneumonitis	5 (0.7)
Interstitial lung disease	4 (0.5)
Skin and subcutaneous tissue disorders	
Rash maculo-papular	3 (0.4)
Rash	2 (0.3)

Source: ADSL 23DEC2020 8:38, ADAE 23DEC2020 9:27,OutputID:T-39-aefrq.
SAR = serious adverse reactions.

The important potential risks include hypersensitivity, irAEs/autoimmune disorders, anemia, rash with hyperkeratosis/keratoacanthomas/SCC of the skin, embryo-fetal toxicity, and impaired wound healing. The important identified risks with bintrafusp alfa observed to data were overall manageable. Mucosal bleeding events of mild to moderate severity were observed in participants treated with bintrafusp alfa in ongoing studies and are a potential risk for bintrafusp alfa. Events may include epistaxis, hemoptysis, gingival bleeding or hematuria amongst others. In general, these reactions resolve without discontinuation of treatment.

In addition, after discussion among NCI investigators on multiple protocols using M7824, multiple 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 and others bleeding events can be attributed to colitis or cystitis which is a known toxicity of anti-PD-L1 agents including M7824. 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 or inflammatory bleeding events described with checkpoint inhibitors like M7824. It is hypothesized that this possible increased bleeding risk may be due to TGF beta inhibition which has an effect on angiogenesis; bleeding has also been observed in participants receiving M7824 and may be drug-related (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool). Accordingly, participants will be notified of the same possible risk in the informed consent document for this study.

Embryofetal toxicities are known risk of the PD 1/PD L1 targeting class and are considered important potential risks for MSB0011359C. Animal models link the PD 1/PD L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue. Embryofetal toxicity is an important potential risk of MSB0011359C. An appropriate contraception warning is provided as part of the inclusion criteria. Pregnant and breastfeeding women are not allowed in the MSB0011359C study, and adequate contraceptive measures are recommended during the study to minimize or eliminate the potential risk to the developing fetus.

14.4.3 Formulation and preparation

MSB0011359C is supplied as a liquid formulation 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 (600mg/mL). The liquid formulation must be diluted with 0.9% sodium chloride for injection solution to a total volume of 250 mL.

14.4.4 Stability and Storage

M7824 drug product must be stored at 2°C to 8°C until use. The storage condition is based on data from ongoing long term stability studies with M7824.

M7824 drug product stored at room (23°C to 27°C) or higher temperatures 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% saline solution 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. If not used immediately, in-use storage times and conditions prior to administration are the responsibility of the user.

No other drugs should be added to the infusion containers containing M7824.

14.4.5 Administration procedures

M7824 is administered as a 1-hour intravenous (iv) infusion once every 2 weeks. Dose, frequency, and administration of M7824 must be in accordance with the respective clinical study protocol.

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16 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Descriptions
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 light or sedentary work (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 > 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 > 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.

17 APPENDIX B: VACCINIA-PSA(L155)/TRICOM PATIENT INSTRUCTION SHEET

1. What vaccination site reactions can you expect?
2. How should you care for the vaccination site?
3. Are there any activities I should avoid?
4. What about contact with other people?
5. Who do I contact when I have a question?

1. What vaccination site reactions can you expect?

A typical reaction in a patient who has been previously vaccinated with vaccinia includes the appearance of a small swelling in 2-3 days that may enlarge to 1-2 inches across, a small blister or pustule after 5-7 days, and healing with little scarring within 2-3 weeks. Some patients may have very little skin reaction. Often the leg that received the vaccine may become swollen. Swollen or tender lymph nodes (“glands”) in the groin may be felt. A fever to 100-101°F may occur on the second or third day. You may notice that you feel tired for 3 or 4 days. The vaccination site may itch for about 2 weeks while the scab is forming. You can take acetaminophen ("Tylenol") if you have any aches or fever but should avoid aspirin. If fever continues for more than a day or two, you should call to speak to the clinic nurse or the research nurse.

In patients who have never received vaccinia or in some who received it a very long time ago, a red swelling is followed by blisters on day 5 to 6 and then formation of a pustule (or "boil") 1-2 inches in diameter on day 9 to 11. A large area of redness may surround this area. A crusted scab usually forms by the second week and falls off by the third week leaving a scar roughly 1/2 inch in diameter. Fever and malaise (the "blahs") may occur during the blister and pustular phases. Swollen and tender lymph nodes may persist for months. Many of the local toxicities described (e.g., pustule and scab formation) are typical of reactions seen when vaccinia is administered via scarification or intradermal administration. These reactions may be seen, but are usually not seen when administered via subcutaneous injection.

2. How should you care for the vaccination site?

Live vaccinia virus is in skin cells at the vaccination site during the 1-2 weeks until healing has occurred. Maximal viral "shedding" from the vaccination site occurs from days 4-14, but can continue until the scab falls off from the skin. After that there is no vaccinia virus in your body. You can spread the virus to other parts of your body or to other people by touching the vaccination site and then touching your eyes, mouth, a cut or some other break in the skin. You do not pass vaccinia virus by coughing or sneezing or by sharing food or cups and dishes.

In general, frequent careful hand washing by you and by any persons in physical contact with you is the best way to prevent transmission of virus. You should also use two types of barriers over your vaccination site at all times until the scab is gone. These barriers are (1) the bandage and (2) clothing (pants or elbow length sleeves depending on the site of vaccination). These barriers should remain in place until the scab has fallen off.

For dressing care, you will have a bag with some no-stick "First-Aid" or "Telfa" pads, disposable gloves, and zip-lock plastic bags. If you run out of supplies between visits, you can use a dry sterile bandage (gauze or "First-Aid" or "Telfa") from the drug store.

The no-stick pad ("First-Aid" or "Telfa" pad) dressing should be worn until the site has healed. If it remains clean and dry and is not coming off, you do not need to change it. If the dressing gets wet either from drainage from the vaccination or from water when you are showering or if it starts coming off, you should remove it and put on a clean bandage. Wear the gloves when handling the old dressings. Put the old dressing and the gloves in the zip-lock bag, then wash your hands, put on the new bandage, and wash your hands again. You do not need to wear gloves for the new bandage. You do not need to wash the vaccination site, but while the dressing is off, you may wash it lightly with a cloth, soap, and water. If you do wash, blot the site dry with a towel (don't rub), then put the wash cloth and the towel in the laundry. Do not let the shower run on the unbandaged site because live virus could be washed onto other areas on your body. Do not put any steroid cream, medicated creams, or other ointments on the vaccination site.

Before you throw away the zip-lock bag with the old dressing and gloves in it, pour a little bleach (about a quarter cup) in the bag to help kill any virus.

Wash your hands after each step.

3. Are there any activities I should avoid or take special care?

You should not go swimming until the vaccination site has healed and you no longer need to wear a bandage on it.

If you wear contact lenses, have removable dentures, have a colostomy or any other "open" area on your body that needs daily care, always wash your hands very well before handling your contact lenses, dentures, dressings, etc. Take care of all of these procedures before changing your vaccination dressing.

4. What about contact with other people?

Remember, frequent careful hand washing by you and by any persons with whom you have physical contact is the best way to prevent transmission of virus. During the time you need to wear a bandage (for 7-14 days after vaccination) there are several kinds of people with whom you should avoid close contact. "Close contact" means that you sleep in the same bed with the person, give the person baths, and/or touch their bare skin to change their clothes (or diapers), apply ointments, or change their bandages.

The individuals you should avoid include children < 3years of age; pregnant women or nursing women; individuals with eczema, history of eczema or other skin conditions such as active cases of extensive psoriasis, severe rashes, generalized itching, infections, burns, chicken pox, or skin trauma; and/or immune suppressed individuals such as individuals with leukemia or lymphoma, with AIDS, or those receiving immunosuppressive treatment (for example, after organ transplant).

5. Who do I contact when I have a question?

If you have any questions at any time, please call. A nurse or a physician is available 24 hours a day by telephone. To speak with your main doctor or with a clinic nurse, call the Hematology/Oncology Clinic between 8 AM and 4:30 PM Monday to Friday. To speak with the research nurses, call the research nurse office during the day; during nights, weekends, and sometimes during the day, when the research office is empty, you may leave a message for the research nurse on the answering machine. You can call Dr. Ravi Madan [redacted] any time during weekday hours. In an emergency on weekends, evenings, or holidays, you can always get in touch with the MEDICAL ONCOLOGY DOCTOR ON CALL (listed below) The on call doctor will call you back. If you have to go to an emergency room near your home, go to the hospital first, and then have the doctors there call for more information.

PHONE NUMBERS

[redacted]

*after clinic hours the NCI Medical Oncology physician On call through NIH page operator [redacted]

18 APPENDIX C: ON STUDY AND FOLLOW-UP EVALUATIONS

18.1 STUDY CALENDAR: BIOCHEMICAL RECURRENCE COHORT

	Screening	**Baseline**	**Surveillance for 4 months[20]**				**Vaccines for 7 months**								**End of treatment[1]**	**Follow-up[14]**
			M -4	M -3	M-2	M-1	C1 D1	C 1 D 15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1		
Informed Consent / Registration	X															
NIH Advance Directives Form[13]	X															
TREATMENT																
Prostvac-V[2]							X									
Prostvac-F[3]								X	X	X	X	X	X	X		
MVA-BN-CV301[4]							X	X								
FPV-CV301[5]									X	X	X	X	X	X		
EVALUATION																
History and PE, Weight	X[8]	X[9]		X		X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Score	X[7]	X[9]														
Pathologic confirmation of dx	X[6]															
Laboratory Testing																
CBC with differential	X[8]	X[9]					X	X	X	X	X	X	X	X	X	X
Acute Care Panel	X[8]															
Hepatic Panel	X[8]															

	Screening	**Baseline**	**Surveillance for 4 months[20]**				**Vaccines for 7 months**								**End of treatment[1]**	**Follow-up[14]**
			M -4	M -3	M-2	M-1	C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1		
Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)		X[9]					X	X	X	X	X	X	X	X	X	X
Serum amylase and lipase		X[9]					X	X	X	X	X	X	X	X	X	X
Serum thyroid stimulating hormone (TSH)		X[9]					X	X	X	X	X	X	X	X	X	X
Urinalysis		X9														X
Serum PSA and Testosterone	X[8]	X[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hep B/Hep C, HIV	X[7]															
Lymphocyte Phenotyping (CD4/CD8)		X[9]					X	X	X	X	X	X	X	X	X	X
PAP (prostatic acid phosphatase)		X[9]					X	X	X	X	X	X	X	X	X	X
ECG		X[10]														
Tc 99 scintigraphy	X[7]						X[21]								X	X[11]
CT-C/A/P	X[7]						X[21]								X	X[11]
[18]F-DCFPyL PET scan			X[19]												X[19]	

	Screening	Baseline	Surveillance for 4 months[20]				Vaccines for 7 months								End of treatment[1]	Follow-up[14]
			M -4	M -3	M-2	M-1	C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1		
NaF PET scan			X[15]													X[15]
Research Bloods		X[16]					X[16]		X						X[12]	X[12]
Research stool sample collection (optional) [18]			X							X					X	
Adverse Events	X			X		X	X →									X
Concomitant Medications	X						X →									X
FACT-P survey		X[17]					X[17]				X[17]			X[17]		
MSK Anxiety Survey		X[17]					X[17]				X[17]			X[17]		

1 cycle = 28 days; C=Cycle; D=Day; M = month (28 days)

Participant visits may be delayed for up to 2 weeks for logistical reasons during the surveillance period. While participant is receiving immunotherapy, visits and treatment may be -/+3 days in the first cycle around the schedule visit. Participants being evaluated after cycle 1 may have their follow-up visits adjusted by +/-7 days for logistical purposes. Laboratory tests specified for each cycle may be performed up to 5 days prior to the start of each cycle for logistical purposes. For participants who do not receive treatment during this window, the dose will be considered missed and the next visit will occur at the next scheduled time.

Footnotes:

[1] End of treatment visit will occur 28 days (-/+14 days) following the last dose of immunotherapy. Willing participants will continue to be followed after cycle 7 until progression or ADT is clinically indicated.
[2] Prostvac-V 2 x 10^8 infectious units/ 0.5 mL by subcutaneous injection.
[3] Prostvac-F 1 x 10^9 infectious units/0.5 mL by subcutaneous injection.
[4] MVA-BN-CV301: one dose is four sites of subcutaneous injection each consisting of 4 x 10^8 infectious units/0.5 mL.
[5] FPV-CV301 1 x 10^9 infectious units/0.5 mL by subcutaneous injection.
[6] Pathologic confirmation will be obtained any time prior to enrollment.
[7] Screening assessments obtained within 8 weeks prior to enrollment.
[8] Screening assessments obtained within 16 days prior to start of enrollment.

[9] Baseline assessments obtained within 16 days prior to treatment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe).
[10] Baseline ECG will be obtained within 8 weeks prior to enrollment
[11] Follow-up Imaging will be done prior to vaccine therapy (cycle 1), and at the end of study visit. During the follow-up period, imaging will be done every 6 months for 1 year then as clinically indicated. A window of +/- 21 days for imaging is allowed. Restaging scans may be completed earlier if clinically indicated.
[12] Optional
[13] As indicated in section **12.3**, all subjects will be offered the opportunity to complete an NIH advance directives 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.
[14] See Section **3.4** for further details on follow up visits.
[15] For participants in the BCR cohort, two NaF PET Scans will be done when logistically feasible. These scans are for research purposes only. These scans will not be used to evaluate for clinical progression of disease.
[16] Can be done any time prior to first treatment.
[17] Surveys are optional at all time points and can be done as noted on study calendar, and every 3 months thereafter while participants are on-study. If surveys are not done, reason will be documented.
[18] Participants will be asked to give stool samples during the surveillance period, after 3-4 months on treatment and after completion of 7 months of treatment (Section **5.2.3**). Note: These are optional.
[19] When logistically feasible, PSMA PET scans should be done in the first two months during surveillance (or within 4 months of treatment initiation if surveillance period is shorter per Amendment v05/10/2023 and within 8 weeks after completing treatment. If PSMA PET is obtained within 8 weeks of starting surveillance, it will not need to be repeated during the surveillance period.
[20] With Amendment v05/10/2023, participants that have at least 3 PSA measurements in the surveillance period, with at least one of them sufficient to provide the doubling time from baseline, will not undergo any further PSA measurements in the surveillance period.
[21] Participants starting treatment per Amendment v05/10/2023 with a surveillance period of 8 weeks or shorter do not need to repeat CT and Tc99 imaging on C1D1

18.2 STUDY CALENDAR: CRPC LEAD-IN COHORT

This cohort is closed as of December 2018.

	Screening	**Baseline**	**Vaccines for 4 months**					**Vaccines plus MSB001359C for 3 months**							**End of treatment[1]**	**Safety Visit[15]**
			C1 D1	C1 D15	C2 D1	C2 D15	C3 D1	C3 D15	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9+		
Informed Consent / Registration	X															
NIH Advance Directives Form[14]	X															
TREATMENT																
Prostvac-V[2]			X													
Prostvac-F[3]				X	X		X		X	X	X	X	X	X		
MVA-BN-CV301[4]			X	X												
FPV-CV301[5]					X		X		X	X	X	X	X	X		
MSB001359C[6]			X	X	X	X	X	X								
EVALUATION																
History and PE, Weight	X[9]	X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Score	X[8]	X[10]														
Pathologic confirmation of dx	X[7]															
Laboratory Testing																
CBC with differential	X[9]	X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin,		X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X

LDH, CK, uric acid, total protein)																
Serum amylase and lipase		X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum thyroid stimulating hormone (TSH)		X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis		X[10]												X		
Serum PSA and Testosterone	X[9]	X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hep B/HepC, HIV	X[8]															
Lymphocyte Phenotyping (CD4/CD8)		X[10]	X	X	X	X	X	X	X	X	X	X		X	X	X
PAP (prostatic acid phosphatase)		X[10]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG		X[11]														
Tc 99 scintigraphy	X[8]		X				X				X			X[12]		X
CT-C/A/P, or MRI	X[8]		X				X				X			X[12]		
Research Bloods		X[16]	X		X		X				X			X	X[13]	X[13]
Adverse Events	X		X →	→	→	→	→	→	→	→	→					X
Concomitant Medications	X		X →	→	→	→	→	→	→	→	→					X

1 cycle = 28 days; C=Cycle; D=Day; M = month (28 days)

Participant visits may be delayed for up to 2 weeks for logistical reasons during the surveillance period. While participant is receiving immunotherapy, visits and treatment may be -/+3 days in the first cycle around the schedule visit. Participants being evaluate after cycle 1 may have their follow-up visits adjusted by +/-7 days for logistical purposes. Laboratory tests specified for each cycle may be performed up to 5 days prior to the start of each cycle for logistical purposes. For participants who do not receive treatment during this window, the dose will be considered missed and the next visit will occur at the next scheduled time.

Footnotes:

[1] End of treatment visit will occur 28 days (+/- 7 days) after last dose of study treatment when logistically feasible and participants are willing.

[2] Prostvac-V 2 x 10^8 infectious units/ 0.5 mL by subcutaneous injection.

[3] Prostvac-F 1 x 10^9 infectious units/0.5 mL by subcutaneous injection.

[4] MVA-BN-CV301: one dose is four sites of subcutaneous injection each consisting of 4 x 10^8 infectious units/0.5 mL.

[5] FPV-CV301 1 x 10^9 infectious units/0.5 mL by subcutaneous injection.

[6] MSB0011359C (anti-PDL1 TGFβ Trap) 1200mg by intravenous infusion over 60 minutes.

[7] Pathologic confirmation will be obtained any time prior to enrollment.

[8] Screening assessments obtained within 8 weeks prior to enrollment.

[9] Screening assessments obtained within 16 days prior to start of enrollment.

[10] Baseline assessments obtained within 16 days prior to treatment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe).

[11] Baseline ECG will be obtained within 8 weeks prior to enrollment.

[12] Restaging will be done every 3 months after cycle 9 and at the safety visit. After the safety visit the participant will be removed from study.

[13] Optional

[14] As indicated in section **12.3**, all subjects will be offered the opportunity to complete an NIH advance directives 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.

[15] See Section **3.4** for further details on follow up visits.

[16] Can be done any time prior to first treatment.

19 APPENDIX D: INSTRUCTIONS FOR PRE-STUDY AND FOLLOW-UP BLOOD TESTS

Blood Studies	Blood Tube/Comments	Destination
CBC with differential	1 light lavender tube	CC Department of Laboratory Medicine (DLM)
Hepatic Panel, Mineral Panel, Acute Care Panel, LDH, CK, Uric Acid, Total Protein	1 4 mL SST	CC DLM
Anti-HIV-1/2	1 8 mL SST	CC TTV lab
Testosterone, total	1 red top tube	CC DLM
Prostate Specific Antigen	4 mL SST	CC DLM
Lymphocyte Phenotyping, TBNK	1 light lavender tube	CC DLM
Immunology Assays	6 10 mL Na Heparin tubes 2 8 ml SST tubes	NCI-Frederick [redacted]

ADL = activities of daily living; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CT = computed tomography; irAE = immune-related adverse event; IV = intravenous; LFT = liver function test; LLN = lower limit of normal; MRI = magnetic resonance imaging; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Events; T4 = free thyroxine; TSH = thyroid-stimulating hormone; ULN = upper limit of normal.

20 APPENDIX F: BROWN TOP SPECIMEN COLLECTION INSTRUCTIONS

1) Wash your hands.
2) Gather your supplies:
    - Stool collection frame/bowl
    - Gloves (optional)
    - Brown top stool containers
    - Garbage bag
3) Label your stool container(s) with date and time of collection.

4) Place the stool collecting frame and bowl into the toilet, securely under the seat.

5) Take a bowel movement into the bowl.
6) Put on gloves (optional).

FOR BROWN TOP CONTAINER

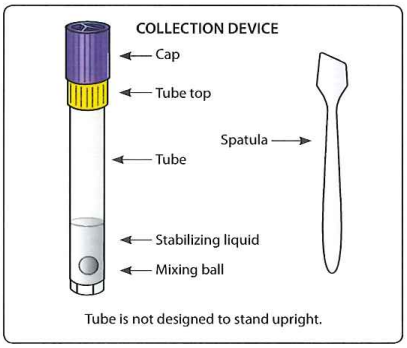
7) Use the small shovel attached to the lid of the brown top container to fill container approximately 1/2 full.
8) Close the brown top container lid and secure the tightly.

9) Dispose of any remaining feces into the toilet.
10) Seal the lid of the plastic bowl and put the whole container in to the plastic garbage bag provided.
11) Throw gloves away (if used) in the same garbage bag.
12) Tie the garbage bag securely and throw away with normal trash.
13) Wash Hands.
14) Put the brown top container in the small plastic biohazard bag provided.
15) Freeze the brown top container for at least 4 hours until you are ready to bring to clinic or ship.
16) To bring to clinic, place specimen in the cooler bag, along with the frozen ice packs provided. To ship, please see shipping instructions.

21 APPENDIX G: PURPLE TOP STOOL COLLECTION INSTRUCTIONS

OMNIgene•GUT

For microbiome



Tube is not designed to stand upright.

Summary and explanation of the kit:
OMNIgene•GUT provides the materials and instructions for collecting and stabilizing microbial DNA from a fecal sample.

Warnings and precautions:
- FOR EXTERNAL USE ONLY.
- Do NOT remove the yellow tube top from the tube.
- Do NOT spill the stabilizing liquid in the tube.
- Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
- If collecting a liquid fecal sample, see separately provided user instructions.
- Small items may pose a choking hazard.

Storage: 15°C to 25°C

Ship in accordance to applicable regulations covering transport of biological specimens.
See MSDS at www.dnagenotek.com

Label legend:

	Collect sample by (Use by)
REF	Catalog number
	Manufacturer
15°C 25°C	Storage instructions
	Caution, consult instructions for use
LOT	Lot number

USER INSTRUCTIONS

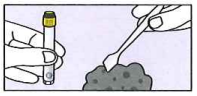
Read all instructions prior to collection

Procedure:

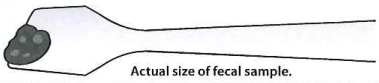
1 **IMPORTANT PREPARATIONS:**
- Empty your bladder before beginning the collection.
- Collect fecal sample free of urine or toilet water.
- Toilet paper or tissues may be required.

2 While holding the yellow tube top, unscrew ONLY the purple cap from the kit and set aside for later use.

IMPORTANT:
Do NOT remove the yellow tube top.
Do NOT spill the stabilizing liquid in the tube.



3 Use the spatula to collect a small amount of fecal sample.



4 Transfer the fecal sample into the yellow tube top. Repeat until the sample fills the yellow tube top.

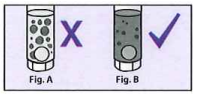
IMPORTANT: Do NOT push sample into the tube.

5 Scrape horizontally across the tube top to level the sample and remove any excess.

Wipe exterior of tube and top with toilet paper or tissue as needed.

6 Pick up the purple cap with the solid end facing down and screw onto the yellow tube top until tightly closed.

7 Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.



8 The fecal sample will be mixed with the stabilizing liquid in the tube; not all particles will dissolve.

IMPORTANT: Continue shaking if **large** particles remain as shown in Figure A.

9 Place spatula in original packaging or wrap in toilet paper and discard in garbage.

IMPORTANT: Send the sample for processing following the delivery instructions supplied separately by the kit provider.

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Superior samples
Proven performance

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DNA Genotek Inc.
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Ottawa, ON, Canada K2V 1C2

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info@dnagenotek.com
www.dnagenotek.com

Australian Sponsor: Emergo Australia, Level 20, Tower II, Darling Park, 201 Sussex Street, Sydney, NSW 2000 Australia

OMNIgene•GUT (OM-200) is not available for sale in the United States.
OMNIgene•GUT (OMR-200) is for research use only, not for use in diagnostic procedures.
*OMNIgene is a registered trademark of DNA Genotek Inc.
Some DNA Genotek products may not be available in all geographic regions, contact your sales representative for details.
All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.

Patent (www.dnagenotek.com/legalnotices)

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22 APPENDIX H: STOOL SPECIMEN SHIPMENT INSTRUCTIONS

Shipping Material:

- There is a white cardboard box that you will be working with.
- There is a white Styrofoam box in the white cardboard box with a piece of bubble wrap on top.
- **Keep these for return shipping**.

- Inside the Styrofoam box is an insulated silver bag with an ice pack inside – **place this entire bag in the freezer**.

Follow specific collection instructions for purple and brown top tubes provided in separate handouts.

PURPLE TOP CONTAINER

- After tightly securing the lid, write the date and time of the collection on the container.
- Wrap the container in the white absorbent material and place in the clear plastic Biohazard specimen bag.
- Zip seal the specimen bag and place in the freezer for at least 4 hours prior to shipping.
- **Keep the purple top container frozen until you ship the whole box back Ship the box back as soon as you can.**

BROWN TOP CONTAINER

- After placing the stool in the brown top container, write the date and time of the collection on the container.
- Wrap the container in the absorbent material inside the specimen bag and place the container inside the specimen bag.
- Seal the specimen bag according to the instructions written on the bag and place in the freezer for at least 4 hours prior to shipping.
- **Keep the brown top container frozen until you ship the whole box back. Ship the box back as soon as you can.**

Just prior to shipping:

- Place the specimen bag containing the FROZEN brown and purple tops inside the insulated silver bag that has been in your freezer.
- This will go inside the Styrofoam container.

- Place the Styrofoam box inside the white cardboard box.
- Place the bubble wrap on top of the Styrofoam box, and tape white cardboard box closed.

- Mail the white cardboard box back.
- The return FedEx label has already been filled out, including our FedEx account number for billing.
- **Fill in the date only** on the return FedEx label.
- Drop the entire box off at a FedEx facility, or call 1-800-463-3339 to schedule a pick-up.
- Do <u>not</u> ship it on a Thursday, Friday, or before a holiday. The box cannot be delivered on the weekend.