PART A2: DATA OR RESULTS FROM ANY PREVIOUS RELEASES OF THE GMO

Overview

PROSTVAC-V and PROSTVAC-F have been evaluated in eight clinical trials in the United States under two separate INDs. These agents have been administered to over 300 men up to a maximum dose of 2×10^8 plaque-forming units (pfu) of PROSTVAC-V and 1×10^9 pfu of PROSTVAC-F. Investigation of PROSTVAC-V and PROSTVAC-F was initiated by the former Therion Corporation in 2002 under BB-IND 10428 (now BB-MF 10428). Therion conducted a Phase 1 trial evaluating the safety and immunogenicity of PROSTVAC-V and PROSTVAC-F in ten patients, and a randomized, placebo-controlled Phase 2 trial evaluating the safety and efficacy (as defined by progression-free survival) of PROSTVAC-V and PROSTVAC-F in 122 men (125 enrolled, 122 received drug) with castration-resistant metastatic prostate cancer (mCRPC).

The NCI initiated its own ongoing investigations of PROSTVAC-V and PROSTVAC-F, alone and in combination with other therapies, in 2003, under NCI BB-IND 10915. These clinical trials cover a range of study designs evaluating PROSTVAC-V and PROSTVAC-F in early and late-stage disease and in combination with other therapies. To date, the NCI has conducted two Phase 1 studies, one Phase 1/2 study and three Phase 2 studies in the United States; no dose-limiting adverse effects have been noted in any of these trials.

Therion completed the randomized Phase 2 study through approximately one year of follow-up. However, in 2006, Therion went out of business. In early 2007, the NCI, which had collaborated with Therion to develop PROSTVAC-V and PROSTVAC-F for the treatment of prostate cancer, acquired all rights to the PROSTVAC-V and PROSTVAC-F technology and to the regulatory files previously owned by Therion. In 2008, BNIT acquired the Therion regulatory files and rights to the PROSTVAC-V and PROSTVAC-F technology from the NCI in a license agreement, and a collaborative research and development agreement (CRADA). BNIT conducted and completed the final overall survival analysis for the Phase 2 trial.

Summaries of Individual Clinical Studies with the GMO Conducted by Therion and BNIT

Therion's BB-IND 10428 was the first IND initiated on the PROSTVAC-V and PROSTVAC-F construct. This IND was initiated in 2002 with a Phase 1 safety trial in 10 patients.
A placebo-controlled, randomized Phase 2 trial in 122 patients was begun in 2003. These studies are summarized in **Table A2-5** and described below.

Table A2-5 PROSTVAC-V and PROSTVAC-F Clinical Studies Conducted by Therion Under Former BB-IND 10428

Protocol No.	Study Type and Regulatory File	Study Title	Study Design	N	Study Duration and Status
P1-4	Pilot Efficacy and Safety	A Phase I Trial of Pox PSA Vaccines (PROSTVAC™) with B7.1, ICAM-1, and LFA-3 Co-stimulatory Molecules (TRICOM) in Patients with Prostate Cancer	Open-label, single arm 1 PROSTVAC-V prime 1 PROSTVAC-F boost	10 active	57 days ------ Complete
TBC-PRO-002	Efficacy and Safety	A Phase II randomized, double blind, controlled study to evaluate the safety and efficacy of PROSTVAC®-VF/TRICOM ™ in combination with GM-CSF in patients with androgen-independent adenocarcinoma of the prostate	Double blind, randomized 2:1 active: placebo 1 PROSTVAC-V prime 6 PROSTVAC-F boosts	122 (82 active, 40 placebo)	168 days, with additional long term follow up ------ Complete

Protocol P1-4: A Phase I Trial of Pox PSA Vaccines (PROSTVAC™) with B7.1, ICAM-1, and LFA-3 Co-stimulatory Molecules (TRICOM) in Patients with Prostate Cancer

Study Description

Therion study P1-4 was a Phase 1, multi-center, open-label, single-arm study to evaluate the safety of PROSTVAC-V and PROSTVAC-F in the treatment of patients with adenocarcinoma of the prostate (DiPaola, 2006). The study population included male patients ≥ 18 years of age who had histologically confirmed adenocarcinoma of the prostate with elevated PSA levels. Study patients had either AIPC with rising PSA, or had androgen-independent metastatic prostate cancer and were not candidates for chemotherapy at the time of enrollment into the trial. Study patients must also have received prior vaccinia (smallpox) immunization with no serious allergic reactions, and have a life expectancy of at least six months. The primary objective of this study was to assess the safety of PROSTVAC-V and PROSTVAC-F when administered to patients with adenocarcinoma of the prostate. The secondary objectives of this study were: 1) to assess immune response to a single prime and boost administration of PROSTVAC-V and PROSTVAC-F, and 2) to detect changes in serum PSA levels and rate of change following vaccination. Eligible patients, after undergoing evaluations consisting of laboratory testing (hematology, chemistry, urinalysis, 24-hour creatinine clearance, PSA levels, serum testosterone), physical examination and electrocardiogram (ECG), received two subcutaneous

injections in the upper arm at Day 1 and Day 29 ± 2 days. Day 1 treatment consisted of one dose of 2 x 10^8 pfu of PROSTVAC-V and Day 29 (± 2) treatment consisted of one dose of 1 x 10^9 pfu of PROSTVAC-F.

Safety parameters evaluated during the course of this study included medical history, vital signs, physical examination, Eastern Cooperative Oncology Group (ECOG) performance status, ECG readings, laboratory tests (hematology, chemistry, urinalysis, 24-hour creatinine clearance), and concomitant medications. Safety was assessed by examining these parameters and tabulating adverse events occurring between baseline and Day 57 (± 2). Changes in ECOG performance status and ECG readings collected at screening and Day 57 (± 2) were evaluated, in addition to abnormalities in laboratory assessments and changes in vital signs and physical examinations collected at baseline, Day 15 (± 2), Day 29 (±2), and Day 57 (±2). Changes in laboratory test results not assessed as being clinically significant were not reported as adverse events. Safety information in this protocol was defined, analyzed and reported using the Common Terminology Criteria for Adverse Events (CTCAE) dictionary, Version 2.

Serious adverse events (SAEs) were defined as 1) any CTC grade 4 event, 2) any event considered a dose-limiting toxicity (DLT), and 3) any death; any life-threatening event *i.e*., an event that places the subject, in view of the Principal Investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death); any event that requires or prolongs in-subject hospitalization; any event that results in persistent or significant disability/incapacity; any congenital anomaly/birth defect diagnosed in a child of a subject who participated in this study and received investigational drug; other medically important events that in the opinion of the Principal Investigator may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Since worsening of prostate cancer was to be expected, disease progression was not reported as an adverse event. However, if the event of disease progression met criteria for seriousness, this event was reported as a SAE. Allergic reactions or complications from the vaccine were graded from CTC Grade 1 through 4.

Results

Ten patients completed the study. The demographic characteristics of these patients are shown in **Table A2-6**.

Table A2-6 Patient Demographics for Study P1-4

Demographic	PROSTVAC-VF, n=10
Age	
Mean (SD)	70 (5.4)
Range	63, 79
Ethnic Origin, No. (%)	
Caucasian	9 (90)
African American	1 (10)

Ten patients received a total of 20 vaccine administrations between January 9, 2003 and March 27, 2003. All ten patients completed all study visits. There were no DLTs and no patient was withdrawn from the study due to a DLT, inoculation-related adverse event, or other adverse event related to the administration of the investigational product.

Two patients were considered withdrawn from the study after, having received both doses of PROSTVAC-V and PROSTVAC-F. One patient received an epidural injection of a corticosteroid, which was prohibited per protocol, as treatment for underlying metastatic disease. The other patient developed progression of metastatic disease unrelated to the investigational product that required treatment with a corticosteroid (refer to SAE narrative below). There were no clinically significant changes from baseline in temperature, pulse or blood pressure noted in any study patient. An ECG at Day 57 (± 2) in one patient revealed sinus bradycardia with a rate of 46 beats per minute (bpm) but this was assessed by the Principal Investigator as not being a clinically significant change from the patient's baseline ventricular rate of 61 bpm. There were no other clinically significant changes from baseline in ECGs observed.

Laboratory tests revealed a clinically significant change from baseline in only one patient whose baseline gamma glutamyl transpeptidase (GGTP) of 165 U/L increased to 298 U/L at Day 57 (± 2). This was most likely due to underlying disease. Abnormal levels of alkaline phosphatase (AP) in four patients and lactate dehydrogenase in five patients that had increased from baseline were assessed by the Principal Investigators as not being clinically significant and were most likely due to underlying disease.

Results from 24-hour urine collections for creatinine clearance determinations were reported as showing no clinically significant changes from baseline. Results from one 66 year-old patient did reveal a decrease in creatinine clearance from 114.8 mL/min at baseline to 50.0 mL/min at Day 57 (± 2). This result was assessed as normal by the Principal Investigator.

A total of 37 AEs were reported in ten patients. Of these, 17 were assessed as being either remotely, possibly, or probably related to the investigational product (**Table 5-10**). All data have been monitored, and data tables were generated from the clinical database. Adverse Events have been coded and are presented using the Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. Two of the 17 were inoculation-related events observed in one patient each. Both were reported as Grade 1 reactions and were mild in severity. There were two events of diarrhea occurring in the same patient and two events of fatigue that occurred in two patients reported as possibly related to the investigational product. Other single adverse events assessed as remotely, possibly, or probably related to the investigational product included anorexia, candidal infection, constipation, cough, dyspepsia, hypoaesthia, lymphadenopathy, myalgia, nausea, pruritis, and paresthesia. All of these adverse events were assessed as mild except for the lymphadenopathy which was assessed as moderate in severity and was described as swollen right cervical nodes. The event resolved without sequelae. The paraesthesia and hypoaesthesia events occurred in the same patient, and were manifested as left sided facial numbness and tingling which were assessed as mild in severity. The patient received the second vaccination without complications, and the symptoms continued. Although this event was reported as remotely related to the investigational product, a causal relation to underlying metastatic disease was subsequently suspected (see below). All other adverse events were reported as not related to the investigational product.

Table A2-7 Adverse Events Remotely, Possibly, or Probably Related to Vaccine Administration in Study P1-4

Body System	Preferred Term	Patient Number Total # (%)*
Blood and lymphatic system disorders		1 (10%)
	Lymphadenopathy	1 (10%)
Gastrointestinal disorders		1 (10%)
	Constipation	1 (10%)
	Diarrhea NOS	1 (10%)
	Dyspepsia	1 (10%)
	Nausea	1 (10%)
General disorders and administration site conditions		3 (30%)
	Fatigue	2 (20%)
	Injection site inflammation	1 (10%)
	Injection site reaction NOS	1 (10%)
Infections and infestations		1 (10%)
	Candidal infection NOS	1 (10%)
Metabolism and nutrition disorders		1 (10%)
	Anorexia	1 (10%)
Musculoskeletal and connective tissue disorders		1 (10%)
	Myalgia	1 (10%)
Nervous system disorders		1 (10%)
	Hypoaesthesia	1 (10%)
	Paraesthesia	1 (10%)
Respiratory, thoracic and mediastinal disorders		1 (10%)
	Cough	1 (10%)
Skin and subcutaneous tissue disorders		1 (10%)
	Pruritus NOS	1 (10%)

*Patients were counted once per preferred term per body system
NOS = not otherwise specified

Deaths

No deaths occurred during this study

Study Discontinuation Due to Adverse Events

One patient, described in SAEs below, discontinued the study prematurely due to an SAE, metastatic disease of sphenoid bone that the investigator judged as unrelated to study vaccine. The patient received both study vaccinations but was prematurely withdrawn from the study on

Day 45 after receiving treatment with corticosteriods, which were prohibited by the protocol. The patient completed all protocol-specified safety assessments.

Serious Adverse Events

One serious adverse event (metastatic disease of the sphenoid bone) assessed as unrelated to investigational product was reported in this study. Patient J-A/005, a 65-year old male with metastatic prostate cancer being treated with leuprolide, received PROSTVAC-V on 05 February 2003 and PROSTVAC-F on 05 March 2003. On Day 30 (06 March 2003), the patient developed mild numbness and tingling on the right side of his head with a trigeminal nerve distribution. He was seen on Day 45 (21 March 2003), was sent to the Emergency Room, and was subsequently admitted to the hospital. MRI of the brain showed probable diffuse metastatic disease to the skull base and sphenoid bones. Epidural components to the metastases were noted along the left greater wing of the sphenoid and possible metastatic lesions toward the right cavernous sinus. The patient was treated with prednisone (20 mg qd) and radiation. The patient received both vaccine administrations but was considered withdrawn from the study on Day 45 after treatment with corticosteroids which were prohibited per the protocol. All safety assessments were completed on this patient. The investigator reported that the event was related to disease progression.

Conclusions

In Study P1-4, there were no DLTs and no patient was withdrawn due to a DLT, inoculation-related event, or other adverse event that was related to the investigational product. Inoculation-related adverse events occurred in two patients and were all Grade 1. No Grade 2 or higher inoculation-related adverse events were observed. One SAE occurred that was unrelated to the investigational product. Based on the available data, one administration of PROSTVAC-V and PROSTVAC-F each were well tolerated, and no clinically significant, serious adverse events causally related to its administration have been observed in this study.

Therion Randomized Phase 2 Study: TBC-PRO-002: A Phase II Randomized, Double-blind, Controlled Study to Evaluate the Safety and Efficacy of PROSTVAC™-VF/ TRICOM™ in Combination with GM-CSF in Patients with Androgen-Independent Adenocarcinoma of the Prostate

Study Description

The objective of this Phase 2 study was to evaluate safety and efficacy of PROSTVAC-V and PROSTVAC-F in combination with recombinant human GM-CSF versus empty vector in combination with placebo in patients with androgen-independent adenocarcinoma of the prostate.

The study was of a randomized (2:1), double-blind, empty vector-controlled design. The study population included male patients ≥ 18 years of age who had histologically confirmed adenocarcinoma of the prostate with evidence of metastatic disease, were refractory to hormone therapy, had castrate testosterone levels, had not received chemotherapy or recent radiation therapy, and had not had an allergy or untoward reaction to prior vaccinia (smallpox). Patients were stratified into treatment groups based on bisphosphonate use (yes or no**).**

Patients were administered study treatment as follows:

- PROSTVAC-V (vaccinia virus, PSA antigen, and TRICOM) 2×10^8 plaque-forming units (PFU) (prime) or empty vector, subcutaneous (sc): Day 0;
- PROSTVAC-F (fowlpox virus, PSA antigen, and TRICOM) 1×10^9 PFU (boost) or empty vector: Days 14, 28, 56, 84, 112, and 140;
- GM-CSF 100 µg or placebo was co-administered on each of the dosing days and consecutively for three days after vaccination at the vaccination site.

The primary endpoint of the study was progression-free survival (pfs), defined as the proportion of patients who remained alive and progression free at the end of the study (Day 168). Progression was defined by central radiology as one or more of the following: 1) identification of two or more new sites of bone metastasis on bone scan compared with the baseline bone scan. Progression on bone scan had to be confirmed by plain radiograph; 2) increase in the sum of all measurable target lymph node metastases on CT scan by greater than 20% according to Response Evaluation Criteria in Solid Tumors (RECIST criteria) compared with baseline scan. The primary efficacy analysis was on proportion of PFS in the intent-to-treat analysis sample. The rate of pfs in the intent-to-treat analysis sample was calculated using the Kaplan-Meier estimate and compared using the log-rank test.

The secondary endpoints of the study were:

- Time-to-progression (TTP) or death. Time-to-progression was defined as the time from registration until progression (as defined for the primary endpoint) or death occurred.
- Overall survival (analyzed using Kaplan-Meier estimates and the log-rank test).
- Tumor responses on target and nontarget lesions, and best overall tumor response according to RECIST criteria. The duration of tumor response (complete or partial response) was to be calculated as well.
- Time to onset of tumor-associated pain diagnosed by the investigator. Tumor-associated pain was to be confirmed by bone scan or CT scan. The time to onset of opioid analgesic use was also calculated.
- Effect of vaccination on PSA levels. A postinduction response in PSA levels was defined as a decrease of 50% or greater in PSA compared with baseline levels. This was to be confirmed by a subsequent PSA value, with a decrease 50% or greater of the baseline value, measured four or more weeks later.
- Safety as measured by AEs.
- Safety data were collected at each vaccination visit and included laboratory tests, physical examination findings, vital sign measurements, AEs, and concomitant medications.

Adverse events were recorded from start of study drug administration until Day 168. Adverse events were graded using the CTCAE v3.0 and were coded according to the MedRA Version 6.0. The relationship of each AE to study treatment was determined by the investigator to be not related, possibly related, or definitely related.

Results

Eighty four patients were enrolled into the active group and 41 patients enrolled into the placebo group. Eighty-two patients in the active group and 40 patients in placebo group received drug. Patient demographics are shown in **Table A2-8.**

Table A2-8 Summary of Demographics and Baseline Characteristics Sample (Therion Phase 2 Study - TBC-PRO-002)

Demographic	PROSTVAC-V and PROSTVAC-F n=82	Control n=40	Total n=122
Age (years)			
Mean (SD)	72.0 (9.0)	76.1 (8.5)	73.3 (9.0)
Range	52, 94	55, 90	52, 94
Ethnic origin, No. (%)			
Caucasian	71 (86.6)	33 (82.5)	104 (85.2)
African American	10 (12.2)	4 (10.0)	14 (11.5)
Hispanic	0	1 (2.5)	2 (1.6)
Asian	0	0	0
Other	0	2 (5.0)	2 (1.6)
Duration of prostate cancer (months)[a]			
Mean (SD)	94.1 (50.89)	113.6 (73.1)	100.4 (59.51)
Range	7, 215	9, 326	7, 326
Gleason score[b]			
Mean (SD)	6.5 (0.88)	6.3 (1.21)	6.4 (1.0)
ECOG status, No. (%)			
0	56 (68.3)	27 (67.5)	83 (68.0)
1	26 (31.7)	13 (32.5)	39 (32.0)
Baseline PSA (ng/mL)			
n	77	40	117
Mean	131.187	183.416	149.043
Range	3.34, 2524.65	5.52, 2729.37	3.34 , 2729.37
Baseline serum LDH (U/L)			
n	83	41	124
Mean (SD)	216.5 (156.05)	227.9 (77.06)	220.2 (134.89)
Range	124, 1379	107, 479	107, 1379
Baseline serum alkaline phosphatase (U/L)			
Mean (SD)	135.9 (121.06)	166.5 (122.05)	145.9 (121.75)
Range	49, 828	67, 555	49, 828
Baseline hemoglobin (g/dL)			
Mean (SD)	13.01 (1.228)	12.75 (1.615)	12.92 (1.366)
Range	9.8, 15.9	9.2, 15.9	9.2, 15.9
Bisphosphonate use[c], No. (%)			
Yes	35 (42.7)	16 (40.0)	51 (41.8)
No	47 (57.3)	24 (60.0)	72 (58.2)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ITT, intent-to-treat; LDH, lactate dehydrogenase; PROSTVAC-VF, vaccinia and fowlpox vectors, PSA, and TRIad of COstimulatory Molecules; PSA, prostate-specific antigen; SD, standard deviation.

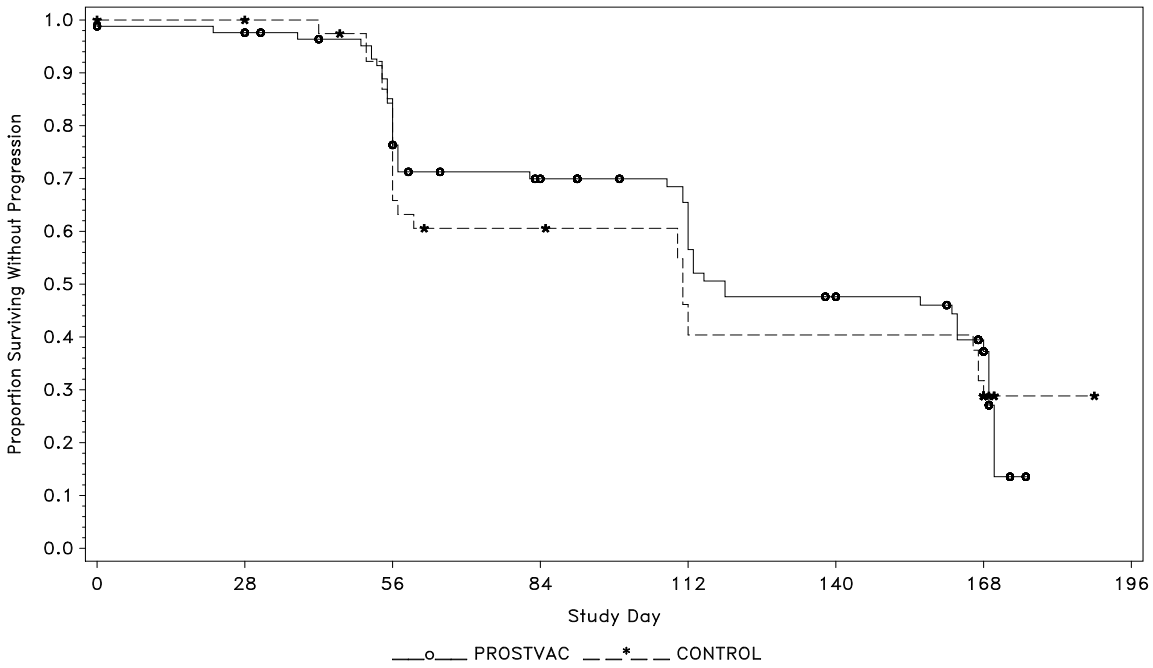
[a] Duration of prostate cancer (months) = (date of registration – date of initial diagnosis + 1)/30.44 days per month. January 1 is imputed for partial date of initial diagnosis on duration calculation.

[b] Since the Gleason score for Patient 003-0036 is recorded as less than or equal to seven in the database, it was set to seven for this analysis.

[c] Actual bisphosphonate use from case report form data is counted in this table. Six patients were misstratified. Bisphosphonate stratification in registration is used for efficacy analyses.

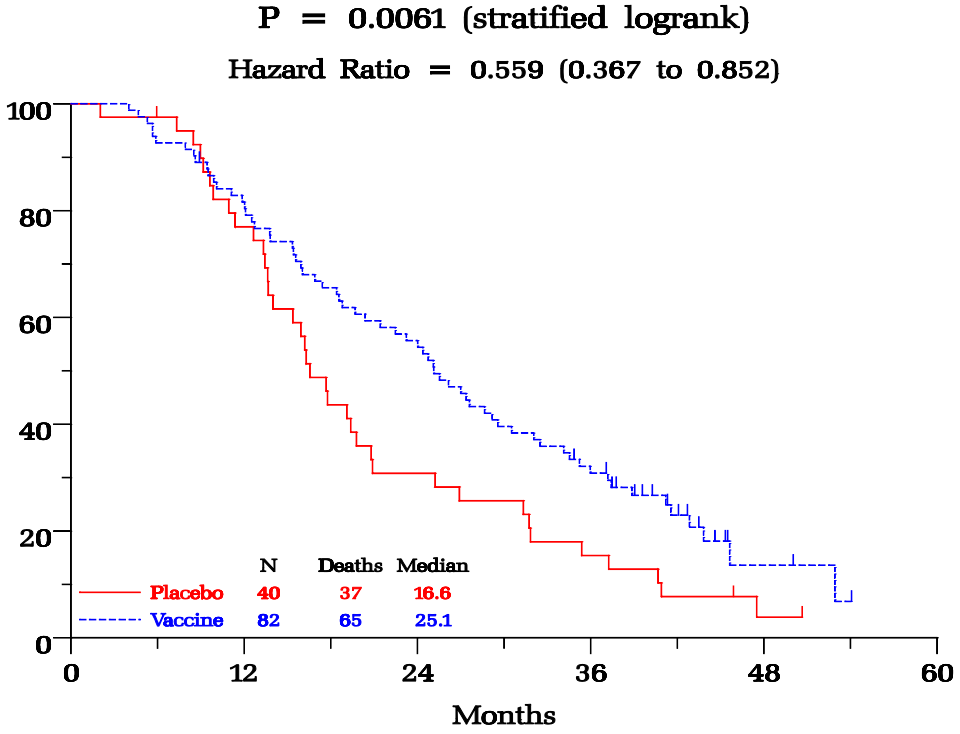
The primary endpoint of the study was progression-free survival at Day 168, which was similar between the PROSTVAC-V and PROSTVAC-F group and control group (**Figure A2-1).** No patients in either group had a partial or complete response (CR), and the majority of patients had stable or progressive disease as of their last visit. Additional secondary measures of efficacy, such as time to onset of tumor-associated pain, time to onset of opioid analgesic use for any pain, and effect of vaccination on PSA levels were similar between the treatment groups and did not reveal any improvement in the PROSTVAC-V and PROSTVAC-F group over the control group.

Figure A2-1 Progression-Free Survival (Therion Phase 2 Study - TBC-PRO-002)



In contrast to the primary endpoint results at Day 168, results for long-term overall survival suggest that the percent survival over time was higher for patients in the PROSTVAC-V and PROSTVAC-F group than for the control group. Median survival in the PROSTVAC-V and PROSTVAC-F group was longer than for the control group (25.1 and 16.6 months, respectively). Additionally, the percentage of patients that survived for longer than 1000 days was approximately twice as high in the PROSTVAC-V and PROSTVAC-F group compared with the control group (29 of 82 patients [35.4%] and 7 of 40 patients [17.5%], respectively). Data were analyzed using the log-rank test and the stratified (by corrected bisphosphonate use) log-rank test. For the log-rank test, the overall hazard ratio was 0.622 (95% CI 0.414 – 0.936), P=0.0216. For the stratified log-rank test, the overall hazard ratio was 0.559 (95% CI 0.367 – 0.852), P=0.0061. Data are presented in **Figure A2-2**.

Figure A2-2 Overall Survival (Therion Phase 2 Study - TBC-PRO-002)



The PROSTVAC-V and PROSTVAC-F group had a safety profile similar to that of the control group, and there were no significant differences in the incidences of AEs or SAEs between the two treatment groups.

Incidence of adverse events with either a possible or definite relationship to treatment is presented in **Table A2-9** by system organ class in order of decreasing frequency. General disorders and administration site conditions were the most common treatment-related system organ class: 67 patients (81.7%) in the PROSTVAC-V and PROSTVAC-F group and 31 patients (77.5%) in the control group. The next most common treatment-related system organ class was gastrointestinal disorders (18.3% of patients in the PROSTVAC-V and PROSTVAC-F group and 10.0% of patients in the control group), nervous system disorders (17.1% of patients in the PROSTVAC-V and PROSTVAC-F group and 15.0% of patients in the control group), skin and sc tissue disorders (14.6% of patients in the PROSTVAC-V and PROSTVAC-F group and 12.5% of patients in the control group), and musculoskeletal and connective tissue disorders (12.2% of patients in the PROSTVAC-V and PROSTVAC-F group and 15.0% of patients in the control group). Individual AEs that occurred in at least 10% of all patients are displayed in **Table A2-10.** The most frequently reported events were injection site-reactions, including injection site erythema, induration, pain, pruritus, swelling, and warmth. These local injection site reactions were balanced in the treatment arms. Other frequently reported events were: fatigue: 35 patients (42.7%); nausea: 17 patients (20.7%), fever: 15 patients (18.3%), chills: 12

patients (14.6%); pain: 10 patients (12.2%) and dizziness: 10 (12.2%). Chills, fatigue, and nausea were more common in the PROSTVAC-V and PROSTVAC-F arm than in the control arm.

Some AEs in this study may have resulted from the administration of GM-CSF. When GM-CSF (Leukine, sargramostim) was used in a placebo-controlled study at 250 μg/m^2 (body surface area) per day, patients in the GM-CSF group had an increased incidence of fluid retention, reported as peripheral edema, pleural effusion, and pericardial effusion (Leukine prescribing information May 2004). The dose used in this study, 100 μg, was lower than the dose in the study cited above. The incidence of peripheral edema was 13.4% in the PROSTVAC-V and PROSTVAC-F group and 10.0% in the control group. There was one case of pleural effusion reported for a patient in the PROSTVAC-V and PROSTVAC-F group, and no cases of pericardial effusion.

Table A2-9 Treatment-Related Adverse Events by System Organ Class in Order of Decreasing Incidence in PROSTVAC-V and PROSTVAC-F Group (Therion Phase 2 Study - TBC-PRO-002)

	PROSTVAC-V and PROSTVAC-F n=82	**Control n=40**
Total Number of Adverse Events	**622**	**197**
System Organ Class	**No. Patients (%)**	**No. Patients (%)**
Patients with at least one adverse event	**70 (85.4)**	**32 (80.0)**
General disorders and administration site conditions	67 (81.7)	31 (77.5)
Gastrointestinal disorders	15 (18.3)	4 (10.0)
Nervous system disorders	14 (17.1)	6 (15.0)
Skin and subcutaneous tissue disorders	12 (14.6)	5 (12.5)
Musculoskeletal and connective tissue disorders	10 (12.2)	6 (15.0)
Metabolism and nutrition disorders	6 (7.3)	1 (2.5)
Vascular disorders	5 (6.1)	1 (2.5)
Infections and infestations	4 (4.9)	4 (10.0)
Respiratory, thoracic, and mediastinal disorders	4 (4.9)	3 (7.5)
Blood and lymphatic system disorders	3 (3.7)	0 (0.0)
Ear and labyrinth disorders	3 (3.7)	0 (0.0)
Investigations	3 (3.7)	2 (5.0)
Psychiatric disorders	3 (3.7)	0 (0.0)
Cardiac disorders	1 (1.2)	2 (5.0)
Renal and urinary disorders	1 (1.2)	0 (0.0)
Reproductive system and breast disorders	0 (0.0)	2 (5.0)
Injury, poisoning, and procedural complications	0 (0.0)	1 (2.5)

Abbreviation: PROSTVAC-V and PROSTVAC-F, vaccinia and fowlpox vectors, prostate-specific antigen, and TRIad of COstimulatory Molecules.
NOTE: The total number of adverse events includes all adverse events. Treatment-related adverse events were those considered by the investigator to be possibly or definitely related to treatment. Patients may have had more than one adverse event per system organ class and preferred term. At each level of summarization, a patient was counted once if he reported at least one event.
Source: Supplemental Table 10.1.2.1 in TBC-PRO-002 Clinical Study Report (not included).

Table A2-10 Treatment-Related Adverse Events Occurring in at Least 10% of all Patients, by System Organ Class – Safety Sample (Therion Phase 2 Study - TBC-PRO-002)

System Organ Class Adverse Event	PROSTVAC-V and PROSTVAC-F n=82 No. Patients (%)	Control n=40 No. Patients (%)
General disorders and administration site conditions		
Chills	12 (14.6)	1 (2.5)
Fatigue	35 (42.7)	8 (20.0)
Injection site erythema	48 (58.5)	22 (55.0)
Injection site induration	10 (12.2)	6 (15.0)
Injection site pain	29 (35.4)	14 (35.0)
Injection site pruritus	17 (20.7)	4 (10.0)
Injection site reaction	10 (12.2)	8 (20.0)
Injection site swelling	23 (28.0)	5 (12.5)
Oedema peripheral	11 (13.4)	4 (10.0)
Pyrexia	15 (18.3)	6 (15.0)
Gastrointestinal disorders		
Constipation	9 (11.0)	6 (15.0)
Diarrhea	7 (8.5)	6 (15.0)
Nausea	17 (20.7)	2 (5.0)
Musculoskeletal and connective tissue disorders		
Arthralgia	10 (12.2)	10 (25.0)
Nervous system disorders		
Dizziness	10 (12.2)	3 (7.5)

Abbreviation: PROSTVAC-V and PROSTVAC-F, vaccinia and fowlpox vectors, prostate-specific antigen, and TRIad of COstimulatory Molecules.

NOTE: At each level of patient summarization, a patient is counted only once if the patient reported one or more events. Adverse events are coded according to the Medical Dictionary for Regulatory Activities Version 6.0.

Source: Supplemental Table 10.1.1.1 in TBC-PRO-002 Clinical Study Report (not included).

Most AEs were CTCAE Grades 1 and 2. The following Grade 3 and Grade 4 treatment related events were reported: PROSTVAC-V and PROSTVAC-F – thrombotic thrombocytopenic purpura, myocardial infarction, asthenia, pyrexia, injection site cellulitis, troponin increased, weight decreased, anorexia, myalgia, renal failure, dypsnea, pleural effusion; Control – injection site induration. The summary of most frequent treatment-emergent AEs by NCI-CTCAE Grade is provided in **Table A2-11.**

Table A2-11 Summary of Most Frequent Treatment-Emergent Adverse Events Severity by NCI-CTCAE Grade (All Adverse Events in Therion Phase 2 Study - TBC-PRO-002)

	PROSTVAC-V and PROSTVAC-F (N=82)				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Total Number of Adverse Events	737	166	27	4	1
General disorders and administration site conditions	44 (53.7%)	24 (29.3%)	3 (3.7%)	0	1 (1.2%)
Musculosceletal and connective tissue disorders	22 (26.8%)	9 (11.0%)	1 (1.2%)	0	0
Gastrointestinal disorders	21 (25.6%)	13 (15.9%)	1 (1.2%)	0	0
Nervous system disorders	15 (18.3%)	5 (6.1%)	3 (3.7%)	0	0
Infections and infestations	13 (15.9)	6 (7.3%)	1 (1.2%)	0	0
Skin and subcutaneous tissue disorders	10 (12.2%)	5 (6.1%)	0	0	0
Respiratory, thoracic and mediastinal disorders	10 (12.2%)	2 (2.4%)	2 (2.4%)	0	0
Vascular disorders	9 (11.0%)	3 (3.7%)	1 (1.2%)	0	0
Metabolism and nutrition disorders	8 (9.8%)	2 (2.4%)	2 (2.4%)	0	0
Psychiatric disorders	7 (8.5%)	6 (7.3%)	0	0	0
Blood and lymphatic system disorders	7 (8.5%)	1 (1.2%)	2 (2.4%)	1 (1.2%)	0
Renal and urinary disorders	6 (7.3%)	1 (1.2%)	0	1 (1.2%)	0
Injury, poisoning and procedural complications	6 (7.3%)	1 (1.2%)	0	0	0
Investigations	5 (6.1%)	3 (3.7%)	2 (2.4%)	1 (1.2%)	0

Source: Supplemental Table 10.1.3.1 in TBC-PRO-002 Clinical Study Report (not included).

There were no treatment-related changes in hematology, chemistry, or urinalysis.

Study Discontinuation Due to Adverse Events

Of the 105 patients treated (82 treatment group, 23 cross-over patients) there were a total of three patients who discontinued study medication early due to PROSTVAC-V and PROSTVAC-F-related adverse events. There were two patients with possibly treatment-related Grade 3 adverse events (injection site cellulitis, and a generalized anorexia/myalgia - possibly due to disease progression). In addition, one patient experienced Grade 4 serious adverse events which led to early discontinuation of study medication (thrombotic thrombocytopenic purpura-TTP, cardiac

infarction). These were felt to be unlikely but possibly related to treatment. This patient is described on the following page.

Serious Adverse Events

A total of 16 treatment-emergent SAEs occurred during the study. These SAEs occurred in five patients (6.1%) in the PROSTVAC-V and PROSTVAC-F group and in two patients (5.0%) in the control group. **Table A2-12** lists all treatment-related SAEs by patient. Of note, there were two SAEs that occurred before treatment in two patients in the PROSTVAC-V and PROSTVAC-F group. Patient 043-0029 experienced chest pain that started four days and resolved two days before receiving his first study treatment. He went on to receive treatment and received six vaccinations throughout the course of the study. Patient 046-0030 committed suicide before receiving any study treatment.

Table A2-12 Patients Who Experienced Treatment-Emergent Serious Adverse Events – Safety Sample (Therion Phase 2 Study - TBC-PRO-002)

Treatment Group Patient Number	MedDRA Preferred Term	CTCAE Grade	Relationship	Outcome
PROSTVAC-V and PROSTVAC-F				
004-0273	Thrombotic thrombocytopenic purpura	4	Possible	Resolved with sequelae
	Myocardial infarction	4	Possible	Resolved
025-0016	Abdominal pain lower	2	None	Resolved
035-0280	Anemia	3	None	Resolved
	Dehydration	3	None	Resolved
	Hyponatremia	3	None	Resolved
	Anemia	3	None	Resolved
046-0033	Urinary retention	1	None	Resolved
	Tachycardia	1	None	Resolved
	Dehydration	1	None	Resolved
054-0283[a]	Disease progression	5	None	Death
CONTROL				
024-0296	Hematuria	2	None	Resolved
	Hydronephrosis	3	None	Resolved
	Bacteremia	3	None	Resolved
035-0051	Asthenia	3	None	Resolved
	Pyrexia	2	Possible	Resolved

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities; PROSTVAC-V and PROSTVAC-F, vaccinia and fowlpox vectors, prostate-specific antigen, and TRIad of COstimulatory Molecules.

[a] Patient 054-0283 died due to progression of prostate cancer approximately four months after he had completed the study. A serious adverse event report was submitted, but no case report form data are available.

Two of the SAEs in the PROSTVAC-V and PROSTVAC-F group, myocardial infarction and thrombotic thrombocytopenic purpura (both in one patient), were possibly but unlikely treatment-related.

The patient, a 74 year old male, was screened for the study on 08 Jul 2004. Medical history included prostate cancer diagnosed in 1994, atrial fibrillation, hyperlipidemia and hypertension. Concomitant medications included clopidogrel, warfarin, hydroclorothiazide, atenolol, losartan, alendronate, doxazosin, and diethylstilbestrol.

The patient received priming dose of PROSTVAC-V on 19 Jul 2004, followed by three biweekly booster doses of PROSTVAC-F. GM-SCF was administered per protocol. The last booster dose of PROSTVAC-F was administered on 14 Sep 2004 and the last dose of GM-CSF on 17 Sep 2004.

On 09 Oct 2004 (Day 83 of the study), patient experienced epigastric pain/heartburn, nausea, dyspnea and diaphoresis. He was hospitalized on 10 Oct 2004, diagnosed with myocardial infarction and thrombotic thrombocytopenic purpura (TTP), and was discontinued from the study. Following admission and evaluation, the patient was found to have multiple abnormalities including anemia, thrombocytopenia, elevated cardiac enzymes, cardiac infarction, and acute renal failure due to TTP. The patient was treated with serial plasmaphoresis, hemodialysis, and other treatments, and discharged on 30 Oct 2004. On 01 Apr 2005 (Day 257) patient was admitted to the hospital with a large left thalamic hematoma, hypertension and an INR of 3.5; his condition deteriorated and he expired on 03 Apr 2005. Intracranial hemorrhage is a very well known complication of anticoagulation therapy with risk of hemorrhage increasing when INR rises above the therapeutic range of 2 to 3. Increase in INR combined with hypertension as another known risk factor is a most likely cause of this patient's death.

Both myocardial infarction and TTP were considered as possibly, but unlikely treatment-related. TTP has not been previously reported in association with vaccinia immunizations.

Conclusions

This randomized and double-blind Phase 2 study provides evidence of a meaningful survival benefit for patients treated with PROSTVAC-V and PROSTVAC-F with a stratified log rank test P = 0.0095. For patients in the intent-to-treat (ITT) analysis set, the median survival was 25.1 months for patients in the PROSTVAC-V and PROSTVAC-F group, compared to 17.7 months for patients in the control group. The percentage of patients who were alive approximately three years after treatment was twice as high for the PROSTVAC-V and PROSTVAC-F group (34.5%) compared with the control group (17.1%). The estimated hazard ratio was 0.581 (95% CI 0.384-0.880). While the primary endpoint of pfs failed to meet statistical criteria, the results from this Phase 2 study provide ample justification for a Phase 3 evaluation of PROSTVAC-V

and PROSTVAC-F and therefore the absence of strong PFS findings serve to suggest important Phase 3 study conduct considerations.

The vaccination regimen was well tolerated and safety profile was similar for the two treatment groups.

Summary of Safety of PROSTVAC-V and PROSTVAC-F in Therion-Sponsored Clinical Studies

PROSTVAC-V and PROSTVAC-F were well tolerated in study TBC-PRO-002. The most frequent adverse events included injection site reactions, chills, fatigue, nausea, pyrexia, peripheral edema, dizziness, althralgia, constipation, and diarrhea. Data beyond this summary are available in the Final Study Report.

Deaths

One patient died while in study TBC-PRO-002. The cause of death was completed suicide. The death occurred after screening and study registration, prior to receiving study drug.

A second patient died due to progression of prostate cancer approximately four months after he had completed study TBC-PRO-002. An SAE report was submitted, but no case report form data are available

Serious Adverse Events

Treatment-related SAEs reported in Therion-sponsored studies P1-4 and TBC-PRO-002 are shown in **Table A2-13 below**.

Table A2-13 Treatment-Related SAEs in BNIT/Therion-Sponsored Studies

Study Number	SAE Term	Study Drug	Relationship
TBC-PRO-002	Thrombotic* thrombocytopenic purpura	PROSTVAC-V and PROSTVAC-F	Possible
TBC-PRO-002	Myocardial infarction*	PROSTVAC-V and PROSTVAC-F	Possible
TBC-PRO-002	Pyrexia	Control	Possible

*These events occurred in the same patient.

Premature Terminations Due to Adverse Events

Five patients treated with PROSTVAC-V and PROSTVAC-F withdrew from BNIT/Therion-sponsored clinical studies. The reasons for withdrawal were unrelated to study treatment for four patients. One patient withdrew because of two SAEs that were defined as possibly related to PROSTVAC-V and PROSTVAC-F. A narrative for this patient's experience is included above.

Summaries of Individual Clinical Studies with the GMO Conducted by the NCI

The NCI studies outlined in **Table A2-1** and summarized below evaluate PROSTVAC-V and PROSTVAC-F under a variety of study designs alone and in combination with various therapies. These are all small studies designed to evaluate the broad potential utility of PROSTVAC-V and PROSTVAC-F for the treatment of prostate cancer, and they are reviewed primarily to further support the safety of this product.

Note that in the NCI clinical trials, PROSTVAC-V and PROSTVAC-F are termed, repectively, PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM, and alternatively termed (Recombinant Vaccinia-PSA(L155)/TRICOM™ and Recombinant Fowlpox-PSA(L155)/TRICOM™).

The study summaries are based on available information from the NCI US IND annual progress reports (APR) and personal communications.

Table A2-1. PROSTVAC-V and PROSTVAC-F Clinical Studies Conducted by the NCI Under BB-IND 10915

Protocol No.	Study Type	Study Title	N	Study Duration and Status
5911	Efficacy, Safety	A Phase 1/II Pilot Study of Sequential Vaccinations with rFowlpox-PSA (L155)-TRICOM (PROSTVAC-F/TRICOM) Alone, or in combination with r Vaccinia-PSA (L155)-TRICOM (PROSTVAC-V/TRICOM), and the Role of GM-CSF, in Men with Prostate Cancer	47	57 days with long term extension ------ Complete
6066	Feasibility	Phase 1 Feasibility Study of Intraprostatic PSA-Based Vaccine in Men with Prostate Cancer and Local Failure Following Radiotherapy or Cryotherapy or Clinical Progression on Androgen Deprivation Therapy in the Absence of Local Definitive Therapy	21	85 days ------ Ongoing, closed to accrual
7207	Efficacy in combination with an Anti-CTLA-4 antibody	Phase 1 Trial of a PSA Based Vaccine and an Anti-CTLA-4 Antibody in Adults with Metastatic Androgen Independent Prostate Cancer	30	Long Term ------ Ongoing, closed to accrual
E9802	Efficacy and Safety	A Phase II Study of PROSTVAC-V (Vaccinia)/TRICOM and PROSVAC-F (Fowlpox)/TRICOM with GM-CSF in Patients with PSA Progression After Local Therapy for Prostate Cancer	50	Long Term ------ Ongoing, Closed to accrual
7354	Efficacy in combination with Flutamide	Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC/TRICOM and Flutamide vs. Flutamide Alone in Men with Androgen Insensitive, Non-Metastatic (D0.5) Prostate Cancer	24	Long Term ------ Ongoing
7678	Efficacy in combination with Quadramet	A Randomized Phase 2.5 Study of 153Sm-EDTMP (Quadramet) With or Without a PSA/TRICOM Vaccine in Men with Androgen-Insensitive Metastatic Prostate Cancer	17	Long Term ------ Ongoing

Protocol 5911: A Phase 1/II Pilot Study of Sequential Vaccinations with rFowlpox-PSA (L155)-TRICOM (PROSTVAC-F/TRICOM) Alone, or in Combination with rVaccinia-PSA (L155)-TRICOM (PROSTVAC-V/TRICOM), and the Role of GM-CSF, in Men with Prostate Cancer

The objectives of this study were to evaluate the safety of PROSTVAC-V and PROSTVAC-F in a prime-and-boost vaccine regimen and to evaluate the effect of GM-CSF and rF-GM-CSF on the immunologic response in patients treated with these vaccines. In addition, data were collected on anti-tumor response, PSA-specific T-cell activation, and vaccinia virus clearance. Of note: this trial most closely resembles the BNIT/Therion randomized, controlled Phase 2 study TBC-PRO-002 in terms of patient population and regimen, although the specific regimens evaluated and the study objectives differ.

Patients with progressive prostate cancer were enrolled in two stages. The vaccines were administered subcutaneously on Days 1, 29, and 57. The maximum tolerated dose (MTD) established in Stage 1 (Arms 2-5) was used in Stage 2 (see below). The study plan called for three to six patients to be entered in the arms of Stage 1.

Stage 1

Arm 1	F^9-F^9-F^9
Arm 2	V^8-F^9-F^9
Arm 3	V^8-F^9-F^9 + G
Arm 4	V^8-F^9-F^9 + (F-G^7)
Arm 5	V^8-F^9-F^9 + (F-G^8)

F^9 = 1 x 10^9 pfu rF-PSA(L155)/TRICOM™
V^8 = 2 x 10^8 pfu rV-PSA(L155)/TRICOM™
G = 100 μg GM-CSF sc at injection site after vaccine daily H 4 days (Days 1-4; 29-32; 57-60)
F-G^7 and F-G^8 = 1 x 10^7 or 1 x 10^8 pfu rF-GM-CSF, respectively, given with F^9

Patients completing Stage 1 of the study who did not have progressive disease and did not experience unacceptable adverse events (AEs) were offered to enter Stage 2, to receive additional monthly vaccinations beginning on Day 85 for three months, and then every 12 weeks as per the treatment arm in which they were enrolled. The second stage of the study followed a randomized Phase 2 design to compare the immunological effects of vaccine alone, or in combination with GM-CSF (G), or rF-GM-CSF (F-G). Patients enrolled in Stage 2 had to have measurable metastatic disease without prior chemotherapy. Eight patients were enrolled randomly in arms 6-9. The treatment dose and schedule for these arms were the same as arms 2-5 in Stage 1 (see above), and were dependent on completion of all dose levels of Stage 1. The maximum accrual was 30 patients for Stage 1 and 32 for Stage 2.

Stage 2

Arm 6	V-F-F
Arm 7	V-F-F + G
Arm 8	V-F-F + (F-G)
Arm 9	V-F-F + (F-G)

A report on the Stage 1 portion of this study detailed the results of the first 15 patients enrolled was published (Arlen, 2007). No DLTs or Grade 3/4 adverse events were observed. Injection site reactions were noted in 8/15 patients (53%), and were more severe in the three patients who received co-immunization with high dose rF-GM-CSF.

Importantly, four patients were followed after vaccinia vaccination by PCR and by plaque assay. While the injection site and PBMC did score positive by PCR (viral DNA fragments), replicating virus was isolated from only one patient, at only one site, the injection site. Interestingly, this patient developed a pock at the injection site and was co-immunized with rF-GM-CSF. None of seven patients with measurable disease, by CT scan, had objective response. Nine of 15 had decreases in PSA velocity, 4/15 had absolute decreases in PSA, and one had a decrease of >30%. Five of six HLA-A2-positive patients had increases of PSA-3a epitope specific T-cells, as measured by 2-4 fold increases in interferon gamma ELISPOTS. This study demonstrated the relative safety of PROSTVAC-V with multiple boosts of PROSTVAC-F in combination with GM-CSF.

The ongoing Stage 2 portion of this study has shown some evidence of short term anti-tumor activity, and correlation with immune responses. Stage 2 was closed to accrual in December 2005. A subgroup of patients were maintained on long-term monthly vaccinations. If patients were stable and experienced no significant adverse events, they were offered the possibility of maintenance therapy with monthly PROSTVAC-F boosts. Five patients were treated for over a year with no adverse events.

In the Stage 2 portion of this study, an attempt was made to compare vaccination with protein based GM-CSF and fowlpox vector encoded GM-CSF as vaccine adjuvants. In that trial, four groups of eight patients each received either, protein GM-CSF, rF-GM-CSF low dose, rF-GM-CSF high dose, or no GM-CSF in conjunction with PROSTVAC-V and PROSTVAC-F vaccination. No clear difference could be discerned in an immunological or clinical outcome; however, the numbers of patients are small, and no definitive conclusion could be drawn.

A report on the 32 patients enrolled in the Phase 2 portion of this trial was published (Gulley, 2010). Two patients out of 12 had CT evidence of reduction in measurable soft tissue disease. One patient experienced a partial response at three months with a 68% decline in his hilar adenopathy, and another had 33% reduction in a pelvic lymph node at six months. Additionally,

one patient had stable disease lasting 11 months, and an additional patient with stable disease for 10 months had a 29% decrease in his soft tissue disease. Five of thirty-two patients demonstrated declines in PSA of >30% and one patient's PSA declined >70%. At the time this report was prepared, 11 of 32 patients were still alive with a median survival of 27 months. Importantly, 13 of 29 patients developed greater than 2-fold increase in PSA-3a epitope specific ELISPOTS, and five patients developed a greater than 6-fold increase in ELISPOTS. Overall survival was correlated with the development of this immune response.

According to NCI BB-IND 10915 20010 APR information there were 47 evaluable patients (47 entered the trial and 47 were treated). Of the 90 adverse events reported, the vast majority were Grade 1 and 2 (87). Injection site reaction was the most frequently observed adverse event (42 patients) and none were greater than a Grade 2 (29 of 42 patients had Grade 2 events). Fatigue and lethargy constituted the second most frequent event reported (9 patients); all were Grade 1. Other individual adverse events constituted less than 5% of the total reported events. There were three Grade 3 adverse events, including bone pain, fever and transfusion (packed red blood cells). No adverse events exceeded Grade 3 in severity. There were no IND Safety Reports (SAEs) related to PROSTVAC-V/F or GM-CSF in this study.

Protocol 6066: Phase 1 Feasibility Study of Intraprostatic PSA-Based Vaccine in Men with Prostate Cancer and Local Failure Following Radiotherapy or Cryotherapy or Clinical Progression on Androgen Deprivation Therapy in the Absence of Local Definitive Therapy

The primary objective of this trial was to evaluate the clinical safety and feasibility of intraprostatic prime/boost vaccine strategy that involves priming with PROSTVAC-V and rF-GM-CSF followed by intraprostatic boosts with PROSTVAC-F with or without subsequent rF-GM-CSF and with or without simultaneous boosts of PROSTVAC-F and rF-GM-CSF. Twenty-one of 30 planned patients have been enrolled in this study as of February 28, 2010. The study includes five cohorts of three to six patients each. According to NCI BB-IND 10915 2009 APR information, there were no DLTs or drug-related serious adverse events (SAEs) reported to date in this study. Of the 106 AEs reported, all but one was Grade 1 and 2. The most commonly reported AE was fever in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L (19 events), followed by injection site reaction/extravasation changes (17 events). One Grade 3 event was reported as related to an obstruction of the prostate. There were no IND Safety Reports (SAEs) related to PROSTVAC-V/F or GM-CSF in this study.

Protocol 7207: Phase 1 Trial of a PSA-Based Vaccine and an Anti-CTLA-4 Antibody in Adults with Metastatic Androgen-Independent Prostate Cancer

The primary objective of this study was to determine the safety and tolerability of a fixed dose of PROSTVAC-V and PROSTVAC-F vaccine in combination with dose escalated anti-CTLA4. The secondary objectives included an evaluation of the immunologic response (as measured by an increase in PSA-specific T-cells by ELISPOT assay in HLA-$A2^+$ patients) and clinical

response (as measured by Response Evaluation Criteria in Solid Tumors [RECIST] and PSA consensus criteria).

This was an open-label, Phase 1 safety trial with sequential cohorts of patients (n = 3 to 6) all receiving a fixed dose of PROSTVAC-V and PROSTVAC-F and GM-CSF, with a dose escalation of MDX-010 (ipilimumab). Monthly boosting vaccinations continued until the patients were off study. The protocol was amended to allow patients who received the initial six doses of MDX-010 to receive a maintenance dose every three months until there is evidence of disease progression or toxicity, for up to an additional 12 months (four additional doses). The protocol was amended to extend biochemical eligibility parameters to patients with Gilbert's syndrome with total bilirubin ≤ 3 mg/dL. Since the majority of patients treated with the highest dose of MDX-010 (10 mg/kg) developed skin rash, the protocol was amended to allow these patients to continue re-treatment with MDX-010 and/or vaccine if the rash was not painful with necrotic keratinocytes on biopsy, the rash does not contain bullous lesions with necrotic keratinocytes on biopsy, the rash resolves to Grade 2 within 28 days of planned administration of MDX-010, and an NCI dermatologist has reviewed the case for any additional risk factors. Because of concerns for progression to toxic epidermal necrolysis, patients with rash who do not meet indicated criteria were not eligible for MDX- 010 retreatment. A protocol amendment excluded patients with autoimmune hemolytic anemia, ulcerative and hemorrhagic colitis, endocrine disorders, sarcoid granuloma, myasthenia gravis, polymyositis, and Guilliain-Barre syndrome. In addition, MDX-010 was withheld for ≥ Grade 3 skin-related AEs regardless of causality.

A preliminary report by Mohebtash *et al.* (2009) on 30 patients indicated there were no dose-limiting toxicities (DLTs) and no >grade 2 toxicities attributed to the PROSTVAC-V/F vaccine. There were no IND safety reports (SAEs) related to PROSTVAC-V/F or GM-CSF in this study.

There was no evidence of clinical benefit in the six patients who had prior chemotherapy at dose level (DL) 1 and 2; therefore, subsequently patients with prior chemotherapy were excluded. The median time to progression for nine chemotherapy-naïve patients treated on DLs 2 and 3 was 6.1 months (range 2.9-11.6 months). Median PSA doubling time increased from 2.2 months at baseline to 3.7 months on study (p=0.17). Five of nine patients had ≥50% declines in PSA from peak during study and one had a sustained decrease in PSA >95% from baseline. Four of these patients had stable disease for ≥6 months and two had unconfirmed partial responses (PR) by RECIST. Of the 15 patients on DL-4, six had stable disease for ≥6 months, six remain on trial and one had a sustained decrease in PSA >99% from baseline. Median PSA doubling time increased >3-fold from 2.6 months at baseline to 8.2 months (p=0.01) at DL-4. The 24 chemotherapy-naïve patients had PSA doubling time increase from 2.5 to 6 months (p=0.003). Fourteen of 30 patients had a PSA drop from baseline on study; two of those 14 had no immune related AEs and 12 showed ≥ Grade 2 immune-related AEs temporarily associated with PSA decline. The investigators concluded that the study combination has clinical activity in chemotherapy-naïve patients and is associated with manageable side effects. They also indicated

that further studies are required to establish if the combination is more effective than either agent alone.

Protocol 7354: A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC/TRICOM and Flutamide vs. Flutamide Alone in Men with Androgen-Insensitive, Non-Metastatic (D0.5) Prostate Cancer

The primary objective of this randomized Phase 2 study is to determine if use of a combination of vaccine plus flutamide may be associated with improvement in time to treatment failure (defined as rising PSA, development of metastatic disease, or removal from treatment due to excessive toxicity) compared with flutamide monotherapy. The secondary objectives are: 1) to determine evidence of patterns of immunologic effects, which differ by treatment; 2) to estimate and compare the toxicity on the two study arms; 3) to evaluate the effect of vaccine on the development of metastatic disease after PSA progression; 4) to evaluate PSA and immune responses in patients who have had flutamide stopped at the time of PSA progression, and either continue vaccine (Arm B) or have vaccine initiated (Arm A) at time of flutamide discontinuation; and 5) to assess response rates in patients randomized to both arms, as well as responses in those patients who crossed-over from the flutamide to the vaccine only arm. The study is designed to randomize 66 patients on a 1:1 basis to receive flutamide with or without the PROSTVAC-V and PROSTVAC-F prime/boost vaccine in combination with GM-CSF. The study is divided into two steps: 1) patients are randomized to flutamide with or without vaccine, and 2) after three months patients who receive flutamide only may cross over to receive vaccine, if their PSA levels are rising and scans show no metastatic disease. The vaccine may start four weeks after flutamide is stopped, if the PSA continues to rise. In case of a decline in PSA after the discontinuation of flutamide, PSA levels will be checked every 28 days and vaccine may commence when the serum PSA levels begin to rise. For patients receiving flutamide and vaccine, after three months flutamide treatment will discontinue but patients may continue to receive vaccine therapy. This study was recently begun and 22 patients have entered as of 30 November 2009.

According to NCI IND 10915 2010 APR information, a total of 41 adverse events have been reported, with the most common event being injection site reaction changes (twelve events). One patient withdrew from the study due to "other" reason and one patient withdrew from the study due to refusal after beginning protocol therapy. There were no IND Safety Reports (SAEs) related to PROSTVAC-V/F or GM-CSF in this study.

Protocol 7678: A Randomized Phase 2.5 Study of 153Sm-EDTMP (Quadramet) With or Without a PSA/TRICOM Vaccine in Men with Androgen-Insensitive Metastatic Prostate Cancer

This is a recently initiated open-label, randomized, two-arm Phase 2.5 study. The primary objective of this trial is to determine if the combination of vaccine and ^{153}Sm EDTMP (Arm B) is potentially able to improve the 4-month progression-free survival (PFS) for patients who have

progressive disease (PD) following docetaxel-based therapy for metastatic androgen-independent prostate cancer (AIPC), over radionuclide therapy alone (Arm A). The secondary goals are to evaluate the PSA outcomes, immunologic and objective responses, toxicity, palliation, overall survival (OS), and PFS.

Patients with AIPC with bone metastasis who have had prior docetaxel will be randomized to receive ^{153}Sm-EDTMP with or without vaccine. GM-CSF is administered sc daily for four days, beginning on the day of each vaccination and within 5 mm of the vaccination site. The accrual goal for this trial is 68 patients; 17 patients have entered as of 30 November 2009.

According to NCI IND 10915 2010 APR information, a total of 20 adverse events ≤ Grade 2 have been reported. The most common events were injection site reaction changes (six events) There have been no IND safety reports (SAEs) related to PROSTVAC-V/F or GM-CSF in this study.

Protocol E9802: A Phase 2 Study of PROSTVAC-V (Vaccinia)/TRICOM and PROSTVAC-F (Fowlpox)/TRICOM with GM-CSF in Patients with PSA Progression After Local Therapy for Prostate Cancer

The primary objectives of this study are to evaluate the effect of PROSTVAC-V followed by PROSTVAC-F and GM-CSF on biochemical PSA progression at six months and to determine the change in PSA velocity pre-treatment to post-treatment. The secondary objectives are to evaluate the percentage of patients experiencing a ≥50% decline in serum PSA at four weeks, to evaluate the tolerability and safety of the PSA/TRICOM^{TM} vaccine in combination with GM-CSF, and to determine the effect of GM-CSF on PSA immediately after treatment (Day 4) compared to a delayed effect (Day 15); PSA nadir and percentage of patients with undetectable PSA will be assessed in patients enrolled in Step 2 of the study. GM-CSF is also administered sc daily for four days, beginning on the day of each vaccination and within 5 mm of the vaccination site. The study is divided into two steps: 1) Patients are treated every four weeks for the first three treatments, then every 12 weeks until biochemical or clinical progression. If patients do not progress, they can be treated maximally for 24 months of PROSTVAC-F following Cycle 4; 2) Upon biochemical or clinical progression, patients are re-registered into the trial and then treated with bicalutamide 50 mg once per day for one month in combination with vaccine therapy (in combination with GM-CSF daily for four days every 12 weeks until biochemical or clinical progression or maximum of 12 months treatment). This trial has completed enrollment of 50 patients.

DiPaola *et al.* (2009) reported preliminary data on 50 patients in this study with median PSA doubling time of 4.4 months following local therapy on Step 1 of the treatment. The overall biochemical response rate (complete response [CR] + partial response [PR]) was 2% and 25 patients had stable disease (SD). Among 29 patients with follow-up >6 months, the PSA progression-free rate at six months was 66%. The authors concluded that a viral PSA vaccine platform can be administered safely to patients with minimal disease volume. The investigators

indicated that the biochemical results on progression-free survival at six months and increase in PSA doubling time in this population support larger randomized studies in patients with micro-metastatic disease.

According to NCI IND 10915 2010 APR information, the most common event was injection site reaction (31 events) followed by fatigue (22 events). One event, fever, was Grade 3. There have been no IND safety reports related to PROSTVAC-V/F or GM-CSF in this study.

Risk Management Procedures to be Utilized in Protocol BNIT-PRV-301

As with the previously conducted clinical studies of recombinant poxviruses conducted in the United States, potential risks for this study will be managed through several avenues:

1) This study will be conducted in accordance with the United States Code of Federal Regulations and ICH guidelines.

    a. All principal investigators and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to to the principles of Good Clinical Practices (GCP) as described in the United States Code of Federal Regulations (CFR) 21 parts 50, 54, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry - E6 Good Clinical Practice: Consolidated Guidance". Further, all study activities will be conducted in keeping with local, state, and federal legal and regulatory requirements and the Declaration of Helsinki 2004.

    b. Clinical sites where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection, processing and storage of human specimens;

    c. All clinical sites will be regularly monitored by BNIT (or its designee) for protocol adherence and compliance with all applicable regulations and guidelines.

2) Clinical site staff will be thoroughly trained on the study protocol prior to initiation of the study.

    a. A thorough study-specific training covering all aspects of the study will occur prior to the initiation of the study via a formal local investigator meeting and/or on-site study initiation visit. All clinical site personnel involved in the handling or administration of the GMO will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals;

b. PROSTVAC-V is considered a BSL-2 agent; PROSTVAC-F is considered BSL-1 (BSL-2 outside the United States). All vaccines in this trial should be handled using standard infection control procedures and study staff should wear personal protection consisting of at least a lab coat, gloves, and protective eyewear when handling PROSTVAC.

c. Clinical site staff with the responsibility of administering PROSTVAC, collecting clinical samples, or clinically evaluating study subjects, are instructed to follow the World Health Organization (WHO) universal precautions for the prevention of transmission of infectious agents in healthcare settings (WHO Standard Precautions 2006).

3) All transport of PROSTVAC will be done according to guidelines for the transport of GMOs and IATA Transportation Regulations;

4) Accountability of Drug Supplies

a. Prior to study start, each Investigator will provide the actual location (facility, room number, and freezer/refrigerator number, if any) and identify the pharmacist, study coordinator, subinvestigators,or other personnel who will have access to the drug, and if different from above, any personnel who will have preparation, administration and or receipt/return/disposal responsibilities of the study drug.

b. The Investigator or his/her designee must maintain accurate records of dates, quantities and the lot numbers of all study drug received, to whom dispensed (patient-by-patient accounting), and accounts of any product accidentally wasted or intentionally destroyed. The Investigator or designee must retain all used, unused, partially used, wasted, or expired study drug until the study monitor has confirmed accountability unless the institution has a policy of immediate disposal/destruction for used experimental products.

c. Local or institutional regulations may require immediate destruction of used study drug. In these cases, it is acceptable for the investigational site staff to destroy any remaining dispensed study drug before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization to destroy study drug prior to monitoring reconciliation must be obtained from the Sponsor or designee at study start-up. Each research center must provide the Sponsor a statement of the institutional policy and procedures for disposing of material with a Biosafety rating of RG2 (Appendix 12.2; RAC Guidelines 2002, Appendix B or equivalent outside the United States) and the institution’s policy and procedures for

disposal of hazardous waste or biologic products. A copy of the policy must be placed in the Pharmacy Manual.

d. At the conclusion of the Treatment phase of the study, all unused vaccine, GM-CSF, and placebo supplies (collectively, study mediation) will be either destroyed on site or by a licensed facility contracted by the site, or returned to the Sponsor or designee following final reconciliation. At the conclusion of the Treatment phase of the study, an overall summary of all study drug received, unused, partially used, wasted, and returned must be prepared.

5) Secondary Transmission

a. Only subjects meeting all protocol-defined eligibility criteria will be enrolled into the study. In particular, the study exclusion criteria outlined in the protocol exclude subjects from participation if they have the potential to come into contact with individuals considered to be at risk for secondary transmission PROSTVAC should a subject shed vaccine virus. These include subjects who are 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 year 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.

b. To minimize the potential for secondary transmission, the site of the PROSTVAC-V or placebo vaccination (Week 1) will be covered with a sterile, nonadherent dressing such as a Telfa bandage until the scab falls off naturally. Patients, and if possible, patient caregivers, will be educated as to the care of the injection site, including proper bandage changing, bathing, possible side effects, and minimization of contact with vulnerable populations. Patients will be provided with injection site care ‘kits’ containing, for example: instruction sheets, disposable gloves, absorbent toweling, alcohol swabs, nonadherent Telfa-type bandages, band-aids, zip-lock biohazard bags for disposal of used bandages and gloves; pre-filled syringes for home injection of GM-CSF or placebo, a cold pack, a sharps container, a digital thermometer, contact information for the study personnel, and a patient diary. Patients will be instructed to return the zip-lock biohazard bag containing all used supplies at their next clinic visit.

c. Accidental transmission of vaccinia virus to a clinic staff member or a member of the patient’s family or friends will be reported on a modified SAE form and the event will be followed by the Principal Investigator until resolved. All such events will be summarized in the annual safety update to the appropriate regulatory authorities. Any accidental or suspected secondary transmission should be reported immediately to the Sponsor’s Medical Monitor or designee.

6) Safety monitoring of all subjects

    a. Subjects will be observed for a minimum of 30 minutes following administration of PROSTVAC or placebo for observation for signs of adverse reactions.

    b. During the 21-week Treatment phase of this study the study visits will occur on Weeks 3, 5, 9, 13, 17 and 21 (± 3 days for each individual visit). Patients will be followed during the Treatment phase of the study for any signs or symptoms oftreatment-emergent toxicity by means of a focused physical exam, hematology, serum chemistry panels, EKG, and recording of AEs and concomitant medications.

    c. In the event of complications due to administration of PROSTVAC-V, including eczema vaccinatum, server generalized vaccinia, progressive vaccinia, and some cases of auto-inoculation, VIG therapy may be provided. In addition, the intravenous anti-viral drug cidofovir (Vistide®) can be used for severe infections, if patient is not responding to VIG.

7) Serious adverse events must be reported by the Investigator within 24 hours of learning of the event. If the event is fatal or life-threatening, the Sponsor or its designee should be notified immediately by phone, as well as by fax or email. The BNIT Medical Monitor will review all SAEs in real time.

8) In addition to the Medical Monitor, a Data Monitoring Committee (DMC), comprised of a multidisciplinary group of experts, will also share responsibility for safety management during the study. The DMC members will have no conflict of interests relative to the sponsor or study, including any financial, intellectual, or operational connection to the Sponsor or any competitor of the Sponsor.

9) The study protocol outlines the events that would discontinue dosing and enrollment of additional subjects until review of the event in question by the Medical Monitor and the DMC

PART A3: DETAILS OF PREVIOUS APPLICATIONS FOR RELEASE

BNIT has applied for approval of deliberate release of the GMO under Directive 2001/18/EC in Belgium, Estonia, France, Germany, Poland, Spain, and the United Kingdom. Approvals for the deliberate release of the GMO have been issued in Estonia (B/EE/12/01), France (B/FR/12/GT01**)**, Spain (B/ES/12/14**)**, and the United Kingdom (England -11/R44/01; Wales - 11/R44/01(W)); approvals are pending in Belgium (B/BE/11/BVW2), Germany, and Poland. Other EU member states in which BNIT anticipates filing applications for deliberate release for the proposed clinical trial (BNIT-PRV-301) include Denmark, Netherlands, and Slovakia.

Outside the European Union, BNIT has applied for approval of deliberate release of the GMO in Australia, Canada, and Iceland. Approvals for the deliberate release of the GMO have been issued in Canada (EAU-666, EAU-667, EAU-668) and Iceland (B/IS/12/01); approval is pending in Australia (DIR116). BNIT also anticipates filing applications for deliberate release of the GMO in Argentina, Brazil, and Chile.

PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

SUMMARY

PROSTVAC™ (PROSTVAC-V/F) is a live attenuated viral vector-based investigational vaccine product that is comprised of two component viral vectors, to be used together in a prime-boost vaccination regimen: (1) PROSTVAC-V: Recombinant vaccinia virus that contains a modified gene encoding human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules: B7.1, intracellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3) (or TRIad of COstimulatory Molecules, TRICOM™); and (2) PROSTVAC-F: Recombinant fowlpox virus that co-expresses the same four human genes as PROSTVAC-V.

PROSTVAC-V/F is being developed for the treatment of metastatic castration-resistant prostate cancer (mCRPC). This agent is the product of more than 15 years of poxviral vector development and evaluation by the NCI, the former Therion Biologics Corporation, and BN ImmunoTherapeutics, Inc. Over 300 patients have been treated with the PROSTVAC-V/F product.

PROSTVAC-V and PROSTVAC-F do not exhibit any known phenotypic changes (e.g., changes in virulence or growth advantage) that would increase their risk to the environment relative to their nonrecombinant parental pox viruses, which are derived from licensed vaccines. Thus, the added human transgenes have not fundamentally altered the inherent properties of the viruses. In addition, the analysis of viral shedding, transmission, persistence in the environment, and other potential issues indicates that the risk of any significant impact of PROSTVAC-V/F on the environment is low.

Viral shedding studies of smallpox vaccine indicate that nonrecombinant vaccinia virus is shed transiently from the site of vaccination and rarely from other sites (*Cooney, 1991; Cummings, 2008; Friedman, 1962, Frey, 2002; Kim,2005; Klote, 2005; Koplan,1975*). Clinical studies of recombinant vaccinia viruses, including PROSTVAC-V, have demonstrated viral shedding only at the site of vaccination (*Arlen, 2007; Brysiowicz, 1996; Cooney, 1991; Graham, 1992; Mukherhjee, 2000; Scholl, 2000; Scholl, 2003*). Subcutaneous vaccination with vaccinia virus results in reduced viral shedding relative to vaccination by scarification (*Henderson, 1939; Cherry, 1977; Connor, 1977*). Bandaging contains the virus at the vaccination site, further minimizing release into the environment (*Talbot, 2006*).

Contact transmission of vaccinia-based smallpox vaccine is rare (*Neff, 2002; CDC, 2004*). No secondary transmission of recombinant pox viruses, including PROSTVAC-V and PROSTVAC-F, to contacts has been reported in humans. However, PROSTVAC-V and PROSTVAC-F are live viruses and, as such, retain the potential for transmission. Consequently,

healthcare personnel who have direct contact with contaminated dressings or other infectious material from participants in clinical studies are instructed to adhere to appropriate infection control measures and can be offered vaccination with vaccinia vaccine. In addition, study exclusion criteria exclude subjects from participation if they have close or household contact with individuals at risk for exposure to vaccinia virus. Use of appropriate infection control measures, such as covering the vaccination site and washing hands after contact with the vaccination site, will prevent transmission.

Fowlpox virus does not replicate in human cells *(Somogyi, 1993)*. Consequently, viral shedding in humans is limited and appears to be confined to the vaccination site. These agents are not known to cause disease in healthy adult humans and are of minimal potential hazard to personnel and the environment under ordinary conditions of use. They can be handled safely in the laboratory without special apparatus or equipment, using techniques generally acceptable for nonpathogenic material.

Pox viruses cannot propagate without a permissive host organism. In a permissive host, pox virus infections are transient, lasting up to several weeks (*Fenner, 1988*). Pox viruses do not integrate into the genome of the infected cell. The pox virus life cycle is carried out in the cytoplasm of infected cells *(Moss, 1996)*. Therefore, the risk of pox virus persistence by integration into the host chromosome is very low, unlike other viruses, such as retroviruses, which integrate and establish permanent infections in the host.

Vaccinia and fowlpox viruses are stable when stored frozen or when lyophilized under carefully controlled conditions (*Fenner, 1988*). Under normal environmental conditions, however, these viruses lose viability within days or weeks (*Essbauer, 2007; Mahnel, 1987; Mahl, 1975; McDevitt, 2007; Newman, 2003; Pastoret, 1996; Sidwell, 1966*). In addition, pox viruses are readily inactivated by a number of common disinfectants and cleaning agents (*Erterpi, 2009*).

Recombinant vaccinia and fowlpox viruses are currently commercially available and widely distributed in the environment as veterinary vaccines (*Meeusen, 2007*). No environmental issues associated with the use of these recombinant vaccines have been reported.

RISK ASSESSMENT

Conclusions on the Potential Environment Impact from the Release of GMOs

Potential Routes for Environmental Exposure

PROSTVAC-V/F will be administered in an appropriately controlled clinic environment by subcutaneous injection into an alcohol-cleansed site in the upper arm (deltoid) or upper outer thigh of men aged 18 years or older with asymptomatic or minimally symptomatic, metastatic CRPC. The PROSTVAC-V or placebo vaccination site will be covered with a sterile, nonadherent dressing such as a Telfa bandage until the scab falls off naturally. For the

PROSTVAC-F or placebo, the injection site will be covered with a Band-aid until a scab (if any) forms or for patient comfort.

Routes by which the product may potentially enter the environment include the following:

- Shedding of virus from study subjects who have received the vaccine;
- Accidental, inappropriate disposal of the product into the sewer, or municipal waste at the site of use;
- Breach of container integrity during shipping and storage
- Accidental exposure to the vaccine by personnel involved in preparation and administration of the vaccine at the site of use or handling during shipping.

An analysis of these potential routes for exposure is described in the sections below, including the appropriate measures in place during the conduct of the study to mitigate the potential for environmental exposure to occur, and mitigation of adverse impacts in the event of any environmental exposure.

PROSTVAC-V and PROSTVAC-F are live vaccines and require a host cell to replicate. They can only remain viable following infection and proliferation in an appropriate host organism. Other than potential host organisms, it is considered that there are no environmental niches or habitats that would be affected, either directly or indirectly, by exposure to PROSTVAC.

i. Likelihood of the genetically modified organism (GMO) becoming more persistent and invasive in natural habitats under the conditions of the proposed release(s).

It is not likely that PROSTVAC-V or PROSTVAC-F will become more persistent or invasive in natural habitats under the conditions of the study.

PROSTVAC-V

Vaccinia virus has no known natural habitat and the origins of vaccinia virus in nature and as a vaccine are unknown. However, although vaccinia virus has no known natural animal reservoirs, some vaccinia strains have been isolated from domestic animals, including several vaccinia strains isolated from dairy cattle throughout Brazil, as well as buffalopox virus, first isolated in India and still associated with sporadic outbreaks in Pakistan, India, Bangladesh, Russia, Indonesia, Egypt, and Italy. Vaccine escape has been hypothesized to account for the presence of vaccinia strains in nature; however, sequence analysis and phylogenetic studies have provided evidence that it is unlikely these isolates have derived from a single vaccine strain used during the past century (*Trindade, 2007*). Thus, despite the extensive worldwide use of smallpox vaccine, vaccine escape into the environment seems to be at best a rare event.

Stability in the Environment

Vaccinia virus is relatively stable when stored at low temperatures. This contributed to the success of the smallpox vaccination program in developed countries where refrigeration was readily accessible. However, vaccine potency rapidly declined after storage at ambient temperatures, resulting in a high rate of vaccination failures in developing countries in the early twentieth century. Major efforts were required to develop a dried vaccine that would be stable at ambient temperatures (*Fenner, 1988*). Simple drying resulted in variable stability. Even complex drying conditions often gave unsatisfactory results. Improvements were gradually introduced and appropriate lyophilization conditions were finally optimized in the 1950s, contributing to the ultimate success of the global smallpox vaccination program.

Pox viruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs. They are also are relatively stable when stored frozen or lyophilized under carefully controlled conditions. However stability decreases significantly as temperature is increased. Under normal environmental conditions, PROSTVAC-V and PROSTVAC-F are expected to lose viability within days or weeks.

There are several published studies on the recovery of non-recombinant vaccinia virus under various laboratory conditions designed to mimic environmental exposure. Virus survival was evaluated, for example on fabric and food (*Sidwell, 1966; Pastoret, 1996; Essbauer, 2007*); dried or in liquid (including saline, drinking water, sterile-filtered lake or river water, storm water, storm water supplemented with fetal calf serum or mixed with potting soil) (*Mahnel, 1977; Mahnel, 1987; Essbauer, 2007*); under different conditions of relative humidity, temperature or pH (*Mahl, 1975; Newman, 2003)*; exposed to air or in airtight containers (*Mahnel, 1987*). There are many differences among these published experiments, including different vaccinia strains, preparation techniques, different starting virus titers, and viral titration methods. However, there are clear data in all of these studies first, that vaccinia is a relatively stable virus under a variety of laboratory conditions, and second, that stability decreases significantly as temperature is increased.

Specific stability studies have been performed on the non-recombinant vaccinia parental virus (TBC-Wy) and on a vaccinia recombinant similar to PROSTVAC-V, designated PANVAC-V (*unpublished data*). PANVAC-V is a recombinant vaccinia virus that, like PROSTVAC-V, expresses the three TRICOM costimulatory molecules; in addition, it expresses two additional tumor-associated antigens (*Petrulio, 2006; Madan, 2007*). Stability of PANVAC-V at dried or in water at various temperatures was compared to that of the non-recombinant parental virus, TBC-Wy. These studies demonstrated that the recombinant virus is comparable to the corresponding non-recombinant parental virus. Both viruses lost viability over a period of weeks when stored in water at 25°C. The same viruses lose viability over a period of days when stored dried at 25°C. These results are consistent with published reports discussed above that

demonstrate that pox viruses are stable at low temperatures but are less stable at higher temperatures without special treatment such as lyophilization under controlled conditions.

Viral Shedding

Release of PROSTVAC-V into the environment as a consequence of shedding after immunization of study subjects is unlikely. Although PROSTVAC-V is a replicating vaccinia virus, it is administered subcutaneously. Consequently, the reactions associated with intradermal administration of traditional vaccinia vaccines (scarring, pustules, vesicle formation) are not typical, and were not observed in either of the BNIT/Therion sponsored clinical studies of PROSTVAC-V/F. Lesion formation at the vaccination site is considered an indirect measure of viral shedding (*Rotz, 2001; Lane, 2003*). Consequently, the lack of lesion formation after administration of PROSTVAC-V is expected to reflect a significantly reduced level of shedding as compared with vaccination by scarification.

This assumption was tested in an NCI-sponsored clinical trial (BB-IND-10915) in which the dose and route of vaccination of PROSTVAC-V were the same as the intended dose and route of vaccination for the proposed study. In the NCI study, viral shedding and lesion formation were evaluated in four patients that had evidence of prior vaccination with vaccinia virus as smallpox vaccine (*Arlen, 2007*). Viral shedding was assessed over a four-week period following administration by PCR analysis and viral plaque assay of injection site swabs, urine, saliva, and blood samples. The results indicated that, while viral DNA is shed and persists in some patient samples, live virus is shed transiently, and probably exclusively, from the vaccination site. Bandaging and proper care of the vaccination site should minimize the likelihood of viral shedding after subcutaneous administration of PROSTVAC-V.

PROSTVAC-F

Avipox viruses are distributed worldwide and cause disease in domestic, pet and wild birds of many species. Transmission of virus can occur through a break in the skin or, more commonly, when vectored by biting insect such as mosquitoes and mites. Aerosols generated from infected birds or the ingestion of contaminated food or water have also been implicated as a source of transmission.

These viruses are highly host specific. Fowlpox virus replication has been demonstrated *in vivo* in certain avian species, namely chickens, turkeys, and pigeons, but not in quail, ducks, or canaries (*Tripathy, 1984; McMillen, 1994*). Productive replication of avipox viruses, including the parental non-recombinant fowlpox virus TBC-FPV, has not been demonstrated *in vivo* in nonavian species. Productive replication *in vitro* has not been observed in human cells, monkey cells, and in most mammalian cells tested (*Somogyi, 1993*). A single exception is a hamster cell line, BHK-21 cells, which is semi-permissive for the growth of fowlpox virus (*Weli, 2004; Weli, 2005*).

Stability in the Environment

There are few published studies on the persistence of avipox viruses in the environment. However, persistence in the environment, adverse sequellae, and other environmental issues have not been reported as a result of the use of licensed recombinant fowlpox and canarypox virus-based products, including veterinary vaccines against Newcastle Disease Virus, avian influenza virus, rabies, feline leukemia virus, canine distemper, and West Nile virus. As part of the licensure procedure, the USDA announced Findings of No Significant Impact with respect to the likelihood of an adverse environmental event using these vaccines (*Payne, 1994*; *Payne, 1995*; *Payne, 1996*; *Federal Register, 1994, 1997a, 1997b, 2003, 2004*).

Specific stability studies have been performed on the non-recombinant fowlpox parental virus (TBC-FPV) and on a fowlpox recombinant similar to PROSTVAC-F, designated PANVAC-F (*unpublished data*). PANVAC-F is a recombinant vaccinia virus that, like PROSTVAC-F, expresses the three TRICOM costimulatory molecules; in addition, it expresses two additional tumor-associated antigens (*Petrulio, 2006; Madan, 2007*). Stability of PANVAC-F at dried or in water at various temperatures was compared to that of the non-recombinant parental virus, TBC-FPV. These studies demonstrated that the recombinant virus is comparable to the corresponding non-recombinant parental virus. Both viruses lost viability over a period of weeks when stored in water at 25°C. The same viruses lose viability over a period of days when stored dried at 25°C. These results indicate that PROSTVAC-F is stable at low temperatures but are less stable at higher temperatures without special treatment such as lyophilization under controlled conditions.

ii. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).

No selective advantage is likely to be conferred to the GMO under the conditions of the proposed release. For both PROSTVAC-V and PROSTVAC-F, comparison of the respective parental and recombinant viruses indicates that the added human transgenes in these recombinant viruses have not fundamentally altered the biologic properties of the viruses with respect to virulence, replicative ability, or stability in the environment.

Generation of PROSTVAC-V and PROSTVAC-F resulted in minimal disruption to the parental genome. In PROSTVAC-V, the human PSA and TRICOM genes are all inserted at a single site in an intergenic region between open reading frames F12L and F13L. No vaccinia genes are interrupted by this insertion. In PROSTVAC-F, the human genes were inserted into the coding sequence of the FPV246, which has homology to the ankyrin repeat gene family. The absence of this gene is not predicted to have an effect on the properties of fowlpox virus.

Growth properties of recombinant and non-recombinant viruses are similar. Under the same set of growth conditions in primary chicken embryo dermal cells, the yield of PROSTVAC-V was

approximately half that obtained with the parental virus. PROSTVAC-F grows approximately as well *in vitro* as its non-recombinant parental fowlpox virus.

Although PROSTVAC-V and PROSTVAC-F have not been directly compared to their respective non-recombinant parental viruses with respect to environmental stability, studies using similar recombinant viruses (PANVAC), which express expresses TRICOM together with two additional tumor-associated antigens, showed that stability of the recombinants dried or in water at various temperatures was comparable to that of their respective non-recombinant parental viruses. When stored at 25°C, both recombinant and non-recombinant viruses lost viability over a period of weeks when stored in water and over a period of days when stored dried.

The most sensitive method for measuring virulence of vaccinia virus *in vivo* has traditionally been the intracranial LD_{50} test in weanling mice. As measured by this neurovirulence assay, PROSTVAC-V is more attenuated that its parental non-recombinant vaccinia virus, which is in turn significantly more attenuated than the Dryvax smallpox vaccine. PROSTVAC-F is also significantly more attenuated than the Dryvax smallpox vaccine.

A randomized (2:1), double-blind, placebo-controlled Phase 2 clinical trial of PROSTVAC-V/F, in which non-recombinant parental viruses were used as placebo, was recently completed (*Kantoff, 2010*). Eighty two patients in the active group and 40 patients in placebo group received drug. The safety profile was similar for the two groups, supporting the similarity between recombinant and non-recombinant viruses with respect to virulence and replicative ability.

The comparison of PROSTVAC-V and PROSTVAC-F with their corresponding non-recombinant parental viruses, which are derived from licensed products currently used as human and veterinary vaccines, respectively, demonstrate that PROSTVAC-V and PROSTVAC-F are not more virulent, growth-advantaged, or stable in the environment. These studies indicate that the added human transgenes in these recombinant viruses have not fundamentally altered the biologic properties of the viruses. Consequently, no selective advantage has been conferred to PROSTVAC-V/F.

iii Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.

The potential for gene transfer to other species under the proposed release of the GMO is extremely low. PROSTVAC-V and PROSTVAC-F cannot infect microbes, insects, cold-blooded vertebrates, or plant cells. The GMO will be released in a hospital examination room and is unlikely to come in contact with other animal species. Furthermore, no dissemination of PROSTVAC-V or PROSTVAC-F outside the injection site has been shown in humans injected by the subcutaneous route.

Recombination between the DNA genome of PROSTVAC-V or PROSTVAC-F with the DNA genome of a host cell is unlikely. The pox virus life cycle is carried out in the cytoplasm (*Moss, 1996*); pox viruses do not integrate into the genome of the infected cell. Therefore, the risk of pox virus persistence by integration into the host chromosome is very low. Although the human genes expressed in PROSTVAC-V and PROSTVAC-F share homology with their counterparts in the human genome, the physical segregation between host and viral genomes renders recombination between PROSTVAC-V or PROSTVAC-F and the human genome an unlikely event. The frequency, already unlikely, of any such recombination events in humans or non-avian species after administration of PROSTVAC-F would be further reduced by the lack of replicative capacity of fowlpox virus in these species.

Recombination between the two PROSTVAC-V/F components, PROSTVAC-V and PROSTVAC-F, is unlikely because the two viruses do not share homology, except with respect to the inserted human genes. No interviral recombination between vaccinia and fowlpox DNA has been observed (*Scheiflinger, 1992*). Furthermore, the two viruses are not expected to be in the same proximity, as vaccinations with PROSTVAC-V and PROSTVAC-F occur at different times and injection sites.

Recombination between PROSTVAC-V or PROSTVAC-F and a wild-type vaccinia or fowlpox virus in an infected host organism is theoretically possible. However, such recombination would not be expected to alter the virulence, growth properties, or environmental persistence of the virus. In addition, the likelihood of recombination between PROSTVAC-V and wild type vaccinia *in vivo* is extremely low because vaccinia is not normally found in nature. Recombination of vaccinia virus with other pox viruses, such as orf, molluscum contagiosum, or shope fibroma virus, has not been observed *in vitro*. Recombination between PROSTVAC-F and a wild or vaccine strain of fowlpox would require release of PROSTVAC-F into an environment containing poultry. Such release is highly unlikely under the proposed conditions of release.

Recombination with other viral genomes is also unlikely due to the lack of homology between different families of viruses as well as to the physical segregation between the genomes of the pox viruses and those of other viruses that replicate in the nucleus. Additionally, in non-avian species susceptible to infection by PROSTVAC-F, few opportunities for genetic recombination with other could occur, since the level of replication that the vector DNA undergoes *in vivo* is low, and limited to cells infected by the inoculum (no generation of infectious particles).

iv. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).

For the purposes of this release, the target organisms are the study subjects, i.e., men with asymptomatic/minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC). These study subjects will be administered treatment with the GMO as follows:

- PROSTVAC-V (vaccinia virus, PSA antigen, and TRICOM) 2×10^8 infectious units (Inf. U) (prime) or empty vector, subcutaneous: Week 1;
- PROSTVAC-F (fowlpox virus, PSA antigen, and TRICOM) 1×10^9 Inf. U (boost) or empty vector, subcutaneous: Weeks 3, 5, 9, 13, 17, and 21;

GM-CSF 100 µg or placebo will be co-administered on each of the dosing days and consecutively for three days after vaccination at the vaccination site.

PROSTVAC-V and PROSTVAC-F are predicted to interact with the immune system of the subjects to generate an immune response to PSA. PROSTVAC-V, a live recombinant vaccinia virus, actively replicates in human cells, resulting in the presentation of high levels of antigen to the immune system over a period of one to two weeks, substantially increasing the potential for immune stimulation. The immune response specific to vaccinia then eliminates the virus. Host cells infected with vaccinia virus are short lived (days) and die by a mixed form of apoptosis/ necrosis. Vaccinia replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA (*Moss, 1996*). Latent infection of humans with vaccinia virus has not been observed (*Fenner, 1988*).

PROSTVAC-F is a live recombinant fowlpox virus. Productive fowlpox virus infection is restricted *in vivo* to avian species and *in vitro* to cells derived from avian species; however, fowlpox-mediated gene expression does occur in infected non-avian cells (*Taylor, 1988; Beukema, 2006*), and a number of studies have shown that immunization of mammalian species by recombinant fowlpox virus can stimulate both humoral and cell-mediated immunity to the expressed transgene. Fowlpox vectors mediate a limited infection in human cells, with early viral and transgene expression, but late gene expression is blocked, and no infectious particles are produced.

Virus Shedding

Shedding of PROSTVAC-V and PROSTVAC-F by study subjects is the primary mechanism by which these subjects would introduce the GMO into the environment. Based on a number of clinical studies with PROSTVAC-V/F and related vaccines, which are described in detail below, viral shedding in this population is expected to be transient and confined to the vaccination site.

In addition, appropriate education of patients regarding care of the injection site (including proper bandage changing, bathing, possible side effects, and minimization of contact with vulnerable populations) will serve to minimize exposure to the environment. Patients will be provided with injection site care 'kits' containing instruction sheets, disposable gloves, absorbent toweling, alcohol swabs, nonadherent Telfa-type bandages, band-aids, and zip-lock biohazard bags for disposal of used bandages and gloves. The zip-lock biohazard bag containing all used supplies will be returned to the clinic for appropriate disposal.

Shedding of both nonrecombinant and recombinant pox viruses, including vaccinia virus, fowlpox virus, and canarypox virus, have been addressed in a number of clinical studies. The data, which are presented below, indicate that: vaccinia viral shedding is transient and occurs at the vaccination site and rarely at other sites; vaccinia viral shedding occurs with high frequency after scarification and with low frequency after subcutaneous vaccination, which is the intended route of administration of PROSTVAC-V; bandaging contains the virus at the vaccination site; shedding of avipox virus after vaccination of nonavian species is confined to the vaccination site.

In studies that assess both a recombinant virus and its nonrecombinant parental virus, the incidence and amount of viral shedding are found to be comparable between the two viruses. That is, no recombinant pox virus has been shown to exhibit greater capacity for shedding relative to its nonrecombinant parental pox virus. These results, combined with the patient precautions with respect to care of the vaccination site, indicate that any potential impact to the environment will be negligible.

Vaccinia Shedding

Vaccinia virus is shed from the primary vaccination lesion of humans and guinea pigs from approximately the third day to the end of the third week after vaccination (*Cooney, 1991; Friedman, 1962*). Limited data are available on viral shedding after vaccination of vaccinia-experienced (previously vaccinated) individuals, but shedding from revaccination sites seems to be shorter by about1 week and possibly of lower titer than shedding from primary vaccination sites (*Seaman, 2010*). This finding is significant, as all study subjects are required to have had prior smallpox vaccination. Viral shedding is further reduced in individuals who are vaccinated by the subcutaneous route (*Henderson, 1939; Cherry, 1977; Connor, 1977*), which is the route that will be used for the administration of PROSTVAC-V.

That airborne or droplet spread of vaccinia virus from the respiratory tract of healthy vaccinees occurs is doubtful (*Lane, 2003*). Although the virus can be found in the bloodstream and pharynx of patients with adverse events involving vigorous viral replication and/or abnormal host defenses, particularly eczema vaccinatum and progressive vaccinia (*Lane, 2003*), epidemiologic evidence for airborne spread is scant. Consequently, airborne infection isolation precautions are not necessary unless procedures that cause aerosolization of the virus are performed.

Experience with Smallpox Vaccine—Lesion Formation and Viral Shedding after Dermal Scarification

A number of vaccinia viral shedding studies were published during the height of the worldwide smallpox eradication program in the 1960s and 1970s, and more recently as a result of anti-bioterrorism initiatives. Viral shedding after vaccination with smallpox vaccine, which is administered by dermal scarification, has been assessed either directly by determining the presence of virus (*Frey, 2003; Frey 2002a; Kim, 2005*) or indirectly by observing lesion

formation as a surrogate for the presence of replicating virus (*Frey, 2002, Hsieh, 2006*) in persons vaccinated with smallpox vaccine.

Lesion formation at the vaccination site is considered an indirect measure of viral shedding (*CDC, 2001; Lane, 2003*). These studies showed that vaccinia viral shedding and lesion formation occur transiently at the injection site in most vaccinees after smallpox vaccination by scarification. Viral shedding at other sites, such as blood, urine, and throat, is rare and is generally associated with more virulent strains of smallpox vaccine or with complications after vaccination. In particular, no viral shedding at sites other than the vaccination site has been demonstrated after the normal course of vaccination with the Dryvax smallpox vaccine. Dryvax and PROSTVAC-V are both derived from the same parental vaccinia virus, Wyeth/NYCBH, which is associated with the lowest frequency of complications among widely used vaccinia strains (*Fenner, 1988*).

Experience with Smallpox Vaccine–Lesion formation and Viral Shedding After Subcutaneous Vaccination

In several studies, lesion formation in individuals who received the smallpox vaccine by the subcutaneous route was compared to that in individuals receiving the smallpox vaccine by intradermal scarification (*Henderson, 1939, Cherry, 1977; Conner, 1977*). In all of these studies, subcutaneous vaccination with smallpox vaccine results in fewer and smaller lesions relative to vaccination by scarification. Viral shedding is associated with the presence of a lesion at the vaccination site. Therefore, viral shedding is reduced after vaccination with vaccinia virus by the subcutaneous route, the intended route of administration for PROSTVAC-V, as compared with vaccination by scarification.

The studies described above that assessed viral shedding by direct assessment of nonrecombinant vaccinia viruses used doses lower than the doses used for recombinant vaccinia viruses, including PROSTVAC-V. However, the results with nonrecombinant vaccinia viruses are consistent with the results observed after vaccination at the higher doses used with recombinant vaccinia viruses (see below).

Recombinant Vaccinia Viral Shedding in Humans

The shedding of four recombinant vaccinia viruses that express HIV-1 envelope, human papilloma virus (HPV) E6/E7, interleukin-2 (IL-2), or MUC-1/IL-2 was evaluated in six published clinical studies (*Cooney, 1991; Graham, 1992; Borysiewicz, 1996; Mukherjee, 2000; Scholl, 2000; Scholl, 2003*). These studies demonstrate that viral shedding profiles after vaccination with recombinant vaccinia viruses are similar to that observed after vaccination with nonrecombinant vaccinia virus. Viral shedding of recombinant vaccinia virus occurs transiently at the vaccination site after scarification and has not been demonstrated to occur at other sites, including nose, throat, urine, and feces after vaccination intramuscularly or intratumorally. Lesion formation at the vaccination site is associated with viral shedding. The frequency of viral shedding is reduced after subcutaneous vaccination with recombinant vaccinia virus. Bandaging contains the virus at the vaccination site. These data suggest that shedding of PROSTVAC-V is

expected to occur transiently and with reduced frequency when administered subcutaneously, its intended route of administration.

PROSTVAC-V and a related vaccine, designated TBC-3B, have been assessed with respect to viral shedding in humans. These two viruses were generated using the same parental vaccinia virus, a derivative of the New York City Board of Health strain. The results are described in detail below.

PROSTVAC-V

A Phase 1 study of PROSTVAC-V/F was conducted in prostate cancer patients by the National Cancer Institute (*Arlen, 2007*). All patients that received PROSTVAC-V in this study were vaccinated subcutaneously with 2 x 10^8 pfu PROSTVAC-V. All patients were boosted four weeks later with 1 x 10^9 pfu PROSTVAC-F. Several groups of patients also received GM-CSF, in the form of either recombinant human GM-CSF (rh-GM-CSF) or recombinant fowlpox expressing GM-CSF (rF-GM-CSF) at the vaccination site at the time of each vaccination.

Viral shedding and lesion formation were evaluated in four patients that had evidence of prior vaccination with vaccinia virus as smallpox vaccine. Viral shedding was assessed over a four-week period following subcutaneous vaccination with PROSTVAC-V. Injection site swabs and urine, saliva, and blood samples were taken on Day 0 (prior to vaccination), then on Days 3 or 4, 7, 14, and/or 28 or 32 post-vaccination. A total of 56 post-vaccination samples were obtained from these four patients.

Each sample, including blood fractionated into serum and peripheral blood mononuclear cell (PBMC) components, was assessed for the presence of viral DNA by PCR, using a primer that detects vaccinia and variola, but not fowlpox virus. Limits of detection were 10^3 pfu in injection site swab, 10^1 pfu in PBMC, 10^3 pfu in serum, 10^6 pfu in urine, and 10^6 pfu in saliva. The results are summarized in **Table A4-1**. Seventeen of 56 post vaccination samples (30%) tested positive for vaccinia DNA. Viral DNA was detected in injection site swabs from all four patients. Viral DNA was also detected in PBMCs from three patients and in serum from two patients. No viral DNA was detected in urine and saliva samples.

Table A4-1: Viral DNA in Patient Samples After Subcutaneous Vaccination with PROSTVAC-V

Sample	**Number of Patients Positive for Viral DNA/ Number of Patients Tested**					
	Day 0	**Day 3/4**	**Day 7**	**Day 14**	**Day 28/32**	**Total**
Urine	0/4	0/3	0/2	0/4	0/3	0/4
Saliva	0/4	0/2	0/1	0/4	0/2	0/4
PBMC	0/4	1/3	0/2	0/4	3/3	3/4
Serum	0/4	1/3	0/2	2/4	2/3	2/4

Injection Site	0/4	1/3	1/2	4/4	2/2	4/4

Four prostate cancer patients were vaccinated with 1 x 108 pfu PROSTVAC-V. Samples were taken prior to vaccination and at various times post-vaccination. DNA extracted from each sample was assessed with respect to the presence of vaccinia viral DNA by PCR.

The presence of viral DNA does not indicate whether live virus is present. Therefore, patient samples were also assessed by plaque assay, which indicates the presence of live virus. The limit of detection in the live virus assay was not determined. The results are summarized in **Table A4-2**. Live virus was detected in only one of the four patients. Live virus was detected at the injection site at Day 7 and Day 14 in this patient. In addition, in one PBMC sample from this patient at Day 3, one single plaque was detected in one out of twelve duplicate wells. Live virus was not detected at the injection site at this time point. Three other PBMC samples from this patient, taken at different time points after vaccination, were negative. All PBMC samples from other patients were negative. No live virus was detected in any sample from any patient after Day14.

Table A4-2: Live Virus in Patient Samples After Subcutaneous Vaccination with PROSTVAC-V

Sample	**Number of Patients Positive for Live Virus/ Number of Patients Tested**					
	Day 0	**Day 3**	**Day 7**	**Day 14**	**Day 28**	**Any Day**
Urine	0/4	0/3	0/2	0/3	0/3	0/4
Saliva	0/1	0/1	0/1	0/3	0/1	0/3
PBMC	0/4	1[a]/3	0/2	0/4	0/3	1[a]/4
Serum	0/3	0/3	0/2	0/4	0/3	0/4
Injection Site	0/3	0/3	1/1	1/4	0/2	1/4

Four prostate cancer patients were vaccinated with 2 x 10^8 pfu PROSTVAC-V. Samples were taken prior to vaccination and at various times post-vaccination. Each sample was assessed with respect to the presence of live vaccinia virus by viral plaque assay.

[a] One plaque was detected in one of twelve duplicate wells from one sample from one patient.

Each patient was monitored with respect to injection site reactions after subcutaneous vaccination with 2 x 10^8 pfu PROSTVAC-V. Injection site reactions are listed in **Table A4-3**. Live viral shedding information from **Table A4-2** is included to assess the association between lesion formation and viral shedding. Only one of four patients formed a lesion (vesicle followed by eschar) after subcutaneous vaccination. Live virus was detected at the vaccination site of this patient only.

Table A4 -3: Injection Site Reactions and Live Viral Shedding after Subcutaneous Vaccination with PROSTVAC-V

Patient Number	Vaccine	Injection Site Reaction	Live Virus Detected At Vaccination Site
3827069	PROSTVAC-V	none	No
3834116	PROSTVAC-V plus 1×10^7 pfu rF-GM-CSF[a]	pruritis, erythema, vesicle, eschar	Yes
3941401	PROSTVAC-V plus 1×10^7 pfu rF-GM-CSF	pruritis, erythema	No
3950165	PROSTVAC-V plus 1×10^8 pfu rF-GM-CSF	pruritis, erythema	No

[a] rF-GM-CSF is recombinant fowlpox virus that expresses GM-CSF.

A randomized, double-blind, placebo-controlled Phase 2 clinical trial of PROSTVAC-VF was recently completed (*Kantoff, 2010*). The objective of this study was to evaluate safety and efficacy of PROSTVAC-V/F in combination with recombinant human GM-CSF versus empty vector in combination with placebo in patients with androgen-independent adenocarcinoma of the prostate. The vaccine route, dosage, and schedule is comparable to that contemplated for the proposed release.

Eighty-four patients were enrolled into the active group and 41 patients enrolled into the placebo group. Eighty-two patients in the active group and 40 patients in placebo group received drug.

General disorders and administration site conditions were the most common treatment-related system organ class: 67 patients (81.7%) in the PROSTVAC-V/F group and 31 patients (77.5%) in the control group. The most frequently reported events were injection site-reactions, including injection site erythema, induration, pain, pruritus, swelling, and warmth. These local injection site reactions were balanced in the treatment arms, indicating that the frequency of lesion formation is comparable after vaccination with nonrecombinant and recombinant vaccines.

TBC-3B

TBC-3B is a recombinant vaccinia virus that co-expresses the env, gag, and pol genes from a Clade B strain of HIV-1. This candidate AIDS vaccine is based on the same parental vaccinia virus as PROSTVAC-V.

Two clinical trials of TBC-3B (BB-IND-4930) were performed by the AIDS Vaccine Evaluation Group (AVEG), sponsored by the National Institutes of Health. Unpublished viral shedding data from these studies indicate that this recombinant vaccinia virus is shed transiently at the

vaccination site after vaccination by scarification. The frequency of TBC-3B viral shedding is reduced after subcutaneous vaccination.

In the first AVEG trial, TBC-3B was administered to fourteen subjects with evidence of prior smallpox (vaccinia) vaccination (vaccinia-immune subjects). The subjects in this first trial were vaccinated with 4.19×10^9 pfu/mL TBC-3B by scarification. The vaccination sites were assessed for the presence of live vaccinia virus by plaque assay. In the second AVEG trial, TBC-3B was administered to twenty healthy subjects with no prior history of smallpox vaccination (vaccinia-naïve subjects). Subjects were vaccinated with 3.67 x 106 pfu TBC-3B either subcutaneously or by scarification.

In these two trials, the vaccination sites were assessed after vaccination for the presence of live vaccinia virus by plaque assay. The outer bandage was assessed for the presence of live vaccinia in one study. The results are summarized in **Table A4-4**. Shedding at the site of vaccination was observed in most subjects that were vaccinated by scarification. In contrast, viral shedding was detected only in a small proportion of subjects after subcutaneous vaccination. All vaccinees that shed virus had lesions at the vaccination site. No virus was detected in the outer bandage, demonstrating that bandaging contained the virus at the vaccination site and effectively prevented spread of virus to the environment.

Table A4-4: Viral Shedding after Vaccination with AIDS Vaccine TBC-3B

Prior Vaccination Status	Route	Dose	Sample(s) Assessed	Number Subjects Positive/Total Tested
Vaccinia-primed[a]	Scarification	4.19×10^9 pfu/mL	Vaccination site	7/14
			Outer bandage	All samples negative[b]
Vaccinia-naive[c]	Scarification	3.67×10^6 pfu	Vaccination site	10/10
	Subcutaneous	3.67×10^6 pfu	Vaccination site	3/10

Vaccinia viral shedding was assessed in two different clinical trials of TBC-3B, a vaccinia-based AIDS vaccine. The presence of live virus at the vaccination site or bandage was measured by infection of susceptible cells.

[a] Evidence of prior vaccination with vaccinia virus (smallpox vaccine)
[b] Thirty-two outer bandages were cultured; number of subjects was not noted
[c] No evidence of prior vaccination with vaccinia virus

Vaccinia viral shedding, lesion frequency, and lesion size at the site of vaccination were assessed in each of the twenty vaccinia-naïve subjects vaccinated subcutaneously or by scarification with TBC-3B. The results are shown in **Table A4-5**. Viral shedding was detected in all subjects vaccinated by scarification. In contrast, viral shedding was detected in only three of ten subjects vaccinated subcutaneously. The last day that virus was detectable at the vaccination site in these three subjects was thirteen, twenty-one, and twenty-eight days, respectively, after subcutaneous vaccination.

All subjects that were vaccinated with TBC-3B by scarification had lesions. The frequency of lesions was lower after subcutaneous vaccination. Viral shedding was observed only in subjects who had lesions at the vaccination site. Thirteen subjects vaccinated with TBC-3B that had detectable virus at the injection site also had lesions at the injection site. The four subjects who had no detectable virus at the injection site had no visible lesions. There were three subjects who had lesions but no detectable virus. In all three of these cases, the lesion size was quite small, ≤ 0.5 cm^2. There were no instances of detectable virus in the absence of a lesion. Thus there is an association between the presence of virus and the presence of visible lesions.

Table A4-5: Viral Titers and Lesions at Site of Vaccination with TBC-3B

Vaccination Route (N)	Number of Vaccinees Lesion-Positive	Number of Vaccinees Virus-Positive[a]	Maximum Viral Titer in All Vaccinees (log10 pfu) Mean ± SD
Scarification (10)	10	10	3.88 ± 1.62
Subcutaneous (10)	6	3	0.84 ± 1.81

Twenty vaccinia-naïve subjects were vaccinated with 3.67×10^6 pfu TBC-3B subcutaneously or by scarification. The amount of live virus in vaccination site swabs was assessed by plaque titration on susceptible cells. The difference between the maximum viral titers observed after scarification versus subcutaneous vaccination was statistically significant (p=0.001, Student's unpaired t-test).
[a] All subjects that shed virus also had lesions at the vaccination site.

A limited study was performed to assess the presence of vaccinia DNA in the blood of subjects in these two clinical trials of TBC-3B. Serum samples were taken on Day 0 prior to vaccination and on Day 56 post-vaccination. In the first trial, peripheral blood lymphocytes (PBL) harvested from the sera of nine vaccinia-primed subjects were subjected to nested PCR analysis for vaccinia DNA. The sensitivity of the assay was 100 copies of DNA per reaction. The limit of detection was ≤ 1 copy per 2500 cells or ≤ 1 copy per 670 cells, depending on the individual sample. No vaccinia DNA was detected in any of the test samples from the nine subjects.

Since TBC-3B contained HIV-1 DNA as well as vaccinia DNA, the blood from seventeen subjects was assessed for the presence of HIV DNA. Serum samples were taken on Day 0 prior to vaccination and on Day 56 post-vaccination. PBLs were harvested and were subjected to nested PCR analysis for HIV DNA sequences that are present in TBC-3B. The sensitivity of this assay was 100 copies of DNA per reaction. No HIV DNA was detected in any of the seventeen subjects. This confirmed the absence of TBC-3B vaccinia genomic DNA in the PBLs of these subjects. Thus, vaccinia viral DNA was not detected in PBLs on Day 56 following vaccination of vaccinia-primed subjects by scarification with a vaccinia-based vaccine.

In the second clinical trial of TBC-3B, serum samples from four subjects vaccinated by each route (scarification or subcutaneous) were taken on Day 0 prior to vaccination and on Day 56 post-vaccination. PBL harvested from the sera of these eight vaccinees were subjected to nested PCR analysis for vaccinia DNA. No vaccinia DNA was detected in any of the test samples from the eight vaccinees.

In addition, the blood from eight vaccinia-naïve subjects vaccinated with TBC-3B (five vaccinated by scarification and three vaccinated subcutaneously) was assessed for the presence of HIV DNA by nested PCR. Serum samples were taken on Day 0 prior to vaccination and on Day 56 post-vaccination. No HIV DNA was detected in any of the eight vaccinees. Since the same HIV DNA sequence was inserted into the genome of the recombinant vaccinia virus

TBC-3B, this confirmed the absence of TBC-3B vaccinia genomic DNA in the PBL of these vaccinees. Thus no vaccinia viral DNA was detectable in blood by Day 56 following vaccination of vaccinia-naïve subjects subcutaneously or by scarification with a recombinant vaccinia-based vaccine.

In summary, viral shedding studies of recombinant vaccinia virus TBC-3B demonstrated that the frequency of viral shedding was lower after subcutaneous vaccination relative to vaccination by scarification. In addition, the few subjects who shed virus after subcutaneous vaccination generally shed lower amounts of virus relative to the amount of virus shed after scarification.

Conclusions-Vaccinia Shedding

The studies described above demonstrate that viral shedding profiles after vaccination with recombinant vaccinia viruses are similar to that observed after vaccination with nonrecombinant vaccinia virus. Viral shedding of recombinant vaccinia virus occurs transiently at the vaccination site after scarification and has not been demonstrated to occur at other sites, including nose, throat, urine, and feces after vaccination intramuscularly or intratumorally. Lesion formation at the vaccination site is associated with viral shedding. The frequency of viral shedding is reduced after subcutaneous vaccination with recombinant vaccinia virus. Bandaging contains the virus at the vaccination site. These data suggest that shedding of PROSTVAC-V is expected to occur transiently and with reduced frequency when administered subcutaneously, its intended route of administration.

Avipox Shedding

Avipox viral shedding has not been detected in blood, saliva, urine, or rectal swabs of nonavian species after vaccination. Avipox viral shedding in humans appears to be confined to the vaccination site and is limited due to lack of viral replication.

PROSTVAC-F is a recombinant fowlpox virus. Recombinant fowlpox-based vaccines have been licensed by the USDA as veterinary vaccines against Newcastle Disease Virus and avian influenza virus. Canarypox virus is an avipox virus related to fowlpox virus. Recombinant vaccines based on canarypox virus have been licensed by the USDA for use in cats, dogs, and horses. These vaccines include rabies, feline leukemia virus, canine distemper, and West Nile Virus. In addition, recombinant fowlpox and canarypox viruses are under investigation as candidate vaccines for a variety of human cancers and infectious diseases.

Avipox Viral Shedding in Humans

Viral shedding studies have been performed after vaccination of humans with recombinant canarypox viruses expressing HIV-1 proteins or MAGE-1/3 epitopes (*Bleijs, 2005; J. Tartaglia and I. Elias, unpublished information*). These studies are summarized below.

In a clinical trial of a canarypox virus-based HIV vaccine, HIV-negative patients were vaccinated intramuscularly with $10^{7.0}$ cell culture infectious doses ($CCID_{50}$, synonymous with $TCID_{50}$) of vaccine. Samples of blood, saliva, and urine were taken at 3, 6, and 9 hours post-vaccination and at 1, 2, 7, and 14 days post-vaccination. Rectal swabs were taken at 1, 2, 7, and 14 days post-vaccination. The samples and swabs from three vaccinees were tested for the presence of viral DNA by nested PCR and were tested for live virus by viral culture. No viral DNA or live virus was detected in these samples.

In another clinical trial of a canarypox virus-based HIV vaccine, HIV-positive patients were vaccinated intramuscularly with $10^{7.08}$ $CCID_{50}$ of vaccine. Samples, including plasma, blood cells, saliva and urine, were collected from twelve patients prior to vaccination and 1, 2, and 6 hours post-vaccination. DNA was extracted from each sample and was then subjected to quantitative PCR (Q-PCR) analysis. All samples were negative for the presence of canarypox viral DNA. Limits of detection were 25 $CCID_{50}$/mL plasma, 200 $CCID_{50}$/mL blood cells, 5-10 $CCID_{50}$/mL saliva, and 25 $CCID_{50}$/mL urine.

In a clinical trial of a canarypox virus-based melanoma vaccine, melanoma patients were vaccinated subcutaneously and intradermally with $10^{7.09}$ $CCID_{50}$ vaccine. Samples of blood, saliva, urine, and injection site swabs were taken from three patients prior to vaccination and 1, 3, 6, and 9 hours post-vaccination. The presence of canarypox viral DNA was assessed by conventional PCR analysis. No canarypox viral DNA was detected in the blood, plasma, saliva, and urine samples. Limits of detection were not specified. Canarypox viral DNA was detected in injection site swabs from all three patients. Patients were boosted with vaccine three weeks after the first vaccination, and vaccination site swabs were taken up to 9 hours post-vaccination. Again, canarypox viral DNA was detected in injection site swabs from all three patients. It is not clear whether live virus was shed from the vaccination site, as the presence of viral DNA does not indicate whether live virus is present.

These studies demonstrated that recombinant avipoxviral shedding has not been detected in blood, saliva, urine, or rectal swabs after vaccination. Viral DNA has been detected at the injection site, but the presence of live virus was not assessed. Therefore, avipox viral shedding in humans, if it occurs, appears to be confined to the vaccination site.

v. Possible immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites, and pathogens.

Possible environmental impact and effects on human health resulting from direct and indirect interactions between PROSTVAC-V/F and non-target humans (e.g., health care workers or contacts of vaccinees) are described in section iv, above and section vi, below. This section will address the impact of contact between PROSTVAC-V/F and non-human organisms.

PROSTVAC-V

Vaccinia virus can infect warm-blooded vertebrates such as mammals, rodents, and birds. Vaccinia virus is not known to infect cold-blooded vertebrates such as fish, amphibians, and reptiles (*Essbauer, 2001*), although vaccinia has been demonstrated to infect certain cells in tissue culture, including kidney cells from the tortoise *Testudo graeca* and liver cells from the lizard *Lacerta viridis* (*Shindarov, 1965*). Other pox viruses, not related to vaccinia virus, have been observed in several cold-blooded species. The appearance of pox virus-like particles has been reported in a small number of these species, such as the frog *Rana temporaria*, the chameleon *Chamaeleo dilepsis*, and several species of crocodiles. Two species of pox viruses, not including vaccinia virus, have been identified that infect reptiles (*Essbauer, 2001*). Therefore, there is little risk to aquatic animals from any potential environmental release of vaccinia viruses such as PROSTVAC-V.

Vaccinia virus has no known natural habitat and the origins of vaccinia virus in nature and as a vaccine are unknown. However, although vaccinia virus has no known natural animal reservoirs, some vaccinia strains have been isolated from domestic animals, including several strains isolated from dairy cattle throughout Brazil (*Trindade 2007*), as well as buffalopox virus, first isolated in India and still associated with sporadic outbreaks in Pakistan, India, Bangladesh, Russia, Indonesia, Egypt, and Italy (*Singh, 2007*). Outbreaks are associated with animal morbidity as well as infection of milkers and animal handlers.

The origin of these circulating vaccinia strains remains unclear. One possibility is that vaccine strains were accidentally introduced into the environment during the smallpox eradication program; however, at least for the Brazilian vaccinia strains, genetic variation among isolates suggests that ancestral strains existed before the beginning of the WHO smallpox eradication vaccination campaigns (*Trindade, 2007*). Thus, it is also possible that vaccinia-like viruses initially existed, undetected, in nature.

The vaccine strains used in India and Brazil were different from the licensed Dryvax smallpox vaccine from which PROSTVAC-V was derived. PROSTVAC-V does not exhibit any known phenotypic changes (e.g., changes in virulence or growth advantage) that would increase its risk to the environment relative to its nonrecombinant parental pox virus. Indeed, PROSTVAC-V is more attenuated that its parental non-recombinant vaccinia virus, which is in turn significantly more attenuated than the licensed Dryvax smallpox vaccine. Thus, the likelihood that PROSTVAC-V would result in the establishment and persistence of vaccinia virus in wild or domestic animals is remote.

The extent of exposure to non-target species is expected to be limited by the fact that vaccine administration occurs in a clinical site under controlled conditions. The administration of PROSTVAC-V via the subcutaneous route, the use of bandaging, which contains virus at the vaccination site, and comprehensive education of healthcare providers and patients all serve to minimize exposure to non-target species. Although the potential exists for secondary

transmission of the virus to non-target organisms, multiple studies indicate that transmission to non-target organisms, either human-to-animal or animal-to-animal, is unlikely except perhaps in the case of transmission of vaccinated or infected animal handlers to cattle.

Potential contact transmission in animals has been studied with the recombinant vaccinia virus-based rabies vaccine, Raboral V-RG. In one study (*Brochier, 1988*), foxes vaccinated orally with $10^{7.2}$ pfu Raboral V-RG were kept in close contact with unvaccinated animals. No contact control seroconverted or showed other evidence of contact transmission. In another study (*Blancou, 1986*), eight foxes were penned in pairs. One animal from each pair was administered 10^8 pfu orally. Serum samples were collected from all animals at Days 14 and 28. All animals were challenged with live rabies at Day 28. All four vaccinated animals had high titers of rabies-neutralizing antibodies and resisted challenge with live rabies. Three of four control contacts had no detectable anti-rabies antibodies and succumbed to rabies challenge. One contact control was positive for antibodies and resisted challenge. Biting behavior had been observed within a few minutes of oral vaccination and was likely the cause of this transmission event.

Contact transmission of Raboral V-RG was assessed in five raccoons housed with non-vaccinated contact controls. Transmission of vaccinia virus was assessed by the presence of anti-vaccinia neutralizing antibodies and survival after challenge with a lethal dose of rabies virus. Two of the five contact controls showed evidence of contact transmission of V-RG. These two raccoons, housed with vaccinated raccoons of the opposite gender, developed low titers of anti-vaccinia neutralizing antibodies and were protected against challenge with rabies virus. Three males housed with vaccinated male raccoons did not develop detectable neutralizing antibodies against vaccinia and were not protected against challenge with rabies virus.

Contact transmission studies were also performed in badgers, ferrets, dogs, cats, mice, and cattle. No evidence of contact transmission of V-RG was observed in these species.

Raboral V-RG was issued a conditional USDA license in 1995 and was fully licensed in 1997. Raboral V-RG is placed in baits that are then distributed by air or by hand into wildlife areas. Over 40 million doses (10^8 pfu per dose) have been distributed since 1995. This represents an extremely large viral burden on the environment ($>10^{15}$ pfu), much greater than any potential amount of PROSTVAC-V that would be introduced under conditions of the proposed release.

One study evaluating potential contact transmission of recombinant vaccinia virus after subcutaneous vaccination of animals has been reported (*Holt, 2002*). No contact transmission was observed. Twelve guinea pigs were vaccinated subcutaneously in the neck three times at monthly intervals with 10^7 pfu of a recombinant vaccinia virus, Connaught strain, expressing Ebola virus protein. Thirty-six control guinea pigs, injected with buffer instead of vaccinia virus, were co-housed in the same room as the vaccinia-vaccinated animals: twelve in the same cages as the vaccinated animals (two vaccinated and two unvaccinated animals per cage), twelve in the cages below the vaccinated animals, and twelve in a rack across the room, near the air exhaust

vent, to assess potential airborne transmission. Blood was taken three weeks following the final vaccination to evaluate seroconversion to vaccinia virus. Eleven of the twelve vaccinated animals seroconverted to vaccinia virus (the vaccinated animal that did not seroconvert had received only one vaccination due to technical issues). The thirty-six unvaccinated animals were negative for the presence of antibodies against vaccinia virus. Thus, there was no evidence of transmission of vaccinia virus from vaccinees to contact controls after subcutaneous vaccination in this study.

PROSTVAC-F

PROSTVAC-F is derived from an attenuated veterinary fowlpox vaccine, POXVAC-TC, which is used by the poultry industry to protect chickens from infection with pathogenic fowlpox virus. No untoward effects on the environment, other bird species, or animal handlers have been reported from the use of POXVAC-TC.

Recombinant fowlpox viruses are currently licensed by the USDA to vaccinate poultry against avian influenza and Newcastle disease. Over two billion doses of recombinant fowlpox virus-based influenza vaccine have been used in Mexico and Central America, with no reported adverse reactions (*Bublot, 2006*).

Fowlpox virus has not been reported to replicate in nonavian species. The probability of PROSTVAC-F spreading to non-target organisms is, therefore, very low. In the unlikely event of viral transmission, no clinical disease is expected in the non-target organism.

Transmission of fowlpox virus is likely to be maximal among poultry, as fowlpox virus replicates in certain avian species. Limited transmission of a vaccine strain of fowlpox virus among chickens, a permissive host for fowlpox virus, has been reported (*McMillen, 1994*). Chicks were vaccinated by wing-web stab and by scarification with 2×10^4 effective infectious doses (EID_{50}) of recombinant fowlpox virus that expresses the HN and F proteins from Newcastle Disease Virus (FPV-NDV) or with the parental fowlpox vaccine virus (FPV-001) used to generate the recombinant virus. Unvaccinated contact controls were co-housed with the vaccinated chicks. In one experiment, vaccinated and unvaccinated chicks were challenged with pathogenic fowlpox virus three weeks after vaccination. In a second experiment, half the vaccinated and unvaccinated chicks were challenged with pathogenic fowlpox virus five weeks after vaccination, and the other half were challenged with pathogenic NDV five weeks after vaccination. All chicks were examined for fowlpox lesions or clinical signs of NDV. All vaccinated animals were protected against challenge with pathogenic fowlpox virus; that is, none developed fowlpox lesions. Most chicks that had been vaccinated with FPV-NDV were protected against challenge with NDV. None of the contact controls were protected against challenge with NDV. A small number of contact controls were protected against challenge with fowlpox virus, indicating contact transmission occurred in a minority of the chicks, a species in which fowlpox virus readily replicates. The low level of contact transmission that occurred was not sufficient to confer protection against challenge with NDV.

The ability of the vaccine strain and the recombinant fowlpox virus to infect other avian species was evaluated. Lesion formation was observed in turkeys but not in quail after inoculation with fowlpox virus. Both parental and recombinant fowlpox virus could be isolated from injection sites of turkeys but not from quail (*McMillen, 1994*). Fowlpox replication occurs in chickens, turkeys, and pigeons, but not ducks or canaries (*Tripathy, 1984*). Therefore, fowlpox virus replicates productively in some but not all avian species, further limiting potential transmission of virus.

vi. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into direct contact with or in the vicinity of the GMO release(s).

Persons working with, coming into direct contact with, or in the vicinity of, the GMO release include (1) study subjects, to whom the GMO will be administered; (2) their close or household contacts; (3) healthcare providers. The possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions with the GMO, as well as the precautions taken to minimize the incidence of adverse effects, are described below.

Clinical Experience

PROSTVAC-V and PROSTVAC-F are products of more than 15 years of poxviral vaccine development and evaluation by the NCI, the former Therion Corporation and BN-ImmunoTherapeutics (BNIT). Since 1991, ten recombinant vaccinia-based vaccines and eight recombinant fowlpox-based vaccines produced by Therion for the treatment of various cancers have been evaluated in human clinical trials sponsored by the NCI. Over 1,000 cancer patients, most with metastatic disease, have been treated to date with these various pox virus-based vaccines in NCI-sponsored or Therion-sponsored clinical trials.

Clinical evaluations of early versions of PSA-containing poxviral vectors involved over 250 patients. The initial constructs tested were vaccinia-PSA, in three Phase 1 studies totaling 81 patients (*Sanda, 1999; Eder, 2000; Gulley, 2002*). Later, a prime boost regimen was devised with boosting with fowlpox-PSA containing vectors, in two studies, one with 64 patients (*Kaufman, 2004*) and another unpublished study of approximately 16 patients. The NCI subsequently began a series of trials using additional admixed vectors encoding a single costimulatory molecule (B7.1/CD80). These studies involved 94 patients (*Gulley, 2005; Arlen, 2005; Madan, 2008*). No significant safety issues were identified in these early studies.

Therion conducted two clinical studies with PROSTVAC-V/F, the product to be administered in the proposed Phase 3 trial: P1-4, a Phase 1 trial evaluating the safety and immunogenicity of PROSTVAC-V/FF in ten patients (*DiPaola, 2006*), and TBC-PRO-002, a randomized, placebo-controlled Phase 2 trial evaluating the safety and efficacy of PROSTVAC-V and PROSTVAC-F in approximately125 men with androgen-independent (hormone-refractory) metastatic prostate

cancer (*Kantoff, 2010*). Therion completed the Phase 2 study through approximately six months of follow-up. Additional long term follow-up has been completed by BNIT.

In 2003, the NCI initiated its own investigations of PROSTVAC-V/FF alone and in combination with other therapies under NCI BB-IND 10915. To date, the NCI has conducted two Phase 1 studies, one Phase 1/2 study and three Phase 2 studies in the United States. These six studies, of which one has been completed, three are fully enrolled and two are open, have treated 188 patients with PROSTVAC-V and PROSTVAC-F.

The most common AEs related to PROSTVAC-V and PROSTVAC-F observed to date have been injection site reactions, all of which were ≤ Grade 2 severity and resolved without sequelae. Typical AEs historically seen with intradermal administration (scarification) of vaccinia virus vaccines (vesicles, pustules, and scaring) have not been observed with PROSTVAC-V. This may reflect the fact that PROSTVAC-V is administered subcutaneously. The most common systemic AEs attributed to PROSTVAC-V and PROSTVAC-F administration were fatigue, nausea/vomiting, fever, chills, arthralgia and dizziness. There have been two SAEs thought to be possibly due to the PROSTVAC-V/FF treatment; both of occurred in the same patient. This patient experienced myocardial infarction and Grade 4 thrombotic thrombocytopenic purpura (TTP), and was discontinued from the study.

Laboratory evaluations likewise did not reveal any untoward effects of treatment. No vaccinia-related complications were noted. Overall, based on clinical information available today, recombinant poxviral vaccines present a very good safety profile.

Potential Toxicities of PROSTVAC-V and PROSTVAC-F

Adverse Reactions associated with smallpox vaccinia vaccinations

Vaccinia virus causes a transient infection, with elimination of viral components over several weeks. Host cells infected with vaccinia virus are short lived (days) and die by a mixed form of apoptosis/necrosis. Vaccinia replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA. Vaccinia virus is known to be shed from the wound site in traditional dermal scarification based vaccination.

The use of vaccinia virus for worldwide eradication of smallpox provides a safety database with the number of observations in the millions. Geographical differences in strains of vaccinia virus used as well as differences in reporting practices, diagnostic and follow-up criteria between countries, are a cause of some discrepancies in the incidences of adverse events reported, but the overall picture of vaccinia virus safety is very well known. An additional set of data is provided by recent vaccination campaign of military and civilian vaccinations in the US.

A number of events post vaccination are expected and considered to be normal: fever, myalgia, headache, fatigue, chills, nausea, soreness and erythema at the vaccination site, local

lymphadenopathy. Satellite lesions around the vaccination site have been reported as well as local edema. These symptoms are self-limiting, last for around three weeks after vaccination and rarely are a cause for serious concern (*Frey, 2002; Fulginiti, 2003*). Mild adverse reactions that can occur post vaccination are bacterial superinfection of vaccination site, erythema multiforme and generalized vaccinia. Superinfection is a rare event with incidence from 0.14 to 55 cases per million according to different reports (*Vellozzi, 2004*).

Erythema multiforme (EM) most often presents as papules, plaques or urticaria which may be symmetrical and may involve palms and soles. EM resolves spontaneously and requires no special care. A development of Stevens-Johnson syndrome with mucosal involvement is extremely rare, with only one case noted in the 2003-2004 vaccination campaign in the US (<1 per 1,000,000) (*Fulginiti, 2003; Neff, 2008*).

Generalized vaccinia results from viremic spread of vaccinia virus from the vaccination site. It presents as generalized rash which behaves like the vaccination site lesion, progressing through papular, vesicular, pustular and scab-forming stages. The incidence is difficult to assess, since historically there was no strict definition to distinguish generalized vaccinia from other conditions where rash is a dominant symptom (severe chickenpox, smallpox, eczema vaccinatum, EM). Retrospective analysis of 2002 – 2004 vaccinations suggests an incidence of ~50 cases per 1,000,000 (*Bryant-Genevier, 2006*). The rash appears within a week after vaccination and resolves within a week. Most instances do not require specific therapy (*Fulginiti, 2003*).

Some of the post-vaccinia adverse events, although very rare, are serious and potentially life-threatening. They include progressive vaccinia (PV), eczema vaccinatum (EV) and postvaccinial encephalitis (PVE).

PV is the most serious complication known. It was almost always fatal prior to the introduction of vaccinia immune globulin (VIG). PV occurs predominantly in persons with T-cell deficiencies or receiving treatments that result in T-cell deficiencies. The primary vaccination site fails to heal, viremic spread of vaccinia leads to generalized appearance of new lesions without reactive immunoinflammatory response (*Bray, 2003; Fulginiti, 2003*). PV is extremely rare; historical incidence is in the order of 1 case per 1,000,000. There were no reports of PV in the military and civilian vaccines in 2002 – 2004 vaccination campaigns (*Neff, 2008*).

EV manifests as rash (popular, vesicular, pustular, erosive) that can be localized or generalized and predominantly occurs in the areas that have been affected by lesions of atopic dermatis or other eczematous skin condition. Historically it occurred at a rate of ~ 1 case per 25,000 vaccinations. EV can occur in a vaccine recipient as well as in susceptible individuals in close contact. Two cases of EV from transmission have been recently reported; both in children of recently vaccinated US military personnel (*Lederman, 2009; Vora, 2008*). In the military vaccination program in the US there were no reports of EV among 450,239 vaccinees, probably due to careful screening for contraindications (*Grabenstein, 2003*). Review of civilian

vaccinations did not detect any cases of EV (*Velozzi, 2005*). EV can be prevented by thorough screening of at-risk individuals and education on importance of avoiding contacts with such persons and proper hygiene.

PVE historical case-fatality rate is 25%. The historical (1963 – 1968) reported frequency of PVE in United States was reported at 2.9 cases per 1,000,000 vaccinations. PVE has higher prevalence and mortality rate in children compared to adults. Higher historical rates were reported in Europe compared to US. Variability is attributed to differences in case definitions, clinical evaluations and differences in vaccine strains used by different countries (*Sejvar, 2005*). Pathogenesis is still under investigation, although several compelling theories focus on autoimmune mechanism. Aside from vaccinia, measles and rabies vaccines have known association with PVE, as well as other viral and bacterial infections (*Bennetto, 2004*; *Menge, 2007*). Review of 2002 – 2004 vaccinations in US reported 3 cases of PVE for the rate of 5 per 1,000,000.

Recent vaccination campaigns in the US revealed a higher than historically observed incidence of myopericarditis in vaccinees. Predominant symptoms were chest pain, shortness of breath and fatigue, typically mild and transient. Among military contingent, 88% of cases occurred in men with the incidence of 16.11 per 100,000 for primary vaccines and 2.07 per 100,000 in revaccinees (*Arness, 2004*). In civilian populations, women accounted for 67% of cases and the majority of events (86%) were reported in revaccinees (*Casey, 2005*; *Sniadack, 2008*). Variability between the two sets of data may be explained by differences in demographics of vaccinees, case detection, ascertainment and reporting practices (*Morgan, 2008*). Myopericarditis has been long associated with a number of viral infections, although there are very few reports of confirmed viremia. A few cases of myopericarditis have been reported following DTP and influenza vaccinations (*de Meester, 2000*; *Boccara, 2001*). It is currently assumed that injury to the heart post viral infection is more of an immune inflammatory reaction than direct nature (*Cassimatis, 2004*; *Feldman, 2000*).

Serious adverse reactions known to be associated with traditional smallpox vaccinations have not been observed in prior clinical studies of PROSTVAC-V or other vaccinia-based vaccines. The risk of these rare events is thought to be further reduced for PROSTVAC-V due to the use of attenuated strain of vaccinia.

Review of data from 2002 – 2004 vaccinations in US reported ~ 1 case of autoinoculation per 6,500 vaccinations with 17% of ocular cases, none with corneal involvement (*Neff, 2008*). Vaccinia keratitis is the most serious consequence of autoinoculation, since lesions on the cornea threaten eyesight. Diseased or injured conjunctiva and cornea may increase the risk of this complication. Vaccinia keratitis will respond to treatment with topical antiviral agents and interferon, and can be prevented with use of occlusive bandages over scarification site and patient education (*Fulginiti, 2003*).

Vaccinia Shedding and Secondary Transmission

Transmission of vaccinia to close contacts is another known complication. Vaccinia virus is shed from the vaccination lesion of vaccinees; transmission of vaccinia virus is rare but does occur. Contact vaccinia may manifest as PV, EV or accidental infection of the eye, mouth, or genital areas. Review of several national and state surveys between 1962 and 1968 gives a frequency for EV at 8 - 27 per 1,000,000, and for accidental infections at 3 - 44 per 1,000,000 (*Neff, 2002*). The rate of contact vaccinia in 2002 – 2004 was <10 cases per 100,000. Education of vaccinees in proper care for the vaccination site, proper hand hygiene, and avoidance of contact with at-risk individuals seems to be a reasonable and effective prophylactic against accidental contact with vaccinia.

Recombinant vaccines based on the same parental virus as that used for PROSTVAC-V have been tested in clinical trials for over a decade. Hundreds of volunteers have been vaccinated by various routes including scarification, intradermal, subcutaneous, and intramuscular administration. Doses have ranged up to 2×10^9 pfu. No evidence of contact transmission of these vaccinia virus-based vaccines has been noted in any clinical trial to date.

Safety of Fowlpox Vaccination

Fowlpox virus is a member of the genus Avipox, which is evolutionarily divergent from vaccinia virus and serologically non-crossreactive (*Taylor, 1988*; *Beukema, 2006*). Immune responses to vaccinia do not block infection and immunization with fowlpox-based vectors. Hence vaccinia-primed immune responses can be boosted with fowlpox vectors. In addition, fowlpox vectors do not replicate in human cells (only in avian cells), and are therefore much less of a safety risk than vaccinia-based vectors. Fowlpox vectors mediate a limited infection in human cells, with early viral and transgene expression, but late gene expression is blocked, and no infectious particles are produced. Thus minimal viral surface antigen is made, and minimal neutralizing antibody immune responses are induced. This enables multiple boosting with the fowlpox-based vectors.

Fowlpox virus has been investigated and used in vaccine design for at least two decades. As with vaccinia virus, it offers the advantages of a large genome but provides an additional safety assurance by not being able to replicate in mammalian cells. Fowlpox virus-based vaccines (HIV, malaria, cancer) have been tested in both animals and humans. No safety concerns have been raised and the adverse events associated with the use of fowlpox vectors have been limited to mild injection site reactions (*Beukema, 2006*; *Essajee, 2004*; *Webster, 2006*). No evidence of transmission of fowlpox virus has been noted in any clinical trial to date. As fowlpox virus is replication-defective in humans, transmission of PROSTVAC-F in humans is an unlikely event.

Procedural Controls

Precautions and Contraindications for Study Patients

Following the priming vaccination with PROSTVAC-V, the vaccination site will be covered by a sterile non-adherent dressing (*e.g.*, Telfa pads), and patients should receive instructions regarding dressing care, proper hands hygiene, bathing, etc. All vaccine vials, applicators, and patient bandages or dressings removed from the vaccination site should be disposed of in appropriate biohazard containers.

PROSTVAC-V will not be administered if any of the following applies to either the patient or the patient's close household contacts for at least three weeks after the PROSTVAC-V vaccination: persons with active or a history of eczema or other eczematoid skin disorders; those with other acute, chronic or exfoliative skin conditions (*e.g.*, burns, impetigo, varicella zoster, severe acne or other open rashes or wounds) until condition resolves; pregnant or nursing women; children less than one year of age; and immunodeficient or immunosuppressed persons (by disease or therapy), including those with HIV infection. Close household contacts are those who share housing or have close physical contact.

Due to information recently issued by the CDC on cardiac events observed in the current smallpox vaccination program, patients with significant cardiovascular abnormalities or diseases will be excluded from treatment.

Because PROSTVAC-V and PROSTVAC-F are manufactured in chicken embryo cells, patients with hypersensitivity to eggs will be excluded from treatment. In addition, there should be no history of allergy or untoward reaction to prior vaccinia (smallpox) vaccination in patients receiving PROSTVAC-V.

Vaccinia-Related Precautions for Healthcare Workers

The risk of transmission of recombinant viruses to exposed healthcare workers is very low. There have been no cases of transmission to healthcare personnel in any of the studies with PROSTVAC-V or related vaccinia-based vaccines. As reported for the handling of vaccinia strains found in smallpox vaccines, if appropriate infection-control precautions are observed, healthcare workers are probably at less risk of infection than laboratory workers because of the smaller volume of lower titer of virus in clinical specimens as compared with laboratory material (*Garner, 1983*; *Bolyard, 1998*). Healthcare workers who are pregnant, have exfoliative skin conditions, or are immunocompromised should avoid exposure to contaminated dressings or to the inoculation site.

Safe Handling of Recombinant Vaccinia Vaccines

Special consideration must be given to the nature of the initial vaccine: a recombinant replicating vaccinia virus. Patient instruction materials will include detailed descriptions for bandaging, bathing, and reporting any possible side effects. Patients will also be educated regarding restrictions on contact with certain classes of individuals (children under the age of 12 months, pregnant or lactating women, immunocompromised individuals).

Procedures for preparation of the vaccine are described in the clinical protocol.

PROSTVAC-V is classified as group 2 biological agent according to the European Economic Community (EEC) classification for the protection of workers with biological agents {Directive 2000/54/EC}. Study staff will be provided specific instructions for storage, use, and destruction of these materials. All patients will be dosed with the vaccines in the clinic or hospital setting according to local regulations. PROSTVAC-V is aseptically vialed at low volume in a sealed vaccine vial. Loading of syringes may be performed using standard aseptic methods in a clinical pharmacy. Routine, standard universal precautions are recommended when directly handling the vaccine vials, including the wearing of a lab coat, eye protection, and gloves. There is no need to sequester patients after dosing. Vials and needles and syringes may be disposed of as for infectious medical waste according to local regulations.

PROSTVAC-F virus is classified as a Biosafety Level 1 organism for practices involving biological materials and containment facilities (as defined by the United States Center for Disease Control and Prevention, Laboratory Biosafety Level Criteria). Because is cannot replicate in mammalian cells, no special precautions beyond standard, universal precautions for infectious materials are required. There are no patient restrictions with respect to activities (such as bathing), personal contact, or bandaging required for vaccination with PROSTVAC-F.

Treatment of Vaccinia Complications

For some very rare complications of vaccinia infection (see end page 25/beginning page 26), early administration of vaccinia immune globulin (VIG) is advised. Recognition of clinical symptoms compatible with eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of auto-inoculation should prompt consideration of VIG therapy. The effectiveness of VIG therapy appears to be time-dependent. VIG is of no benefit in the treatment of post-vaccinial encephalitis, and is **contraindicated** for the treatment of vaccinial keratitis. VIG is available in United States through CDC and in several other countries through appropriate health authorities. Despite the very low risk for any complication requiring VIG administration, BNIT has secured a supply of VIG for the countries where it is not available internally.

Although there is no recognized alternative to VIG in treating severe complications resulting from vaccinia vaccination, in vitro and animal model data in several poxvirus infections models demonstrate the activity of cidofovir at clinically relevant doses. Subjects who experience severe vaccinia complications may be treated with cidofovir. Treatment with cidofovir will be recommended primarily after clinical failure following treatment with vaccinia immune globulin. Vistide® is generally available through hospital pharmacies.

vii. Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain resulting from consumption of the

GMO and any product derived from it if it is intended to be used as animal feed.

Not applicable. PROSTVAC-V/F will not be utilized as animal feed.

viii. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

PROSTVAC-V/F has not been shown and is not anticipated to have any involvement in biogeochemical processes.

ix. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different for those used for non-GMOs.

All techniques used within the study are considered standard procedures for monitoring, collection sampling, and disposal of waste during clinical studies, regardless of whether the study involved a GMO.

PART A5: JUSTIFICATION FOR INFORMATION CONSIDERED TO BE CONFIDENTIAL

Not applicable.

PART A6: STATEMENT ON WHETHER INFORMATION ABOUT GMO AND PURPOSE OF THE RELEASE HAVE BEEN PUBLISHED

Final study reports for the completed clinical studies using PROSTVAC are available upon request.

Relevant publications available for reference of previously conducted studies with PROSTVAC-V and PROSTVAC-F include the following:

Arlen, P. M., L. Skarupa, et al. (2007). "Clinical safety of a viral vector based prostate cancer vaccine strategy." J Urol **178**(4 Pt 1): 1515-20.

DiPaola, R. S., M. Plante, et al. (2006). "A phase I trial of pox PSA vaccines (PROSTVAC-VF) with B7-1, ICAM-1, and LFA-3 co-stimulatory molecules (TRICOM) in patients with prostate cancer." J Transl Med **4**: 1.

DiPaola, R. S., Y. Chen, et al. (2009). "A phase II study of PROSTVAC-V (vaccinia)/TRICOM and PROSTVAC-F (fowlpox)/TRICOM with GM-CSF in patients with PSA progression after local therapy for prostate cancer: Results of ECOG 9802". ASCO Genitourinary Cancers Symposium .

Gulley, J. L., P. M. Arlen, et al. (2010). "Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer." Cancer Immunol Immunother **59**(5): 663-74.

Kantoff, P. W., T. J. Schuetz, et al. (2010). "Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer." J Clin Oncol **28**(7): 1099-105.

Mohebtash, M., R. A. Madan, et al. (2009). "Phase I trial of targeted therapy with PSA-TRICOM vaccine (V) and ipilimumab (ipi) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC)." J Clin Oncol **27**(15s): (suppl; abstr 5144).

In addition to clinical studies of PROSTVAC, there have been a number of studies that investigated related recombinant vaccinia and fowlpox viruses that express prostate specific antigen (PSA). These are listed below.

Arlen, P. M., J. L. Gulley, et al. (2005). "Antiandrogen, vaccine and combination therapy in patients with nonmetastatic hormone refractory prostate cancer." J Urol **174**(2): 539-46.

Arlen, P. M., J. L. Gulley, et al. (2006). "A randomized phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer." Clin Cancer Res **12**(4): 1260-9.

Cavacini, L. A., M. Duval, et al. (2002). "Evidence of determinant spreading in the antibody responses to prostate cell surface antigens in patients immunized with prostate-specific antigen." Clin Cancer Res **8**(2): 368-73.

Eder, J. P., P. W. Kantoff, et al. (2000). "A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer." Clin Cancer Res **6**(5): 1632-8.

Gulley, J., A. P. Chen, et al. (2002). "Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer." Prostate **53**(2): 109-17.

Gulley, J. L., P. M. Arlen, et al. (2005). "Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer." Clin Cancer Res **11**(9): 3353-62.

Hodge, J. W., J. Schlom, et al. (1995). "A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate." Int J Cancer **63**(2): 231-7.

Kaufman, H. L., W. Wang, et al. (2004). "Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group." J Clin Oncol **22**(11): 2122-32.

Lechleider, R. J., P. M. Arlen, et al. (2008). "Safety and immunologic response of a viral vaccine to prostate-specific antigen in combination with radiation therapy when metronomic-dose interleukin 2 is used as an adjuvant." Clin Cancer Res **14**(16): 5284-91.

Madan, R. A., J. L. Gulley, et al. (2008). "Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy." Clin Cancer Res **14**(14): 4526-31.

Sanda, M. G., D. C. Smith, et al. (1999). "Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer." Urology **53**(2): 260-6.