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Title: A Randomized, Prospective, Phase II Study to Determine the Efficacy of Bacillus Calmette-Guerin (BCG) given in combination with PANVAC™ versus BCG given alone in Adults with High Grade Non-Muscle Invasive Bladder Cancer (NMIBC) who failed at least 1 Induction Course of BCG

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CTEP Supplied Investigational Agents:

Note: Study closed with CTEP as of 5/1/2019

Agent name	IND #	IND Sponsor	Manufacturer
PANVAC-V [Recombinant Vaccinia-CEA(D609)/MUC1(L93)/TRICOM] NSC 727026	IND 11660	CTEP	Therion Biologics
PANVAC-F [Recombinant Fowlpox-CEA(D609)/MUC1(L93)/TRICOM] NSC 727027	IND 11660	CTEP	Therion Biologics

Commercially available agents:

TICE Bacillus Calmette-Guerin (BCG), NSC 614388

PRÉCIS

Background:

- High grade (G3) non-muscle invasive urothelial carcinoma of the bladder (stages Ta, T1, and CIS) has a high rate of recurrence and progression
- The standard of care therapeutic agent is a single induction course of bacillus Calmette-Guerin (BCG)
- Although a second induction course can be used in patients who fail a single induction course of BCG, only 35% of patients who failed an initial induction course will experience 12 month disease-free survival after receiving a second induction course
- For those patients failing a second induction course, radical cystectomy with pelvic lymphadenectomy is the recommended treatment, although it has a high morbidity rate and a small but real mortality rate
- Therefore, there is an unmet need for localized treatment for patients who fail an initial induction course of BCG that can potentially improve upon the poor results of a second induction course of BCG
- Recently, a unique pox viral vector-based vaccine, PANVAC™, has been shown to induce a CD4 and CD8 antigen-specific immune response against the tumor-associated antigens (TAAs), carcinoembryonic antigen (CEA) and mucin-1 (MUC-1). This vaccine also contains transgenes for three human T cell co-stimulatory molecules that can potentially augment an immune response
- We hypothesize that the combined administration of BCG and PANVAC™ may augment the BCG-induced cytotoxic T lymphocyte response against bladder cancer cells expressing MUC-1 and/or CEA and potentially reverse BCG failure in patients that have failed one induction course of BCG

Objectives:

- To determine if there is an improvement in disease-free survival (DFS*) with BCG + PANVAC compared with BCG alone in a phase II study in non-muscle invasive high-grade urothelial carcinoma of the bladder who have failed to respond to intravesical BCG.

*within 1 year post treatment

Eligibility:

- Individuals who have failed at least one previous induction course of intravesical BCG, defined as histologically confirmed persistent or relapsing tumor present on post-BCG endoscopic evaluation. All BCG failures will be considered for inclusion into the study, including BCG-refractory, -resistant, and -relapsing, as defined in the “Rationale and Background.” For the purposes of the study, “BCG-refractory” and “BCG-resistant” subjects will be considered to have “BCG-persistent” disease.
- Patients who are not currently candidates for radical cystectomy (e.g. patient refuses surgery, comorbidities preclude major surgery, etc.).
- Normal organ function, ECOG 0-2.

Design:

- This is a randomized, open label prospective, Phase II study in subjects with non-muscle invasive high grade urothelial carcinoma of the bladder who have failed at least one induction course of intravesical BCG, randomized to one of the following arms: TICE BCG +PANVACTTM or TICE BCG alone. Randomization is stratified by BCG treatment subgroup.
- All subjects will receive intravesical TICE BCG (50mg) as per usual standard of care once weekly (+/- 2 days) starting in week 3 for a total of 6 weeks.
- The combination arm will receive the pox viral vaccines that contain the transgenes for CEA and MUC-1 (both with modified HLA-A2 agonist epitopes) as well as 3 human T-cell costimulatory molecules, B7-1, ICAM-1, and LFA-3 [rV-PANVACTTM (vaccinia) and rF-PANVACTTM (fowlpox)] as follows:
 - rV-PANVACTTM 2 x 10⁸ pfu SQ at week 0 (+/- 7 days) only.
 - rF-PANVACTTM 1 x 10⁹ pfu SQ at weeks (+/- 7 days) 3, 7, 11, and 15.
- For this Phase II study, we will test the hypothesis that subjects in the TICE BCG +PANVACTTM arm have better disease-free survival than subjects in the TICE BCG alone arm.
- Patient accrual is targeted at one patient per month during the first 6 months and 1-2 patients every 1-2 months afterwards, and follow-up period after completing accrual will be 12 months post treatment.
- Based on a power of 84% and type 1 error (1-sided) of 0.15, a total of 49 subjects will need to be accrued.
- Allowing for a proportion of the subjects not being evaluable, a maximum of 54 subjects will be accrued.

SCHEMA

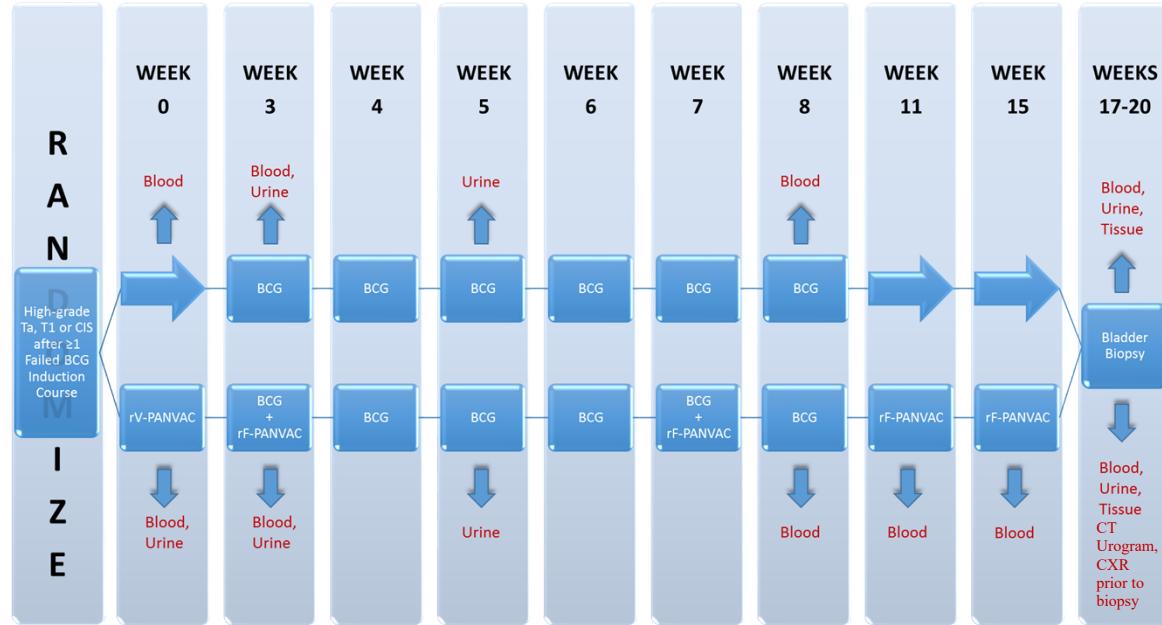


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1 OBJECTIVES

1.1 PRIMARY OBJECTIVE

1.1.1 To determine if there is an improvement in disease-free survival (DFS*) with BCG + PANVAC compared with BCG alone in a phase II study in non-muscle invasive high-grade urothelial carcinoma of the bladder who have failed to respond to intravesical BCG. *within 1 year post treatment

1.2 SECONDARY OBJECTIVES

1.2.1 To estimate tumor upgrading and tumor upstaging rates for each study arm.
1.2.2 To determine the safety of the combination therapy.

1.3 EXPLORATORY OBJECTIVES

1.3.1 To assess the expression of CEA and MUC-1 antigens in bladder tumor specimens pre- and post-treatment in both arms
1.3.2 To assess the presence of CD4 and CD8 T cells in bladder tumor specimens pre- and post-treatment in both arms
1.3.3 To assess the presence of Tregs by double staining with FoxP3 and CD4 in bladder tumor specimens pre- and post-treatment in both arms
1.3.4 To assess the presence of myeloid derived suppressor cells (MDSC) in bladder tumor specimens pre- and post-treatment in both arms
1.3.5 To measure the CD4 antigen-specific response to CEA in peripheral blood mononuclear cells (PBMCs) obtained from blood
1.3.6 To measure the CD8 antigen-specific response to CEA, MUC-1, and Brachyury in all subjects who have the HLA-A2 allele in PBMCs obtained from blood
1.3.7 To analyze all PBMC samples by flow cytometry for the following cell types: CD4, CD8, Tregs, MDSCs, and Natural Killer (NK) cells
1.3.8 To analyze sera samples obtained from blood at all time points for antibodies to CEA, MUC-1, and Brachyury
1.3.9 To assess production of urinary cytokines and chemokines in collected samples

2 BACKGROUND

Bladder cancer accounts for the 4th most common cancer in men and the 12th most common cancer in women. An estimated 73,510 people living in the United States will be diagnosed with

bladder cancer in 2012, with approximately 14,880 dying of bladder cancer in the same year.¹ Most of these patients (>75%) will be diagnosed with non-muscle invasive bladder cancer and approximately 50% of these patients will present with high grade disease.² High grade non-muscle invasive bladder cancer (Ta, T1, and/or CIS) is difficult to treat because of high rates of recurrence and progression. The last major advance in bladder cancer therapy was the approval of bacillus Calmette-Guerin (BCG) in 1990 by the FDA.³ Currently, intravesical BCG given as an induction course (6 weeks of intravesical therapy) is the standard of care^{4,5} followed by maintenance therapy. BCG has an initial failure rate of ~35% in terms of disease recurrence or progression. In ~20-35% of cases that failed an initial induction course of BCG, a second course of induction BCG may be beneficial, but patients who fail two courses are often best served by radical cystectomy.⁶⁻⁸ Patients with CIS alone may respond at a higher rate to a second induction course, however, cystectomy may still be required in at least 1/3 of these patients. This surgery involves the en bloc removal of the bladder and adjacent organs (prostate in males and uterus in females) and concomitant creation of a urinary diversion, which can affect a patient's quality-of-life significantly. Moreover, Shabsigh et al. reported that 64% of patients who undergo cystectomy suffer at least one complication within the first 90 days after surgery⁹ and the operation has been reported to have at least a 2.5% perioperative mortality rate even at a high-volume center.¹⁰ This fact is reflected in a 2003 survey, which showed that 81% of urologists were reluctant to refer their patients to radical cystectomy despite failing 2 courses of intravesical therapy.¹¹ In summary, radical cystectomy has the potential for significant morbidity and therefore, an unmet clinical need for intravesical therapy exists for these high grade non-muscle invasive bladder cancers that fail BCG.

BCG failures can be subdivided into several groups based on the time to tumor response and/or recurrence.¹² BCG-refractory disease is defined as the inability to be disease-free by 6 months after an induction course of BCG. In contrast, BCG-resistant disease is defined as the inability to be disease-free by 3 months after an induction course of BCG. Finally, BCG-relapsing disease is defined as recurrence of disease after being disease-free for at least 6 months after receiving BCG therapy. Unfortunately, disease-free survival rates at 12 months are not as well-defined based on these subclassifications. Thus, clinical endpoint trials involving this population should be randomized and stratified according to these subgroups to help better predict responders to therapy.

BCG works by an unclear immunologic mechanism that promotes activation and infiltration of immune cells within the bladder wall. The immune system is critical as the administration of T cells to athymic animals has been shown to restore their ability to respond to BCG therapy.^{13,14} A hypothesized mechanism for BCG is that it induces macrophage cytotoxicity and that Th1 immune system cytokines (e.g. TNF- α , IFN- γ , IL-12, and IL-18) play a positive role in the macrophage cytotoxicity while Th2 immune system cytokines (e.g. IL-4, IL-10) and T regulatory cells (Tregs) are potentially inhibitory to this BCG-induced macrophage cytotoxicity.¹⁵ T cell infiltration appears important as the degree of infiltration (CD3, CD4, and CD8) has been shown to be significantly greater in patients with a complete response to BCG in comparison to patients with a partial response or treatment failure.¹⁶

Modulation of the immune system is one strategy employed in cancer therapeutics. Tumor associated antigens (TAAs), which are unique molecules minimally expressed by normal tissues and over-expressed in cancer cells, can be used as immunologic targets. Recently, researchers at

the NIH have developed a poxvirus cancer vaccine therapy called PANVAC™ that has demonstrated therapeutic efficacy against a variety of carcinomas. PANVAC™ consists of a primary vaccination with a replication competent recombinant vaccinia (rV-) viral vector followed by multiple booster vaccinations with a replication-incompetent recombinant fowlpox (rF-) viral vector. These vectors contain transgenes for both TAAs and human T cell co-stimulatory molecules. Although TAAs are weakly immunogenic, a method of disrupting immune tolerance is to introduce TAAs to the immune system through poxviral vectors.¹⁷ Antigen-specific active immunotherapy is designed to generate immune responses, particularly cell-mediated responses, against specific TAAs using vaccines that express one or more of these antigens.

The TAAs in PANVAC™ are epithelial mucin-1 (MUC-1) and carcinoembryonic antigen (CEA) which are altered and overexpressed in many cancers.¹⁸ MUC-1 is a glycosylated transmembrane protein that is overexpressed in almost all human carcinomas. Furthermore, it has been demonstrated that the c-terminus of MUC-1 functions as an oncogene.^{19,20} According to one study, MUC-1 is present in all cases of malignant urothelium, but increased expression is noted with higher grade and stage tumors.²¹ Another study demonstrated MUC-1 expression in 93% of bladder tumors studied.²² CEA is a glycosylated cell surface protein expressed in several tumors as well and in particularly in colorectal, ovarian, bladder, breast, and gastric tumors.²³ CEA is expressed in 76% of high grade tumors and in 59% of pT1 bladder tumors.²⁴

Co-stimulatory molecules are critical in the generation of T-cell responses especially against weak antigens such as TAAs. The initiation of an immune response requires at least two signals for activation of naïve T cells by antigen-presenting cells. The first signal is antigen specific, delivered through the T-cell receptor via the peptide/MHC, and causes the T cell to enter the cell cycle. The second, “co-stimulatory,” signal is required for cytokine production and proliferation. At least three distinct molecules normally found on the surface of professional antigen-presenting cells have been reported to be capable of providing the second signal critical for T-cell activation: B7-1, ICAM-1, and LFA-3. Both antigen and co-stimulatory molecules must be expressed in the same cell to properly engage the TCR and co-stimulatory receptor. In order to achieve this, multigene constructs using poxviral vectors (avipox and vaccinia) have been generated. The co-stimulatory molecules are B7.1 (CD80), ICAM01 (CD54), and LFA-3 (CD58) and are referred to as TRICOM (triad of co-stimulatory molecules).²⁵ Each of these co-stimulatory molecules binds to a different ligand, and the second messenger pathways of each ligand are unique, raising the potential for synergy of these molecules. The vaccines are therefore, summarized as rV-CEA-MUC-1-TRICOM (rV-PANVAC™) and rF-CEA-MUC-1-TRICOM (rF-PANVAC™).

PANVAC™ is injected subcutaneously, processed by antigen-presenting cells (APCs), and then induces CEA- and MUC-1-specific cytotoxic T lymphocyte responses that are then potentiated by the co-stimulatory molecules. The pox viral vectors have a high rate of cellular infection, can carry large amounts of transgenes, process genes in the cytoplasm of infected cells with a low risk of incorporation within the host genome, and have an established history of clinical safety in patients. In fact, several hundred patients have already been treated on clinical trials with pox viral vectors and there have been no dose limiting toxicities attributable to these vaccines.¹⁸

Initial studies with PANVAC showed that it was well tolerated and was associated with objective responses in some patients. One patient with metastatic ovarian cancer and symptomatic ascites had complete resolution of her ascites and normalization of her CA-125 (from 300 to <20) for 18 months.²⁶ A patient with metastatic breast cancer has an ongoing complete response of her disease for over 3 years.²⁷ The final results of a randomized phase II study were recently reported. This study enrolled 48 patients with metastatic breast cancer and randomized them to weekly docetaxel with PANVAC monthly vs. docetaxel alone. The primary endpoint was PFS. The median PFS for patients randomized to the combination arm was 6.6 months vs. 3.8 months in the chemotherapy alone arm.²⁸

A phase II randomized controlled study involved 74 patients with completely resected metastases from colorectal cancer, who had received perioperative chemotherapy.²⁹ Patients were randomized to receive 4 vaccinations with either PANVAC or dendritic cells modified with PANVAC. There was no significant difference in 2-year recurrence-free survival among the DC arm (50%), the PANVAC arm (56%), and a contemporary control arm (55%). However, at a median follow-up of 40 months, the survival rate for vaccinated patients was 81%, which far exceeded that of the unvaccinated controls.

Recently, a PSA specific vaccine, rV-PSA TRICOM and rF-PSA-TRICOM, was evaluated in a randomized, placebo-controlled, blinded Phase II study for minimally symptomatic metastatic castration-resistant prostate cancer. Although no difference in progression free survival was noted, patients receiving the vaccine had longer median survival by 8.5 months with hazard ratio of 0.56 (95% CI, 0.37 to 0.85), p=0.0061.³⁰ Other studies have demonstrated safety of the vaccine in solid organ tumors when administered subcutaneously.^{18,26,27} These initial studies suggest safety and activity in a broad range of tumor types.

High grade non-muscle invasive bladder cancer tends to respond well to BCG and when it does not, there is evidence to suggest an incomplete or inefficient immune response. Given that CEA and MUC-1 are overexpressed in bladder cancer, we hypothesize that PANVAC™ will augment the BCG-induced cytotoxic T lymphocyte response against bladder cancer cells expressing MUC-1 and/or CEA, which will clinically result in a greater disease-free survival rate than BCG alone in patients who have failed at least one previous course of intravesical BCG. We also hypothesize that the PANVAC+BCG group will demonstrate decreased tumor upgrading and tumor upstaging, particularly with regards to invasion of the detrusor musculature, and predict that the combination treatment will not demonstrate greater treatment-related toxicities to intravesical BCG alone. Finally, we hypothesize that the immune response will be augmented in the PANVAC+BCG group compared to BCG alone as measured by secondary immunologic correlates described below.

3 PATIENT SELECTION

3.1 ELIGIBILITY CRITERIA

3.1.1 Inclusion Criteria

- 3.1.1.1 Patients must have histologically confirmed localized high grade (G3) transitional cell carcinoma (urothelial carcinoma) of the bladder that is stage Ta, T1, and/or CIS

confirmed by the Laboratory of Pathology, NCI 90 days prior to study entry. This can be obtained at an outside hospital prior to entry into the study or at the NCI. However, all outside pathology specimens will require that the formalin-fixed paraffin embedded tissues be re-read by the Laboratory of Pathology, NCI. For patients enrolled at collaborating trial sites, diagnosis must be confirmed by the Department of Pathology at the institution where the patient is enrolled on the trial. Pathology can also be reviewed by the Laboratory of Pathology at the NCI if the participating trial site prefers another pathologic evaluation.

- 3.1.1.2 Patients have failed at least one previous induction course of intravesical BCG, defined as histologically confirmed persistent or relapsing tumor present on post-BCG endoscopic evaluation. All BCG failures will be considered for inclusion into the study, including BCG-refractory, -resistant, and -relapsing, as defined in the “Rationale and Background.” For the purposes of the study, “BCG-refractory” and “BCG-resistant” subjects will be considered to have “BCG-persistent” disease.
- 3.1.1.3 Patients who are not currently candidates for radical cystectomy (e.g. patient refuses surgery, comorbidities preclude major surgery, etc.).
- 3.1.1.4 Age ≥ 18 years.

Because no dosing or adverse event data are currently available on the use of BCG in combination with PANVAC™ in patients <18 years of age, children are excluded from this study.

- 3.1.1.5 ECOG performance status ≤ 2 (see Appendix A, Section 17.1).

- 3.1.1.6 Patients must have normal organ and marrow function as defined below:

- absolute neutrophil count	$\geq 1,500/\text{mcL}$
- platelets	$\geq 50,000/\text{mcL}$
- total bilirubin	$\leq 1.5 \times$ institutional upper limit of normal
- AST(SGOT)/ALT(SGPT)	$\leq 3 \times$ institutional upper limit of normal
- estimated GFR (calculated using CKD-EPI equation)	$\geq 30 \text{ mL/min}/1.73 \text{ sq.m.}$

- 3.1.1.7 Computerized Tomography (CT) urogram or Magnetic Resonance Imaging (MRI) urogram. If urogram protocol not available or contrast allergy/poor renal function preclude such imaging, then noncontrast CT or MRI of the abdomen/pelvis within 45 days of study entry will suffice.
- 3.1.1.8 Chest x-ray negative for metastatic disease.
- 3.1.1.9 Ability of patient to understand and the willingness to sign a written informed consent document.

3.1.2 Exclusion Criteria

- 3.1.2.1 Previous pelvic radiation for bladder or prostate cancer if performed <12 months prior to enrollment into the study.
- 3.1.2.2 Patients who are receiving any other concurrent investigational agents (patients are eligible to enroll 4 weeks after completion of prior agent).
- 3.1.2.3 Patients who have had chemotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier. There will be at least a 3 week delay from the time of a previous bladder biopsy/TURBT to allow for adequate bladder healing prior to enrollment.
- 3.1.2.4 Patients with a history of encephalitis, multiple sclerosis, or seizures within the last year (from seizure disorder or brain metastasis) should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- 3.1.2.5 History of allergy or untoward reaction to prior vaccination with vaccinia virus
- 3.1.2.6 Patients should have no evidence of being immunocompromised as listed below:
 - Human immunodeficiency virus positivity due to the potential for decreased tolerance and risk for severe side effects
 - Active autoimmune diseases requiring treatment or a history of autoimmune disease that might be stimulated by vaccine treatment. This requirement is due to the potential risks of exacerbating autoimmunity. Patients with endocrine disease that is controlled by replacement therapy including thyroid disease and adrenal disease and vitiligo may be enrolled.
 - History of splenectomy
- 3.1.2.7 Uncontrolled intercurrent illness which would interfere with the ability of the patient to carry out the treatment program, including, but not limited to, active second malignancy other than a cancer that has been successfully treated resulting in a high likelihood of long-term survival (e.g. completely resected basal cell or squamous cell carcinoma of the skin, stage 1 renal cell carcinoma treated with partial nephrectomy, treated low risk prostate cancer, etc.), inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), active diverticulitis, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.1.2.8 Pregnant women are excluded from this study because the vaccines used in the study

may have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the vaccine, breastfeeding should be discontinued if the mother is treated with vaccines. These potential risks may also apply to other agents used in this study. Patients must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately

- 3.1.2.9 Concurrent use of systemic steroids, except for physiologic doses of systemic steroids for replacement or local (topical, nasal, or inhaled) steroid use. Limited doses of systemic steroids to prevent IV contrast, allergic reaction, or anaphylaxis (in patients who have known contrast allergies) are allowed. Although topical steroids are allowed, steroid eye-drops are contraindicated
- 3.1.2.10 Altered immune function, including immunodeficiency or history of immunodeficiency; eczema; history of eczema, or other eczematoid skin disorders; or those with acute, chronic or exfoliative skin conditions (e.g. atopic dermatitis, burns, impetigo, varicella zoster, severe acne, or other open rashes or wounds). There is an increased risk to patients or contacts with eczema, atopic dermatitis, and other immune deficiencies who are at risk for eczema vaccination.
- 3.1.2.11 Medical conditions which, in the opinion of the investigators, would jeopardize the patient or the integrity of the data obtained
- 3.1.2.12 Serious hypersensitivity reaction to egg products
- 3.1.2.13 Chronic hepatitis infection, including B and C, because of potential immune impairment
- 3.1.2.14 Clinically significant cardiomyopathy or cardiac complications, including recent myocardial infarction or cerebrovascular accident within one year, and/or unstable or uncontrolled angina
- 3.1.2.15 Previous intolerance to BCG intravesical therapy suggested by development of systemic BCG infection in the past and/or grade 4 or greater adverse effect by CTCAE v5.0.
- 3.1.2.16 Patients unable to avoid close contact or household contact with the following high-risk individuals for three weeks after the Day 1 vaccination: (a) children \leq 3 years of age, (b) pregnant or nursing women, (c) individuals with prior or concurrent extensive eczema or other eczematoid skin disorders, or (d) immunocompromised individuals, such as those with HIV.

3.1.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

3.2 RECRUITMENT STRATEGIES

This study will be listed on available websites (www.clinicaltrials.gov and others) and participants will be recruited from the current patient population at NIH.

3.3 SCREENING EVALUATION

- 3.3.1 Clinical Evaluation (within 28 days of before starting treatment).
 - 3.3.1.1 History and physical examination
 - 3.3.1.2 ECOG performance status
- 3.3.2 Viral Studies and Prior Vaccination Status
 - 3.3.2.1 HIV test within 8 weeks of initial visit and repeat if clinically indicated
 - 3.3.2.2 Hepatitis B and C titers within 8 weeks of initial visit and repeat if clinically indicated
 - 3.3.2.3 Document known prior vaccinia vaccination (small pox vaccine)

3.4 BASELINE EVALUATION

- 3.4.1 Laboratory studies (within 28 days before starting treatment)
 - 3.4.1.1 Complete blood count plus differential and platelet count
 - 3.4.1.2 Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, pre-albumin) with BUN and creatinine
 - 3.4.1.3 PT, PTT
 - 3.4.1.4 6 green top tubes of blood will be drawn for PBMCs, HLA Typing, and sera for correlative/exploratory research aims (e.g. ELISPOT, CD4+ Antigen-specific responses, etc.) on day 1 of study enrollment
 - 3.4.1.5 Beta-HCG for women of child-bearing age (within 48 hours prior to day 1). In addition, patients, both male and female, should be willing to practice effective birth control during the study and four months following the last study treatment, unless they have had a prior hysterectomy, bilateral oophorectomy, or vasectomy
- 3.4.2 Urine studies
 - 3.4.2.1 Urinalysis with urine culture (prior to operative procedures and within 2-21 days of operative procedures)
 - 3.4.2.2 Urine cytology (prior to operative procedures and within 2-21 days of operative procedures or at time of operative procedure)
 - 3.4.2.3 Urine collection for correlative/exploratory research aims (e.g. inflammatory cells, cytokines) at the time of operative procedure
- 3.4.3 Imaging (within past 45 days and of adequate quality)
 - 3.4.3.1 Chest X-ray
 - 3.4.3.2 Computerized Tomography (CT) urogram or Magnetic Resonance Imaging (MRI) urogram. If urogram protocol not available or contrast allergy/poor renal function preclude such imaging, then CT or MRI of the abdomen/pelvis will suffice
- 3.4.4 Pathology

3.4.4.1 Formal review of available previous bladder tumor specimens by the Laboratory of Pathology, NCI, to confirm the bladder cancer diagnosis prior to enrollment into the study. For patients enrolled at collaborating trial sites, diagnosis must be confirmed by the Department of Pathology at the institution where the patient is enrolled on the trial. Pathology can also be reviewed by the Laboratory of Pathology at the NCI if the participating trial site prefers another pathologic evaluation.

3.4.4.2 Tissues obtained at the NCI will be used to confirm diagnosis and for study of immune-related markers. With preoperative patient permission, extra tissue not sent to Laboratory of Pathology, NCI, will be frozen for future analysis of immune-related markers and tumor infiltrates. This additional harvested tissue will be used for research purposes only and is not required for a candidate to participate in this protocol. Tissue will also be collected at collaborating trial sites as specified by Appendix E (Section **17.5**).

3.5 ACCRUAL ESTIMATES

Accrual Targets					
Ethnic Category	Sex				
	Females		Males		Total
Hispanic or Latino	4	+	4	=	8
Not Hispanic or Latino	23	+	23	=	46
Ethnic Category: Total of all subjects	27	+	27	=	54
Racial Category					
American Indian or Alaskan Native	1	+	1	=	2
Asian	1	+	1	=	2
Black or African American	4	+	4	=	8
Native Hawaiian or other Pacific Islander	1	+	1	=	2
White	20	+	20	=	40
Racial Category: Total of all subjects	27	+	27	=	54

Accrual Rate: 2-3 patients/month

Total Expected accrual: Min: 49 Max: 54

Projected Start Date of study: December 1, 2013

Anticipated Primary Completion Date: December 1, 2017

4 REGISTRATION PROCEDURES

4.1 GENERAL GUIDELINES

Eligible patients will be entered on study centrally at the CCR by the Study Coordinator.

Issues that would cause treatment delays should be discussed with the Principal Investigator.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

4.2 REGISTRATION PROCEDURES

4.2.1 Registration at the CCR

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-1@mail.nih.gov.

After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

4.2.2 Participating Site Registration

Registration will be a two part process as patients are screened on this protocol. A protocol registration form will be supplied by the CCR study coordinator and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To initially register a subject, after the participant has signed consent, complete the top portion of the form and send to CCR study coordinator. Once eligibility is confirmed, after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and send to CCR study coordinator. In addition, source documents supporting the eligibility criteria must be sent to the CCR study coordinator. The CCR study coordinator will notify you either by e-mail or fax that the protocol registration form has been received which will include the unique patient/subject ID number. Questions about eligibility should be directed to the CCR study coordinator or PI. Questions related to registration should be directed to the CCR study coordinator.

Subjects that do not meet screening criteria should be removed from the study following the procedure in section [5.8.3](#).

4.3 RANDOMIZATION PROCEDURES

There are two arms to this study and subjects will be randomized equally to either the TICE BCG arm or the combination arm of TICE BCG + PANVAC™. The study arms are described in detail below in Section [5.1](#). The arms will be stratified during randomization for TICE BCG

subgroup based on number of previous induction courses and time of recurrence. This is described in more detail along with the statistical analysis in the statistical section (Section 14).

5 TREATMENT PLAN

5.1 STUDY DESIGN

This is a phase II study in subjects with non-muscle invasive (stages Ta, T1, and/or CIS) high grade urothelial carcinoma of the bladder who have failed at least one course of intravesical BCG. The study will be halted for 28 days after the first six patients with combination therapy are accrued and successfully treated. If any Grade 3 or greater toxicities are observed that do not resolve with conservative management, then they will be discussed with CTEP. With preliminary evidence of safety, the remaining subjects will be accrued to the trial. (See **SCHEMA**)

- 5.1.1 All subjects will receive BCG (TICE strain, 50 mg) intravesically as per usual standard of care once weekly (+/- 2 days) starting in week 3 for a total of 6 weeks (last dose in week 8).
- 5.1.2 Subjects in the combination arm will receive the pox viral vaccines that contain the transgenes for CEA and MUC-1 (both with modified HLA-A2 agonist epitopes) as well as 3 human T-cell costimulatory molecules, B7-1, ICAM-1, and LFA-3 [rV-PANVAC™ (vaccinia) and rF-PANVAC™ (fowlpox)] as follows:
 - 5.1.2.1 rV-PANVAC™ 2 x 10⁸ pfu SQ at week 0 (+/- 7 days) only.
 - 5.1.2.2 rF-PANVAC™ 1 x 10⁹ pfu SQ at weeks (+/- 7 days) 3, 7, 11, and 15.
- 5.1.3 All subjects will provide blood, urine, and tissue (if available) at study entry and at the end of the study. Tissue at study entry can be obtained either from outside FFPE or from previous TURBT performed at NIH (see section 3.4.4.1). This tissue is required for diagnosis. All subjects will also have blood drawn at weeks 3 and 8 to coincide with the start and end of BCG treatment. Urine samples will also be obtained before and after the 1st and 3rd instillation of BCG as defined in Section 10.1.2.
- 5.1.4 All subjects will undergo tuberculin skin testing using purified protein derivative (PPD) at study entry (prior to receiving BCG instillation #1) and around the time of the week 17-20 cystoscopy/EUA. The associated skin reaction will be evaluated by a health care provider within 48-72 hours after injection. Skin induration greater than 10mm in diameter will be considered a positive result. Initial PPD results do NOT preclude subsequent treatment with BCG – these results will only be used to assess the immunologic response to BCG (see section 10.1.1).
- 5.1.5 Between weeks 17-20 (+/- 7 days), a CT urogram and chest x-ray for re-staging purposes prior to biopsy, a planned cystoscopy, exam under anesthesia, and biopsy of the previous tumor area and/or any new areas of tumor to evaluate for the presence/recurrence of disease.

5.1.6 After the initial 20 week study course, subjects will be followed for up to 1 year at the study center every 3 months per routine standard of care for high grade urothelial cancer of the bladder with cystoscopy and cytology. If recurrence is noted, it will be treated and if confirmed to be a recurrence on pathology, the patient will come off study. Surveillance and/or treatment for recurrences beyond the initial 12 months may be performed by outside urologists.

5.2 TRIAL OUTLINE

5.2.1 After meeting all entry criteria based on screening evaluation, subjects will be enrolled into the study. Of note, there will be at least a 3 week delay from the time a previous bladder biopsy/TURBT to allow for adequate bladder healing prior to enrollment.

5.2.1.1 6 green top tubes of blood will be drawn for PBMCs, HLA Typing, and sera for correlative/exploratory research aims (e.g. ELISPOT, CD4+ Antigen-specific responses, etc.) on day 1 of study enrollment

5.2.1.2 Beta-HCG for women of child-bearing age (within 72 hours prior to day 1). In addition, patients, both male and female, should be willing to practice effective birth control during the study and four months following the last study treatment, unless they have had a prior hysterectomy, bilateral oophorectomy, or vasectomy.

5.2.2 TICE BCG arm:

5.2.2.1 Weeks 3-8: Receive weekly (+/- 2 days) intravesical doses of BCG (TICE strain, 50 mg) as per usual standard of care for a total of 6 weeks. Urine will be tested weekly prior to each BCG administration and the presence of gross hematuria or evidence of a urinary tract infection will result in holding that week's BCG dose and postponing therapy until the following week. Also, at the beginning of each administration, patients will be queried about urinary symptoms.

- Complaints of persistent dysuria, urgency, bladder spasms, and/or frequency from previous week's administration that is consistent with Grade 3 or greater toxicity will result in holding BCG administration for that week, supportive care (e.g. medications and hydration), and/or BCG dose reduction the following week at 1/3 normal dose as this can result in less toxicity with similar results for recurrence and progression.³¹
- Patients will be maintained at this reduced dose if they tolerate therapy.
- Future inability to tolerate this reduced dose will result in holding BCG for that week and postponing the BCG until the following week and dropping to 1/6 of the normal dose as this dose may still be effective in prolonging progression-free and cancer-specific survival times.³²

5.2.2.2 Blood and urine will be obtained for correlative studies as described in Section 10.1.

5.2.2.3 Between weeks 17-20 (+/- 7 days), a CT urogram and chest x-ray prior to biopsy, a planned cystoscopy, EUA, and TURBT will be done of previous tumor area and of any new areas of tumor. Tissue will be sent for pathology and be formalin-fixed paraffin embedded. If possible, some tissue will also be frozen.

- If a suspicious lesion is identified, it will be biopsied. If recurrent tumor is not found, then the subject will return every 3 months for a surveillance cystoscopy as per standard-of-care. If recurrent tumor is found, the patient has met the primary endpoint of recurrence and will be taken off study.

5.2.2.4 At every surveillance cystoscopy post-treatment up to 12 months, urine may be collected for cytology as well as for immune-related markers along with blood through 6 green top tubes for correlative immunologic aims.

5.2.3 TICE BCG + PANVAC™ ARM

5.2.3.1 Enroll into study with baseline evaluation and studies as described in Section 3.4. Of note, there will be at least a 3 week delay from the time a previous bladder biopsy/TURBT to allow for adequate bladder healing prior to enrollment.

5.2.3.2 Week 0 (+/- 7 days) – all subjects in this arm receive single vaccinia priming vaccine - rV-PANVAC™ 2 x 10⁸ pfu SQ after undergoing phlebotomy with 6 green top tubes for immunologic correlates.

5.2.3.3 Weeks 3-8: Receive weekly (+/- 2 days) intravesical doses of BCG (TICE strain, 50 mg) as per usual standard of care for a total of 6 weeks. Urine will be tested weekly prior to each BCG administration and the presence of gross hematuria or evidence of a urinary tract infection will result in holding that week's BCG dose and postponing therapy until the following week. Also, at the beginning of each administration, patients will be queried about urinary symptoms.

- Complaints of persistent dysuria, urgency, bladder spasms, and/or frequency from previous week's administration that is consistent with Grade 3 or greater toxicity will result in holding BCG administration for that week, supportive care (e.g. medications and hydration), and/or BCG dose reduction (BCG can be given 1/3rd of the normal dose on the following week as this can result in less toxicity with similar results for recurrence and progression³¹).
- Subjects will be maintained at this reduced dose if they tolerate therapy.
- Future inability to tolerate this reduced dose will result in holding BCG for that week and postponing the BCG until the following week and dropping to 1/6 of the normal dose as this dose may still be effective in prolonging progression-free and cancer-specific survival times.³²

5.2.3.4 PANVAC administration will continue as planned regardless of any delays in BCG administration. Research sample collection of urine will coincide with the first and third week of BCG instillation regardless of any delays with BCG administration. Research sample collection of blood will coincide with the first and sixth week of BCG instillation regardless of any delays with BCG administration. [For example, if BCG is first given on week 3 of the study, but held on week 4 and resumed on week 5, then

urine collection would be collected on weeks 0, 3, 6 (previously week 5), 18-21 (previously 17-20), and optionally on follow-up visits. In this scenario, blood would be collected on weeks 0, 3, 9 (previously week 8), 18-21 (previously 17-20), and optionally on follow-up visits. The timing of PANVAC administration will NOT change in this scenario.] Week (+/- 7 days) 3, 7, 11, and 15 – all patients in this arm receive boost fowlpox vaccines - rF-PANVACTM 1 x 10⁹ pfu SQ

- 5.2.3.5 Blood and urine will be obtained for correlative studies as described in Section 10.1.
- 5.2.3.6 Between weeks 17-20 (+/- 7 days), a CT urogram and chest x-ray prior to biopsy, a planned cystoscopy, EUA, and TURBT will be done of previous tumor area and of any new areas of tumor. Tissue will be sent for pathology and be formalin-fixed paraffin embedded. If possible, some tissue will also be frozen.
- 5.2.3.7 Also at the time of the end-of-study cystoscopy, urine will be collected for cytology and for immune-related markers along with blood for correlative aims.

5.3 AGENT ADMINISTRATION

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

CTEP IND Agents:

rV-PANVAC™ (vaccinia priming vaccine)
rF-PANVAC™ (fowlpox boosting vaccine)

Other Agents:

Bacillus Calmette-Guerin (BCG) – TICE strain

The administration of the agents is described below based on the treatment arms:

5.3.1 TICE BCG alone arm:

Weeks 3-8

Bacillus Calmette-Guerin (BCG) – TICE strain

- Attenuated live strain of *Mycobacterium bovis*
- Induction course of 6 weekly (+/- 2 days) instillations
- Instillation volume (50 mg in 50 mL sterile saline).
- Dose – 50 mg vial (TICE) is reconstituted in accompanying diluent and then further diluted in sterile, preservative-free saline according to manufacturer guidelines
- Each week, RNs will steriley insert a urethral catheter atraumatically into the subject's bladder and administer the reconstituted BCG. Following instillation of the 50 mL of BCG, the urethral catheter will be removed.

- BCG will then be held in the bladder for 1-2 hours depending on patient ability to hold the medication in the bladder. The patient will then void in a toilet and rinse the toilet with bleach prior to flushing.
- All equipment in contact with BCG will be treated as chemotherapeutic waste and disposed of as such in a MPW container.

5.3.2 TICE BCG + PANVAC™ arm:

Week 0

rV-PANVAC™ (vaccinia priming vaccine) 2×10^8 pfu subcutaneously (+/- 7 days).

Week 3

BCG (+/- 2 days see details above).

rF-PANVAC™ (fowlpox boosting vaccine) 1×10^9 pfu subcutaneously (+/- 7 days).

Weeks 4-6

BCG (+/- 2 days see details above).

Week 7

BCG (+/- 2 days see details above).

rF-PANVAC™ (fowlpox boosting vaccine) 1×10^9 pfu subcutaneously (+/- 7 days).

Week 8

BCG (+/- 2 days see details above).

Week 11

rF-PANVAC™ (fowlpox boosting vaccine) 1×10^9 pfu subcutaneously (+/- 7 days).

Week 15

rF-PANVAC™ (fowlpox boosting vaccine) 1×10^9 pfu subcutaneously (+/- 7 days).

5.3.3 Precautions for the vaccines

- Prior to administration of the drugs, safe-handling precautions should be thoroughly reviewed (see Section 9, precautions and special handling subsections of “Pharmaceutical Information”).
- The proper procedure for disposing the live vaccine is a critical part of drug administration (see Section 9, disposal sections of “Pharmaceutical Information”).

5.4 DOSE LIMITING TOXICITY

TICE BCG will be given in the standard dose and schedule as described above. All toxicities observed will be tabulated by maximum grade per subject. A dose limiting toxicity (DLT) will be any grade 3 or greater adverse effect (AE) by CTCAE v5.0 related to the investigational agent and persisting for more than one week and not improving by routine standard of care. In

addition, any grade 3 or greater AE as per a previously published vaccinia toxicity grading scale³³ related to the investigational agent will also be considered a DLT.

Intravesical BCG therapy can lead to common side effects that are usually self-limited, particularly with dose reduction. These side effects include, but are not limited to, gross hematuria, dysuria, frequency, urgency, bladder spasms, and generalized cystitis. These side effects will not be considered a DLT if it is related to BCG and resolves with BCG dose reduction and/or postponing therapy by 1-2 weeks and treating symptoms with conservative management. In contrast, persistent side effects despite dose reduction/postponing of BCG therapy or the development of systemic BCG infection (aka “BCG-osis”) will be considered a DLT.

Severe adverse events due to local instillation of BCG are uncommon. In a retrospective analysis of 2602 patients reported in 1989, the overall rate of serious complications was less than five percent.³⁴ The most common complication was fever >103°F in 2.9 percent followed in decreasing frequency by:

- Significant hematuria (1 percent)
- Granulomatous prostatitis (0.9 percent)
- Pneumonitis and/or hepatitis (0.7 percent)
- Arthralgia (0.5 percent)
- Epididymitis (0.4 percent)
- Sepsis (0.4 percent)
- Rash (0.3 percent)
- Ureteral obstruction (0.3 percent)
- Contracted bladder (0.2 percent)
- Renal abscess (0.1 percent)
- Cytopenia (0.1 percent)

Systemic sepsis and even death can also develop early following local instillation of BCG. During the initial clinical experience with BCG as an intravesical agent, most cases of sepsis could be traced back to recognized errors in BCG administration such as traumatic catheterization, administration too early after transurethral surgery, or instillation during a concomitant UTI. All these conditions result in physical disruptions in the urothelial blood barrier. Sepsis has been estimated to occur in approximately one in every 15,000 patients treated with intravesical BCG.³⁴ Prompt recognition and administration of multidrug, antibiotic therapy is necessary if true BCG sepsis occurs.

- 5.4.1 For the purpose of protecting subject safety, we implement the following stopping rule for safety monitoring of subjects who receive the combination therapy.
- 5.4.2 If one subject experiences a grade 5 event, thought to be possibly or probably due to the combination therapy (PANVAC + BCG), the study will be stopped. For grade 3 or 4

events probably related to the investigational agent (PANVAC) and persisting for more than one week and not improving by routine standard of care, the study will be stopped according to the stopping rule described in Section 14.

5.4.2.1 The exceptions to what constitute true adverse events/DLTs are previously noted.

5.4.3 Formal dose escalation/Phase I study is not planned as the TICE BCG doses are consistent with standard of care and the TICE BCG is being administered in an intravesical fashion while the PANVAC™ is given subcutaneously and in doses that have been shown to be safe in multiple Phase I and Phase II studies in a variety of different cancers.

5.5 GENERAL CONCOMITANT MEDICATION AND SUPPORTIVE CARE GUIDELINES

For the vaccine administration, anti-emetics and anti-diarrheal agents may be administered as required, but are not anticipated to be needed and should not be used prophylactically on the first cycle. The selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens should not include dexamethasone or other steroids.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Any subjects taking antibiotics for any reason must complete that course of therapy and be free of evidence of further infection before receiving any dose of vaccine. To avoid blunting of the immune response, we will avoid administering corticosteroids or other immunosuppressive agents unless medically indicated.

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

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

5.5.1 Treatment of Vaccinia Vaccination Complications

5.5.1.1 Vaccinia Immune Globulin (VIG)

Vaccinia Immune Globulin (VIG): First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in patients that are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG. VIG is contraindicated, however, for the treatment of isolated vaccinia keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is only available through the CDC's Strategic National Pharmaceutical Stockpile by contacting the CDC's Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100. Upon receipt of a call from a patient or upon direct observation of a patient or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible: 1) to initiate review of the clinical case, 2) to seek consultation on the

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

5.5.1.2 Cidofovir (Vistide®, Gilead Sciences)

Cidofovir (Vistide®, Gilead Sciences): Cidofovir is an FDA-approved antiviral drug for the treatment of CMV retinitis among patients with AIDS. Cell-based *in vitro* studies and animal model studies have demonstrated this agent's antiviral activity against certain orthopoxviruses. Currently, its efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be used when VIG therapy is not effective [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated September 28, 2009. Available at: <http://www.bt.cdc.gov/agent/smallpox/vaccination/mgmt-adv-reactions.asp>]. The CDC has informed the NCI/CTEP that cidofovir will not be supplied through Strategic National Pharmaceutical Stockpile to investigators involved in CTEP-sponsored protocols utilizing recombinant vaccinia-based vaccines. Thus, investigators should obtain cidofovir for second-line therapy through commercial sources if necessary. Investigators should consult the CDC Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100 regarding appropriateness of therapy and guidance.

5.6 DURATION OF THERAPY

In the absence of treatment delays due to adverse event(s), therapy will continue as per the aforementioned schema. Off therapy criteria is listed in Section 0.

5.7 DURATION OF FOLLOW UP

Subjects will be followed for 1 year after the initial 20 week study course is completed with surveillance cystoscopy or until death, whichever occurs first.

5.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

5.8.1 Criteria for removal from protocol therapy

- Intercurrent illness or medical circumstances: if at any time the constraints of this protocol are detrimental to the subject's health, the subject may be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator (investigator discretion).
- Participant requests to be withdrawn from active therapy
- Enroll on another protocol or receives standard of care.
- Unacceptable treatment-related toxicity (DLT) as defined in Section [5.4](#).
- Grade 4 toxicity after vaccinia injection per table below³⁵:

Vaccinia-Related Toxicity Grading	
Grade 1	
	Cutaneous reaction extending to not more than 10 cm from vaccination site
Grade 2	
	Cutaneous reaction extending to more than 10 cm from the vaccination site or any autoinoculation that resolves without sequelae
Grade 3	
	Not applicable
Grade 4	
	Autoinoculation syndrome with sequelae: postvaccinia encephalitis, vaccinia gangrenosum, eczema gangrenosum, or Steven-Johnson syndrome

5.8.2 Off-Study Criteria

- Completed study follow-up period (1 year post treatment)
- Disease progression to muscle-invasive urothelial carcinoma of the bladder
- Subject's request to be taken off study. In this event, the reasons for withdrawal will be documented.
- If subjects are non-compliant with the protocol guidelines, they may be removed from the study at the discretion of the principal investigator.
- Death

5.8.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off study. A Participant Status Updates Form from the web site ([https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-\(ADCR\).aspx?Mode=Edit](https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit)) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-1@mail.nih.gov.

For participating sites, the Participant Status Updates Form will be supplied by the CCR study coordinator. Send the completed form to the CCR study coordinator.

6 DOSING DELAYS/DOSE MODIFICATIONS

6.1 TICE BCG

The need for BCG dose modification may arise and has been described previously. To review, subjects will receive weekly (+/- 2 days) intravesical doses of BCG (TICE strain, 50 mg) as per usual standard of care for a total of 6 weeks. Urine will be tested weekly prior to each BCG administration and the presence of gross hematuria or a urinary tract infection by urinalysis will result in holding that week's BCG dose and postponing therapy until the following week. Also, at the beginning of each administration, subjects will be queried about urinary symptoms. Complaints of persistent dysuria, urgency, bladder spasms, and/or frequency from previous week's administration that is consistent with Grade 3 or greater toxicity will result in holding BCG administration for that week, supportive care (e.g. medications and hydration), and/or BCG dose reduction the following week at 1/3 normal dose as this can result in less toxicity with similar results for recurrence and progression.³¹ Complaints of non-infective cystitis or hematuria consistent with Grade 2 or higher will result in holding BCG administration for that week. Therapy will resume the following week at if these symptoms are at less than or equal to Grade 1. Subjects will be maintained at this reduced dose if they continue to fail to tolerate full dose therapy.

Future inability to tolerate this reduced dose will result in holding BCG for that week and postponing the BCG until the following week and dropping to 1/6 of the normal dose (details in Section 5.2). Of note, weekly BCG postponement or dose reductions will not alter the vaccination schedule. For example, if the week 5 dose of BCG is withheld, the subject will still receive the next dose of PANVAC™-F on week 7, but the last dose of BCG would be given on week 9 instead of week 8 (assuming that no other BCG postponement is necessary).

6.2 PANVAC™-V (VACCINIA) AND PANVAC™-F (FOWLPOX)

6.2.1 Dosing delay

- Subjects must have recovered to \leq grade 1 toxicity related to vaccine for constitutional symptoms (that are not related to the BCG or medications related to BCG treatments) in order to receive a subsequent vaccination. These constitutional symptoms are listed in Section 8.1.1.1.

- Subjects with \leq grade 2 toxicity not related to vaccine treatment (i.e. attributed to BCG) will remain eligible to receive vaccine.
- Subjects that recover from grade 3 toxicity unrelated to vaccine treatment (i.e. related to BCG) will remain eligible for the vaccine. However, subjects that experience a grade 4 toxicity unrelated to vaccine treatment (i.e. attributed to BCG) will not remain eligible for further vaccine administration.
- If BCG is permanently discontinued, the patient will remain eligible for further vaccine administration in the absence of a grade 4 toxicity.
- Vaccination will be held for $>$ grade 2 proteinuria measured initially by a urine dipstick but confirmed by a 24 hour urine collection.
- If \geq grade 2 nonautoimmune toxicity attributable to the vaccine persists for $>$ 42 days, the subject will not receive further vaccine inoculations and will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- Subjects who develop grade 3 injection site reactions will have their vaccine held until injection site reaction resolves to grade 2 or less.
- Subjects who develop \geq a grade 2 allergic or autoimmune disease that threatens vital organ function or any \geq grade 3 autoimmunity, not related to a therapeutic response, will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints. Follow-up will be performed during each vaccination and blood draw interval for immunologic testing as described in Section 11
- Subjects who develop any grade 4 toxicity attributable to the study drug(s) will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- If a scheduled vaccine dose is missed due to scheduling or logistical issues, the vaccine may be given within 7 days of the appointed time (which resets the appointed date for further vaccinations) or be considered a missed dose. If the subject has a delay in vaccination not due to toxicity during the core phase, the vaccine may be delayed for up to 42 days without removal of the subject from study. BCG administration will not be affected by missed/delayed vaccine administrations.

6.2.1.1 Dose modifications

No dose modifications are allowed with this vaccine

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

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

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found

<https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements>. Note: Only IND Safety

Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

If the study is under review by an IC specific IRB, the following should be noted:

- Reports referenced in policy 801 are made to the IC Specific IRB rather than to the OHSRP Office of Compliance and Training.
- Section 5.2 of the policy 801 does not apply
- Policy 802 does not apply

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found

<https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements>.

7.3 NCI CLINICAL DIRECTOR REPORTING

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

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

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NCI GUIDANCE FOR REPORTING EXPEDITED ADVERSE EVENTS FOR MULTI-CENTER TRIALS

The site PI must immediately report to the coordinating center PI any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event within 24 hours of PI awareness of the event. The Site PI must also report any protocol deviations to the coordinating center PI within 7 days of PI awareness as per the form provided in Appendix D (Section 17.4). Participating centers must also submit the report to their IRB in accordance with their institutional policies. The appendices will be made available to other participating sites.

7.5 NIH OFFICE OF SCIENCE POLICY (OSP)/INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 Serious Adverse Event Reports to OSP/IBC

The Principal Investigator (or delegate) will notify OBA via email (HGTprotocols@mail.nih.gov) and IBC of any unexpected fatal or life-threatening experience associated with the use of the vaccine as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the vaccine, but are not fatal or life-threatening, much be reported to NIH OSP/IBC as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information. Adverse events may be reported by using the Adverse Event Reporting template available on the NIH OSP website at: <http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/gemcris> or by using the FDA Form 3500a.

7.5.2 Annual Reports to OSP/IBC

The study Principal Investigator will submit to OSP via email (HGTprotocols@mail.nih.gov) and IBC a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect unless the IND sponsor has been authorized to submit this report.

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the FDA annual report can be sent to OSP/IBC in lieu of a separate report. Please include the OSP/IBC protocol number on the annual report, and the updated clinical protocol.

7.5.2.1 Clinical Trial Information

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

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

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

7.5.2.2 Progress Report and Data Analysis

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

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

7.6 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.6.1 Principal Investigator/Research Team

All data will be abstracted in a timely manner and entered into C3D. Adverse events will be reported as required above. Any safety concerns, or new information that might affect either the ethical and or scientific conduct of the trial will be reported to the IRB using iRIS to the Sponsor.

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

7.6.2 Safety Monitoring Committee

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 ADVERSE EVENTS: LIST AND SPONSOR REPORTING REQUIREMENTS

Study closed with CTEP as of 5/1/2019. No further reporting is required.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 8.1) and the characteristics of an observed AE (Section 8.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition to** routine reporting.

8.1 COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISKS LIST(S) (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and *italicized* text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements'
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously via CTEP-AERS. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

8.1.1 CAEPRs for CTEP IND Agents

For protocols with CAEPRs not including a "SPEER" category, protocol-specific exceptions to the CTEP-AERS reporting table can be found in the CAEPR's "ASAEL" category instead. This protocol-specific exception is limited to Grade 1 and Grade 2 ASAEL events, *i.e.* Grade 3 through Grade 5 ASAEL-listed events are NOT exceptions to CTEP-AERS reporting.

8.1.1.1 PANVACTM-V and PANVACTM-F

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and *italicized* text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements'
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. Below is the CAEPR for PANVAC-VF/TRICOM.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

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Adverse Events with Possible Relationship to PANVAC-VF/TRICOM (CTCAE 4.0 Term)	Specific Protocol Exceptions to Expedited Reporting (SPEER)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	
Anemia	
GASTROINTESTINAL DISORDERS	
Diarrhea	
Nausea	
Pancreatitis ²	
Vomiting	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Chills	<i>Chills (Gr 2)</i>
Fatigue	<i>Fatigue (Gr 2)</i>
Fever	<i>Fever (Gr 2)</i>
Flu like symptoms	<i>Flu like symptoms (Gr 2)</i>
Injection site reaction	<i>Injection site reaction (Gr 2)</i>
Pain	
INVESTIGATIONS	
Alanine aminotransferase increased	
METABOLISM AND NUTRITION DISORDERS	
Anorexia	
Hypoalbuminemia	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	
Arthralgia	

Back pain	
Bone pain	
Generalized muscle weakness	
Myalgia	Myalgia (Gr 2)
Pain in extremity	
NERVOUS SYSTEM DISORDERS	
Headache	Headache (Gr 2)
Syncope	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	
Cough	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	
Hyperhidrosis	
Pruritus	
Skin induration	

¹This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

²Transient pancreatitis was observed in at least one patient who received endoscopic ultrasound (EUS)-guided intra-pancreatic tumor injection.

Non-serious adverse events also reported on PANVAC-VF/TRICOM trials but with the relationship to PANVAC-VF/TRICOM still undetermined due to low frequency (i.e., <3%):

EYE DISORDERS - Eye pain

GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis; Gastrointestinal disorders - Other (fluid around pancreas); Mucositis oral; Stomach pain

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema limbs; Non-cardiac chest pain

IMMUNE SYSTEM DISORDERS - Allergic reaction

INVESTIGATIONS - Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased

METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypomagnesemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Neck pain

NERVOUS SYSTEM DISORDERS - Dysgeusia

PSYCHIATRIC DISORDERS - Agitation; Insomnia

RENAL AND URINARY DISORDERS - Proteinuria

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Erythema multiforme; Rash maculo-papular

VASCULAR DISORDERS - Hot flashes; Hypotension

Notes:

1. PANVAC-V [Recombinant Vaccinia-CEA(D609)/MUC1(L93)/TRICOM] and PANVAC-F [Recombinant Fowlpox-CEA(D609)/MUC1(L93)/TRICOM] when used in combination with other agents, either commercial or investigational, could be associated with changes in the frequency or severity of known events or the emergence of new patterns of events.
2. Other potential risks or complications associated with the use of the vaccinia vaccine strain from which the attenuated recombinant vector is derived, include those observed during the smallpox vaccination programs:
 - Inadvertent inoculation (autoinoculation and direct contact transmission)
 - Non-specific erythematous or urticarial rashes (generally self-limiting) and rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome)
 - Generalized vaccinia (disseminated maculopapular or vesicular rash of varying extent on any part of the body)
 - Eczema vaccinatum (vaccinia lesion development on areas of the skin that are, or had at one time been, eczematous)
 - Progressive vaccinia (local vaccination lesion fails to heal and develops progressive necrosis, with destruction of large areas of skin, subcutaneous tissue, and underlying structures. Progressive lesions may spread to other skin surfaces and to bone and viscera)
 - Post-vaccinia encephalitis/encephalomyelitis
 - Fetal vaccinia
 - Myocarditis/pericarditis
3. The inclusion of co-stimulatory molecules in these agents may theoretically stimulate autoimmunity or exacerbate existing disease in susceptible individuals.
4. Thrombotic thrombocytopenic purpura (TTP) occurred with closely related agents, PROSTVAC-V/TRICOM [Recombinant Vaccinia-PSA(L155)/TRICOM] and PROSTVAC-F/TRICOM [Recombinant Fowlpox-PSA(L155)/TRICOM].

8.1.2 Adverse Event List(s) for Commercial Agents

8.1.2.1 TICE Bacillus Calmette-Guerin (BCG)

(See package insert for complete list of side effects)

Symptoms of bladder irritability, related to the inflammatory response induced, are reported in approximately 60% of patients receiving TICE® BCG. The symptoms typically begin 4–6 hours after instillation and last 24–72 hours. The irritative side effects are usually seen following the third instillation, and tend to increase in severity after each administration.

The irritative bladder adverse effects can usually be managed symptomatically with products such as pyridium, propantheline bromide, oxybutynin chloride and acetaminophen. The mechanism of action of the irritative side effects has not been firmly established, but is most consistent with an immunological mechanism. There is no evidence that dose reduction or antituberculous drug therapy can prevent or lessen the irritative toxicity of TICE® BCG. “Flu-like” symptoms (malaise, fever, and chills) which may accompany the localized, irritative toxicities often reflect hypersensitivity reactions which can be treated symptomatically. Antihistamines have also been used. Adverse reactions to TICE® BCG tend to be progressive in frequency and severity with subsequent instillation. Delay or postponement of subsequent treatment may or may not reduce the severity of a reaction during subsequent instillation. Although uncommon, serious infectious complications of intravesical BCG have been reported. The most serious infectious complication of BCG is disseminated sepsis with associated mortality. In addition, *M. bovis* infections have been reported in lung, liver, bone, bone marrow, kidney, regional lymph nodes, and prostate in patients who have received intravesical BCG. Some male genitourinary tract infections (orchitis/epididymitis) have been resistant to multiple drug antituberculous therapy and required orchiectomy. **If a patient develops persistent fever or experiences an acute febrile illness consistent with BCG infection, BCG treatment should be discontinued and the patient immediately evaluated and treated for systemic infection.** The local and systemic adverse reactions reported in a review of 674 patients with superficial bladder cancer, including 153 patients with carcinoma *in situ*, are summarized.

SUMMARY OF ADVERSE EFFECTS SEEN IN 674 PATIENTS WITH SUPERFICIAL BLADDER CANCER, INCLUDING 153 WITH CARCINOMA *IN SITU*

Adverse Event	N	Overall (Grade ≥ 3)	Adverse Event	N	Overall (Grade ≥ 3)
Dysuria Urinary	401	60% (11%)	Arthritis/Myalgia	18	3% (<1%)
Frequency Flu-Like Syndrome	272	40% (7%) 33%	Headache/Dizziness	16	2% (0) 2%
Hematuria Fever	224	(9%) 26% (7%)	Urinary Incontinence	16	(0) 2%
Malaise/Fatigue	175	20% (8%) 7%	Anorexia/Weight Loss	15	(<1%) 2%
Cystitis Urgency	134	(0) 6% (2%) 6%	Urinary Debris Allergy	15	(<1%) 2%
Nocturia	50 40	(1%) 5% (1%)	Cardiac (Unclassified)	15	(<1%) 2%
Cramps/Pain	39 30	4% (1%) 3%	Genital Inflammation/ Abscess	14	(<1%) 2%
Rigors	27 22	(1%) 3% (<1%)	Respiratory (Unclassified)	13	(1%) 2%
Nausea/Vomiting	20		Urinary Tract Infection	12	(<1%) 2%
			Abdominal Pain	11	(<1%) 2%
				10	(1%) 2%
				10	(1%)

The following adverse events were reported in $\leq 1\%$ of patients: anemia, BCG sepsis, coagulopathy, contracted bladder, diarrhea, epididymitis/prostatitis, hepatic granuloma, hepatitis, leukopenia, neurologic (unclassified), orchitis, pneumonitis, pyuria, rash, thrombocytopenia, urethritis, and urinary obstruction.

In SWOG study 8795, toxicity evaluations were available on a total of 222 TICE® BCG-treated patients and 220 MMC-treated patients. Direct bladder toxicity (cramps, dysuria, frequency, urgency, hematuria, hemorrhagic cystitis, or incontinence) was seen more often with TICE® BCG, with 356 events compared to 234 events for MMC. Grade ≤ 2 toxicity was seen significantly more frequently following TICE® BCG treatment ($p=0.003$). No life-threatening toxicity was seen in either arm. Systemic toxicity with TICE® BCG was markedly increased compared to that of MMC, with 181 events for TICE® BCG compared to 80 for MMC. The frequency of toxicity was increased in all grades, particularly for grades 2 and 3. The most common complaints were malaise, fatigue and lethargy, fever, and abdominal pain. Thirty-two TICE® BCG patients were reported to have been treated with isoniazid. Five TICE® BCG patients had liver enzyme elevation, including two with grade 3 elevations. Eighteen of the 222 (8.1%) TICE® BCG patients failed to complete the prescribed protocol compared to 6.2% in the MMC group.

8.2 DEFINITIONS

8.2.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

8.2.1.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized **death on study requires both** for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
- **For expedited reporting purposes only:**
 - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 8.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
 - Other AEs for the protocol that do not require expedited reporting are outlined in Section 8.4.

- **Attribution** of the AE:

- Definite – The AE is *clearly related* to the study treatment.
- Probable – The AE is *likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE is *doubtfully related* to the study treatment.
- Unrelated – The AE is *clearly NOT related* to the study treatment.

8.3 EXPEDITED ADVERSE EVENT REPORTING TO CTEP

Study Closed with CTEP on 5/1/2019. No further reporting is required

8.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (<http://ctep.cancer.gov>). The reporting procedures to be followed are presented in the “NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs” which can be downloaded from the CTEP Web site (<http://ctep.cancer.gov>). These requirements are briefly outlined in the tables below (Section 8.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

8.3.2 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

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

Death due to progressive disease should be reported as **Grade 5 “Disease progression”** in the system organ class (SOC) “General disorders and administration site conditions.”. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Pregnancy loss

- Pregnancy loss is defined in CTCAE as “Death in utero.”

- Any Pregnancy loss should be reported expeditiously, as Grade 4 “Pregnancy loss” under the Pregnancy, puerperium and perinatal conditions SOC.
- A Pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEPAERS recognizes this event as a patient death.

A neonatal death should be reported expeditiously as Grade 4, “Death neonatal” under the General disorders and administration SOC.

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention^{1,2}

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

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

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization \geq 24 hrs		10 Calendar Days		24-Hour 5 Calendar Days
Not resulting in Hospitalization \geq 24 hrs	Not required		10 Calendar Days	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- “24-Hour; 5 Calendar Days” - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.

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- “10 Calendar Days” - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

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

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

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

Effective Date: May 5, 2011

8.3.3 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section [8.4](#)):

CTCAE SOC	Adverse Event	Grades	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Renal and urinary disorders	Gross hematuria	1 & 2	This AE should not lead to hospitalization except in the event of continuous bladder irrigation (CBI)	Intravesical BCG	Can be managed with oral hydration and possible BCG dose reduction (per PI discretion)
Renal and urinary disorders	Cystitis (non-infective)	1 & 2	This AE should not lead to hospitalization except in the event of continuous bladder irrigation (CBI)	Intravesical BCG	Can be managed with oral anti-cholinergics or analgesics and possible BCG dose reduction (per PI discretion)
Renal and urinary	Frequency/urgency	1 & 2	This AE should not lead to hospitalization	Intravesical BCG	Can be managed with oral anti-cholinergics or analgesics and possible BCG dose reduction (per PI discretion)
Renal and urinary	Bladder pain/spasms	1 & 2	This AE should not lead to hospitalization	Intravesical BCG	Can be managed with oral anti-cholinergics or analgesics and possible BCG dose reduction (per PI discretion)

8.4 ROUTINE ADVERSE EVENT REPORTING TO CTEP

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

8.5 SECONDARY MALIGNANCY

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

8.6 SECOND MALIGNANCY

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

9 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents or commercial agents administered in this study can be found in Section **8.1**.

9.1 CTEP IND AGENTS

9.1.1 PANVAC™-V (NSC 727026)

Other Names: Recombinant-Vaccinia-CEA(D609)/MUC-1(L93)/TRICOM™

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

Description: PANVAC-V is a recombinant vaccinia virus vector vaccine containing genes for human CEA, MUC-1 and three co-stimulatory molecules (designated TRICOM™): B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The CEA gene coding sequence is modified to code for a single amino acid substitution (aspartic acid, instead of asparagine at amino acid position 609) in one 9-mer, HLA-A2-restricted, immunodominant epitope designed to enhance immunogenicity. The MUC-1 gene coding sequence is also modified to code for a single amino substitution (leucine, instead of threonine at amino acid position 93) in one 10-mer, HLA-A2-restricted, immunodominant epitope designed to enhance immunogenicity. TBC-vTRICOM, is used as the parental virus for this recombinant vaccine, and is generated by insertion of the genes for the three co-stimulatory molecules into an attenuated, live, derivative of the Wyeth (New York City Board of Health) strain of vaccinia virus. A plasmid vector containing the modified CEA and MUC-1 genes is used to transfect primary chicken embryo dermal (CED) cells infected with the parental vaccinia virus to generate the recombinant vaccinia virus. The final PANVAC-V recombinant vaccine is generated by infection of primary CED cells with the recombinant vaccinia virus. Vaccinia virus can infect mammalian cells and express the inserted transgenes, and is a potent immune stimulator, eliciting both a strong humoral and cellular immune

response. Vaccinia virus is replication competent in mammalian cells, making systemic infections possible.

How Supplied:

Lot: 2-050103: PANVAC-V is supplied by the Pharmaceutical Management Branch, CTEP, NCI in vials containing 0.3 mL of the vaccine at a final viral concentration titer of 2.1×10^9 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol (total vial contents = 6.3×10^8 pfu's).

All other lots: PANVAC-V is supplied by the Pharmaceutical Management Branch, CTEP, NCI in vials containing 0.75 mL of the vaccine at a final viral concentration titer of 4×10^8 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol (total vial contents = 3×10^8 pfu's).

Note: The PANVAC-V concentration varies between lots, requiring changes to dose preparation instructions. **Use extreme caution when preparing each dose.**

Preparation: Thaw vials completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds prior to dose preparation. Perform all dilutions of the vaccine with 0.9% sodium chloride for injection, USP and vortex all dilutions vigorously again for at least ten seconds prior to withdrawing the final dose. **Note the concentration of the current supply of PANVAC-V on your institutional preparation guidelines to avoid potentially serious dosing errors.**

PANVAC™-V (vaccinia) Lot: 2-050103 (2.1×10^9 pfu/mL, 0.3 mL vial):

Allow the contents of one vial to thaw completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds. Add 0.7 mL of 0.9% sodium chloride, USP to the thawed vial to yield 1 mL of PANVAC™-V (vaccinia) at a concentration of 6.3×10^8 pfu/mL. Vortex vigorously at high power for at least ten seconds. Withdraw 0.32 mL (2×10^8 pfu) for subcutaneous administration.

PANVAC™-V (vaccinia) all other lots:

Allow the contents of one vial to thaw completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds. Withdraw 0.5 mL (2×10^8 pfu) for subcutaneous administration.

Storage: Store intact vials of PANVAC-V at -70°C or colder.

Stability: Shelf-life studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at $2-8^{\circ}\text{C}$. Do not re-freeze thawed vials. Vials of PANVAC-V are for single-use only and do not contain a preservative. Administer prepared doses as soon as possible following preparation (*i.e.*, within one hour). If necessary, store prepared doses at $2-8^{\circ}\text{C}$ for up to 4 hours following preparation.

Route of Administration: PANVAC-V is administered by subcutaneous injection.

Special Handling and Precautions

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

Preparation, Handling and Disposal Recommendations

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

12. Place an empty biohazard sharps container in the biosafety cabinet to dispose of all waste generated.
13. Transport the agent from the -70°C freezer to the work area in leak proof bag.
14. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
15. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
16. Place all waste into a sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
17. Place all waste and protective apparel in a leak proof biohazard bag, and place the bag inside a biohazard sharps container for incineration according to institutional policy and according to local, state, and federal regulations.
18. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
 - Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
 - Use protective apparel, eyewear, mask, and gloves.
 - Cover spills with disposable absorbent towels.
 - Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
 - Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

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

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

Precautions for Healthcare Workers

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

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

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

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

Avoid exposure to the recombinant vaccinia vaccine, any contaminated dressings, or other infectious materials from patients, or the patient's inoculation site if you are pregnant or breast-feeding; have a history or presence of active eczema or atopic dermatitis; have acute, chronic or exfoliative skin conditions; or, are immunocompromised. More information on vaccinia precautions for healthcare workers can be obtained from

<http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm#tab2> and
<http://www.cdc.gov/mmwr/PDF/rr/rr5207.pdf>.

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

Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

Patient Care Implications and Contraindications

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

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (*e.g.*, burns, impetigo, varicella zoster, severe acne, contact dermatitis, psoriasis, herpes or other open rashes or wounds), until the condition resolves.
- are pregnant or intend to become pregnant (due to the potential risk of fetal vaccinia); or are breast-feeding (due to the potential risk of contact transmission and inadvertent inoculation). It is currently unknown if vaccinia virus or antibodies are excreted in breast milk. Because there is no safety data available, patients (*i.e.*, vaccinees) should avoid becoming pregnant, fathering a child, or breast-feeding for at least 4 months following completion of therapy with the recombinant vaccine.
- are in close contact with children less than 3 years of age (due to the potential risk of contact transmission and inadvertent inoculation).
- are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection.

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

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

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

Due to the method of manufacturing (virus grown in primary chicken embryo dermal cells), do not administer the recombinant vaccinia vaccine to patients with a history of allergy to eggs or egg products. Do not administer the recombinant vaccinia vaccine to patients with a history of allergy or serious reaction to prior vaccinia vaccination (e.g., smallpox vaccination).

Potential Complications Associated With Recombinant Vaccinia Vaccination

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

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

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

There have been a number of complications reported in the literature associated with vaccinia vaccination for smallpox. Reported complications from vaccinia vaccine when given by scarification include: a) auto-inoculation of other sites with vaccinia, b) generalized vaccinia, c) eczema vaccinatum, d) progressive vaccinia (vaccinia necrosum), or e) post-vaccinal

encephalitis. In a 1968 ten-state survey, cases of these complications per million vaccinations in adult recipients (≥ 20 years of age) of vaccinia primary vaccination and revaccination were:

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

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

Information has been reported by the US Department of Defense (DoD) during the post-vaccination surveillance assessment of adverse events in military personnel following implementation of a smallpox vaccination program from the period of December 13, 2002 through May 28, 2003. Although not directly comparable to historical numbers, due to differences in multiple population variables, estimated cases (number of cases per million vaccinations based on vaccination of 450,293 personnel, with a median age of 26 years and 70.5% as primary vaccinees) of these same complications per million vaccinations were:

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

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

1. **Non-specific erythematous or urticarial rashes:** These rashes can appear approximately 10 days after vaccination and may sometimes be confused with generalized vaccinia, but are generally self-limiting. Patients are usually afebrile and

these benign rashes usually resolve spontaneously within 2-4 days. Erythema multiforme can present as different types of lesions, including macules, papules, urticaria, and bull's eye lesions (dark papule or vesicle surrounded by a pale zone and an area of erythema). These lesions may be extremely pruritic, lasting up to four weeks. Rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome) may occur, requiring hospitalization. Vaccinia Immune Globulin (VIG) therapy is not indicated to treat these rashes. Supportive care measures are warranted since these rashes are likely manifestations of an immune response or hypersensitivity reaction to the vaccine and are not likely to contain vaccinia virus.

2. **Bacterial Infection:** Vaccination site infection, most likely due to staphylococcus and streptococcus normal skin flora, is rare. Onset is approximately 5 days post-vaccination and is more common in children. Appropriate antibiotic therapy is required.
3. **Inadvertent Inoculation:** This can occur in the vaccinee (autoinoculation) as well as in close contacts (contact transmission). Accidental infection is the most common complication of vaccinia vaccination, accounting for approximately 50% of all complications associated with vaccination and revaccination. This usually results from autoinoculation of vaccinia virus transferred from the site of the vaccination. Sites typically involved include the face, eyelids, nose, mouth, genitalia, or rectum, but can also involve the arms, legs, and trunk. Contact transmission of vaccinia, with accompanying toxicities, may occur when a recently vaccinated individual has contact with a susceptible individual. In a 1968 ten-state survey, contact transmissions were reported to occur at a rate of 27 infections per million vaccinations. The age group in which contact transmission occurred most commonly was in children \leq 5 years. Eczema vaccinatum as a result of contact transmission may result in a more severe syndrome than that seen in vaccinees, perhaps because of multiple simultaneous inoculations. About 30% of eczema vaccinatum cases reported in the 1968 ten-state survey were a result of contact transmission. It is possible that the number of cases of contact transmission would be greater in today's population, due to a largely unvaccinated patient population against smallpox. Contact transmission rarely results in postvaccinal encephalitis or progressive vaccinia. Most cases of inadvertent inoculation usually resolve without specific therapy and resolution of lesions follow the same course as the vaccination site in immunocompetent individuals. VIG can be used for severe cases involving extensive lesions or if comorbid conditions exist. VIG is contraindicated in the presence of isolated keratitis due to the risk of increased corneal scarring. VIG use can be considered in cases of ocular implantation, with keratitis, if vision-threatening or if other life-threatening vaccinia-related complications exist that require VIG therapy.
4. **Generalized vaccinia:** Generalized vaccinia is characterized by a disseminated maculopapular or vesicular rash of varying extent on any part of the body and typically develops 6-9 days after vaccination. The lesions follow the same course as the vaccination site lesion. The lesions are hematogenously spread and may contain vaccinia virus. In immunocompetent individuals, the rash is generally self-limiting and requires supportive care therapy. VIG treatment can be used in severe cases for patients who are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses. The differential diagnosis of GV includes eczema vaccinatum, erythema multiforme, inadvertent inoculation at multiple sites, and

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

5. **Eczema vaccinatum:** Eczema vaccinatum is a serious complication in persons with eczema and other types of chronic or exfoliative skin conditions. It can also occur among eczematous contacts of recently vaccinated persons. Vaccinia lesions (generalized papular, vesicular or pustular lesions) develop on areas of the skin that are, or had at one time been, eczematous. These areas become highly inflamed and lesions may spread to healthy skin. The rash is often accompanied by fever and individuals are systemically ill. The fatality rate for untreated cases (prior to availability of VIG) has been reported from 30-40%. Following availability of VIG, mortality was reduced to approximately 7%. Early diagnosis and prompt treatment with VIG is necessary to reduce mortality.
6. **Progressive vaccinia:** Progressive vaccinia is the most serious cutaneous complication, occurring when the local vaccination lesion fails to heal and develops progressive necrosis, with destruction of large areas of skin, subcutaneous tissue, and underlying structures. Progressive lesions may spread to other skin surfaces and to bone and viscera. Progressive vaccinia is associated with a high mortality rate. This complication has been seen in patients with a compromised immune system due to a congenital deficiency, lymphoproliferative disease, immunosuppressive treatment, or HIV infection. Management should include aggressive VIG therapy.
7. **Post-Vaccinial Encephalitis/Encephalomyelitis:** Vaccinia complications affecting the CNS are unpredictable. Post-vaccinial encephalitis typically affects children < 2 years of age and is characterized by an onset of symptoms 6-10 days following vaccination, which include seizures, hemiplegia, aphasia, and transient amnesia. Histopathological changes include generalized cerebral edema, mild lymphocytic meningeal infiltration, ganglion degenerative changes and perivascular hemorrhages. Older children and adults can

develop encephalitis or encephalomyelitis characterized by an onset of symptoms 11-15 days following vaccination, which include fever, vomiting, headache, malaise, and anorexia, progressing to loss of consciousness, amnesia, confusion, disorientation, restlessness, delirium, drowsiness, seizures and coma. Histopathological changes include demyelination with lymphocytic infiltration, but limited cerebral edema. Mortality rates have ranged from 15-25%, with 25% of patients who recover being left with varying degrees and types of neurological deficits. VIG has not been shown to be effective in treating CNS disease and is not recommended. Post-vaccinal encephalitis/encephalomyelitis are diagnoses of exclusion and are not believed to be a result of replicating vaccinia virus. Although no specific therapy exists, supportive care, anticonvulsants, and intensive care might be required. A review of vaccinia-related deaths (68) during a 9-year period (1959–1966 and 1968) revealed that among first-time vaccines, 36 (52%) patients died as a result of post-vaccinal encephalitis.

8. **Fetal Vaccinia:** Fetal vaccinia is a rare, but serious complication following vaccinia vaccination during pregnancy or shortly before conception (e.g., within four weeks). To date, fewer than 50 cases have been reported and often result in fetal or neonatal death. Efficacy of VIG therapy in a viable infant or used prophylactically in women during pregnancy is unknown. The CDC has established a National Smallpox Vaccine in Pregnancy Registry. This registry will follow women during their pregnancies and their babies, after they are born, to determine the outcome of such pregnancies. The CDC can be contacted at (404) 639-8253.
9. **Myocarditis/Pericarditis:** The CDC has recommended a temporary medical deferral to the voluntary Smallpox Vaccination Program for persons with heart disease or cardiovascular risk factors (March 25, 2003) and issued “interim supplementary information” regarding evidence that smallpox vaccination may cause myocarditis and/or pericarditis (March 31, 2003) in people recently vaccinated with the smallpox vaccine. The cardiac events reported include myocardial infarction, angina, myocarditis, pericarditis, and myopericarditis. Although the CDC believes that there is no evidence to conclude that the licensed vaccinia vaccine causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends that individuals with underlying heart disease be excluded from participation in the current Smallpox Vaccination Program. While it is currently not possible to fully evaluate the risk of cardiac events or the risk of myocarditis, pericarditis, or myopericarditis associated with vaccinia vaccination, it is reasonable to inform patients participating in studies using recombinant vaccinia virus of these reports and provide relevant guidance for evaluating these events. Further investigation from the ongoing vaccine program may provide additional data regarding an association or lack of association with cardiovascular disease. Patients are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice. At this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare

event. If not otherwise excluded, patients with known CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility.

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

Treatment of Vaccinia Vaccination Complications

Vaccinia Immune Globulin (VIG): First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in patients that are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG. VIG is contraindicated, however, for the treatment of isolated vaccinia keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is only available through the CDC's Strategic National Pharmaceutical Stockpile by contacting the CDC's Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100. Upon receipt of a call from a patient or upon direct observation of a patient or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible: 1) to initiate review of the clinical case, 2) to seek consultation on the appropriateness of VIG therapy, 3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and 4) to determine how to access and have the appropriate doses of VIG delivered. Early institution of VIG therapy is advised following recognition of clinical symptoms compatible with some vaccinia complications (eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of inadvertent inoculation). The effectiveness of VIG therapy appears to be time dependent. VIG has not proven to be of benefit in the treatment of post-vaccinia encephalitis, and is contraindicated for treatment of isolated vaccinia keratitis due to the increased risk of corneal scarring. A new IV formulation of VIG that has a lower level of aggregated protein allowing it to be used by either the IM or IV route is available through the CDC. This formulation will most likely be preferred for administration and the CDC will instruct investigators regarding appropriate dosing and

method of administration based on the formulation and availability. There is no guarantee that VIG will successfully treat complications. At present, there are no other anti-viral therapies of proven benefit for the treatment of vaccinia-related complications.

Cidofovir (Vistide®, Gilead Sciences): Cidofovir is an FDA-approved antiviral drug for the treatment of CMV retinitis among patients with AIDS. Cell-based *in vitro* studies and animal model studies have demonstrated this agent's antiviral activity against certain orthopoxviruses. Currently, its efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be used when VIG therapy is not effective [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated February 21, 2003. Available at: <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5204a1.htm>]. The CDC has informed the NCI/CTEP that cidofovir will not be supplied through Strategic National Pharmaceutical Stockpile to investigators involved in CTEP-sponsored protocols utilizing recombinant vaccinia-based vaccines. Thus, investigators should obtain cidofovir for second-line therapy through commercial sources if necessary. Investigators should consult the CDC Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100 regarding appropriateness of therapy and guidance.

9.1.2 PANVAC™-F (NSC 727027)

Other Names: Recombinant-Fowlpox-CEA(D609)/MUC-1(L93)/TRICOM™

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

Product Description: PANVAC-F is a recombinant fowlpox virus vector vaccine containing genes for human CEA, MUC-1 and three co-stimulatory molecules (designated TRICOM™): B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The CEA gene coding sequence is modified to code for a single amino acid substitution (aspartic acid, instead of asparagine at amino acid position 609) in one 9-mer, HLA-A2-restricted, immunodominant epitope designed to enhance immunogenicity. The MUC-1 gene coding sequence is also modified to code for a single amino substitution (leucine, instead of threonine at amino acid position 93) in one 10-mer, HLA-A2-restricted, immunodominant epitope designed to enhance immunogenicity. An attenuated, live, plaque-purified isolate from the POXVAC-TC strain of fowlpox virus was used as the parental virus for this recombinant vaccine. A plasmid vector containing the modified CEA and MUC-1 genes and a plasmid vector containing the genes for the three co-stimulatory molecules were used to transfect primary chicken embryo dermal (CED) cells infected with the parental virus to generate the recombinant fowlpox virus. The final PANVAC-F recombinant vaccine is manufactured by infection of primary CED cells with the recombinant fowlpox virus. Fowlpox virus can infect mammalian cells and express the inserted transgenes to stimulate both humoral and cellular immunity, but cannot replicate in non-avian species, making systemic infections unlikely.

How Supplied:

Lot: 3-052203: PANVAC-F is supplied by the Pharmaceutical Management Branch, CTEP, NCI in vials containing 0.3 mL of the vaccine at a final viral concentration titer of 5.8×10^9 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol (total vial contents = 1.74×10^9 pfu's).

All other lots: PANVAC-F is supplied by the Pharmaceutical Management Branch, CTEP, NCI in vials containing 0.75 mL of the vaccine at a final viral concentration titer of 2×10^9 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol (total vial contents = 1.5×10^9 pfu's).

Note: *The PANVAC-F concentration may vary between lots, requiring changes to dose preparation instructions. Use extreme caution when preparing each dose.*

Preparation: Thaw vials completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds prior to dose preparation. Perform all dilutions of the vaccine with 0.9% sodium chloride for injection, USP and vortex all dilutions vigorously again for at least ten seconds prior to withdrawing the final dose. **Note the concentration of the current supply of PANVAC-F on your institutional preparation guidelines to avoid potentially serious dosing errors.**

PANVAC-F (fowlpox), Lot 3-052203 (5.8×10^9 pfu/mL, 0.3mL vial):

Thaw one vial completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least 10 seconds. Withdraw 0.18mL (1×10^9 pfu) from the thawed vial for subcutaneous injection.

PANVAC-F (fowlpox) All other lots:

Thaw one vial completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least 10 seconds. Withdraw 0.5mL (1×10^9 pfu) from the thawed vial for subcutaneous injection.

Storage: Store intact vials of PANVAC-F at -70°C or colder.

Stability: Shelf-life stability studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at $2\text{-}8^{\circ}\text{C}$. Do not re-freeze thawed vials. Vials of PANVAC-F are for single-use only and do not contain a preservative. Administer prepared doses as soon as possible following preparation (*i.e.*, within one hour). If necessary, store prepared doses at $2\text{-}8^{\circ}\text{C}$ for up to 4 hours following preparation.

Route of Administration: PANVAC-F is administered by subcutaneous injection. Other routes of administration have been studied. Refer to protocol for specific information.

Special Handling

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

Preparation, Handling and Disposal Recommendations

1. Strictly adhere to standard microbiological practices and techniques.
2. Limit/restrict access to preparation areas while dose preparation is in progress.
3. Use appropriate infection control measures (e.g., thorough hand washing) after handling any materials.
4. Institute and follow policies for safe handling of sharps.
5. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [e.g., minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eye wear, hair cover].
6. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
7. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
8. Place an empty biohazard sharps container lined with a leak-proof biohazard bag in or near the biosafety cabinet to dispose of all waste generated.
9. Transport the agent from the -70°C freezer to the work area in leak proof bag.
10. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.

11. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
12. Place all waste into the sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
13. Incinerate waste according to institutional policy and according to local, state, and federal regulations.
14. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
 - Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
 - Use protective apparel, eyewear, mask, and gloves.
 - Cover spills with disposable absorbent towels.
 - Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
 - Dispose of all waste as biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
15. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

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

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

Patient Care Implications

Cover vaccination sites with a sterile dry dressing (e.g., Telfa pad). Once the injection site is healed, no further barrier is necessary. As a precaution, protect injection sites that are exhibiting evidence of weeping, oozing or ulceration with a sterile dry dressing. In these circumstances, instruct patients to avoid direct contact of the injection site with susceptible individuals (e.g.; those who may be immunocompromised by disease or therapy). Instruct patients to avoid becoming pregnant, breast feeding or fathering a child for at least 4 months following therapy

completion with the recombinant vaccine. Instruct patients receiving fowlpox vaccines to avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site.

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

9.1.3 Agent Procurement

9.1.3.1 Agent Ordering and Agent Accountability

NCI-supplied PANVAC™-V (NSC 727026) and PANVAC™-F (NSC 727027) may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form, and Financial Disclosure Form. If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (<https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx>). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (<https://eapps-ctep.nci.nih.gov/iam/>) and the maintenance of an “active” account status and a “current” password. For questions about drug orders, transfers, returns, or accountability, call 240-276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

9.1.3.2 Agent Inventory Records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator’s Handbook for Procedures for Drug Accountability and Storage.)

9.2 COMMERCIAL AGENT

9.2.1 TICE Bacillus Calmette-Guerin (BCG)

(Please see package insert for complete drug information)

9.2.1.1 Indications and Usage

TICE® BCG is indicated for the treatment and prophylaxis of carcinoma *in situ* (CIS) of the urinary bladder, and for the prophylaxis of primary or recurrent stage Ta and/or T1 papillary

tumors following transurethral resection (TUR). TICE® BCG is not recommended for stage TaG1 papillary tumors, unless they are judged to be at high risk of tumor recurrence.

TICE® BCG is not indicated for papillary tumors of stages higher than T1.

9.2.1.2 Dosage and Administration

The dose for the intravesical treatment of carcinoma *in situ* and for the prophylaxis of recurrent papillary tumors consists of one vial of TICE® BCG suspended in 50 ml preservative-free saline.

Do not inject subcutaneously or intravenously.

9.2.1.3 Contraindications

TICE® BCG should not be used in immunosuppressed patients or persons with congenital or acquired immune deficiencies, whether due to concurrent disease (e.g., AIDS, leukemia, lymphoma) cancer therapy (e.g., cytotoxic drugs, radiation) or immunosuppressive therapy (e.g., corticosteroids).

Treatment should be postponed until resolution of a concurrent febrile illness, urinary tract infection, or gross hematuria. Seven to 14 days should elapse before BCG is administered following biopsy, TUR, or traumatic catheterization.

TICE® BCG should not be administered to persons with active tuberculosis. Active tuberculosis should be ruled out in individuals who are PPD positive before starting treatment with TICE® BCG.

10 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

10.1 LABORATORY CORRELATIVE STUDIES

Please refer to Appendix E (Section 17.5) for more information.

10.1.1 Tuberculin Skin Testing

PPD results will be used to assess the immunologic response to BCG therapy. This premise is based on a previous report that correlates PPD positivity with improved tumor recurrence-free intervals after BCG treatment (Lufotenegger et al, J Urol, Feb 1996, Vol 155, 1483-7).

10.1.2 Urine

For the BCG alone arm, urine may be collected at weeks 3, 5, and at the end of the study.

For the BCG + PANVAC vaccine arm, urine may be collected at weeks 0, 3, 5, and at the end of the study.

Collection volume will range from 30 to 100 mL with each collection. An attempt will be made to collect urine prior to BCG instillation and 2 hours after BCG drainage, based on previous

study demonstrating urinary cytokine changes during these time points (Bisiaux et al, J Urol, Vol 181, April 2009, 1571-80).

The aliquot will be stored in the Linehan's laboratory at -80°C for future proteomic and/or biomarker discovery studies depending on availability. Studies have shown minimal degradation of urinary peptides upon thawing and analysis of specimens ³⁶. Urinary levels of certain cytokines can be prognostic for response to bladder immunotherapy. For example, large amounts of TRAIL are noted in the urine of patients who have responded to BCG therapy ³⁷. Therefore, we will potentially check urinary levels of the following cytokines and chemokines by ELISA: TRAIL, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, IFN- γ , and TNF- α at week 0, week 3, week 5 (2-8 hours after last BCG dose has best yield) ³⁸ and at the end of the study.

10.1.3 Blood

For the BCG alone arm, blood samples for research analysis will be obtained

- at week 0 or at randomization by 1 10cc purple top tube
- prior to treatment at weeks 3 and 8 by 2 10cc red top tubes and 6 10cc green top tubes
- at the end of study (between weeks 17-20) by 1 10cc purple top tube, 2 10cc red top tubes and 6 10cc green top tubes.

For the BCG + PANVAC vaccine arm, blood samples for research analysis will be obtained

- at week 0 by 1 10cc purple top tube
- prior to treatment at weeks 0, 3, 8, 11, and 15 by 2 10cc red top tubes and 6 10cc green top tubes
- at the end of study (between weeks 17-20) by 1 10cc purple top tube, 2 10cc red top tubes and 6 10cc green top tubes.

Blood may also be collected on follow-up visits for up to 12 months following end of study cystoscopy. No more than 10.5 mL/kg or 550 mL, whichever is smaller, will be obtained from adult subjects over an 8-week period. The studies and method of storage for these specimens are described in Sections **10.1.5** and **10.2**.

10.1.4 Tissue (if clinically available)

For subjects at the NIH: If tissue is clinically available, the Urologic Oncology Branch procedures for tissue collection will be followed. A procurement form will be completed. The tissue procurement nurse or a UOB-designated procurement staff member will work with an NCI pathologist following resection of the specimen to obtain tumor tissue from the resected specimen. This will then be delivered by the procurement nurse or UOB Procurement staff member to the Urologic Oncology Branch Research Laboratory for individual processing. Specimens will be placed in cryovials and also be snap frozen in OCT for frozen tissue sections. Additional tissue will be placed into antibiotic-enriched culture media in order to establish an urothelial cancer cell line. Patients will have the opportunity to opt out of cell line creation from

their tissues. Tissue that is used solely for research purposes will be obtained only if it can be obtained with minimal risk of complications from the procedure and only after the procedure has been explained to the patient and informed consent obtained. In that event, the patient will be invited to participate in a tissue procurement protocol such as 97-C-0147.

cDNA arrays may be performed on tumor tissues to compare gene expression profiles in tumor cells before and after treatment with BCG or BCG and PANVAC. Immunohistochemical and or cytogenetic analyses may be performed, depending on the tumor of origin. Cell lines may be characterized by karyotyping, interphase fluorescence in situ hybridization (FISH) mapping, immunohistochemistry, and/or flow cytometry. Differences between cell lines will be manipulated to study the various molecular pathways active in nutrient sensing, cell cycle, cell signaling, proliferation, apoptosis, angiogenesis, and metastatic potential in urothelial cancer. Tumor-produced factors may be evaluated in serum or urine or a search for new tumor markers may be performed. We may evaluate proteomic profiles of tissues or serum or urine and correlate these with disease and stage. This may potentially allow for the detection of non-invasive predictive and/or prognostic biomarkers from urine specimens.

Pathologic material, such as fixed tissue blocks from surgery performed at the NIH Clinical Center, outside surgeries or autopsy materials, may be obtained at the request of, and with written permission from the subject, or appropriate relatives. These samples may undergo pathologic analysis by individuals in the NCI Laboratory of Pathology to confirm diagnosis and may undergo histo-immunologic or other analysis. In particular to the current study, we would like to study the T-cell infiltration pattern within bladder tumors pre- and post-treatment. Specifically, we will look at:

- The expression of CEA and MUC-1 antigens in bladder tumor specimens pre- and post-treatment in both arms
- The presence of CD4 and CD8 T cells in bladder tumor specimens pre- and post-treatment in both arms
- The presence of regulatory T cells (Tregs) by double staining with FoxP3 and CD4 in bladder tumor specimens pre- and post-treatment in both arms
- The presence of myeloid derived suppressor cells (MDSC) in bladder tumor specimens pre- and post-treatment in both arms

10.1.5 Collection of immunologic blood samples

For the BCG alone arm:

- at week 0 or at randomization by 1 10cc purple top tube
- prior to treatment at weeks 3 and 8 by 2 10cc red top tubes and 6 10cc green top tubes
- at the end of study (between weeks 17-20) by 1 10cc purple top tube, 2 10cc red top tubes and 6 10cc green top tubes.

For the BCG + PANVAC vaccine arm:

- at week 0 by 1 10cc purple top tube

- prior to treatment at weeks 0, 3, 8, 11, and 15 by 2 10cc red top tubes and 6 10cc green top tubes
- at the end of study (between weeks 17-20) by 1 10cc purple top tube, 2 10cc red top tubes and 6 10cc green top tubes.

Additionally, blood may be collected on follow-up visits for up to 12 months following end of study cystoscopy/ bladder biopsy. From this blood, PBMCs and sera will be isolated, when appropriate samples are available, for the following immune correlates:

Immunologic testing will include:

1. Circulating Tumor Cytokines (CTC) analysis
2. IFN-gamma ELISPOT assays for CD8 T-cell responses specific for CEA, MUC-1, and Brachyury (Limited to patients with HLA-A2 allele)
3. Measure CD4 antigen-specific response to CEA
4. Sera samples will be analyzed for the development of antibodies to CEA, MUC-1 and Brachyury. (Samples from baseline and at end of study)
5. Flow cytometry analysis using 10 markers of all PBMC samples for each of the following cell types: CD4, CD8, Tregs, MDSCs, and NK.
6. Serum for NCA (CEA-CAM) titers will be drawn anytime that a patient exhibits new onset neutropenia (ANC <1000)
7. Immunologic studies will be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined to not be treatment-related or until the participant's primary medical care is transferred from the principal investigator.

10.1.6 The immunologic testing samples for CTC analysis will be processed at:

Dr. William Linehan's Lab
10 Center Drive, 1W-5888
Bethesda, MD 20892

For patients at CINJ, research samples consisting of 2 purple top tubes (week 0 and week 17-20) will be drawn. These tubes will be further processed as per Appendix E, Section **17.5** and then shipped via Fed-Ex to Dr. Linehan's lab:

William Linehan, MD
c/o Julian Custer or Quentin Li
Urologic Oncology Branch
10 Center Drive, 1W-5888
Bethesda, MD 20892

10.1.7 The other immunologic testing samples will be processed at:

Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B

Frederick MD 21702
301-846-1000

On days samples are drawn, Jen Bangh at CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same day courier delivery of the specimens drawn at NIH.

For patients at CINJ, research samples consisting of 6 green top tubes and 2 red top tubes will be drawn at multiple time points (see Study Calendar, Section 11). The green top tubes will be sent via overnight Fed-Ex to the NCI Frederick Repository at the address below (see Appendix E, Section 17.5):

Leidos Biomedical Research
Attn: Bill Kopp/Theresa Burks
1050 Boyles Street
Bldg. 469/Room 121
Frederick, MD 21702
Phone 301-846-5125 or 301-846-1707

The red top tubes will be spun down and stored in a -80°C freezer at CINJ. These serum samples will be batched and then shipped to the NCI Frederick Repository (address above) at intervals.

Once a patient's treatment schedule has been determined, Caroline Jochems at the Laboratory of Tumor Immunology and Biology/NIH should be notified at jochemscm@mail.nih.gov for planning purposes.

10.1.7.1 Biopsies

Biopsies will be obtained at the end of therapy solely for diagnostic purposes as per standard of care to ensure that cancer is no longer present. After pathologic diagnosis, each biopsy specimen will be further analyzed by immunohistochemistry for the following: (a) expression of CEA and MUC-1 antigens on tumor cells, (b) the presence of CD4 and CD8 T cells, (c) the presence of regulatory T cells (Tregs) by double staining with FoxP3 and CD4, and (d) the presence of myeloid derived suppressor cells (MDSC).

Satisfactory analytical performance characteristics (specificity, sensitivity and reproducibility at the minimum) of individual antibodies or affinity ligands for use in immunohistochemistry will be demonstrated prior to analyses of glycosylated CEA and MUC-1 on tumor cells, CD4 and CD8 T cells, Tregs by double staining with FoxP3 and CD4, and MDSC.

10.1.7.2 PBMCs, sera, and urine

The following time points will be analyzed: baseline (prior to vaccination in vaccination arm), week 3 (prior to BCG for both arms), week 8 (prior to last BCG for both arms), and at end of study (between weeks 17-20).

PBMCs

All patients who have the HLA-A2 allele will be analyzed by ELISPOT for CD8 T-cell responses specific for CEA, MUC-1, and a cascade antigen Brachyury. All samples will be evaluated for CD4 responses specific for CEA protein. All immune assays will have appropriate antigen controls. All PBMC samples will be analyzed by flow cytometry using 10 markers for each of the following cell types: CD4, CD8, Tregs, MDSCs, and NK.

- Satisfactory analytical performance characteristics of individual antibodies or affinity ligands for use in ELISPOT, flow cytometry, or ELISA will be demonstrated prior to analyses of the proposed protein markers, cytokines and chemokines.
- Analyses of sera samples for quantitation of antibodies to CEA, MUC-1 and a cascade antigen Brachyury will be described in detail to show clinically recapitulated tumor-associated antigens, including glycosylated proteins, are used in measuring autoantibodies in sera.

Background: Our studies in the LTIB have demonstrated the ELISPOT assay for IFN- γ production to be quantitative and reproducible as a measure of human T-cell responses to vaccination (26). The continued use of one reproducible assay has been instrumental in our ability to evaluate and compare patients' immune responses using different vaccines and vaccine strategies in the same institution, and among different cancer centers. ELISPOT assays employing the CEA agonist peptide and the MUC-1 agonist peptide have already been developed.

Sera

Sera samples at all time points will be analyzed for the development of antibodies to CEA, MUC-1, and a cascade antigen Brachyury.

Urine

Urine samples may be collected at baseline and at the end of therapy (week 17-20) for the presence of cytokines and chemokines. In addition, an effort will be made to collect urine at the time of BCG instillation #1 and #3 to assess for cytokine production in response to BCG therapy (as described earlier in section **10.1.2** and in Section **17.5** (Appendix E)).

For patients at CINJ, research samples consisting of urine (week 0, week 3, week 5, and week 17-20) will be drawn. These tubes will be further processed as per Appendix E, Section **17.5** and then shipped via Fed-Ex to Dr. Linehan's lab:

William Linehan, MD
c/o Julian Custer or Quentin Li
Urologic Oncology Branch
10 Center Drive, 1W-5888
Bethesda, MD 20892

Bladder cancer is believed to be an immune-modulated malignancy and therefore the immune correlates will be used to establish whether or not there is an immune basis for bladder cancer. If tumors are found to have a certain proportion of immune subsets, then the correlates may be used as potential markers of disease in the future.

10.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

All specimens obtained for research purposes will be coded upon arrival in the UOB research laboratory to maintain patient confidentiality. All samples will be accessioned and entered into a database (such as LabMatrix) with access limited to the PI and UOB research data managers. The location of all samples will be carefully tracked in the database. All stored samples will be coded and identifying patient information will not be placed on sample containers. Stored samples will be kept in freezers/refrigerators or secure containers located in the Urologic Oncology Branch research laboratories or in approved storage facilities. The research samples collected will be used for the correlative/biomarker studies mentioned above in Section 10.1. Tissue slides/blocks may be obtained in order to determine protocol eligibility. Tissues are submitted to the NCI Laboratory of Pathology at the NIH Clinical Center for clinical determination of histologic features. Specialized stains/assays may be used to clarify tissue phenotype. After completion of analysis, tissue slides/blocks are either returned to the originating pathology department or archived in the Laboratory of Pathology or Urologic Oncology Branch and tracked in the UOB database (LabMatrix). Archived material will be retained for the duration of the study unless the originating pathology department requests return.

Coded/anonymous samples with minimal clinical information may be shared with qualified NIH and non-NIH researchers who have IRB-approved protocols with similar research objectives. No personal identifying information such as name, address, or date of birth will be provided to the non-UOB investigator. In the event a subject is eligible to participate in a clinical trial based on tumor characteristics, coded tissue samples may be sent to an outside laboratory. Samples will be ordered and tracked through CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical records. Samples will not be sent outside the NIH without IRB notification and an executed MTA.

Subjects will be given the option of consenting to current and future use of their research samples per the informed consent process with their option declared in the consent document. Samples will be destroyed at the completion of the study on those subjects who decline future use of their samples. Otherwise, any specimens that are remaining at the completion of the protocol will be stored for an indefinite amount of time. These specimens may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of any new questions were covered in the consent document and protocol. Any new uses of the samples other than those defined in this protocol will be submitted prospectively for IRB review and approval. The IRB will be notified in the event research samples are inadvertently lost or destroyed and meet the criteria in section 7.2.1.

10.2.1 Storage and Tracking of Collected Blood Samples

All data associated with the patient samples is protected by using a secure database. All samples drawn at the NIH Clinical Center will be stored in the laboratories of the Urologic Oncology Branch as previously described.

Serum samples will be stored in -80°C freezers and PBMC samples will be stored in vapor phase liquid nitrogen at the NCI Frederick Central Repository in Frederick, MD. Samples drawn at the NIH Clinical Center will be transported by the Leidos couriers; samples from CINJ will be sent via Fed-Ex, as previously described.

Samples will be tracked and managed by the Central Repository database. Leidos Biomedical Research, Inc. manages the NCI Frederick Central Repositories. NCI Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

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

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

10.2.2 Protocol Completion/Sample Destruction

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

Samples, and associated data, will be stored permanently unless the patient withdraws consent. Loss or unanticipated destruction of samples will be reported per section **7.2.1**.

11 STUDY CALENDARS

Evaluations below may be performed within \pm 5 days of indicated time in order to accommodate patient schedules, holidays and weather emergencies.

A) STUDY CALENDAR FOR BCG + PANVAC ARM

	Baseline (On study)	Week 0	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 11	Week 15	Week 17- 20	Follow- up ¹⁰
BCG			X	X	X	X	X	X				
PANVAC-V ¹		X										
PANVAC-F ¹			X				X		X	X		
NIH Advanced Directives Form ¹¹	X											
History and Physical ²	X		X	X	X	X	X	X	X	X	X	X
CBC with differential ³	X										X	X ^o
Chemistry ^{3,4}	X										X	X ^o

Abbreviated Title: Vaccine and BCG for bladder ca

Version Date: 05/22/2025

	Baseline (On study)	Week 0	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 11	Week 15	Week17- 20	Follow- up ¹⁰
PT, PTT ³	X										X	X ^o
PPD (Research)	X										X	
Urinalysis ⁵	X		X	X	X	X	X	X		X	X	
Urine Cytology ⁶	X										X	X
Urine (Research) ⁷		X	X*		X*						X	X ^o
Immunology (Research blood) ⁸		X	X					X	X	X	X	X ^o
Serum Beta- HCG ⁹	X											
Biopsy											X	X
Chest X-ray	X										X	
CT urogram	X										X	

B) STUDY CALENDAR FOR BCG ONLY ARM

	Baseline (On study)	Week 0	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 11	Week 15	Week17- 20	Follow- up ¹⁰
BCG			X	X	X	X	X	X				
NIH Advanced Directives Form ¹¹	X											
History and Physical ²	X		X	X	X	X	X	X		X	X	X
CBC with differential ³	X										X	X ^o
Chemistry ^{3,4}	X										X	X ^o
PT, PTT ³	X										X	X ^o
PPD (Research)	X										X	
Urinalysis ⁵	X		X	X	X	X	X	X			X	X

	Baseline (On study)	Week 0	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 11	Week 15	Week 17- 20	Follow- up ¹⁰
Urine Cytology ⁶	X										X	X
Urine (Research) ⁷			X*		X*						X	X ^o
Immunology (Research blood) ⁸		X	X					X			X	X ^o
Serum Beta- HCG ⁹	X											
Biopsy											X	X
Chest X-ray	X										X	
CT urogram	X										X	

¹ Applies only to treatment arm 2 (BCG + PANVACTM). Panvac administration continues as planned regardless of any delays in BCG administration.

*These time periods should correspond to BCG instillations #1 and #3, respectively. If a postponement of an instillation occurs, urine collection will occur after instillations #1 and #3 irrespective of the trial week. On these weeks, urine will be collected twice: first collection will be prior to BCG instillation and second collection of urine will be 2 hours after drainage of BCG.

² Baseline: H & P and laboratory studies should be completed within 28 days of initiating treatment. This will include determination of ECOG performance status.

³ Baseline laboratory studies should be completed within 28 days of initiating treatment.

⁴ Chemistry panel: Na+, K+, Cl-, CO₂, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, pre-albumin, and LDH.

⁵ Urinalysis: If prior to an operative procedure, then will be obtained within 2-21 days of surgery and with an accompanying urine culture. If prior to BCG administration, a simple urinalysis will be performed in the hospital on the day of administration. ***Urine will be tested weekly prior to each BCG administration and the presence of gross hematuria or a urinary tract infection by urinalysis will result in holding that week's BCG dose as specified in the protocol.

⁶ Urine cytology: will be obtained from voided urine within 2-21 days of operative procedures OR through instrumentation at the time of an operative procedure

⁷ Urine (research): Urine will be obtained for correlative immune studies checking for chemokine and cytokine levels

⁸ Blood will be obtained for immunologic/research assays. CTC analysis, plasma and sera will be isolated to look for CD4 antigen-specific responses, CD8 antigen-specific responses, and flow cytometry profiles of various immune cell types.

⁹ In females of child-bearing age, Beta-HCG to be done at baseline within 48 hours prior to receiving vaccinia

¹⁰ These patients will be followed every 3 months for 1 year after the completion of the initial 20 week study course with standard of care for non-muscle invasive high grade bladder cancer. This includes urinalysis, urine cytology, cystoscopy, possible bladder biopsy, and medical assessments. For patients unable to complete every 3 month follow-up at the NIH or CINJ after week 20, we will keep them on study and obtain their outside pathologic records and labs from their outside urologists who will perform these evaluations.

¹¹ As indicated in section **15.3**, all subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required. Participating sites should adhere to appropriate procedures specific to their site.

X^o = optional

12 MEASUREMENT OF EFFECT

12.1 RESPONSE CRITERIA

12.1.1.1 Evaluable for toxicity

All patients who receive at least one dose of protocol therapy will be evaluable for toxicity from the time of their first treatment with TICE BCG or rV-PANVAC™. All observed toxicities will be tabulated using CTCAE v4.1 and a previously published, vaccinia toxicity grading scale used with this vaccine³³ by the highest grade experienced for each patient and presented for each study arm.

12.1.1.2 Evaluable for objective response

Only those patients who have biopsy-proven disease present at baseline, have received at least one cycle of therapy (6 or fewer weekly intravesical doses of BCG), and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

In order to compare the secondary outcomes between study arms, all durations will be measured from the start of TICE BCG therapy (week 3). This is the start of therapy for the TICE BCG arm alone. BCG is not started at week 0 for two reasons: 1) to allow adequate healing from previous TURBT and 2) allow vaccinia priming vaccine to be given to combination group at week 0 with enough recovery time to possibly make the administration of BCG in week 3 safer in these patients. Furthermore, by following this schema, patients in each group will start BCG at the same time after study entry.

12.1.1.3 Recurrence-free survival

The recurrence-free survival duration will be measured from the start of TICE BCG therapy (week 3) until disease recurrence or death due to any cause in each arm. Recurrence is suspected and/or determined by urine cytology and/or cystoscopic exam and then confirmed pathologically after a TURBT. Positive cytology in the absence of pathologic confirmation is not considered to be a recurrence.

12.1.1.4 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first. The duration of progression-free survival is measured from the start of TICE BCG therapy (week 3) until progression or death due to any cause in each study arm. Progression is defined as upstaging from a lower stage to a higher stage (e.g., Ta to T1 or T1 to T2-4; or any N+ or M+ in these high grade tumors).

12.1.1.5 Time to Tumor Recurrence

The duration of time measured from the start of TICE BCG therapy (week 3) until recurrence is noted.

13 DATA COLLECTION AND REPORTING/REGULATORY REQUIREMENTS

13.1 DATA COLLECTION

An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.

All data will be stored by a patient code identification number only, without personal identifiers, and linked to samples by that identification number in Labmatrix. Clinical Data will be entered into the NCI C3D database. Data will be collected using protocol-specific case report forms, and verified for accuracy and completeness. Data should be entered into the data base in a timely manner. Hard copies of data will be stored in locked secured areas and data will be entered onto a secured electronic data base. The following protocol-specific study forms will be complete and stored: eligibility checklist (developed by Central Registration Office, CRO). A copy of all serious AE forms will be kept in the research record.

Data will be verified to ensure that:

- Eligible patients are confirmed and eligibility checklist was completed. Consent form must be signed prior to registration with Central Registration.
- Treatment is given according to protocol (dated notes about doses given, complications, and clinical outcomes).
- Toxicity is assessed according to protocol (laboratory report slips, etc.)
- Response is assessed according to protocol (X-ray, scan, lab reports, and date noted on clinical assessment, as appropriate).
- Drug Accountability Records are kept for each patient.

All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for 30 days after removal from study treatment or until off-study, whichever comes first.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.

- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

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

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

13.2 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting until March 31, 2018. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

13.2.1 Toxicity Grading for Vaccinia Toxicity ³³

Grade 1: Cutaneous reaction extending no more than 10 cm from the vaccination site (i.e., limited to the upper arm)

Grade 2: Any autoinoculation syndrome that resolves without sequelae; Generalized vaccinia extending more than 10 cm from the vaccination site

Grade 3: Any toxicity that is between grade 2 and 4

Grade 4: Autoinoculation syndrome (e.g. blindness); post vaccinia encephalitis; vaccinia gangrenosum; eczema gangrenosum; Stevens-Johnson syndrome

13.3 DATA REPORTING

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section [7](#).

13.3.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site

(<http://ctep.cancer.gov/reporting/cdus.html>).

Note: If your study has been assigned to CDUS-Complete reporting, all adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS

13.3.2 Responsibility for Data Submission

Participating study sites are responsible for submitting CDUS data and/or data forms to either the Coordinating Center or to the Lead Organization on the study quarterly. The date for submission to the Coordinating Center or to the Lead Organization will be set by them. CDUS does not accept data submissions from the participants on the study. When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see Section **13.3**).

Either the Coordinating Center or the Lead Organization is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

13.4 CTEP MULTICENTER GUIDELINES

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix B (Section **0**).

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.
- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

13.5 COLLABORATIVE AGREEMENTS LANGUAGE

The PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) (hereinafter referred to as Agents) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between BNIT (Bavarian Nordic ImmunoTherapeutics) (hereinafter referred to as “Collaborator(s)”) and the NCI. Therefore, the following obligations/guidelines, in addition to the provisions in the “Intellectual Property Option to Collaborator”

(http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: <http://ctep.cancer.gov>.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):
 - a) NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
 - b) Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.
 - c) Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

13.5.1 Material Transfer Agreement (MTA)

A Material Transfer Agreement (MTA) is in process in order to receive specimens from participating sites for storage and future analysis.

14 STATISTICAL CONSIDERATIONS

14.1 PRIMARY ENDPOINT, STRATIFICATION, AND SAMPLE SIZE JUSTIFICATION

The study is to test the hypothesis that patients in the TICE BCG+PANVAC arm have better recurrence-free survival than patients in the TICE BCG alone arm. Patients to be accrued to the study are adults with high grade non-muscle invasive bladder cancer who failed at least 1 induction course of BCG. These patients comprise 3 subgroups, namely, BCG persistent with one failure of BCG induction course, BCG persistent with 2 or more failures of BCG induction courses, and BCG relapsing that can behave like BCG naïve. The ratio of the composition of the three subgroups is expected to be 1:5:1 with the majority of the patients being BCG persistent and having failed 2 or more BCG induction courses. In order to achieve the balance of treatment in each subgroup, stratified randomization will be implemented in this study. However, although the three subpopulations have different recurrence-free survival experience and may respond to treatment benefit differently, the study is powered to test the overall treatment benefit of BCG+PANVAC on the entire patient population.

In the first subgroup of BCG persistent patients with one failure of BCG induction course, it is assumed that the 1-year recurrence-free survival rate is 37.5% ^{7,8} in the TICE BCG alone arm and 65% in the TICE BCG+PANVAC arm.^{38,39} Under the assumption of exponential distribution for the recurrence-free survival in both arms, the difference in recurrence-free at 1-year corresponds to hazard ratio (HR) of 2.277. In the second subgroup of BCG persistent patients with 2 or more failures of BCG induction courses, the 1-year recurrence-free survival rate in the TICE BCG alone arm is expected to be 20%.⁴⁰ Assume that the treatment benefit of TICE BCG+PANVAC in the second subgroup is lower than in the first subgroup and HR is set at 2, which corresponds to a one-year recurrence-free survival of 44.7% in the TICE BCG+PANVAC arm. In the third subgroup of BCG relapsing patients, it is expected that the 1-year recurrence-free survival rate is 48% and 65% in the TICE BCG alone and TICE BCG+PANVAC arm, respectively. This corresponds to HR=1.70.

Patient accrual is targeted at one patient per month during the first 6 months and 2 patients per month afterwards, and follow-up period after completing accrual will be 12 months. The power calculation was based on one-sided log-rank test at 15% significance level. Sample size, power and duration of the study are presented in the table below. With a total number of 49 patients, the power is 84% (see table below). Under this scenario, the expected number of events (recurrence or death) is 40. In order to allow for the possibility of a small number of unevaluable patients, the accrual ceiling will be set at 54 patients.

	Subgroup 1 1-year recurrence-free survival for BCG persistent patients who failed 1 BCG induction course	Subgroup 2 1-year recurrence-free survival for BCG persistent patients who failed 2 or more BCG induction courses	Subgroup 3 1-year recurrence-free survival for BCG naïve patients	Total sample size	Power (%)	Duration of accrual (months)	Expected number of events (recurrence or death)
Treatment benefit	HR=2.277 1-year RFS: BCG alone: 37.5% BCG+PANVAC: 65%	HR=2 1-year RFS: BCG alone: 20% BCG+PANVAC: 44.7%	HR=1.7 1-year RFS: BCG alone: 48% BCG+PANVAC: 65%	49	84	28	40

One interim analysis for futility will be performed according to the stopping procedure.³⁹ Specifically, the interim analysis will be performed when half of the expected events (20) have been observed. At that time, if the observed event rate of the TICE BCG+PANVAC arm is higher than that of the TICE BCG alone arm (i.e. the hazard ratio ≥ 1), we would consider terminating the trial and concluding that an advantage for the BCG+PANVAC regimen has not been established.

For the purpose of protecting patient safety, we implement the following stopping rule for safety monitoring of patients who receive the combination therapy.

If one patient experiences a grade 5 event, thought to be possibly or probably due to the combination therapy, the study will be stopped. For grade 3 or 4 event, the study will be stopped according to the following stopping rule. These adverse events are described in more detail in Section 7.

Number of patients	No. of grade 3 or 4 serious adverse events
3-9	3
10-18	4
19-26	5

The operating characteristic of the stopping rule was assessed by simulations. Based on 10,000 simulations, the probability of stopping early for an excess of serious adverse event over the course of trial is 0.04 when the serious adverse event rate is at the anticipated level of 6% and 0.94 when the serious adverse event rate is 30%. This is deemed reasonable for protecting patient safety.

14.2 SECONDARY ENDPOINTS

Using Kaplan-Meier methods, we will estimate the probability distribution of progression-free survival and the time to recurrence. Results will be summarized by point estimates (12 months) with 95% CI. Exploratory analyses of these secondary objectives will compare the two treatment arms using the log rank test. Fisher's exact test will be used to compare frequency distributions of toxicity grades between the two arms.

14.3 EXPLORATORY/CORRELATIVE ENDPOINTS

14.3.1 To characterize the expression of MUC1 and CEA in bladder tumor specimens pre-(week 0) and the change in expression post-vaccination (on tissue obtained between weeks 17-20) for patients treated with TICE BCG+PANVAC™.

14.3.1.1 MUC1 expression will be evaluated using IHC. A positive result is defined if >5% of tumor cells reacted with any intensity. Intensity will be arbitrarily scored on a 4 point scale: 0 (no staining), 1+ (weak staining), 2+ (moderate staining), and 3+ (strong staining). Potential antibody will be clone Ma695 dilution 1:100 (Novocastra, Newcastle upon Tyne, England) but has to be analytically validated using appropriate positive and negative controls prior to use.²²

14.3.1.2 CEA expression in FFPE pre- and post-treatment tissues will be evaluated by IHC. A positive result is defined when the cytoplasmic staining is equal in intensity to glycocalyceal staining. Tumors will be scored as 0 (no signal), 1+ (signal in <25% sampled tumor area), 2+ (26-75% of tumor cells), and 3+ (>75% of tumor cells). The potential antibody will be CD66e (clone 12-140-10; Novocastra, Newcastle upon-Tyne, England; dilution 1:500; Ventana NexES immunostainer; protease digestion).

14.3.1.3 **Statistical Analysis:** For both MUC1 and CEA expression the distributions of IHC staining intensity at baseline and post-vaccination as well as the change in score will be summarized with descriptive statistics. The results will be summarized by calculating the proportion and 95% confidence interval of positive staining tissue samples at baseline and the distribution of pre and post-vaccination combinations (--, -+, +-, ++) characterizing the change with treatment. Results will be presented separately for each treatment arm. The distributions for the two treatment arms will be compared using a chi square test.

14.3.2 To explore the CD4 Antigen-Specific response to MUC1 and CEA

14.3.2.1 The CD4 antigen-specific response will be assessed by culturing CD4+ T cells obtained from PBMCs pre- and post-treatment with irradiated APCs in the presence of MUC1 and CEA peptides/proteins. The IFN- γ levels will be measured by ELISA. Flu protein (positive control) and myoglobin (negative control) will serve as controls.

14.3.2.2 **Statistical Analysis:** Descriptive statistics will be calculated to summarize the CD4 antigen-specific response to MUC1 and CEA determined pre-treatment as well as the change post-vaccination. The same methods for analysis for each treatment arm and the comparison of arms as described for evaluation of the immune response to the vaccines will be applied.

14.3.3 To explore the CD8-mediated immune response to MUC1 and CEA

14.3.3.1 Assess CD8 T cell response by ELISPOT assay in pre- and post-treatment PBMCs using CEA and MUC1-specific T lymphocytes. We will be using an overlapping peptide library that will allow for the ability to test ~65% of HLA groups for MUC1 and ~80% of HLA groups for CEA

14.3.3.2 **Statistical Analysis:** A positive CD8 T cell response is defined as ≥ 2 -fold increase post treatment compared with the pretreatment value in IFN- γ -secretion. Descriptive statistics will be calculated to summarize the CD8 T cell response as continuous variables to MUC1 and CEA at both time points for each study arm. In addition, the change from pre- to post-treatment will be summarized by the proportion of patients achieving a positive response. The proportion with 95% confidence intervals will be presented for each treatment arm along with additional descriptive statistics (e.g. mean change, the mean percent change). Fisher's exact test will be performed to compare the positive CD8 T cell response between the two treatment arms.

14.4 REPORTING AND EXCLUSIONS

14.4.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with rV-PANVACTM and rF-PANVACTM.

14.4.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible.

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol deviations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

15 HUMAN SUBJECTS PROTECTIONS

15.1 RATIONALE FOR SUBJECT SELECTION

15.1.1 Selection Based on sex, Ethnicity, and Race

Subjects from all racial/ethnic groups and both sex are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism, immune response or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore sex and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

15.1.2 Justification for Exclusions

Due to impaired cellular immunity, HIV patients are at an increased risk of serious side effects from vaccinations with infectious agents and are excluded. This is based on recommendations from the CDC and FDA. In addition, pregnant women are also excluded due to potentially increased risks of serious side effects from vaccinations with infectious agents.

15.2 PARTICIPATION OF CHILDREN

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

15.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section 15.5), will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively

impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

Participating sites should adhere to appropriate procedures specific to their site.

15.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

BCG is a standard of care treatment and will be used in this study. The biggest risk of using this agent after failing an initial induction course of BCG is progression of disease to muscle-invasive disease. However, this occurs after multiple, failed BCG induction treatments and occurs more often after 2 years of such therapy⁴⁰. In addition, patients will be accurately staged for disease with imaging, TURBT, and in some cases, repeat TURBT. They will be followed quite rigorously throughout the study and thereafter with a planned biopsy between weeks 17-20 to ensure that no progression of disease has occurred during the treatment period. Patients who progress will promptly undergo radical cystectomy. Radical cystectomy and pelvic lymphadenectomy is also considered a standard of care treatment for high grade BCG refractory NMIBC. However, since enrollment into this study requires patients to refuse radical surgery or not be medically fit for surgery, we believe the risk of proceeding with another induction course of BCG is outweighed by its benefit.

Other risks of the use of BCG include local bladder symptoms such as blood in the urine, urinary frequency, urgency of urination, dysuria (pain), and bladder spasms which are often self-limiting and usually resolve within a week by withholding BCG and administering standard of care medications (e.g. anti-inflammatories, anti-spasmodics, hydration, etc.). Finally, a rare potential risk of BCG administration is development of “BCG-osis” which is a form of bacteremia/urosepsis or systemic BCG infection that occurs with BCG administration on very rare occasions. In this event, patients will be taken off the study and treated with anti-microbials as per recommendations by infectious disease consultants. The addition of PANVACTTM is unknown but given the relative safety of both agents and different routes of administration, we presume that the risk is minimal.

15.4.1 Alternative Approaches or Treatments

Patients will be consented verbally and in writing regarding the risks and benefits of this trial, the treatment requirements, and alternative approaches to entering on this trial.

15.4.2 Procedure for Protecting Against or Minimizing any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will have blood tests, examinations and CT scans of the chest/abdomen/pelvis as described in the monitoring schedule as described in Appendix C (Section 17.3). Patients will also be required to have a local physician to provide long-term care and to monitor for complications. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Warren Grant Magnuson Clinical Center, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and

treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

15.4.3 Provisions for Monitoring Data Collection to Ensure Safety of Subjects

As information is gathered from this trial, clinical results will be shared with patients. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a patient's willingness to participate further, will be explained. Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants or material identifying participants will not be released without permission, except as such release is required by law. Records at the Clinical Center are maintained according to current legal requirements, and are made available for review by Cancer Therapy Evaluation Program, the Food and Drug Administration, or other authorized users, as stated under the guidelines established by the Federal Privacy Act.

15.5 RISKS/BENEFITS ANALYSIS

The patients we will be enrolling have high grade NMIBC that has already failed the standard of care therapy, BCG induction. These patients are on the verge of developing muscle-invasive disease which requires potentially morbid surgery and change in quality of life. Therefore, they are very interested in strategies to preserve the bladder. Repeat induction BCG therapy is a standard of care and is being given to all patients in this study. The addition of PANVAC™ may potentially improve upon the poor RFS seen with BCG alone and hopefully this study will bear that out. Alternative treatments besides surgery include other intravesical agents that have not shown to be that effective. Whether the vaccine will have any clinical effect upon improving the outcome with BCG is unknown, therefore, benefit cannot be promised nor can the chance of benefit be accurately predicted. Patients' participation in this study is voluntary and refusal will not result in penalty or loss of benefit to which patient is otherwise entitled.

Participation may be discontinued at any time without penalty and the patient can ask questions.

15.6 CONSENT PROCESS AND DOCUMENTATION

The investigational nature and objectives of this trial, the procedures involved, and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be explained to the patient and a signed informed consent document obtained. All listed associate investigators are permitted to obtain informed consent.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

15.6.1 Telephone Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

15.6.2 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), (*If a study with an IND or IDE, also cite 21 CFR 50.27 (b) (2)*). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

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APPENDICES

17.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

17.2 APPENDIX B: CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating

sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
 - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
 - The Coordinating Center must be designated on the title page.
 - Central registration of patients is required. The procedures for registration must be stated in the protocol.
 - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
 - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
 - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

17.3 APPENDIX C: PANVAC [RECOMBINANT VACCINIA-CEA(D609)/MUC1(L93)/TRICOM] PATIENT INSTRUCTION SHEET

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

1. What vaccination site reactions can you expect?

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

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

2. How should you care for the vaccination site?

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

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

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

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

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

Wash your hands after each step.

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

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

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

4. What about contact with other people?

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

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

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

If you have any questions at any time, please call. A nurse or a physician is available 24 hours a day by telephone. To speak with your main doctor or with a clinic nurse, call the Urology Clinic between 8 AM and 4:30 PM Monday to Friday. To speak with the research nurses, call the research nurse office during the day; during nights, weekends, and sometimes during the day, when the research office is empty, you may leave a message for the research nurse on the answering machine. You can call the principal investigator any time during weekday hours. In an emergency on weekends, evenings, or holidays, you can always get in touch with the on-call urologic oncology doctor (fellow) (listed below). This doctor can be contacted by calling the NIH Clinical Care Center operator, who will page the on call doctor. The on call doctor will then call you back. If you have to go to an emergency room near your home, go to the hospital first, and then have the doctors there call the NIH for more information.

PHONE NUMBERS

Urology Outpatient Clinic (3 rd floor)	(301) 496-5484
Sonia Bellfield, RN	(240) 760-6118
Rebecca Dolan, CRNP	(301) 827-1007
Raju Chelluri	(240) 858-3700*

*after clinic hours the NCI Urology Oncology physician on call through NIH page operator (301) 496-1211

17.4 APPENDIX D: NIH PROBLEM REPORT FORM

NCI Protocol #:	Protocol Title:
	Report version: (select one) <input type="checkbox"/> Initial Report <input type="checkbox"/> Revised Report <input type="checkbox"/> Follow-up
Site Principal Investigator:	
Date of problem:	Location of problem: (e.g., patient's home, doctor's office)
Who identified the problem? (provide role (not name of person): nurse, investigator, monitor, etc...)	
Brief Description of Subject (if applicable) <i>(Do NOT include personal identifiers)</i>	Sex: <input type="checkbox"/> Male <input type="checkbox"/> Female Age: <input type="checkbox"/> Not applicable (more than subject is involved)
Diagnosis under study:	
Name the problem: (select all that apply)	
<input type="checkbox"/> Adverse drug reaction <input type="checkbox"/> Abnormal lab value <input type="checkbox"/> Death <input type="checkbox"/> Cardiac Arrest/ code <input type="checkbox"/> Anaphylaxis <input type="checkbox"/> Sepsis/Infection <input type="checkbox"/> Blood product reaction <input type="checkbox"/> Unanticipated surgery/procedure <input type="checkbox"/> Change in status (e.g. increased level of care required)	

- Allergy (non-medication)
- Fall
- Injury/Accident (not fall)
- Specimen collection issue
- Informed consent issue
- Ineligible for enrollment
- Breach of PII
- Tests/procedures not performed on schedule
- Other, brief 1-2 word description: _____

Detailed Description of the problem: (*Include any relevant treatment, outcomes or pertinent history*):

*Is this problem unexpected? (*see the definition of unexpected in the protocol*) YES NO Please explain:

*Is this problem related or possibly related to participation in the research? YES NO Please explain:

*Does the problem suggest the research places subjects or others at a greater risk of harm than was previously known or recognized? YES NO Please explain:

Is this problem? (*select all that apply*)

- An Unanticipated Problem* that is: Serious Not Serious
- A Protocol Deviation that is: Serious Not Serious
- Non-compliance

*Note if the 3 criteria starred above are answered, “YES”, then this event is also a UP.

Is the problem also (*select one*) AE Non-AE

Have similar problems occurred on this protocol at your site? YES NO

If “Yes”, how many? _____ Please describe:

Describe what steps you have already taken as a result of this problem:

In addition to the NCI IRB, this problem is also being reported to: (select all that apply)

Local IRB

Study Sponsor

Manufacturer : _____

Institutional Biosafety Committee

Data Safety Monitoring Board

Other: _____

None of the above, not applicable

INVESTIGATOR'S SIGNATURE:

DATE:

17.5 APPENDIX E: SPECIMEN COLLECTION

The following protocols are used in the Linehan Laboratory and efforts will be made at collaborating centers to collect bio-specimens in a similar fashion. When feasible and when an appropriate materials transfer agreement is in place specimens used for correlative endpoints can be transferred from collaborating centers to the NIH for storage and future analysis. Until then, specimens will be stored at the collaborating sites under the following conditions.

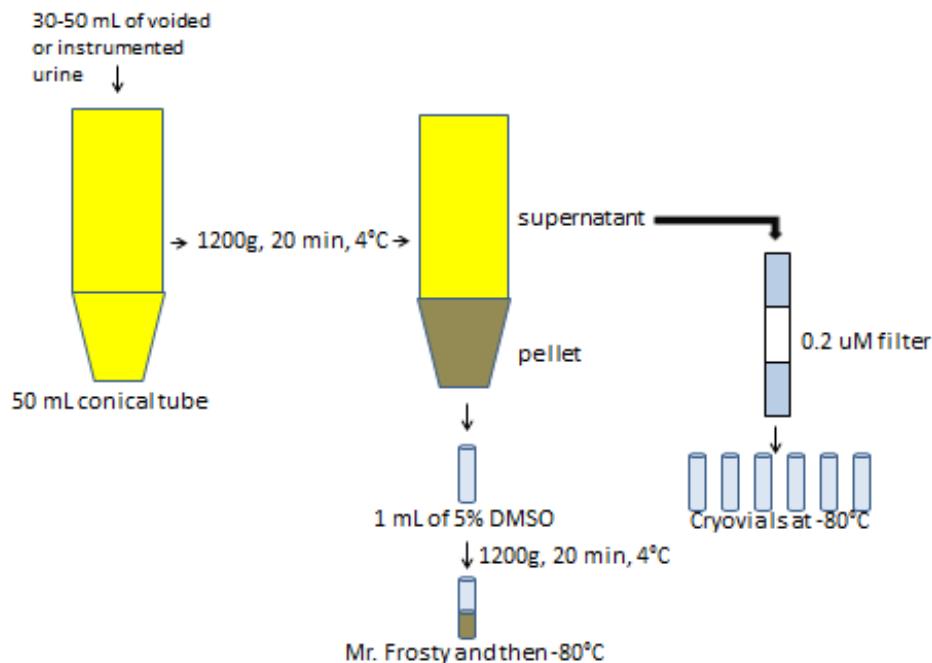
Urine Protocol

- A. Urine will be collected at room temperature from patients through voiding or instrumentation and transported to laboratory staff on ice. Collection will be 30-100 mL of urine in a sterile urine container.
- B. Time points for collection

BCG/PANVAC arm	BCG arm
<ul style="list-style-type: none">• Week 0 – collect before vaccine administration• Week 3 – Two samples:<ul style="list-style-type: none">○ Pre-BCG urine prior to BCG instillation○ Post-BCG urine after BCG is drained from bladder. This sample is the first voided urine obtained after the BCG instillation is completed and all of the BCG is first drained.	<ul style="list-style-type: none">• Week 3 – Two samples:<ul style="list-style-type: none">○ Pre-BCG urine prior to BCG instillation○ Post-BCG urine after BCG is drained from bladder. This sample is the first voided urine obtained after the BCG instillation is completed and all of the BCG is first drained.• Week 5 – Two samples:<ul style="list-style-type: none">○ Pre-BCG urine prior to BCG

<p>drained.</p> <ul style="list-style-type: none">• Week 5 – Two samples:<ul style="list-style-type: none">○ Pre-BCG urine prior to BCG instillation○ Post-BCG urine after BCG is drained from bladder. This sample is the first voided urine obtained after the BCG instillation is completed and all of the BCG is first drained.• Week 17-20 – collect urine prior to bladder biopsy.	<p>instillation</p> <ul style="list-style-type: none">○ Post-BCG urine after BCG is drained from bladder. This sample is the first voided urine obtained after the BCG instillation is completed and all of the BCG is first drained.• Week 17-20 – collect urine prior to bladder biopsy.
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- C. Centrifuge urine at 1200xg (2491 rpm for 20 minutes using our 5810R Eppendorf Centrifuge) at 4°C in 50 mL conical tube.
- D. Filter the supernatant (cell-free urine) with .45 µm filter to remove large debris. Save cell pellet.
- E. Store filtered urine as cell-free, filtered urine in 6 vials (1.8 mL cryovial each) at -80°C without any cryopreservative.
- F. Resuspend urine cell pellet in 1 mL of 5% DMSO (95% MEM media) and transfer to 1.8 mL cryovial tube.
- G. Spin this cryovial at 1200xg for 20 minutes at 4°C and leave cell pellet packed.
- H. Do slow freeze of cell pellet with Mr. Frosty and then store at -80.
- I. Schematic:



Urine will be processed at:

William Linehan, MD
c/o Julian Custer or Quentin Li
Urologic Oncology Branch
10 Center Drive, 1W-5888

Bethesda, MD 20892 For patients at CINJ, research samples consisting of urine (week 0, week 3, week 5 and week 17-20) will be obtained and processed as above and then shipped via Fed-Ex to the above address.

Blood Protocol

- Blood will be collected at various time points before any treatments and in various types of tubes and processed as below.
- Time points for collection

BCG/PANVAC arm	BCG arm
<ul style="list-style-type: none">Week 0 – 1 purple top tube (contains EDTA), total volume 10 mL; 6 green top	<ul style="list-style-type: none">Week 0 or at randomization – 1 purple top tube (contains EDTA), total volume 10 mL

<p>tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL</p> <ul style="list-style-type: none">• Week 3 - 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL• Week 8 - 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL• Week 11 - 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL• Week 15 - 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL• Week 17-20 - 1 purple top tube (contains EDTA), total volume 10 mL; 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL	<ul style="list-style-type: none">• Week 3 - 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL• Week 8 - 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL• Week 17-20 - 1 purple top tube (contains EDTA), total volume 10 mL; 6 green top tubes (contains sodium heparin), total volume 60 mL; 2 red top tubes, total volume 20 mL
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C. Red Top tubes and Green Top Tubes will be processed as follows:

Clinical Services Program (CSP)
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick MD 21702
301-846-1000

On days samples are drawn, Jen Bangh at CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same day courier delivery of the specimens drawn at NIH. For samples drawn at outside sites, please refer to the Research Sample Shipment section below.

Once a patient's treatment schedule has been determined, Caroline Jochems should be notified at jochemscm@mail.nih.gov for planning purposes.

D. Purple Top Tubes will be processed in Dr. Linehan's Lab as follows:

- a. Centrifuge blood at 4,000 rpm for 10-20 minutes using our 5810R Eppendorf Centrifuge at 4°C.
- b. Transfer plasma to 1.8 mL cryovial tubes. Store the tubes with plasma at -80C.

- c. Collect buffy coat layer (white blood cells) using individually packaged 3 mL transfer pipets.
- d. Extract DNA from buffy coat layer (white blood cells) with Promega MAXWELL 16 instrument using the Maxwell Blood DNA Purification Kit as follows:
 - i. Remove the seal from each cartridge.
 - ii. Place plunger in well #7 and sample (white blood cells) in well #1.
 - iii. Open the door. Press “Run/Stop” to extend platform.
 - iv. Transfer cartridges to platform. Add elution tubes and 400 μL elution buffer to each tube.
 - v. Close the door. Press “Run” to perform purification for 50 min.
 - vi. Run completed. Verify that plungers are clear.
 - vii. Transfer elution tubes to magnetic elution rack and transfer DNA samples to storage tubes.
 - viii. Label and store the tubes at -80C.
- e. For patients at CINJ, research samples consisting of 2 purple top tubes (week 0 and week 17-20) will be drawn, processed as above, and then shipped via Fed-Ex to Dr. Linehan’s lab.

Tissue Protocol

Tissue will be collected for diagnostic and therapeutic purposes at the beginning of the trial and in weeks 17-20 of the trial following BCG or BCG/PANVAC therapy. Additional tissue that is safely obtained and not needed for pathologic diagnosis can be placed in a sterile container and processed in the following manner:

- i. Remove samples to be frozen from the container with sterile forceps and gently dab on sterile gauze to remove excess fluid.
- ii. Once dry, place sample on a piece of tin foil above dry ice to freeze samples
- iii. Once frozen, move the samples to empty labeled vial.
- iv. If there are enough specimens in the sample to freeze in Tissue Tek O.C.T. Compound (OCT), then place one sample on plastic OCT plate instead of tin foil
- v. Add OCT medium to the OCT plate. Then place the plate on top of dry ice with the plastic side down (the OCT medium and embedded tissue should be facing you).
- vi. If tissue culture is going to be performed on sample, leave at least one piece of tissue in the original container (without freezing it) and deliver this sample to the tissue culture lab.
- vii. Once labeled and placed on dry ice, store samples in a -80 freezer.

Research Sample Shipment

For samples drawn at outside sites to be sent to the NCI Frederick Repository, please FED-EX Overnight to the shipping address below. PBMCs are to be sent on the day of draw; serum is to be frozen and batch-shipped at intervals.

USE NIH/NCI/LTIB FedEx Account Number: 281312103

1. Materials

Pack blood vials in the shipping kit provided to the site by the NCI research team.

Contents of the kit:

EXAKT-PAK for Vials Category B D-pak MD8702V06 (Accommodates 6 vials)

Includes Inner pack (Ambient) and Insulated Cooler

With 2 cool packs per cooler, part #CP1003, slightly cooled (refrigerated, not frozen)

2. Address

Leidos Biomedical Research

Attn: Bill Kopp/Theresa Burks

1050 Boyles Street

Bldg. 469/Room 121

Frederick, MD 21702

Phone 301-846-5125, or 301-846-1707

- Please notify the Frederick laboratory when specimens are being shipped. Please email Frederick prior to shipping to notify the lab.
- Emails should be sent to the following individuals:
Bill Kopp, koppw@mail.nih.gov
Theresa Burks, burkst@mail.nih.gov
Caroline Jochems, jochemscm@mail.nih.gov
William Linehan, wl3e@mail.nih.gov
James Gulley gulleyj@mail.nih.gov
- Additionally, **NO** specimens should be shipped on Fridays or the day before a Federal holiday.

3. Labeling of Blood Samples

Please label research tubes with a coding mechanism. List the patient's enrollment number first (will be provided by the Central Registration Office at time of registration), followed by the patient's initials, and lastly followed by the enrollment site.

Example: 01-ABC-CINJ

4. Supply for shipping kits

SHIPPING PAKS to be ordered by the NCI and shipped to participating cancer center.

Abbreviated Title: Vaccine and BCG for bladder ca
Version Date: 05/22/2025

Please contact the NCI team for requesting additional shipping kits.

EXAKT Technologies, Inc.
Home office: 7002 N. Broadway Extension
Oklahoma City, OK 73116-9006
405-848-5800

17.6 APPENDIX F: SAMPLE SHIPPING MANIFEST

Ship From: Address: Phone: Contact Name: Clinical Site:	Shipping Manifest	Ship To: Attn: Phone: E-mail:		
Shipping Date:		Carrier:		
Clinical Protocol: CTEP 9539				
In Package	Unique Patient ID*	Description	Number of items	Comments
Total number of items =				