

Amendment

Protocol Number:	04C0246-U	Reference Number:	370747
Principal Investigator:	James L Gulley, MD PhD	NCI	GMB 301.480.8870 gulleyj@mail.nih.gov
(NIH Employee Name, Institute/Branch, Telephone and e-mail)			
Protocol Title:	An Open Label Pilot Study to Evaluate the Safety and Tolerability of PANVAC(TM)-V (Vaccinia) and PANVAC (TM)-F (Fowlpox) in Combination with Sargramostim in Adults with Metastatic Carcinoma		

SIGNATURES

Principal Investigator (*):

James L Gulley, MD PhD - applied signature on 06/20/2017 5:10 PM EDT

Accountable Investigator:

PI is the Accountable Investigator

Branch Chief/CC Department Head (**):

James L Gulley, MD PhD - applied signature on 06/20/2017 5:10 PM EDT

Medical Advisory Investigator (if applicable):

N/A

Lead Associate Investigator signature:

N/A

Referral Contact signatures:

N/A

Associate Investigators signatures:

N/A

For Institute/Center Scientific Review Committee:

N/A

Other IC Clinical Director signatures:

N/A

APPROVALS

IRB Chair:

Michael Hamilton - applied signature on 06/29/2017 12:39 PM EDT

Clinical Director:

N/A

CONCURRENCE

OPS Protocol Specialist:

Kim Mitchell

07/10/17

Signature

Print Name

Date

* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: Expedited

DEC Clearance Date: N/A

Protocol Version Date: 05/04/2017

Abbreviated Title: Vaccine for metastatic cancer

NCI Protocol #: 04-C-0246

CTEP #: 6536

NCT #: NCT00088413

Version Date: 05/04/2017

Amendment: U

OSP #: 0403-636

IBC #: RD-04-VI-08

Title: An open label pilot study to evaluate the safety and tolerability of PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) in combination with sargramostim in adults with metastatic carcinoma

NCI Principal Investigator:

James Gulley, M.D., Ph.D.^{A, B, C, D, E, F}
Genitourinary Malignancies Branch
National Cancer Institute
10 Center Drive
Building 10, Room 13N240
Bethesda, MD 20892
Phone: (301) 480-7164
Email: gulleyj@mail.nih.gov

Statistician:

*Seth Steinberg, Ph.D.^{E, F}

NIH Associate Investigators:

William Dahut, M.D. GMB, CCR, NCI^{A, B, C, D, E, F}
Ravi Madan, M.D. GMB, CCR, NCI^{A, B, C, D, E, F}
Myrna Rauckhorst, R.N. OCD, CCR, NCI^{A, B, C, E, F}
Sheri McMahon, R.N. OCD, CCR, NCI^{A, B, C, E, F}

Responsible Research Nurses:

Myrna Rauckhorst, R.N. OCD, CCR, NCI^{A, B, C, E, F}
Sheri McMahon, R.N. OCD, CCR, NCI^{A, B, C, E, F}

Referral Contact/Study Coordinator:

Myrna Rauckhorst, R.N. OCD, CCR, NCI^{A, B, C, E, F}
10 Center Drive
Building 10, Room 13N210
Bethesda, MD 20892
Phone: (240) 760-6069
Email: mrauckhorst@mail.nih.gov

Non-NIH Associate Investigator:

Philip M. Arlen, M.D. GMB, CCR, NCI^{A, B, C, D, E, F}

* Indicates Associate Investigators who will not be making protocol related medical decisions.

Abbreviated Title: Vaccine for metastatic cancer

Version Date: 05/04/2017

CTEP #: 6536

Investigator Roles:

- A. Obtain information by intervening or interacting with living individuals for research purposes*
- B. Obtaining identifiable private information about living individuals*
- C. Obtaining the voluntary informed consent of individuals to be subjects*
- D. Makes decisions about subject eligibility*
- E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes*
- F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes*
- G. Some/all research activities performed outside NIH*

IND Information:

Drug Name: PANVAC™-V[Recombinant-Vaccinia-CEA(D609)/MUC-1(L93)/TRICOM]

NSC Number: 727026

IND Number: 11660

Sponsor: Cancer Therapy Evaluation Program (CTEP)
 National Cancer Institute (NCI)

Drug Name: PANVAC™-F [Recombinant-Fowlpox-CEA (D609)/MUC-1(L93)/TRICOM]

NSC Number: 727027

IND Number: 11660

Sponsor: Cancer Therapy Evaluation Program (CTEP)
 National Cancer Institute (NCI)

Drug Name: Leukine® (Sargramostim)

NSC Number:

IND Number:

Sponsor: Manufactured by Berlex Laboratories, Inc., Richmond, CA

PRÉCIS

Background:

- CEA and MUC-1 are overexpressed in multiple adenocarcinomas
- Pox viral vectors can induce a strong immune response to CEA and MUC-1
- The use of agonist epitopes within the TAA can induce a better immune response than native peptides and have been associated with clinical responses
- Heterologous prime and boost regimens are superior in terms of generalizing immune responses; and this may translate into improved clinical responses
- The use of GM-CSF does not add significant toxicity and in pre-clinical models is essential for induction for optimal immune responses
- It is possible by using vectors directed against TAA that there may be additive or synergistic immune responses and this may be important in overcoming antigenic escape variance
- Evidence of clinical benefit has been noted in some patients treated with this vaccine

Objectives:

- For the first two arms (colorectal cancer and non-colorectal cancer): To evaluate the safety and tolerability of the vaccine
- For the Ovarian Cancer and Breast Cancer arms: To evaluate clinical response to the vaccine

Eligibility:

- In the first two arms (colorectal and non-colorectal cancer), histologically confirmed adenocarcinoma that is CEA or MUC-1 positive described as metastatic disease (measurable or evaluable) or metastatic disease documented by biopsy but not evaluable by imaging (e.g. small volume peritoneal disease)
- For the ovarian and breast cancer arms, patients must have evaluable disease
- Normal organ function, ECOG 0-1

Design:

- This is a non-randomized four arm, pilot trial of 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 [PANVACTTM-V (vaccinia) and PANVACTTM-F (fowlpox)] in patients with metastatic carcinoma that express CEA or MUC-1 antigen
- The first arm will enroll 10 patients with metastatic colorectal adenocarcinoma
- The second arm will consist of 10-15 patients with any metastatic non-colorectal carcinoma that expresses either CEA or MUC-1
- The third arm will consist of about 12 patients with metastatic breast carcinoma. The fourth arm will consist of about 12 patients with metastatic ovarian carcinoma
- All patients will receive PANVACTTM-V (vaccinia) subcutaneously (s.c.) scheduled on day 1, followed by PANVACTTM-F (fowlpox) s.c. scheduled on days 15, 29, and 43 (Core Phase)

Abbreviated Title: Vaccine for metastatic cancer

Version Date: 05/04/2017

CTEP #: 6536

- Sargramostim (100 µg) will be given at the site of the vaccination on each vaccination day and for three consecutive days thereafter
- Up to 12 additional monthly boosting vaccinations (Extension Phase) will be offered to patients who have completed the Core Phase of the study and who have not experienced disease progression
- Following the 12 monthly vaccinations, patients without disease progression will be allowed to receive vaccine every 3 months
- Patients who have radiographic evidence of progressive disease, but who are otherwise clinically stable may revert back to monthly vaccinations

SCHEMA

Core Phase

Day 1	PANVAC™-V (vaccinia) (2×10^8) pfu s.c.
On or about days 15, 29, 43	PANVAC™-F (fowlpox) (1×10^9) pfu s.c.

The day of and for 3 consecutive sargramostim 100 μ g s.c. at vaccination site. days following vaccine (on or about days 1- 4, 15-18, 29-32, and 43-46)

Safety assessments will be scheduled on days 1, 15, 29, 43, and 71.

PANVAC™-V (vaccinia) is a recombinant vaccinia virus that contains 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 (designated TRICOM). PANVAC™-F (fowlpox) is a recombinant replication defective fowlpox virus that contains the identical transgenes as PANVAC™-V (vaccinia).

Optional Extension Phase (Up to 12 additional monthly boosting immunizations)

About every 28 days PANVAC™-F (fowlpox) (1×10^9) pfu s.c.

Days 1- 4 with monthly sargramostim 100 μ g s.c. at vaccination site.
PANVAC™-F (fowlpox) injection

Safety assessments will continue about every 28 days during the Extension phase.

Maintenance Phase

Every 3 months boosting immunization
subcutaneously PANVAC™-F (fowlpox) (1×10^9) pfu

Days 1- 4 with each sargramostim 100 μ g subcutaneously at
PANVAC™-F (fowlpox) injection vaccination site

Restaging scans may be performed at PI discretion at an interval not to exceed one year
Clinic visits for clinical assessment will take place prior to each vaccination at a 3-month interval

*This identical schema (including identical dose and dose schedule) has been approved by the FDA under a Therion Biologics Corporation IND.

TABLE OF CONTENTS

Summary of Protocol Changes	Error! Bookmark not defined.
PRÉCIS	3
SCHEMA	5
1 INTRODUCTION	8
1.1 Study Objectives	8
1.2 Background and Rationale	8
2 ELIGIBILITY ASSESSMENT AND ENROLLMENT	16
2.1 Eligibility Criteria	16
2.2 Research Eligibility Evaluation	19
2.3 Patient Registration and Treatment Randomization	19
3 STUDY IMPLEMENTATION	20
3.1 Study Design	20
3.2 Drug Administration	22
3.3 Treatment Modifications	22
3.4 On Study Evaluation: (See APPENDIX C)	24
3.5 Concurrent Therapies	27
3.6 Off Study Criteria	27
3.7 Post Study Evaluation (see APPENDIX C)	28
4 SUPPORTIVE CARE	28
4.1 Treatment of Vaccinia Vaccination Complications	28
5 DATA COLLECTION AND EVALUATION	30
5.1 Data Collection	30
5.2 Response Criteria	31
5.3 Toxicity Criteria	33
5.4 Statistical Considerations	34
5.5 Data Safety Monitoring Plan	36
6 HUMAN SUBJECTS PROTECTIONS	37
6.1 Rationale for Subject Selection	37
6.2 Participation of Children	38
6.3 Participation of NIH Subjects Unable to Give Consent	38
6.4 Evaluation of Benefits and Risks/Discomforts	38
6.5 Risks/Benefits Analysis	39
6.6 Consent and Assent Process and Documentation	39
7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN	40
7.1 Definitions	40
7.2 NCI-IRB and Clinical Director Reporting	42
8 DATA REPORTING	43
8.1 Provisions for Monitoring to Ensure Safety of Subjects	43
8.2 Routine Adverse Event Reporting	47
8.3 Secondary AML/MDS	47

8.4	Record Keeping.....	47
8.5	Regulatory Issues	47
9	PHARMACEUTICAL INFORMATION.....	52
9.1	PANVAC™-V (NSC 727026)	52
9.2	PANVAC™-F (NSC 727027).....	64
9.3	Sargramostim (GM-CSF, LEUKINE®)	69
10	REFERENCES	71
11	APPENDIX A: Performance Status Criteria	76
12	APPENDIX B: Instructions for Care of the Vaccine Site.....	77
13	APPENDIX C: Treatment and Monitoring Schedule: Pre, During and Post Therapy.....	80
14	APPENDIX D: Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference	82
15	APPENDIX E: ELISPOT ASSAY	88

1 INTRODUCTION

1.1 Study Objectives

1.1.1 Primary

- For the first two arms (colorectal cancer and non-colorectal cancer): To evaluate the safety and tolerability of PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) in combination with sargramostim in patients with metastatic carcinoma that express CEA or MUC-1 antigen
- For the Ovarian Cancer and Breast Cancer arms: To evaluate clinical response to the vaccine

1.1.2 Secondary

- For the first two arms: To document any objective anti-tumor responses that may occur
- For the Ovarian Cancer and Breast Cancer arms: To evaluate the safety and tolerability of PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) in combination with sargramostim
- To evaluate immune response generated by this combination therapy as measured by ELISPOT assay

1.2 Background and Rationale

1.2.1 Preclinical Support Data

Colorectal cancer is the third most common malignancy and the second most common cause of cancer death in this country. According to the American Cancer Society, approximately 150,000 new diagnoses of, and about 60,000 deaths from colorectal cancer will occur in the United States in 2003. Current therapeutic strategies have had limited success in the metastatic colorectal setting. A promising approach for the treatment of human malignancies is the development of vaccines that target specific tumors. This immunotherapeutic approach to the treatment of cancer is based on the observation that human tumor cells express a variety of tumor-associated antigens (TAAs) that are not expressed or are minimally expressed in normal tissues. These antigens, which include viral tumor antigens, cellular oncogene proteins, and tissue-specific differentiation antigens, can serve as targets for the host immune system and elicit responses that result in tumor destruction. Because tumors have developed a variety of mechanisms to evade immune detection and activation, the development of effective therapeutic vaccines for cancer will hinge on the ability to activate cellular immune responses. 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 identification and isolation of genes encoding TAAs has allowed the development of recombinant anti-tumor vaccines designed to elicit immune responses to one or more antigens

known to be expressed by a particular tumor type.

CEA

Carcinoembryonic antigen (CEA) is an 180,000-dalton glycoprotein that is overexpressed on most adenocarcinomas of the colon, rectum, stomach, and pancreas, as well as on breast cancers and non-small-cell lung cancers. In particular, CEA is overexpressed in > 90% of adenocarcinomas of the colon. It has also been identified in fetal gut and in small amounts in normal adult colonic mucosa. The CEA gene family belongs to the immunoglobulin superfamily and resides on the long arm of chromosome 19. CEA shares approximately 70% amino acid homology with non-specific cross-reacting antigen, which is found on normal granulocytes.(1) The clinical experience with CEA based vaccines is described below.

MUC-1

Mucin-1 (MUC-1) is a glycosylated transmembrane protein that is uniquely characterized by an extracellular domain that consists of a variable number of tandem repeats of 20 amino acids.(2) Differential expressions of MUC-1 and MUC-2 have been reported in colorectal adenomas (3-5) and colorectal cancers.(3;6;7) MUC-1 is overexpressed in > 90% of adenocarcinomas of the colon. Several earlier studies in colorectal neoplasia have demonstrated that higher levels of expression of MUC-1 in colorectal cancer were correlated with increased incidence of regional lymph node metastasis and liver metastasis.(8-10) A few studies in colorectal neoplasia (10;11) as well as in other human malignancies (10;12), have suggested that increased expression of the core peptide of MUC-1 is associated with poor prognosis. The clinical experience with MUC-1 based vaccines is described below.

Thus, a vaccine directed against both CEA and MUC-1 targets may well be additive or even synergistic in terms of the breadth of immune responses generated, and perhaps will be important in overcoming antigenic escape variants from vaccine therapy.

TRICOM

Costimulatory 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, “costimulatory,” 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 costimulatory molecules must be expressed in the same cell to properly engage the TCR and costimulatory receptor. In order to achieve this, multigene constructs using poxviral vectors (avipox and vaccinia) have been generated. These vectors contain the costimulatory molecule transgenes B7-1, ICAM-1, and LFA-3, and have been given the designation TRICOM (TRIad of COstimulatory Molecules), i.e., rV-TRICOM and rF-TRICOM.(13) Each of these Costimulatory molecules binds to a different ligand, and the second messenger pathways of each ligand are unique, raising the potential for synergy of these molecules.

1.2.2 Experimental Studies

Pox virus vectors

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile with over 1 billion people inoculated. This virus actively replicates in human cells, resulting in the presentation of high levels of antigen to the immune system over a period of one to two weeks, substantially increasing the potential for immune stimulation. The immune response specific to vaccinia then eliminates the virus. As a result of its safety profile and ability to elicit both humoral and cell-mediated immunity in humans, the vaccinia virus (genus *Orthopoxvirus*) was chosen as one of the vectors to deliver MUC-1, CEA, and TRICOM.(14;15)

Fowlpox virus, like vaccinia, is a member of the Poxviridae family (genus Avipoxvirus) that can infect mammalian cells and express inserted transgenes to stimulate both humoral and cellular immunity.(16;17) Avipox vectors such as fowlpox cannot replicate in non-avian species, making the possibility of human systemic infections extremely remote thus making it potentially safer than a replicative virus.(18) Fowlpox virus can be given multiple times and unlike vaccinia, immune responses to the tumor-associated antigen continue to improve with each subsequent vaccination. Results from NCI-sponsored Phase 1 studies of other fowlpox-based vaccines support the safety of this vector (see section [1.2.3](#)).

Immunization with these live recombinant pox virus vectors that have been genetically engineered to express one or more antigens allows expression of TAAs and subsequent co-presentation of antigenic peptides with host histocompatibility antigens, a strategy that favors the induction of cell-mediated immune responses. Recombinant pox viruses can infect antigen-presenting cells, including dendritic cells and macrophages, resulting in efficient expression of TAAs simultaneously with costimulatory molecules required for the elicitation of T cell responses. TAAs expressed by recombinant pox viruses are presented to the immune system together with highly immunogenic virus proteins, which may act as adjuvants to enhance immune responses to the TAAs. Thus, the use of recombinant pox virus vectors for the presentation of TAAs to the immune system results in the generation of cytotoxic T cells that can specifically destroy the selected tumor with little incremental toxicity.

Murine models

CEA transgenic mice have been developed in which the human CEA transgene, under the control of its endogenous promoter, is expressed in both fetal and adult tissues in a manner similar to that of humans (Eades-Perner Ca. Research 54: 4169-4179, 1994). Experimental studies have demonstrated that no anti-CEA immune responses can be generated in these mice when CEA protein in adjuvant is used as an immunogen.(19) However, when either recombinant vaccinia CEA (rV-CEA) vector or recombinant avipox CEA vector is used to vaccinate CEA transgenic mice, a vigorous CEA-specific T-cell response can be generated. (20)

Experimental studies have also demonstrated that the use of rV-MUC-1 as a vaccine can lead to

the elimination of established experimental lung metastases in mice.(21) Preclinical studies in double transgenic mice that develop pancreatic tumors and contain MUC-1 as a self molecule that is expressed on these tumors, have demonstrated that adoptively transferred MUC-1-specific CTL can eliminate pancreatic tumors.(22)

TRICOM

Preclinical studies performed in the Genitourinary Malignancies Branch (GMB) and Laboratory of Tumor Immunology and Biology (LTIB) using these TRICOM constructs have shown them to be superior to those constructs that do not contain the costimulatory molecules.(23-25) In CEA transgenic mice, which contain CEA as a self-antigen, much greater anti-tumor activity against established CEA-expressing tumors was seen when CEA/TRICOM vectors were used as opposed to CEA vectors devoid of TRICOM. *In-vitro* studies using TRICOM vectors containing human costimulatory molecules have shown them to greatly enhance the activation of antigen-specific human T cells including dendritic cells. (26) Recent studies have also shown that infection of antigen-presenting cells with TRICOM vectors leads to the generation of higher avidity T cells.(27)

Experimental studies have also shown that vaccination with rV-CEA/TRICOM and rF-CEA/TRICOM leads to the generation of even more vigorous T-cell responses to CEA and to improved anti-tumor immunity of established tumors when compared to the identical vaccine without the added costimulatory molecules.(28-30) Preclinical studies have also demonstrated that vaccination with CEA-TRICOM vectors can eliminate spontaneous tumor development.(31)

Agonist epitopes

Cancer immunity in humans may rest on the development of an effective immune response directed to "self" molecules that are common to tumor and normal cells. This of course has the inherent problem of breaking tolerance in order to generate and propagate tumor-associated specific T cells. In an attempt to circumvent this problem, novel peptides have been constructed to increase the immune response directed against "self" antigens. The advantage of using agonist epitopes has now been demonstrated in clinical trials in both melanoma and carcinoma patients.(32;33) Agonist epitopes have now been generated for both CEA and MUC-1. The PANVAC vectors contain the entire CEA and MUC-1 genes, respectively, with the modifications for the agonist epitopes.

Protein antigens are presented to cytotoxic T lymphocytes as small peptides (approximately 9-10 amino acids long) bound to class I molecules of the major histocompatibility (MHC) complex. One strategy to increase the immunogenicity of a self-antigen such as CEA is to modify selected epitopes within the protein sequence to enhance their binding to MHC class I alleles. One such epitope, designated CAP-1, which is specific for the MHC class I A2 allele, was modified by the introduction of a single amino acid change (asn to asp) at position 6 in the epitope (designated 6D).(34) The modified epitope, CAP-1(6D), was shown to be 100-1000 times more efficient than the native CAP-1 peptide in the induction of CAP-1-specific cytotoxic T lymphocytes (CTLs). In contrast to the native peptide, CAP-1(6D) was able to induce CD8+ CTLs from normal peripheral blood mononuclear cells that were able to recognize both the modified and native

peptides. In addition, these CTLs recognized and lysed tumor cell lines expressing native CEA. These studies indicate that CEA glycoprotein containing the modified peptide may be more efficient in and capable of eliciting and sustaining antitumor responses than unmodified glycoprotein.

As described above for CEA, a selected epitope within the MUC-1 protein sequence was modified to increase its binding to the MHC class I A2 allele in order to enhance the immunogenicity of the polypeptide. This epitope, designated P92, was modified by the introduction of a single amino acid change (thr to leu) at position 2 in the epitope. The modified epitope, designated P93L, was shown to be more efficient than the native P92 peptide in the stimulation of gamma-interferon production by MUC-1-specific T cell lines.(35) P93L was also able to induce CD8+ CTLs from peripheral blood mononuclear cells collected from pancreatic patients that could recognize and lyse tumor cell lines expressing native MUC-1. These studies indicate that MUC-1 glycoprotein containing the modified peptide may be more efficient in and capable of eliciting and sustaining antitumor responses than unmodified glycoprotein.

GM-CSF

GM-CSF has been shown to be an effective vaccine adjuvant because it enhances antigen processing and presentation by dendritic cells. Experimental and clinical studies suggest that recombinant GM-CSF can boost host immunity directed at a variety of immunogens.(36-38)

Using murine tumor models, several researchers have now shown that modification of tumor cells to enhance GM-CSF expression, using retroviral vectors (16) or vaccinia virus vectors (39;40), results in enhanced tumor-specific immune responses capable of effecting tumor destruction. Furthermore, this immune response is effective against not only the engineered, GM-CSF-expressing tumors, but also against unaltered tumor cells. We have shown that with vaccine strategy, GM-CSF is a vital component with a significant decrease in anti-tumor efficacy in murine models when GM-CSF is deleted from the regimen. We will use GM-CSF locally, at the vaccination site, to enhance immune responses elicited by the recombinant vaccines.

Diversified prime and boost regimens

Several preclinical studies and now clinical studies have demonstrated the advantage of a prime vaccination with recombinant vaccinia and boosting with a recombinant avipox as compared to the continued use of either vector alone.(41;42) These and other studies showed one could boost with a recombinant avipox vaccine without the induction of host-neutralizing immunity.(43-45)

In-vitro human studies

PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) have both been evaluated *in-vitro*. Infection of human dendritic cells with either vector was shown to result in faithful expression of CEA, MUC-1, B7-1, ICAM-1 and LFA-3, as measured by both Northern Blot analysis and FACS analysis. Human dendritic cells were also infected with PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) and tested for their ability to activate both CEA-specific and MUC-1-specific human T-cell lines. Results demonstrated that the PANVAC vectors were as efficient as CEA/TRICOM or MUC/TRICOM vectors in activating CEA- and MUC-1-specific human T

cells, respectively.

Preclinical studies performed in the Laboratory of Tumor Immunology and Biology, CCR, NCI were designed to look at the effect of the addition of trastuzumab to a breast cancer tumor cell line to determine its effects on the ability of specific T-cells to lyse this cell line. MCF-7 is a breast cancer cell line that is HLA-A2 +, HER-2 neu +, and positive for MUC-1 expression. We have developed a cytotoxic T-cell line, T1191, that is HLA-A2 + and MUC-1 specific. We performed a CTL (cytotoxic T-lymphocyte) killing assay using MCF-7 as target cells. T-1101 cells were used as CTL in this assay to determine the percent lysis of this breast cancer cell line with and without the addition of trastuzumab (at a concentration of 100 μ g/ml). A 16 hr ^{111}In release assay was performed for the CTL assay. The T-cell specific killing was increased by 1.5 fold (21% killing to 36.5 % killing) in those cells treated with the trastuzumab. This suggests that the use of trastuzumab may augment the ability of cytotoxic T-cells to kill tumor cells.

Preclinical toxicology studies

The above murine studies with CEA/TRICOM also demonstrated the absence of long-term toxicity, including autoimmunity, in mice that have eliminated established tumors.(46)

A non-human primate toxicology study has been carried out by Therion involving priming with rV-CEA(6D)/TRICOM admixed with rV-MUC-1 and boosting with rF-CEA(6D)/TRICOM admixed with rF-MUC-1/TRICOM. All vaccinations also included human GM-CSF. Complete necropsies were performed on all animals and gross lesions, bone marrow smears and kidneys were examined microscopically. No test article-related lesions in the kidneys or bone marrow were observed in this study. Hematology, serum chemistry, urinalysis, food consumption, and body weights were also assessed. Test article related lesions were observed only at the vaccine administration sites.

1.2.3 Clinical Studies

CEA Vaccines

The immunogenicity of CEA in humans has been demonstrated in several clinical trials. Foon and coworkers reported the development of humoral and T cell immunity to CEA as a result of immunization with a CEA anti-idiotype vaccine.(47) In addition, a number of clinical trials using recombinant vaccinia and/or avipox viruses expressing CEA have been conducted.(48-53) These trials demonstrated for the first time that CEA, when expressed by a recombinant pox virus, can elicit or enhance human immune responses capable of recognizing and destroying tumor cells that express CEA.

Pilot Study studies using rV-CEA (39) or avipox-CEA (40;54) vaccines demonstrated that both are capable of inducing T-cell responses specific for CEA in patients with metastatic carcinoma. These T cells were then shown to be capable of lysing human tumor cells expressing CEA. A subsequent clinical trial in collaboration with John Marshall at Georgetown University has indicated better immune responses and preliminary evidence of clinical benefit of priming with rV-CEA and giving multiple boosts with avipox-CEA vaccine compared with the reverse

sequence of vaccinations. (55) Patients were randomized to receive vaccinations priming with vaccinia-CEA (V) and boosting with 3 monthly avipox-CEA (A) vaccinations (designated VAAA regimen), or they received the 3 monthly avipox-CEA vaccinations followed by a fourth rV-CEA vaccine (designated AAAV regimen). In each group, patients were evaluated for immunologic responses using the ELISPOT assay.(56) Patients on the VAAA arm had statistically significantly increases in their numbers of CEA specific T cells. Patients with stable disease or with clinical responses to their regimen continued to receive multiple boosts with avipox-CEA. This group of patients, with metastatic colorectal, pancreatic, lung, and breast carcinomas, who had failed multiple prior therapies, would be expected to have a median survival of approximately 6–12 months. Although there were only 9 patients in each randomized arm, patients were followed for survival with the intent of having a hypothesis generating analysis of survival in the 2 arms. After 2+ years of follow-up, 6 out of 9 patients randomized to the VAAA regimen exhibited stable disease with some patients receiving up to 24 monthly vaccinations. All 9 of the patients randomized to the AAAV arm had progressed at the 2-year follow-up. The results of the comparison in survival of these two groups were statistically significant ($p=0.05$). Furthermore, there was a statistically significant correlation between CEA-specific immunologic responses and overall survival.(57)

In another study, the agonist peptide epitope to CEA (designated CAP1-6D) has been shown to have activity in patients with CEA-expressing tumors. Patients received 2 monthly vaccinations with dendritic cells loaded with the CEA agonist peptide. Two of 12 patients experienced complete responses (CR), one patient had a mixed response, and 2 had stable disease. Clinical response correlated with CEA-specific T-cell responses.(58)

A Phase I clinical study has recently been completed in collaboration with Georgetown University (John Marshall, PI) employing rV-CEA/TRICOM (V), avi-CEA/TRICOM (A), and both vaccines in a diversified prime and boost regimen. Fifty-nine patients with advanced CEA positive cancers have been accrued to 8 cohorts. Cohorts 1-3 received AAAA alone, 4–6 received VAAA, 7–8 received VAAA+ sargramostim, 8 with divided doses of vaccine. Vaccines were administered every month for the 6 doses and then every 3 months. Most patients had GI cancers and were heavily pre-treated. No significant toxicity was observed. Mild fevers, skin reactions at the vaccination site and regional adenopathy were observed. One pathologic complete response (pCR), 5 decreasing serum CEA, 25 stable disease (SD) (>4 months) were observed; 7 of these patients have been stable for >12 months and 18 for >4 months. Significant CEA-specific immune responses were observed in all patients tested. This study thus demonstrated that the TRICOM vaccines are safe, generate a significant CEA-specific immune response in all A2+ patients, and may have significant clinical benefit in patients with advanced cancer.(59) Three other trials employing TRICOM vectors are ongoing at Fox Chase Cancer Center, Duke University Cancer Center, and Columbia Presbyterian. There have been no dose limiting toxicities (DLT) attributed to vaccine on any of these studies.

MUC-1 vaccines

In a Phase I study at the Dana-Farber Cancer Center, employing three vaccinations of rV-MUC-1 in patients with advanced breast cancer, no serious adverse events were noted; drops in serum

markers were observed in some patients. In related studies, an rV-MUC-1 IL-2 vaccine was administered in a Phase I trial to patients with inoperable breast cancer. MUC-1-specific T-cell responses were detected in 2 of 9 patients.(60) In a multi-center Phase II study using this vaccine, 2 of 31 patients with metastatic breast cancer, who had visceral metastases and progressive disease after intensive chemotherapy, had objective tumor regression following vaccination.(61) Other vaccine trials are ongoing employing MUC-1-based vaccines with excellent safety profiles. Many have shown the ability of the vaccine to generate MUC-1-specific T-cell responses. None of the above MUC-1-based vaccines, however, contain costimulatory molecule, an agonist MUC-1 epitope, or are being used in diversified prime and boost regimens.

Vaccines containing MUC-1 and CEA

As a precursor study for the PANVAC vaccines, an admixture study was carried out at Columbia Presbyteran (sponsored by Therion Biologics Corp.). In this safety and tolerability study, pancreatic cancer patients received a s.c. priming dose of rV-CEA(6D)-TRICOM admixed with rV-MUC-1 on day 0, followed by a s.c. boost dose of rF-CEA(6D)-TRICOM on days 14, 28, and 42. sargramostim was given s.c. at the injection site on the day of each vaccine and for 3 consecutive days thereafter. Twelve patients were vaccinated. No serious adverse events related to the treatment regimen have been identified, and no dose limiting toxicities have occurred. Three of 12 patients remained on trial with stable disease for more than 6 months.

1.2.4 Summary

In this pilot trial we will seek to translate the following observations from the laboratory and prior clinical trials into the clinic in patients with incurable tumors.

- CEA and MUC-1 are overexpressed in adenocarcinomas of the colon. It is estimated that > 99% of patients with colorectal cancer have over-expression of one or both TAA.
- Pox viral vectors can induce a strong immune response to TAA such as CEA and MUC-1.
- The use of agonist epitopes within the TAA can induce a better immune response than native peptides and have been associated with clinical responses
- Heterologous prime and boost regimens are superior in terms of generalizing immune responses; and this may translate into improved clinical responses
- The use of GM-CSF does not add significant toxicity and in pre-clinical models is essential for induction for optimal immune responses.
- A precursor study with viruses containing MUC-1, CEA and TRICOM showed no evidence of vaccine related toxicity and 3 of 12 advanced pancreatic cancer patients with stable disease for more than 6 months
- It is possible by using vectors directed against TAA that there may be additive or synergistic immune responses and this may be important in overcoming antigenic escape variance

- Several hundred patients have been treated on clinical trials with pox viral vectors made in collaboration with Therion Biologics Corp., and there have been no dose limiting toxicities attributable to vaccines
- This trial is identical in design to one approved by the FDA under a Therion Biologics Corp. IND that will be performed in pancreatic cancer patients. This trial will allow us to provide initial evaluation of CEA and MUC-1 immune responses with this vaccine strategy.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

- A. Histologically confirmed carcinoma that for patients in the first two arms (colorectal and non-colorectal cancer) is CEA or MUC-1 positive. Tumor that has been shown to express CEA or MUC-1 ($\geq 20\%$ of cells) by immunohistochemical techniques or patients that have had an elevated serum CEA ($> 5 \mu\text{g/L}$) at any point during their disease course. For patients in the ovarian and breast cancer arms, as $>95\%$ of these express MUC-1 or CEA, we will not require staining prior to coming onto trial.
- B. Patients must have completed at least one 5-FU containing chemotherapy regimen (e.g. 5-FU/LV with or without either irinotecan or oxaliplatin) for the colorectal cancer arm, or either failed or not be a candidate for therapy of proven efficacy for their disease in the non-colorectal, breast, and ovarian cancer arms.
- C. 18 years of age or greater.
- D. All patients on the colorectal adenocarcinoma cohort must be HLA-A2 positive.
- E. At least 10 patients on the non-colorectal carcinoma cohort must be HLA-A2 positive.
- F. Patients on the breast and ovarian arms are not required to be HLA-A2 positive.
- G. For the colorectal and non-colorectal cancer arms (the initial two arms), patients will be required to have: metastatic disease (measurable or evaluable), metastatic disease documented by biopsy but not evaluable by imaging (e.g. small volume peritoneal disease), and patients with surgically resected metastatic disease at high risk of relapse. For the ovarian and breast cancer arms, patients will be required to have evaluable disease.
- H. Able to understand and give informed consent.
- I. Able to avoid close household contact (close household contacts are those who share housing or have close physical contact) for at least three weeks after recombinant vaccinia vaccination with persons with active or a history of

eczema or other eczematoid skin disorders; those with other acute, chronic or exfoliative skin conditions (e.g., atopic dermatitis, burns, impetigo, varicella zoster, severe acne, or other open rashes or wounds) until condition resolves; pregnant or nursing women; children 3 years of age and under; and immunodeficient or immunosuppressed persons (by disease or therapy), including HIV infection.

- J. ECOG performance status of 0 – 1.
- K. Serum creatinine not above the institution limits of normal, and AST \leq twice the upper limits of normal OR creatinine clearance on a 24 hour urine collection of \geq 60 mL/min.
- L. Total bilirubin within the institution limits of normal OR patients with Gilbert's syndrome, a total bilirubin \leq 3.0
- M. Recovered completely from any reversible toxicity associated with recent therapy. Typically this is 3-4 weeks for patients who most recently received cytotoxic therapy except for the nitrosoureas and mitomycin C for which 6 weeks is needed for recovery.
- N. Hematological eligibility parameters (within 16 days of starting therapy):
 - Granulocyte count \geq 1,500/mm³
 - Platelet count \geq 100,000/mm³
 - Hgb \geq 10 Gm/dL
- O. Prior immune therapy (e.g. related vaccinia and fowlpox vaccines or antigen-specific peptides) is allowed.
- P. Men and women must agree to use effective birth control or abstinence during and for a period of 4 months after the last vaccination therapy.
- Q. Patients with prostate cancer must continue to receive GnRH agonist therapy (unless orchiectomy has been done).
- R. Patients should appear clinically stable (in the opinion of the principal investigator) to complete the full 3 month course of vaccination with an anticipated survival of 6 months or longer

2.1.1.1 Inclusion Criteria for Extension or Maintenance Phase

- A. Completion of Core phase of the protocol.
- B. Stable or responding disease (PR, CR).
- C. No dose limiting toxicity (see below) in Core phase possibly, probably or definitely related to the vaccine.

Dose limiting toxicities include:

- Any Grade 2 generalized urticaria or Grade 3 or greater allergic reaction.
- Any Grade 2 or greater autoimmune response
- Any Grade 3 or greater hematologic or non-hematologic reaction, including injection-site reaction.

2.1.2 Exclusion Criteria

- A. 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.
- B. Concurrent use of systemic steroids, except for physiologic doses for systemic steroid replacement or local (topical, nasal, or inhaled) steroid use. Limited doses systemic steroids to prevent IV contrast, allergic reaction, or anaphylaxis (in patients who have known contrast allergies) are allowed
- C. History of allergy or untoward reaction to prior vaccination with vaccinia virus
- D. Pregnant or breast-feeding women
- E. 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)
- F. Serious intercurrent medical illness which would interfere with the ability of the patient to carry out the treatment program, including, but not limited to, inflammatory bowel disease, Crohn's disease, ulcerative colitis, or active diverticulitis
- G. Patients with a history of cardiomyopathy or symptomatic congestive heart failure (unless stable on treatment), symptomatic arrhythmia not controlled by medication. Unstable atherosclerotic heart disease (e.g. unstable angina) who require active intervention and history of myocardial infarction or embolic stroke within the past 6 months.
- H. Clinically active brain metastasis, or a history of encephalitis, multiple sclerosis, or seizures within the last year (from seizure disorder or brain metastasis)
- I. Medical conditions, which, in the opinion of the investigators would jeopardize the patient or the integrity of the data obtained
- J. Concurrent chemotherapy; an exception to this is to allow for patients with breast cancer who are receiving trastuzumab, to continue therapy with trastuzumab while receiving the vaccine treatment
- K. Serious hypersensitivity reaction to egg products
- L. Clinically significant cardiomyopathy requiring treatment

- M. Chronic hepatitis infection, including B and C, because of potential immune impairment
- N. Although topical steroids are allowed, steroid eye-drops are contraindicated
- O. Cardiac complications, including recent myocardial infarction or cerebrovascular accident within one year, and/or unstable or uncontrolled angina

2.2 Research Eligibility Evaluation

- A. Clinical Evaluation (within 16 days of before starting treatment).
 - History and physical examination
 - ECOG performance status (see Appendix A)
- B. Laboratory studies (within 16 days before starting treatment)
 - Complete blood count plus differential and platelet count
 - Serum chemistries (Na⁺, K⁺, Cl⁻, CO₂, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH)
 - with BUN and creatinine
 - Serum CEA,
 - ANA titer, CD4:CD8 ratio, CD3, 4, 8 subsets
 - 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 or bilateral oophorectomy.
- C. Urinalysis
- D. HIV test within the past 8 weeks
- E. Electrocardiogram (EKG) within the past 28 days.
- F. Computerized Tomography (CT) of the chest/abdomen/pelvis, or Magnetic Resonance Imaging (MRI) within the past 28 days.
- G. HLA-A2 profile
- H. Hepatitis B and C within the past 8 weeks
- I. Document known prior vaccinia vaccination (small pox vaccine).

2.3 Patient Registration and Treatment Randomization

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing the consent. A registration 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 (HOIS) ncicentralregistration-1@mail.nih.gov. After confirmation of eligibility, the Central Registration 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.

3 STUDY IMPLEMENTATION

3.1 Study Design

This is a four arm, open label safety trial in which all enrolled patients receive 2×10^8 pfu PANVAC™-V (vaccinia) subcutaneously on Day 1, followed by (1×10^9) pfu PANVAC™-F (fowlpox) or about days 15, 29, and 43 (Core phase). 100 µg sargramostim will be given subcutaneously at the site of the vaccination on each vaccination day and for three consecutive days thereafter. The four arms will consist of 10 colorectal cancer patients, 10-15 non-colorectal cancer patients, about 12 breast cancer patients, and about 12 ovarian cancer patients. The enrollment on the latter two arms is based on preliminary evidence of clinical benefit from the first two arms. Enrollment to these arms (ovarian cancer and breast cancer) will not begin until the first two arms have been enrolled. The first 9 patients (total from the initial two arms) will be used to assess safety. While the initial few non colorectal patients do not need to be HLA-A2 positive, all patients enrolled after the first 9 (from both arms combined) must be HLA-A2 positive in the colorectal and non-colorectal arm. As we will be primarily assessing for clinical responses, patients in the breast and ovarian arms do not need to be HLA-A2 positive.

An optional provision of up to 12 additional monthly boosting immunizations (Extension phase) with (1×10^9) pfu PANVAC™-F (fowlpox) subcutaneously in combination with sargramostim will be offered to patients who have completed the Core phase of the study and have not experienced disease progression, have not experienced unacceptable toxicity, and may benefit from continuing treatment. If additional boosts are administered, patients must continue to follow study-prescribed criteria for remaining on trial (e.g., no concurrent chemotherapy until the last safety visit is completed, no concurrent steroid use, etc.). Safety assessments will continue as during the Core phase.

Patients who have completed the extension phase without evidence of progressive disease may continue vaccination every 3 months (Maintenance phase) with PANVAC™-F (fowlpox) (1×10^9) pfu in combination with Sargramostim until vaccine is no longer available. From the end of the extension phase, patients must continue to follow study-prescribed criteria for remaining on trial (e.g., no concurrent chemotherapy until the last safety visit is completed, no concurrent steroid use, etc.). Safety and clinical assessments will continue at each regularly scheduled visit. Patients who are in the Maintenance phase (vaccines every 3 months) who show no radiographic evidence of metastatic cancer, may have their restaging exams performed at the discretion of the PI as clinically warranted on a per patient basis at an interval not to exceed one year. These patients will have interval clinical evaluation every 3 months prior to each vaccination.

Patients who have radiographic evidence of progressive disease during the maintenance phase who are otherwise clinically stable may revert back to monthly vaccinations as in the extension phase (see above).

Scans documenting progressive disease on the maintenance phase will be used as a baseline scan to assess for subsequent evaluation of disease response. Patients having further progression will be taken off study per off-study procedure (Section **3.6.1**).

3.1.1 Trial Outline

Core Phase

Day 1	PANVAC™-V (vaccinia) (2×10^8) pfu subcutaneously
On or about days 15, 29, 43	PANVAC™-F (fowlpox) (1×10^9) pfu subcutaneously
The day of and for 3 consecutive days following vaccine (on or about days 1- 4, 15-18, 29-32, and 43-46)	Sargramostim 100 μ g s.c. at vaccination site.
Optional Extension Phase	(Up to 12 additional monthly boosting immunizations)
About every 28 days	PANVAC™-F (fowlpox) (1×10^9) pfu subcutaneously
Days 1- 4 with monthly PANVAC™-F (fowlpox) injection	sargramostim 100 μ g subcutaneously at vaccination site
The monthly vaccinations of PANVAC™-F and sargramostim will begin about 28 days following completion of the last fowlpox vaccination of the core phase. The extension phase will be divided a 56 day cycle each containing vaccines (at day 1 and day 29).	

Maintenance Phase

Every 3 months	boosting immunization PANVAC™-F (fowlpox) (1×10^9) pfu subcutaneously
Days 1- 4 with each PANVAC™-F (fowlpox) injection	sargramostim 100 μ g subcutaneously at vaccination site

Restaging scans may be performed at patient and PI discretion, at least every 12 months.

Clinic visits for clinical assessment will take place prior to each vaccination at a 3-month interval.

3.2 Drug Administration

3.2.1 Study drugs

Study drugs will be prepared and placed in syringes by the Warren Magnuson Clinical Center pharmacy personnel. Both vaccine and sargramostim are given subcutaneously at the same site (see Section 9).

This pilot study will be conducted by delivering a priming dose of (2×10^8) pfu PANVAC™-V (vaccinia) subcutaneously, followed 2 weeks later by a boosting dose of (1×10^9) pfu PANVAC™-F (fowlpox) administered subcutaneously. Boosting doses of (1×10^9) pfu PANVAC™-F (fowlpox) administered subcutaneously will be repeated at two week intervals, for a total of three vaccinations. Sargramostim (100 μ g) will be given subcutaneously at the site of the vaccine injection as an adjuvant, at the time of each immunization and for three consecutive days thereafter. Up to 51 patients will be enrolled into this study. Patients will be seen at each vaccination visit and four weeks following the final boosting vaccination for physical examination and collection of laboratory data and adverse event information. A preliminary assessment of immune response will also be performed at the end of the study. Continued boosting immunizations of PANVAC™-F (fowlpox) will be available to patients who do not experience disease progression (see Trial Outline section 3.1.1). Patients will continue to receive the vaccine as long as drug is available.

3.2.2 Precautions

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

3.3 Treatment Modifications

Patients must have recovered to < grade 2 toxicity for the parameters used to assess levels of organ function required for eligibility (see section 2) after each vaccination in order to receive a subsequent vaccination. If > grade 1 toxicity persists for > 42 days, the patient will not receive further vaccine inoculations and will be removed from protocol. Follow-up will be performed during each vaccination and blood draw interval for immunologic testing as described in section

3.4.5. Vaccination will be held for > grade 2 proteinuria.

If a scheduled dose of the vaccine is missed, 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 patient 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 patient from study. If the patient has a delay in vaccination not due to toxicity during the extension or maintenance phase, the vaccine may be delayed for up to 3 months without removal of the patient from study.

Dosing Delay: Patients should have resolution to < grade 2 or return to baseline of all toxicities prior to the start of the next injection of PANVACTM-F (fowlpox).

Vaccine Dose Modification: None

3.3.1 PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) (Vaccine)

Patients must have recovered to < Grade 2 toxicity or to levels of organ function required for eligibility in Section **2** after each vaccination in order to receive a subsequent vaccination. No dose modifications will be made. If > Grade 1 toxicity persists for > 42 days, the patient will not receive further vaccine inoculations and patients will be removed from protocol. This study will utilize the CTCAE version 4.0 for grading systemic toxicity starting August 1, 2010 (all AE's before August 1, 2010 will be graded using the CTCAE version 3). In addition, patients who develop \geq grade 2 allergic disease to the vaccine or \geq grade 2 autoimmune disease that may threaten vital organ function or any grade 3 or greater autoimmunity will be removed from the protocol. Any grade 3 or greater toxicity to sargramostim lasting more than 48 hours with the exception of fever, local reactions, rash, headache and adenopathy, and any grade 4 toxicity to sargramostim would require discontinuation of the sargramostim. Grade 3 toxicities not attributed to sargramostim lasting more than 48 hours or any grade 4 toxicity not attributed to sargramostim will require removal of the patient from protocol.

Sargramostim (GM-CSF)

If reversible grade \geq 3 toxicity attributable to sargramostim administration is encountered, sargramostim will be reduced by 50% for the following cycle. If similar reversible grade \geq 3 toxicity attributable to sargramostim recurs in more than two cycles, it will be discontinued and patient will be allowed to receive vaccinations without further sargramostim.

In addition, patients who develop \geq grade 2 allergic disease to the vaccine or \geq grade 2 autoimmune disease that may threaten vital organ function or any grade 3 or greater autoimmunity will be removed from the protocol. Any grade 3 or greater toxicity to sargramostim lasting more than 48 hours with the exception of fever, local reactions, rash, headache and adenopathy, and any grade 4 toxicity to sargramostim would require discontinuation of the sargramostim. Grade 3 toxicities not attributed to sargramostim lasting more than 48 hours or any grade 4 toxicity not attributed to sargramostim will require removal of

the patient from protocol.

3.4 On Study Evaluation: (See APPENDIX C)

- 3.4.1 All patients who are deemed eligible and who sign the informed consent form will be enrolled onto this trial.
- 3.4.2 Medical assessment, performance status will be completed within 3 days prior to vaccination.
- 3.4.3 Consideration for work up of any history of symptoms of myopericarditis and coronary atherosclerotic heart disease following vaccinia vaccination. This should include ECG, cardiac enzymes, echocardiogram as clinically appropriate. All patients should be encouraged to minimize cardiovascular risk factors including encouraging evaluation and management of blood pressure, diabetes, hyperlipidemia, smoking cessation, and maintaining healthy diet and exercise.
- 3.4.4 Laboratory studies: (within 16 days prior to on-study date)

Baseline only:

- Hepatitis B, C and HIV panel (within 8 weeks prior to day 1)
- HLA-A2 profile
- 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

Baseline and during study:

- CBC/differential, with platelet count.
- Serum chemistries (Na⁺, K⁺, Cl⁻, CO₂, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH)
- PT and PTT
- Serum CEA
- ANA titer, CD4:CD8 ratio, CD3, 4, 8 subsets (baseline and around day 71 prior to vaccination)
- Serum for NCA (CEA-CAM) titers will be drawn anytime that a patient exhibits new onset neutropenia. (ANC <1000)
- Urinalysis with dipstick protein prior to each vaccination, 4 weeks after the last vaccination, and monthly thereafter for 3 months where feasible

These laboratory tests will be drawn prior to each vaccination (about days 1, 15, 29 and 43) and then again on around day 71, and thereafter in the follow-up phase for as long a period as

possible at the discretion of the Principal Investigator, for all patients. At a minimum we will obtain serum creatinine, electrolytes, and a dipstick protein prior to each vaccination and 4 weeks after the last vaccination. Laboratory 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.

3.4.5 Collection of immunologic blood samples

Baseline and around day 71 blood samples will be obtained via apheresis as described in section **5.2**. In addition, prior to all other vaccinations on protocol, we will obtain 6 green top tubes. Prior to each vaccine, 2 “tiger” top tubes will be obtained.

Immunologic testing will include:

IFN-gamma ELISPOT assays for CEA-specific T lymphocytes and MUC-1-specific T lymphocytes. (Samples from baseline and about day 71.)

Antibodies to CEA, vaccinia, fowlpox, MUC-1 and ANA titer. (Samples from baseline and about around day 71.)

CD3, CD4, and CD8 subsets, and CD4:CD8 ratio, will be done at baseline and about day 71 prior to vaccination while the patient remains on trial. Serum for NCA (CEA-CAM) titers will be drawn anytime that a patient exhibits new onset neutropenia. (ANC <1000) 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.

Blood samples may be used for other research studies which may include phenotypic and functional analysis of immune cell subsets, and analysis for cytokines, chemokines, antibodies, tumor-associated antigens and / or other markers.

3.4.6 For all patients, appropriate imaging with computerized tomography (CT) of the chest/abdomen/pelvis or magnetic resonance imaging (MRI), to reassess tumor extent and to determine tumor measurements will be done every 2 months.

3.4.7 Monitoring after the initial phase of vaccinations

All patients who continue on treatment with PANVAC™-F (fowlpox) after the initial phase of four vaccinations will continue to have clinical / immunologic monitoring.

3.4.8 The samples will be processed at

Clinical Services Program (CSP)

Abbreviated Title: Vaccine for metastatic cancer

Version Date: 05/04/2017

CTEP #: 6536

NCI Frederick Cancer Research and Development Center
PO Box B
Frederick MD 21702
301-846-1000

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

Once a patient's treatment schedule has been determined, it should be faxed to the Laboratory of Tumor Immunology and Biology/ NIH (Fax: [301] 496-2756; phone: [301] 496-9573) for planning purposes.

3.4.9 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 transported to the NCI Frederick Central Repository by the Leidos couriers.

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

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 System II (BSI). 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, three types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdraw request. Vials are labeled with a unique BSI ID which is printed in both eye readable and bar coded format. No patient specific information is encoded in this ID.

Investigators are granted view, input and withdraw authority only for their specimens. They may

not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

3.4.10 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. The PI will report destroyed samples to the IRB if samples become unsalvageable or destroyed by environmental conditions (ex. Broken freezer or lack of dry ice in shipping container) or if a patient chooses to withdraw his/her consent. Samples will also be reported as lost if they are lost in transit or misplaced by a researcher.

3.5 Concurrent Therapies

Concurrent anticancer treatment with chemotherapy, hormonal therapy, systemic glucocorticoids (topical and inhaled steroids allowed), major surgical procedures or radiation therapy or immunotherapy is not permitted. Patients with prostate cancer must continue to receive GnRH agonist therapy (unless orchectomy has been done). Patients with breast cancer may continue to receive trastuzumab.

3.6 Off Study Criteria

- Progression of disease as described in section [5.2](#). (Please note radiographic progression on maintenance phase may not be an off study criteria as outlined in Section [3.1](#)) If during the core phase of the study, patients are determined to have minimal progression of disease such as 1 or 2 small lesions and do not require additional therapy, they may remain on study until evaluation for progression of disease after the core phase of the study.
- Unacceptable treatment-related toxicity (dose limiting toxicity) as described in sections [2.1.1.1](#) and [5.3](#).
- Intercurrent illness or medical circumstances: if at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.
- Patient's request to be taken off study. In this event, the reasons for withdrawal will be documented.
- If patients are non-compliant with the protocol guidelines, they may be removed from the study at the discretion of the principal investigator.
- Development of proteinuria $>1\text{g}$ per 24 hours.

3.6.1 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. An Participant Status Updates Form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-1@mail.nih.gov.

3.7 Post Study Evaluation (see APPENDIX C)

Patients will be referred to local physician for follow-up care off study. The follow-up data will be collected during the 3 month period after patient comes off study if available:

- A. History and physical
- B. Height, weight, BSA
- C. Performance status
- D. CBC and differential
- E. Creatinine
- F. AST, ALT, T.bili
- G. Blood for immunologic tests
- H. Urinalysis
- I. Serum ANA

The Biologic Response Modifiers Advisory Committee has recommended that long-term follow-up extend over a period of 15 years. This will be accomplished by signing patients onto protocol 04-C-0274 "Follow-Up Study of Subjects Previously Enrolled in Poxviral Vector Gene Transfer Studies." Patients will receive annual history and physical examinations for the first 5 years following the last vaccine. Additional data will be obtained annually for years six through fifteen via telephone contacts. These inquiries will focus on clinical information pertaining to development of de novo cancer, neurologic, autoimmune, and hematologic disorders. In addition, medical problems including information on unexpected hospitalizations and medications will be collected. Information regarding the findings will be reported to the FDA.

4 SUPPORTIVE CARE

4.1 Treatment of Vaccinia Vaccination Complications

4.1.1 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 Vaccinia Immune Globulin (VIG). VIG is contraindicated, however, for the treatment of isolated vaccinal keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated

with vaccinia vaccine. VIG is an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. Upon receipt of a call from a 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 intravenous formulation of VIG is available through the CDC, which has a lower level of aggregated protein, allowing it to be used by either the IM or IV route. This formulation will most likely be preferred for administration and investigators will be instructed by the CDC regarding appropriate dosing and method of administration based on formulation and availability. There is no guarantee that VIG will successfully treat complications. At present, there are no other anti-viral therapies of proven benefit for the treatment of vaccinia-related complications

4.1.2 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 antiviral activity of this agent against certain orthopoxviruses. Currently, efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, "VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be released by the CDC after all inventories of VIG have been exhausted, after a patient fails to improve with VIG treatment, or as a last effort for a patient who is otherwise near death." [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated February 21, 2003. Available at URL: <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. This agent will only be provided by the CDC in the occurrence of an emergency public health event. Thus, investigators should obtain cidofovir for second-line therapy through commercial sources if necessary. NCI/CTEP investigators may use the CDC cidofovir IND protocol as a "guideline" when providing cidofovir for treatment under an off-label use. The CDC will provide their IND protocol for the use of cidofovir related to adverse reactions post vaccinia vaccination to NCI/CTEP for distribution to investigators of NCI-sponsored protocols. The CDC Clinician Information Line at 1-877-554-4625 should still be consulted regarding

appropriateness of therapy and guidance.

For the vaccine administration, antiemetics 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 patients 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.

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 patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of >50,000/mm³.

5 DATA COLLECTION AND EVALUATION

5.1 Data Collection

5.1.1 Eligible patients must be confirmed and checklist completed. Consent form must be signed prior to registration with Harris Technical Services.

5.1.2 For adverse event reporting see sections **7** and **8**.

In addition, all serious adverse events should be reported to the Office of Science Policy (OSP), NIH per their requirements. 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/gemcrisor> by using the FDA Form 3500a.

5.1.3 Data will be collected using protocol-specific case report forms, verified for accuracy and completeness, and submitted to Theradex electronically every 2 weeks. 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 Harris Technical Services), Theradex forms, and blood sample flow sheets. A copy of all serious AE forms will be kept in the regulatory binder.

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.

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 breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

5.1.4 Treatment is given according to protocol (dated notes about doses given, complications, and clinical outcomes).

5.1.5 Toxicity is assessed according to protocol (laboratory report slips, etc.)

5.1.6 Response is assessed according to protocol (X-ray, scan, lab reports, date noted on clinical assessment, as appropriate).

5.1.7 Drug Accountability Records are kept for each patient.

5.2 Response Criteria

All patients in this study must be assessed for response to treatment, even if there are major treatment deviations. Each patient will be assigned one of the following categories: 1) complete response; 2) partial response; 3) stable disease; 4) progressive disease; and 5) not evaluable (early death from malignant disease, early death from toxicity, early death due to other causes, or unknown-not assessable, insufficient data). This protocol will use CTEP's Response Evaluation Criteria on Solid Tumors (RECIST) for assessing response. A quick reference to the RECIST guidelines can be downloaded at the following URL:

<https://ctep.cancer.gov/protocolDevelopment/default.htm>. A copy of this quick reference is found in **APPENDIX D**.

The minimum restaging evaluation a patient who has measurable or non-measurable, evaluable disease to be considered for response assessment will be about 71 days. Patients who experience

rapid disease progression mandating discontinuation of therapy prior to completing two treatment cycles will be considered treatment failures. The same imaging studies used to define the extent of tumor at baseline upon study entry will be used for restaging. The time to disease progression will be defined as from the first date of therapy until the first notation of clinical progression.

5.2.1 Criteria for Response Assessment

Measurable disease will include any lesion with clearly defined borders that can be measured with rulers or calipers on physical exam or radiographically on X-rays or Computerized Tomography (CT) of the chest/abdomen/pelvis, or Magnetic Resonance Imaging (MRI) scans. Measurement of lesions by ultrasound is not generally recommended for obtaining reproducible tumor measurements but is acceptable. Previously irradiated lesions (prior to study), malignant hepatomegaly and lesions visible on bone scan will not be considered measurable. The measure should consist of the longest diameter only for all target lesions. Photographs (which include a centimeter scale, the date and the patient's initials in the photographed field) should be obtained at the time of each tumor measurement for visible lesions.

At the time of each assessment of tumor response, the measurement of the longest diameter only for all target lesions should be obtained, and the sum of all of these computed.

Complete Response:

Disappearance of all clinical and laboratory signs and symptoms of disease for a minimum of 4 weeks during which no new lesions may appear. Specifically, all tumor masses must disappear. There must be no cancer-associated deterioration in weight ($>10\%$), performance status or symptoms. For bony metastases, CR means the re-calcification of all lytic lesions or the biopsy-proven absence of tumor cells. Normalization of the bone scan is not necessary for the patient to be considered to have a CR; however, any worsening of the bone scan needs to be evaluated.

Partial Response:

A minimum of 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter. If the bone scan was abnormal due to metastatic disease, it must show improvement; malignant hepatomegaly, if present, must decrease by 30%. There may be no new lesions and the response must last for at least 4 weeks during which time there should be no cancer-associated deterioration in weight, performance status or symptoms.

Stable Disease:

Neither sufficient shrinkage to qualify for partial response nor progressive disease, taking as reference the smallest sum longest diameter since the treatment started. This condition should persist for at least 3 months. Patients who at study entry are without radiographic or clinical evidence of disease will be considered having stable disease if there is no further evidence of disease by clinical assessment and surveillance radiographic studies.

Progressive Disease:

A minimum of 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new measurable lesions.

In the case of identification of progressive disease prior to end of the initial phase of vaccinations, patients may complete their initial 4 vaccinations, with subsequent re-staging and reassessment, if the investigator feels further treatment is safe and clinically appropriate.

5.2.2 Assays for Immunologic Response

Our studies in the GMB and 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. (52-54) 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. (See Appendix E for details).

The primary immunologic endpoint for evaluating the immune response will be the frequency of interferon gamma-releasing T cells specific to CAP-1-6D, an HLA-A2 restricted epitope of CEA, or MUC-1 as measured by the ELISPOT assay. It is planned that all patients will undergo exploratory analysis of the ability to detect CD4 positive responses using a whole protein CEA assay.

In all patients undergoing apheresis, 5×10^8 to 2×10^9 mononuclear cells will be obtained by a single-access (single venipuncture) "four-pass" mononuclear cell procedure on the Haemonetics V-50 instrument, during which 2.0 liters of whole blood would be processed at a flow rate of about 70-80 ml/min. The total duration of the procedure is 20 minutes per pass or about 80 minutes. Patients will be required to have a minimum HCT of 28% and a platelet count of at least 75,000 to undergo a Haemonetics procedure.

5.3 Toxicity Criteria

Common Terminology Criteria for Adverse Events, Version 4.0(CTCAE)

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 beginning August 1, 2010. All AE's before August 1, 2010 will be graded using the CTCAE version 3. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

Toxicity Grading for Vaccinia Toxicity (49) (see Section [8.1.2](#))

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

5.4 Statistical Considerations

The primary objectives of this pilot study are to determine whether PANVACTM-F (fowlpox) in combination with sargramostim is safe when administered to patients with colorectal adenocarcinoma or non-colorectal metastatic carcinoma, and to preliminarily assess immune response to the investigational vaccine by determining CEA-specific and MUC-1 specific cell-mediated immune responses.

Sample Size and Power

A total of up to 51 patients may be enrolled onto this trial. All 10 patients with colorectal adenocarcinoma will be HLA-A2 positive, while at least 10 of 15 non-colorectal patients will also be HLA-A2 positive. Patients in the breast and ovarian arm will not need to be HLA-A2 positive.

A safety evaluation will take place in the first 9 patients to be enrolled on the trial, regardless of disease type. This number of patients was chosen to provide an adequate initial demonstration of human safety and tolerability. Based upon the stopping rule for DLT attributable to the agents (see below; not taking into the account stopping due to a death or 2 Grade 4 toxicities), the following are the probabilities of stopping early:

Probability (DLT in a given patient)	Probability (early termination due to DLT)
--------------------------------------	--

0.1	0.13
0.3	0.65
0.6	0.96

Thus, this study has a moderate probability of terminating early in the first 9 total patients if the probability of a DLT is 0.3 or greater. These probabilities will increase if allowance is made for the chance that a death or 2 grade 4 toxicities are noted.

The primary endpoints based on laboratory assays will be assessed with exploratory intent. All colorectal patients will be HLA-A2 positive, as well as at least 10 of the non-colorectal carcinoma patients, and these HLA-A2 positive patients will undergo analysis of immune response MUC-1 and CEA by ELISPOT assay. If possible, up to three individual assay values will be determined from each patient for each type of assay (ELISPOT for CEA and MUC-1) at each time point to help determine the inherent variability in the marker value determinations. Differences of (average) assay values between baseline and post-vaccination will be estimated, and the standard deviation determined. This information will be used to help identify the number

of patients which would be needed in a subsequent trial in order to detect a statistically significant difference in the change in the assay values from baseline to the two time points defined above. Although this is intended to be a pilot study, for any of the assays, 10 patients would provide 80% power to declare a single change of 1 standard deviation in magnitude as statistically significant at the two-sided 0.05 level. However, if such a difference were noted, we would declare it to be an exploratory finding since such differences were not expected to be identified in this intentionally small trial. Thus, both patients with colorectal adenocarcinoma and patients with non-colorectal carcinoma will be evaluated in this fashion, in separate cohorts of 10 patients apiece.

In the cohort of patients with non-colorectal, metastatic carcinoma, the initial few patients enrolled (up until a total of 9 have been enrolled, from both disease categories combined) do not need to be HLA-A2 positive, as they will be enrolled in order to obtain preliminary information about toxicity and evidence of clinical response in different disease states that are refractory to standard treatment. However, once the toxicity evaluation phase has concluded, all remaining patients with non-colorectal adenocarcinoma must be HLA-A2 positive in order to undergo analysis of immune response MUC-1 and CEA by ELISPOT assay as an exploratory analysis of the magnitude of immune responses in non-colorectal cancer patients given this vaccine.

Stopping Rules

One occurrence of Grade 5 toxicity by the NCI-CTCAE (Version 4 starting August 1, 2010; All AE's before August 1, 2010 will be graded using the CTCAE version 3) attributable to the treatment regimen will result in study termination. In the first 10 patients, if there are two occurrences of Grade 4 toxicity that are attributed to the treatment regimen, the study will be terminated.

If > 1 of the first 3 patients
or
≥ 2 of the first 6 patients
or
≥ 3 of the first 9 patients

experience a dose limiting toxicity attributed to the treatment regimen, the maximum tolerable dose has been exceeded and the study must be terminated.

Dose limiting toxicities include:

- Any Grade 2 allergic reaction of asymptomatic bronchospasm or generalized urticaria or any other Grade 3 or greater allergic reaction.
- Any Grade 2 or greater autoimmune response.
- Any Grade 3 or greater hematologic or non-hematologic reaction, including injection-site reaction.

These stopping rules apply only to the Core phase and will not apply to the Extension or Maintenance phase (optional additional monthly boosts). All serious adverse events (SAEs) attributed to the treatment regimen occurring in the Extension and/or Maintenance phase will be reported to the FDA and to the NIH.

5.4.1 Statistical considerations for pilot portion of trial

Amendment I will allow enrollment of patients with either breast cancer or ovarian cancer to determine, in a pilot fashion, if early indications of clinical benefit from the agents administered may suggest that further investigation is warranted.

Among patients initially enrolled in the trial, the one patient with ovarian cancer experienced a large reduction of ascites and substantial improvement in clinical symptoms as well as CA125. A patient with breast cancer also showed a modest reduction of tumor volume, although less than required to be considered a partial response.

In order to determine if the vaccine approach studied in this trial is worth pursuing in a phase II trial, this amendment will allow accrual of 12 additional, eligible patients with ovarian cancer and 12 additional, eligible patients with breast cancer. In each category separately, with 12 patients, there is 84% power to rule out a 5% PR rate in favor of a 25% PR rate, assuming that an exact binomial test with a one-sided 0.15 alpha level is used.

By so doing, if 1/12 patients in either category has a clinical response, this has an associated one-sided lower 85% confidence bound of 1.3%, while 2/12 with clinical response has an associated one-sided lower 85% confidence bound of 5.8%. The information obtained in this pilot evaluation will be used to determine if a phase II trial is warranted and to help establish the parameters for such a trial.

It is expected that approximately 1 year will be required to enroll the 12 patients in each category. Since there may be an occasional patient who is inevaluable, the ceiling of 26 additional breast and ovarian cancer patients combined will be used. As a result, the accrual ceiling for the trial will be expanded from the original 25 to 51 after the amendment is in effect.

5.5 Data Safety Monitoring Plan

5.5.1 The clinical research team will meet weekly at each clinic to review all adverse events for each subject in this trial. Unexpected adverse events and/or serious adverse events will be reported to the NCI's Institutional Review Board (IRB) and Cancer Therapy Evaluation Program (CTEP) (see section 7). If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent will be modified accordingly. In addition, the drug monitor at CTEP will review the data regularly. The NCI/CCR DSMB will monitor the study at its meetings, which are held twice yearly.

5.5.2 Submission of Data to NCI's Clinical Trials Monitoring Service (CTMS) contractor:

This protocol will be monitored by the NCI's CTMS (contract held by Theradex, Princeton, N.J.) according to guidelines. Data will be submitted to CTEP every two weeks. The NCI/DCT Case Report Form and the NCI database will be used to report to CTEP.

Below are some of the services Theradex® provides under this contract:

- Confirming fulfillment of all regulatory requirements
- Verification of secure and appropriate pharmacy procedures
- Adequacy of institutional review and consent procedures
- Verification of laboratory competency
- Verification of study data submitted to CTMS by investigators
- Evaluation of eligibility of all patients
- Evaluation of all course of treatment
- Evaluation of investigator compliance
- Quality assurance evaluation of incoming data from Theradex®'s proprietary electronic data capture system, ACES®
- Quality assurance evaluation of case report forms submission

6 HUMAN SUBJECTS PROTECTIONS

6.1 Rationale for Subject Selection

6.1.1 Selection Based on Gender, Ethnicity, and Race

Subjects from all racial/ethnic groups and both genders 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 gender 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.

6.1.2 Strategies/Procedures for Recruitment

Patient accrual for this protocol would be facilitated by the Clinical Center Support Center (CCSC), developed to increase the accrual to clinical studies via community outreach as well as recruitment letters to referring physicians.

This protocol will be available through the physicians' data query (PDQ) database.

6.1.3 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.

6.2 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.

6.3 Participation of NIH 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 6.4), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the “NIH Advance Directive for Health Care and Medical Research Participation” form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team 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.

6.4 Evaluation of Benefits and Risks/Discomforts

There is no standard treatment for second line chemotherapy for this group of patients. Preliminary results of studies using a similar vaccine have shown promising early immunologic responses and indication of clinical benefit.

6.4.1 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.

6.4.2 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. 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 National Naval Medical Center, Bethesda, Maryland or 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.

6.4.3 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 National Naval Medical 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.

6.5 Risks/Benefits Analysis

The patients we will be enrolling will have metastatic colorectal cancer, for which no known cure exists. This study involves clinical research with an experimental vaccine designed to generate an immune response against antigens found in colon cancer. Patients will undergo multiple vaccinations and will be asked to undergo apheresis on at least two time points for investigational analysis immune responses. Alternative treatments include chemotherapy, other clinical trials, or supportive care. The side effects of the vaccines and apheresis are outlined elsewhere (see section 8 and 5.2 respectively.) Whether the vaccine will have any clinical effect 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.

6.6 Consent and Assent 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. A screening consent form will also be provided to the referring physician to screen patients for eligibility to incorporate such tests as HLA testing for HLA-A2 positivity as well as CEA staining of the

patient's tumor. Moreover, any experimental invasive procedure will require a separate consent form. All listed associate investigators except those listed on the cover sheet as not being able to make clinical decisions are permitted to obtain informed consent.

Outside Screening Sample Consent: Telephone consent may be employed in order to screen outside samples from prospective subjects for CEA expression and/or HLA-A2 expression. In such cases, 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 subjects will sign and date the informed consent form. 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.

Prospective subjects who consent to send such samples for outside testing will NOT be registered with the NCI Central Registration Office unless they are subsequently enrolled on protocol. Subjects and their referring medical team will be notified of the results and records will be maintained with the protocol research files.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 Definitions

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2** and **8.2**.

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.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
 - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
 - (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB and Clinical Director Reporting

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
 - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
 - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
 - All Grade 5 events regardless of attribution;
 - All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

8 DATA REPORTING

8.1 Provisions for Monitoring to Ensure Safety of Subjects

8.1.1 Data will be submitted to NCI's Clinical Trials Monitoring Service Contractor (CTMS--contract held by Theradex, Princeton, N.J.). Data will be submitted to CTMS by express mail at least once every other Friday on standard Theradex reporting forms.

8.1.2 Serious Adverse Event (SAE; formerly known as Adverse Drug Reaction). This protocol will follow CTEP, DCTD, NCI's expedited reporting requirements for Investigational New Drugs sponsored by NCI. Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (<https://eapps-ctep.nci.nih.gov/ctepaers>). 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/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 8.1.3).

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.

- A list of agent-specific expected adverse events can be found in the Pharmaceutical **Section 9**.
- All life-threatening events (Grades 4 and 5) and the first occurrence of any previously unknown reactions (regardless of grade) will be reported to Dr. Philip Arlen (Building 10, Room 13N210; Tel: 301-496-4251), Dr. James Gulley (Building 10, Room 13N208; Tel: 301-480-7164), or Dr. Ravi Madan (Building 10, Room 12N226; Tel: 301-480-7168) immediately by telephone or 301-496-1211 (after hours).

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

Phase 1 and Early Phase 2 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 electronic submission within the timeframes detailed in the table below.

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

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:

- o “24-Hour; 5 Calendar Days” - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- o “10 Calendar Days” - A complete expedited report on the AE must be submitted electronically 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 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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.

Note: All deaths on study must be reported using expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:

- “24 hours; 5 calendar days” – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
- “10 calendar days” - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.
- An expedited AE report for all protocols utilizing agents under a CTEP IND must be submitted electronically to CTEP-AERS.
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm
- 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.
- All expedited AE reports must also be sent to the local IRB according to local IRB policy and procedure.
- All AEs reported via CTEP-AERS must also be reported via the routine AEs reporting defined by the protocol.
- A list of agent-specific expected adverse events can be found in the Pharmaceutical section 9).

In addition, all serious adverse events should be reported to OSP at NIH per their requirements. 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/gemcrisor> by using the FDA Form 3500a.

- All life-threatening events (Grades 4 and 5) and the first occurrence of any previously unknown reactions (regardless of grade) will be reported to Dr. James Gulley (Building 10, Room 5B52) immediately by telephone (301) 480-7164 or 301-496-1211 (after hours).

8.2 Routine Adverse Event Reporting

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.**

8.3 Secondary AML/MDS

AML/MDS events must be reported via CTEP-AERS (in addition to routine AE reporting mechanisms). In CTCAE v 4.0, the event(s) may be reported as either: 1) Leukemia secondary to oncology chemotherapy, 2) Myelodysplastic syndrome, or 3) Treatment-related secondary malignancy.

8.4 Record Keeping

Data will be CTMS monitored. The NCI/DCT Case Report Form and the NCI database will be used to report to CTEP.

All patients must have signed an Informed Consent and an on-study confirmation of eligibility form will be filled out before entering on the study.

Data will be submitted to CTEP at least every 2 weeks. Data will be CTMS monitored. The NCI/DCT Case Report Form and the NCI database will be used to report to CTEP.

Summary of completed study will be submitted to IDB/CTEP within 2 months of study completion. A status report will be submitted and presented at upcoming NCI meetings as requested.

Complete records must be maintained on each patient, which will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on a computer database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flow sheets, records of adverse events, specialty forms for pathology, radiation, or surgery; and off-study summary sheets, including a final assessment by the treating physician.

8.5 Regulatory Issues

The agent(s) (hereinafter referred to as Agent) to the NCI under a Clinical Trials Agreement (CTA) or a Cooperative Research and Development Agreement (CRADA) between BN Immuno Therapeutics (BNIT) (hereinafter referred to as “Collaborator(s)”) and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines apply to the use of the PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) in this study:

PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) may not be used outside the scope of this protocol, nor can PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data from PANVACTM-V (vaccinia) and PANVACTM-F (fowlpox) are confidential and proprietary to Collaborator(s) and should be maintained as such by the investigators.

For a clinical protocol in which an investigational Agent is used in combination with (an) other investigational Agent(s), each the subject of different CTAs or CRADAs, 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”):

The NCI must provide all Collaborators with written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict the NCI’s participation in the proposed combination protocol.

Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

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 investigational Agent.

The NCI encourages investigators to make data from clinical trials fully available to Collaborator(s) for review at the appropriate time. Clinical trial data developed under a CTA or CRADA will be made available exclusively to Collaborator(s), and not to other parties.

When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, which will then notify the appropriate investigators (Group Chair for cooperative group studies, or Principal Investigator for other studies) of Collaborator’s wish to contact them.

Any data provided to Collaborator(s) 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.

Any manuscripts reporting the results of this clinical trial must be provided to CTEP 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. An additional 30 days may be requested in order to ensure that confidential and proprietary data, in addition to

Collaborator(s) intellectual property rights, are protected. Copies of abstracts must be provided to Collaborator(s) for courtesy review as soon as possible and preferably at least 3 days prior to submission, but 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 and/or abstract 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.

- 8.5.1 The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 and a CV.
- 8.5.2 The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all drugs received from DCTD using the NCI Drug Accountability Record (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)
- 8.5.3 Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
PANVAC-V [Recombinant Vaccinia-CEA(D609)/MUC1(L93)/TRICOM] (NSC 727026)
PANVAC-F [Recombinant Fowlpox-CEA(D609)/MUC1(L93)/TRICOM] (NSC 727027)

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/adverse_effects.htm 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.

Version 1.3, May 30, 2014¹

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	<i>Myalgia (Gr 2)</i>
Pain in extremity	
NERVOUS SYSTEM DISORDERS	
Headache	<i>Headache (Gr 2)</i>
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 maculopapular

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].

9 PHARMACEUTICAL INFORMATION

9.1 PANVAC™-V (NSC 727026)

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

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

9.1.3 **Product Description:**

PANVAC™-V is a recombinant vaccinia virus vector vaccine containing genes for human CEA, MUC-1 and three co-stimulatory molecules: B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The CEA gene has a single amino acid substitution (aspartic acid, instead of asparagine at protein amino acid position 609) in one 9-mer, HLA-A2-restricted, immunodominant epitope to enhance immunogenicity. The MUC-1 gene also contains a single amino substitution (leucine, instead of threonine at protein amino acid position 93) in one 10-mer, HLA-A2-restricted, immunodominant epitope to enhance immunogenicity. TBC-vTRICOM, which was used as the parental virus for this recombinant vaccine, was 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 was used for insertion into the TBC-vTRICOM viral genome to generate the final recombinant vaccine. 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. PANVAC™-V is manufactured by plasmid transfection of primary chicken embryo dermal cells infected with the recombinant parental vaccinia virus (TBC-vTRICOM).

9.1.4 **How Supplied:**

Lot: 1-013003: PANVAC™-V (vaccinia) is supplied in vials containing 0.3 mL of the vaccine at a final viral concentration titer of 1.29×10^9 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol (total vial contents = 3.87×10^8 pfu's).

Lot: 2-050103: PANVAC™-V (vaccinia) is supplied 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).

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

9.1.5 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 (vaccinia) on your institutional preparation guidelines to avoid potentially serious dosing errors.**

PANVAC™-V (vaccinia) Lot: 1-013003 (1.29 x 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. Withdraw 0.16 mL (2×10^8 pfu) from the thawed vial for subcutaneous administration.

PANVAC™-V (vaccinia) Lot: 2-050103 (2.1 x 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.

9.1.6 Storage:

Intact vials of PANVAC™-V should be stored at -70°C or colder.

9.1.7 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 $2\text{-}8^{\circ}\text{C}$. Thawed vials should not be re-frozen. Vials of PANVAC™-V are for single-use only and do not contain a preservative. It is recommended that prepared doses be administered as soon as possible following preparation (*i.e.*, within one hour). If absolutely necessary, prepared doses may be stored at $2\text{-}8^{\circ}\text{C}$ for up to 4 hours following preparation.

9.1.8 Route of Administration:

PANVAC™-V is administered by subcutaneous injection.

9.1.9 Toxicities and Complications Associated with Vaccinia Vaccination

Expected local reactions to vaccinia inoculation by scarification in patients who 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, soreness at the vaccination site, myalgia, local lymphadenopathy, and intense erythema surrounding the vaccination site.

Expected local reactions to vaccinia inoculation by scarification in patients who have previously been vaccinated with vaccinia 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. A milder local reaction is expected when recombinant vaccinia vaccines are administered by intradermal, intralesional, subcutaneous, or intramuscular routes of injection.

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

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

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

Recent 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. 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:** Infection of the vaccination site, 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 postvaccinial encephalitis or progressive vaccinia. Most cases of inadvertent inoculation usually resolve without specific therapy and resolution of lesions follow the same course as the vaccination site in

immunocompetent individuals. VIG can be used for severe cases involving extensive lesions or if comorbid conditions exist. VIG is contraindicated in the presence of isolated keratitis due to the risk of increased corneal scarring. VIG use can be considered in cases of ocular implantation, with keratitis, if vision-threatening or if other life-threatening vaccinia-related complications exist that require VIG therapy.

4. **Generalized vaccinia:** Generalized vaccinia 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. Treatment with VIG can be utilized in severe cases of this condition in patients that are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.
5. **Eczema vaccinatum:** Eczema vaccinatum is a serious complication in persons with eczema and other types of chronic or exfoliative skin conditions. It can also occur among eczematous contacts of recently vaccinated persons. Vaccinial lesions (generalized papular, vesicular or pustular lesions) develop on areas of the skin that are, or had at one time been, eczematous. These areas become highly inflamed and lesions may spread to healthy skin. The rash is often accompanied by fever and individuals are systemically ill. The fatality rate for untreated cases (prior to availability of VIG) has been reported from 30-40%. Following availability of VIG, mortality was reduced to approximately 7%. Early diagnosis and prompt treatment with VIG is necessary to reduce mortality.
6. **Progressive vaccinia:** Progressive vaccinia is the most serious cutaneous complication that occurs 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 therapy with VIG.
7. **Post-Vaccinial Encephalitis/Encephalomyelitis:** Vaccinial complications affecting the CNS are unpredictable. Post-vaccinial encephalitis typically affects children < 2 years of age and is characterized by an onset of symptoms 6-10 days following vaccination, which include seizures, hemiplegia, aphasia, and transient amnesia. Histopathological changes include generalized cerebral edema, mild lymphocytic meningeal infiltration, ganglion degenerative changes and perivascular hemorrhages. Older children and adults can develop encephalitis or encephalomyelitis characterized by an onset of symptoms 11-15 days following vaccination, which include fever, vomiting, headache, malaise, and anorexia, progressing to loss of consciousness, amnesia, confusion, disorientation, restlessness, delirium, drowsiness, seizures and coma. Histopathological changes include demyelination with lymphocytic infiltration, but limited cerebral edema. Mortality rates have ranged from 15-25%, with 25% of patients who recover being left with varying degrees and types of neurological deficits. VIG has not been shown to be effective in treating CNS disease and is not recommended.

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, less 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 follow 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. While the vaccinia strain used to prepare recombinant vaccinia virus vaccines is derived from the same NYC Board of Health strain as Dryvax® used in the current Smallpox Vaccination Program, the attenuation, preparation, quality control, and storage of the products are markedly different. The NCI/CTEP experience with recombinant vaccinia vectors reveals no reports of myocardial infarcts or angina associated with vaccinia vaccination, one patient with pericarditis associated with a malignant pleural effusion, and five patients with sudden death of unknown etiology. None were thought to be associated with recombinant vaccinia vaccination. Although the CDC believes that there is no evidence to conclude that Dryvax® 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 receiving 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. Because patients are being immunized with recombinant vaccinia vaccines with therapeutic intent, currently it is not recommended excluding patients with known CAD, previous heart attack, history of angina, or other evidence of risk factors for coronary artery disease who are otherwise eligible for the study, but the patient’s cardiac disease should be controlled. 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 at this time.

9.1.10 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 Vaccinia Immune Globulin (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 an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. Upon receipt of a call from a 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 intravenous formulation of VIG is available through the CDC, which has a lower level of aggregated protein, allowing it to be used by either the IM or IV route. This formulation will most likely be preferred for administration and investigators will be instructed by the CDC regarding appropriate dosing and method of administration based on formulation and availability. There is no guarantee that VIG will successfully treat complications. At present, there are no other anti-viral therapies of proven benefit for the treatment of vaccinia-related complications.

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 antiviral activity of this agent against certain orthopoxviruses. Currently, efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, "VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be released by the CDC after all inventories of VIG have been exhausted, after a patient fails to improve with VIG treatment, or as a last effort for a patient who is otherwise near death." [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated February 21, 2003. Available at URL: <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. This agent will only be provided by the CDC in the occurrence of an emergency public health event. Thus, investigators should obtain cidofovir for

second-line therapy through commercial sources if necessary. NCI/CTEP investigators may use the CDC cidofovir IND protocol as a "guideline" when providing cidofovir for treatment under an off-label use. The CDC will provide their IND protocol for the use of cidofovir related to adverse reactions post vaccinia vaccination to NCI/CTEP for distribution to investigators of NCI-sponsored protocols. The CDC Clinician Information Line at 1-877-554-4625 should still be consulted regarding appropriateness of therapy and guidance.

9.1.11 Precautions (Healthcare workers)

The risk of transmission of recombinant vaccinia viruses 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 for transmission of vaccinia or recombinant vaccinia viruses to such persons, it is suggested that healthcare personnel who are involved with the preparation or administration of doses, or have direct contact with contaminated dressings or other infectious material from participants in clinical studies, should adhere to appropriate infection control measures and be offered vaccination with vaccinia vaccine. Routine, non-emergency vaccination with vaccinia vaccine should not be administered to healthcare workers if any of the following apply to either recipients, 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):

- individuals with active eczema or a history of eczema or atopic dermatitis, or individuals with Darier's disease
- individuals with 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
- individuals who are pregnant or intend on becoming pregnant within 4 weeks of vaccination
- individuals who are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection

Additionally, routine, non-emergency vaccination with vaccinia vaccine should not be administered to healthcare workers if any of the following apply to the vaccinee only:

- individuals with moderate or severe acute illnesses, until the illness resolves
- individuals less than 18 years of age, unless specifically indicated
- individuals who are breast-feeding
- individuals undergoing topical steroid therapy for inflammatory eye diseases or undergoing therapy with systemic steroids due to the potential for immune suppression and increased risk for vaccinia-related complications. Localized topical steroid use and inhaled steroid use may be permissible.

- As a precaution, the CDC has recommended 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 vaccinia vaccine while a possible causal relationship between vaccination and cardiac events is being evaluated.

Healthcare workers with a prior history of allergy or serious reaction to prior vaccinia vaccination or any of its components should not receive vaccinia vaccine. Healthcare workers who are pregnant; who have a history or presence of active eczema or atopic dermatitis; that have acute, chronic or exfoliative skin conditions; or, who are immunocompromised should avoid exposure to the recombinant vaccinia vaccine, and any contaminated dressings, or other infectious materials from patients, or the patient's inoculation site.

For more information on vaccinia precautions for healthcare workers, see the following website: <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm#tab2>.

The CDC is the only source of 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 vaccinia vaccine, contact Drug Services, National Center for Infectious Diseases, CDC at (404) 639-3670.

9.1.12 Special Handling

Vaccinia virus is classified as a Biosafety Level 2 organism (agents that are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available). The recombinant product is a preparation of a live virus affecting humans and contains DNA sequences derived from the human genome. The product should be handled as an infectious hazardous biological substance and waste materials should be disposed of as infectious hazardous biological waste and incinerated according to local institutional policies and according to any local, state, or federal regulations. For more information regarding biohazard risk group classification and biohazard safety levels see NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines): April, 2016. Available at URL: <http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines> and Biosafety in Microbiological and Biomedical Laboratories; 4th Edition. U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Available at URL: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

Biosafety Level 2 General Requirements Related to Vaccinia Virus

At a minimum, the following procedures should be adhered:

1. All dose preparations and procedures (e.g., vortexing) with high potential for creation of aerosols are to be performed in an appropriately certified Class II biological safety cabinet. In general, procedures and guidelines (e.g., minimizing

creation of aerosols during dose preparation; no eating, drinking or applying cosmetics in the work area), and personal protective apparel utilized for preparation of antineoplastic agents [e.g, gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eyewear, hair cover] should be utilized during preparation of recombinant vaccinia products for patient administration.

2. Access to preparation areas should be limited or restricted while dose preparation is in progress.
3. Appropriate infection control measures (e.g, thorough hand washing) should be utilized after handling any materials.
4. All procedures are performed carefully to minimize creation of aerosols.
5. An autoclave for decontaminating waste is available.
6. All contaminated liquid or solid wastes are to be decontaminated prior to disposal according to any local, state, or federal regulations. Contaminated materials that are to be decontaminated at a site away from the preparation area should be placed in a durable leak-proof container prior to being transported.
7. Established policies and procedures are in place whereby only personnel who have been advised of the potential hazards and meet any specific requirements (e.g, immunization) should be allowed entry into areas where product is prepared or agents are stored.
8. A biosafety manual is prepared whereby personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
9. Warning hazard signs should be posted on the access door identifying the agents, the name and phone number of the Principal Investigator or other responsible person, and indicates any special requirements for entry.
10. Only needle-lock syringes and needles should be utilized for preparation. Extreme caution should be used to prevent autoinoculation. Needles should not be bent, sheared, replaced in the needle guard, or removed from the syringe following use. Needles and syringe should be promptly placed in puncture-resistant containers and decontaminated prior to disposal.
11. Spills and accidents resulting in overt exposure to recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/OBA (Office of Biotechnology Activities). Reports should be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838. Medical evaluation, surveillance, and treatment should be provided as appropriate and written records should be maintained.

Preparation and Disposal Procedure Recommendations

- a. All necessary supplies should be on hand prior to beginning the preparation procedure. A detailed worksheet outlining all supplies, dose calculations and preparation procedures should be readily available prior to beginning the preparation procedure.
- b. Agent for dose preparation should be transported from the -70°C freezer to the work

area in leak proof bag.

- c. All dose preparations are to be performed in an appropriately certified Class II biological safety cabinet. In general, procedures and guidelines (e.g., minimizing creation of aerosols during dose preparation; no eating, drinking or applying cosmetics in the work area), and personal protective apparel utilized for preparation of antineoplastic agents [e.g., gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eyewear, hair cover] should be utilized during preparation of recombinant vaccinia products for patient administration.
- d. Prior to dose preparation, all surfaces of the biological safety cabinet should be decontaminated by wiping down with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, then wiped with sterile gauze soaked in 70% alcohol. Manufacturer's recommendations, with respect to disinfectant concentration, contact time and method of application, should be consulted.
- e. An empty biohazard sharps container lined with a leak-proof biohazard bag should be placed in or near the biosafety cabinet to dispose of all waste generated in preparation of the final dose for patient administration.
- f. Dose preparation should be performed using aseptic technique in a sterile barrier field within the biological safety cabinet. Any items to be used for dose preparation should be sprayed or wiped with 70% alcohol prior to being placed in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Caution should be exercised to avoid exposure of the virus to the disinfectants.
- g. The final prepared dose should be sprayed with 70% alcohol prior to removal from the biological safety cabinet and transported in a leak proof bag or container labeled with a biohazard symbol.
- h. All waste should be placed into the biohazard sharps container lined with the leak proof biohazard bag and the biological safety cabinet decontaminated again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, followed by sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in a biohazard safety bag.
- i. Ultimately, all waste contained within the biohazard bags should be placed in a large autoclave bag labeled with biohazard symbols. After a 30-minute steam autoclave sterilization cycle at 121°C, the autoclave bag should be placed in a biohazard sharps container for incineration according to institutional policy and according to any local, state, or federal regulations. If autoclaving is not possible, all waste and protective apparel should be placed in a leak proof biohazard bag, and the bag placed inside a biohazard sharps container for incineration according to institutional policy and according to any local, state, or federal regulations.
- j. Accidental spills should be handled similarly to antineoplastic spills according to institutional policy:
 1. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
 2. Use protective clothing, eyewear, mask, and gloves.

3. Spill should be covered with absorbent towels.
4. Area should be decontaminated with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
5. All waste should be decontaminated prior to disposal and disposed of as biohazardous waste by incineration according to institutional policy and according to any local, state, or federal regulations.

9.1.13 Patient Care Implications and Contraindications

A sterile dry dressing (e.g., Telfa pad) should cover vaccination sites and patients should receive instruction regarding proper hand-hygiene, sterile dressing care, bathing, laundering of clothing, etc. Patient bandages or dressings removed from the vaccination site should be treated as infectious waste and disposed of in appropriate biohazard containers. The recombinant vaccinia vaccine should not be administered if any of the following apply to either recipients, or for at least three weeks after vaccination (i.e., until the scab has separated from the skin and the underlying skin has healed), their close household contacts (close household contacts are those who share housing or have close physical contact):

- Individuals with active eczema or a history of eczema or atopic dermatitis.
- Individuals with Darier's disease.
- Individuals with 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.
- Individuals who are pregnant or intend on becoming pregnant (due to the potential risk of fetal vaccinia). 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.
- Individuals in close contact with children less than 3 years of age (due to the potential risk of contact transmission and inadvertent inoculation).
- Individuals who are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection

Additionally, the recombinant vaccinia vaccine should not be administered if any of the following apply to vaccinees only:

- Individuals with moderate or severe acute illnesses, until the illness resolves.
- Individuals who 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.
- Individuals undergoing topical steroid therapy for inflammatory eye diseases, or undergoing therapy with systemic steroids due to the potential for immune suppression and increased risk for vaccinia-related complications. Localized topical steroid use and inhaled steroid use may be permissible.
- At this time, until a more definitive causal relationship is determined, it is

recommended that patients with known CHF or clinically significant cardiomyopathy should 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 Dryvax® used in the Smallpox Vaccination Program causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends that individuals with underlying heart disease be excluded from participation in the current Smallpox Vaccination Program. Because patients are being immunized with recombinant vaccinia vaccines with therapeutic intent, it is currently not recommended to exclude patients with known CAD, previous heart attack, history of angina, or other evidence of risk factors for coronary artery disease who are otherwise eligible for the study, but patients should be informed of the potential risks and the patient's cardiac disease should be controlled.

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. Patients with a prior history of allergy or serious reaction to prior vaccinia vaccination (e.g., smallpox vaccination) should not receive the recombinant vaccinia product.

9.2 PANVAC™-F (NSC 727027)

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

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

9.2.3 **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 asparagines 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.

9.2.4 How Supplied:

Lot: 4-060503: PANVAC-F is supplied in vials containing 0.3 mL of the vaccine at a final viral concentration titer of 6.6×10^9 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol (total vial contents = 1.98×10^9 pfu's).

Lot: 3-052203: PANVAC-F is supplied 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).

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

9.2.5 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. If necessary, 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.**

Preparation Instructions for PANVAC-F, Lot 4-060503 (6.6 x 10⁹ 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 0.16mL (1×10^9 pfu) from the thawed vial for subcutaneous injection.

Preparation Instructions for PANVAC-F, Lot 3-052203 (5.8 x 10⁹ 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.

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

9.2.7 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 four days when stored at 2-8°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). Prepared doses may be stored at 2-8°C for up to four hours following preparation.

9.2.8 Route of Administration: PANVAC™ – F is administered by subcutaneous injection.

9.2.9 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 eyewear, 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 procedure, 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 -70°C freezer to the work area in a 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 disinfectant.
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 contained 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 the 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, or 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

For more information about biohazardous risk group classification and biohazard safety levels see NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines): April, 2016 (<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>) and biosafety in Microbiological and Biomedical Laboratories; 4th Edition. U.S. Department of Health and Human Services Center for Disease Control and Prevention and National Institutes of Health ([Http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm)).

9.2.10 Patient Care Implications and Contraindications

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

9.2.11 Agent Procurement

9.2.11.1 Availability

PANVAC™-V (NSC 727026) and PANVAC™-F (NSC 727027) are manufactured by Therion Biologics Corporation and supplied by the Pharmaceutical Management Branch, CTEP, DCTD, NCI.

9.2.11.2 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.

Agent may be requested by completing a Clinical Drug Request (NIH-986) and mailing it to the Drug Management and Authorization Section, PMB, DCTD, NCI, 9000 Rockville Pike, EPN Room 7149, Bethesda, MD 20892-7422 or faxing it to (301) 480-4612. For questions call (301) 496-5725.

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 (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

9.3 Sargramostim (GM-CSF, LEUKINE®)

9.3.1 Product Description

Refer to the FDA-approved package insert for complete product information. Sargramostim is a recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) produced by recombinant DNA technology in yeast (*Saccharomyces cerevisiae*). Sargramostim is a 127 amino acid glycoprotein, altered from the native, natural human GM-CSF molecule; the position 23 arginine has been replaced with a leucine to facilitate the expression of the protein in yeast.

9.3.2 How supplied

Sargramostim used in this protocol is a commercially available drug, manufactured by Berlex Laboratories and will be purchased from commercial sources. Sargramostim is available as a sterile, preserved injectable solution in a 500- μ g multidose liquid vial.

9.3.3 Formulation and Preparation:

LEUKINE Liquid (Sargramostim, GM-CSF) is formulated as a sterile, preserved (containing 1.1% benzyl alcohol) solution containing 500 mcg/mL, 1mL per vial. Lyophilized LEUKINE is formulated as a sterile, white preservative-free powder containing 250 mcg per vial. Each mL of preserved solution (LEUKINE Liquid) and reconstituted Lyophilized LEUKINE contains 40 mg/mL mannitol, USP, 10 mg/mL sucrose, NF, and 1.2 mg/mL tromethamine, USP.

Reconstitute each 250 mcg vial of lyophilized LEUKINE with 1 mL of Sterile Water for Injection, USP or 1 mL of Bacteriostatic Sterile Water for Injection, USP containing 0.9% benzyl alcohol to yield a 250 mcg/mL solution. The diluent should be directed against the side of the vial to avoid excess foaming. Avoid vigorous agitation of the vial; do not shake.

9.3.4 Stability and Storage:

LEUKINE Liquid and Lyophilized LEUKINE should be stored refrigerated at 2-8°C. Each vial bears an expiration date. LEUKINE Liquid may be stored for up to 20 days at 2-8°C once the vial has been entered. Any remaining solution should be discarded after 20 days. Lyophilized LEUKINE reconstituted with Sterile Water for Injection, USP should be discarded within 6 hours of preparation. Lyophilized LEUKINE reconstituted with Bacteriostatic Sterile Water for Injection, USP (containing 0.9% benzyl alcohol) may be stored for up to 20 days at 2-8°C once the vial has been reconstituted. Any remaining solution should be discarded after 20 days.

9.3.5 Administration procedures

Route of administration: Sargramostim will be administered subcutaneously in a dose of 100 μ g/day for 4 days starting on the day of the vaccination. This will be done in one site which will be marked with a pen to identify. Subsequent sargramostim injections will be given subcutaneously in that site.

9.3.6 Adverse Events, Contraindications and/or Toxicities

Toxicities described in patients receiving sargramostim include: fever, chills, diaphoresis, myalgia, fatigue, malaise, headache, dizziness, dyspnea, bronchospasm, pleural effusion,

Abbreviated Title: Vaccine for metastatic cancer

Version Date: 05/04/2017

CTEP #: 6536

anorexia, indigestion, nausea, vomiting, diarrhea, injection site tenderness, urticaria, pruritus, hypersensitivity reaction, bone pain, thromboembolic events, phlebitis, hypotension, peripheral edema, leukocytosis, thrombocytosis, or thrombocytopenia, hepatic enzyme abnormalities, and bilirubin elevation. The first administration of sargramostim has provoked a syndrome of dyspnea and hypotension within two hours after sargramostim injection in a single patient received yeast sargramostim; this type of reaction has more been observed in patients receiving sargramostim produced in *E. coli*. One report of vascular leak syndrome occurring after autologous bone marrow transplant in a patient receiving continuous IV infusion of sargramostim has been recorded. All these toxicities were seen at much higher dose than what the patients will be receiving on this protocol, as explained above.

10 REFERENCES

Reference List

- (1) Scheithauer W, Kornek GV, Brugger S, Ullrich-Pur H, Valencak J, Raderer M et al. Randomized phase II study of irinotecan plus mitomycin C vs. oxaliplatin plus mitomycin C in patients with advanced fluoropyrimidine/leucovorin-pretreated colorectal cancer. *Cancer Invest* 2002; 20(1):60-68.
- (2) Falcone A, Masi G, Allegrini G, Danesi R, Pfanner E, Brunetti IM et al. Biweekly chemotherapy with oxaliplatin, irinotecan, infusional Fluorouracil, and leucovorin: a pilot study in patients with metastatic colorectal cancer. *J Clin Oncol* 2002; 20(19):4006-4014.
- (3) Carlonagno C, Lauria R, De Laurentiis M, Arpino G, Massarelli E, Ferrara C et al. Second-line chemotherapy with a hybrid-alternating regimen of bolus 5FU modulated by methotrexate and infusional 5FU modulated by folinic acid in patients with metastatic colorectal cancer pretreated with 5FU. A phase 2 study. *Oncology* 2002; 63(3):219-225.
- (4) Hegewisch-Becker S, Gruber Y, Corovic A, Pichlmeier U, Atanackovic D, Nierhaus A et al. Whole-body hyperthermia (41.8 degrees C) combined with bimonthly oxaliplatin, high-dose leucovorin and 5-fluorouracil 48-hour continuous infusion in pretreated metastatic colorectal cancer: a phase II study. *Ann Oncol* 2002; 13(8):1197-1204.
- (5) Chau I, Webb A, Cunningham D, Hill M, Waters JS, Norman A et al. Oxaliplatin and protracted venous infusion of 5-fluorouracil in patients with advanced or relapsed 5-fluorouracil pretreated colorectal cancer. *Br J Cancer* 2001; 85(9):1258-1264.
- (6) Hodge JW. Carcinoembryonic antigen as a target for cancer vaccines. *Cancer Immunology Immunotherapy* 1996; 43(3):127-134.
- (7) Foon KA, Chakraborty M, John WJ, Sherratt A, Kohler H, Bhattacharyachatterjee M. Immune-Response to the Carcinoembryonic Antigen in Patients Treated with An Antiidiotype Antibody Vaccine. *J Clin Invest* 1995; 96(1):334-342.
- (8) Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J et al. Molecular-Cloning and Expression of Human Tumor-Associated Polymorphic Epithelial Mucin. *Journal of Biological Chemistry* 1990; 265(25):15286-15293.
- (9) Blank M, Klussmann E, Kruger-Krasagakes S, Schmitt-Graff A, Stolte M, Bornhoeft G et al. Expression of Muc2-Mucin in Colorectal Adenomas and Carcinomas of Different Histological Types. *International Journal of Cancer* 1994; 59(3):301-306.
- (10) Ajioka Y, Watanabe H, Jass JR. MUC1 and MUC2 mucins in flat and polypoid colorectal adenomas. *Journal of Clinical Pathology* 1997; 50(5):417-421.
- (11) Biemer-Huttmann AE, Walsh MD, McGuckin MA, Ajioka Y, Watanabe H, Leggett BA et al. Immunohistochemical staining patterns of MUC1, MUC2, MUC4, and MUC5AC mucins in hyperplastic polyps, serrated adenomas, and traditional adenomas of the colorectum. *Journal of Histochemistry & Cytochemistry* 1999; 47(8):1039-1047.
- (12) Ajioka Y, Xing PX, Hinoda Y, Jass JR. Correlative histochemical study providing evidence for the dual nature of human colorectal cancer mucin. *Histochemical Journal* 1997; 29(2):143-152.
- (13) Hodge JW, Schlom J, Donohue SJ, Tomaszewski JE, Wheeler CW, Levine BS et al. A recombinant

vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer* 1995; 63(2):231-237.

(14) Morrissey PJ, Bressler L, Park LS, Alpert A, Gillis S. Granulocyte-Macrophage Colony-Stimulating Factor Augments the Primary Antibody-Response by Enhancing the Function of Antigen- Presenting Cells. *Journal of Immunology* 1987; 139(4):1113-1119.

(15) Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K et al. Vaccination with Irradiated Tumor-Cells Engineered to Secrete Murine Granulocyte-Macrophage Colony-Stimulating Factor Stimulates Potent, Specific, and Long-Lasting Antitumor Immunity. *Proceedings of the National Academy of Sciences of the United States of America* 1993; 90(8):3539-3543.

(16) Vieweg J, Rosenthal FM, Bannerji R, Heston WDW, Fair WR, Gansbacher B et al. Immunotherapy of Prostate-Cancer in the Dunning Rat Model - Use of Cytokine Gene Modified Tumor Vaccines. *Cancer Research* 1994; 54(7):1760-1765.

(17) McLaughlin JP, Abrams S, Kantor J, Dobrzanski MJ, Greenbaum J, Schlom J et al. Immunization with a syngeneic tumor infected with recombinant vaccinia virus expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) induces tumor regression and long-lasting systemic immunity. *J Immunother* 1997; 20(6):449-459.

(18) Taylor J, Meignier B, Tartaglia J, Languet B, Vanderhoeven J, Franchini G et al. Biological and Immunogenic Properties of A Canarypox-Rabies Recombinant, Alvac Rg (Vcp65) in Non-Avian Species. *Vaccine* 1995; 13(6):539-549.

(19) Kass E, Schlom J, Thompson J, Guadagni F, Graziano P, Greiner JW. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. *Cancer Res* 1999; 59(3):676-683.

(20) Aoki R, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G et al. MUC-1 expression as a predictor of the curative endoscopic treatment of submucosally invasive colorectal carcinoma. *Diseases of the Colon & Rectum* 1998; 41(10):1262-1272.

(21) Scholl SM, Balloul JM, Le Goc G, Bizouarne N, Schatz C, Kieny MP et al. Recombinant vaccinia virus encoding human MUC1 and IL2 as immunotherapy in patients with breast cancer. *Journal of Immunotherapy* 2000; 23(5):570-580.

(22) Schlom J, Panicali D. Recombinant poxvirus vaccines. In: Rosenberg SA, editor. *Biologic Therapy of Cancer: Principles and Practices*. Philadelphia: J.B. Lippincott Co, 2000: 686-694.

(23) Hodge JW, Schlom J, Donohue SJ, Tomaszewski JE, Wheeler CW, Levine BS et al. A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer* 1995; 63(2):231-237.

(24) Mukherjee P, Ginardi AR, Madsen CS, Sterner CJ, Adriance MC, Tevethia MJ et al. Mice with spontaneous pancreatic cancer naturally develop MUC- 1-specific CTLs that eradicate tumors when adoptively transferred. *Journal of Immunology* 2000; 165(6):3451-3460.

(25) Salazar E, Zaremba S, Arlen PM, Tsang KY, Schlom J. Agonist peptide from a cytotoxic T-lymphocyte epitope of human carcinoembryonic antigen stimulates production of TC1-type cytokines and increases tyrosine phosphorylation more efficiently than cognate peptide. *International Journal of Cancer* 2000; 85(6):829-838.

- (26) Zaremba S, Barzaga E, Zhu M, Soares N, Tsang KY, Schlom J. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 1997; 57(20):4570-4577.
- (27) Grosenbach DW, Barrientos JC, Schlom J, Hodge JW. Synergy of vaccine strategies to amplify antigen-specific immune responses and antitumor effects. *Cancer Res* 2001; 61(11):4497-4505.
- (28) Hiraga Y, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G et al. Immunoreactive MUC1 expression at the deepest invasive portion correlates with prognosis of colorectal cancer. *Oncology* 1998; 55(4):307-319.
- (29) Zotter S, Hageman P, Lossnitzer A, Mooi W, Higers J. Tissue and tumor distribution of human polymorphic epithelial mucin. *Cancer in Review* 1988; 11:55-101.
- (30) Yonezawa S, Sato E. Expression of mucin antigens in human cancers and its relationship with malignancy potential. *Pathology International* 1997; 47(12):813-830.
- (31) Ramanathan R, Lee K, Mckolanis J, Hiltbold E, Dyky M, Alvelo-Rivera M et al. Phase 1 Study of a MUC-1 Synthetic Vaccine Admixed with SB-AS2 Adjuvant in Resected and Locally Advanced Pancreatic Cancer. ASCO Abstract No 1791 2000.
- (32) Hodge JW, McLaughlin JP, Kantor JA, Schlom J. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. *Vaccine* 1997; 15(6-7):759-768.
- (33) Hodge JW, Grosenbach DW, Rad AN, Giuliano M, Sabzevari H, Schlom J. Enhancing the potency of peptide-pulsed antigen presenting cells by vector-driven hyperexpression of a triad of costimulatory molecules. *Vaccine* 2001; 19(25-26):3552-3567.
- (34) Scheithauer W, Kornek GV, Schuell B, Ulrich-Pur H, Penz M, Raderer M et al. Second-line treatment with oxaliplatin + raltitrexed in patients with advanced colorectal cancer failing fluoropyrimidine/leucovorin-based chemotherapy. *Ann Oncol* 2001; 12(5):709-714.
- (35) Tsang K, Palena C, Gulley J, Arlen P, Schlom J. Identification and characterization of a human cytotoxic T-lymphocyte epitope and agonist epitope from the non-variable number of tandem repeat sequence of MUC-1 tumor antigen. *Clin Cancer Res*. In press.
- (36) Hodge JW, Grosenbach DW, Rad AN, Giuliano M, Sabzevari H, Schlom J. Enhancing the potency of peptide-pulsed antigen presenting cells by vector-driven hyperexpression of a triad of costimulatory molecules. *Vaccine* 2001; 19(25-26):3552-3567.
- (37) Zhu MZ, Terasawa H, Gulley J, Panicali D, Arlen P, Schlom J et al. Enhanced activation of human T cells via avipox vector-mediated hyperexpression of a triad of costimulatory molecules in human dendritic cells. *Cancer Research* 2001; 61(9):3725-3734.
- (38) Oh S, Hodge JW, Ahlers JD, Burke DS, Schlom J, Berzofsky JA. Selective induction of high avidity CTL by altering the balance of signals from APC. *Journal of Immunology* 2003; 170(5):2523-2530.
- (39) Kass E, Parker J, Schlom J, Greiner JW. Comparative studies of the effects of recombinant GM-CSF and GM-CSF administered via a poxvirus to enhance the concentration of antigen-presenting cells in regional lymph nodes. *Cytokine* 2000; 12(7):960-971.

- (40) Moss B. Replicating and host-restricted non-replicating vaccinia virus vectors for vaccine development. *Developments in Biological Standardization* 1994; 82:55-63.
- (41) Aarts WM, Schlom J, Hodge JW. Vector-based vaccine/cytokine combination therapy to enhance induction of immune responses to a self-antigen and antitumor activity. *Cancer Res* 2002; 62(20):5770-5777.
- (42) Greiner JW, Zeytin H, Anver MR, Schlom J. Vaccine-based therapy directed against carcinoembryonic antigen demonstrates antitumor activity on spontaneous intestinal tumors in the absence of autoimmunity. *Cancer Research* 2002; 62(23):6944-6951.
- (43) Grosenbach DW, Barrientos JC, Schlom J, Hodge JW. Synergy of vaccine strategies to amplify antigen-specific immune responses and antitumor effects. *Cancer Res* 2001; 61(11):4497-4505.
- (44) Aarts WM, Schlom J, Hodge JW. Vector-based vaccine/cytokine combination therapy to enhance induction of immune responses to a self-antigen and antitumor activity. *Cancer Res* 2002; 62(20):5770-5777.
- (45) Kass E, Panicali DL, Mazzara G, Schlom J, Greiner JW. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res* 2001; 61(1):206-214.
- (46) Hodge JW, Grosenbach DW, Aarts WM, Poole DJ, Schlom J. Vaccine Therapy of Established Tumors in the Absence of Autoimmunity. *Clinical Cancer Research* 2003; 9(5):1837-1849.
- (47) Scheithauer W, Kornek GV, Schuell B, Ulrich-Pur H, Penz M, Raderer M et al. Second-line treatment with oxaliplatin + raltitrexed in patients with advanced colorectal cancer failing fluoropyrimidine/leucovorin-based chemotherapy. *Ann Oncol* 2001; 12(5):709-714.
- (48) Marshall JL, Hawkins MJ, Tsang KY, Richmond E, Pedicano JE, Zhu MZ et al. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 1999; 17(1):332-337.
- (49) Marshall JL, Hoyer RJ, Toomey MA, Faraguna K, Chang P, Richmond E et al. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 2000; 18(23):3964-3973.
- (50) Zhu MZ, Marshall J, Cole D, Schlom J, Tsang KY. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. *Clin Cancer Res* 2000; 6(1):24-33.
- (51) Arlen P, Tsang KY, Marshall JL, Chen A, Steinberg SM, Poole D et al. The use of a rapid ELISPOT assay to analyze peptide-specific immune responses in carcinoma patients to peptide vs. recombinant poxvirus vaccines. *Cancer Immunol Immunother* 2000; 49(10):517-529.
- (52) von Mehren M, Arlen P, Tsang KY, Rogatko A, Meropol N, Cooper HS et al. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin Cancer Res* 2000; 6(6):2219-2228.
- (53) von Mehren M, Arlen P, Gulley J, Rogatko A, Cooper HS, Meropol NJ et al. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 2001; 7(5):1181-1191.
- (54) Moss B. Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety.

Abbreviated Title: Vaccine for metastatic cancer

Version Date: 05/04/2017

CTEP #: 6536

Proceedings of the National Academy of Sciences of the United States of America 1996; 93(21):11341-11348.

- (55) Taylor P, Weinberb R, Langue B. Fowlpox virus based recombinant vaccines. Advances in Vaccine Development 1988; 33:321-324.
- (56) Somogyi P, Frazier J, Skinner MA. Fowlpox Virus Host-Range Restriction - Gene-Expression, Dna-Replication, and Morphogenesis in Nonpermissive Mammalian-Cells. Virology 1993; 197(1):439-444.
- (57) Tsang KY, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Schlom J. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. J Natl Cancer Inst 1995; 87(13):982-990.
- (58) Hodge JW, McLaughlin JP, Kantor JA, Schlom J. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. Vaccine 1997; 15(6-7):759-768.
- (59) Zhu MZ, Marshall J, Cole D, Schlom J, Tsang KY. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. Clin Cancer Res 2000; 6(1):24-33.
- (60) Nakamori S, Ota DM, Cleary KR, Shirotani K, Irimura T. Muc1 Mucin Expression As A Marker of Progression and Metastasis of Human Colorectal-Carcinoma. Gastroenterology 1994; 106(2):353-361.
- (61) Marshall JL, Hawkins MJ, Tsang KY, Richmond E, Pedicano JE, Zhu MZ et al. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. J Clin Oncol 1999; 17(1):332-337.

11 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

12 APPENDIX B: INSTRUCTIONS FOR CARE OF THE VACCINE

Appendix B

Instructions for Care of the Vaccine Site

1. What vaccination site reactions can you expect?
2. How should you care for the vaccination site?
3. How should I dispose of the used needle and syringe?
4. Are there any activities I should avoid?
5. What about contact with other people?
6. Whom do I contact when I have a question?

1. What vaccination site reactions can you expect?

In patients who have never received vaccinia intradermally (under the skin), or in some who received it a very long time ago, a red swelling may occur followed by a blister 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.

Patients in this study will be receiving injections subcutaneously in their arm or leg. Based on your previous exposure, most patients will have little skin reaction, except that the arm or leg that received the vaccine may be swollen. Swollen or tender lymph nodes ("glands") in the armpit may also 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 two weeks while the scab is forming. If you have any aches or fever, you can take acetaminophen ("Tylenol"), but should avoid aspirin. If fever continues for more than a day or two, or exceeds 102°F you should call to speak to the clinic nurse or the research nurse.

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 (until your next inoculation). 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 with physical contact with you is the best way to prevent transmission of the virus. While you are receiving care in the Outpatient Cancer Center for your first cycle, you will be placed in a private room. You should also use two types of barriers over your vaccination site **at all times until the scab has fallen off**. These barriers are (1) the bandage and (2) long sleeves if the vaccination site is in your arm, or long pants (including nightclothes) if the site is in your leg. For dressing care you will receive some no-stick dressings, disposable gloves, and zip-lock plastic bags. If you should run out of supplies between visits, you can obtain some from your local store. If your clothes become soiled, please remove them and wash in hot soapy water with bleach.

The no-stick 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 disposable cloth, soap, and water. If you do wash, blot the site dry with a disposable soft towel (don't rub), then dispose of the washcloth and the towel in a zip lock bag. **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. How should I dispose of the used needle and syringe?

The needle and syringe that you use to inject GM-CSF into the vaccine site should be discarded in a closed container. Use a sturdy empty container with a secure lid. **DO NOT RECAP THE NEEDLE.**

After you inject the GM-CSF, drop the syringe AS IS in the bottle. Pour a small amount of bleach in the bottle (about 2 inches deep) and close securely. You may return the bottle on your next visit to the clinic and we will dispose it for you.

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

You should not go swimming or bathing (bath tub, hot tub, etc.) until the vaccination site has healed and you no longer need to wear a bandage on it. If you wear contact lenses,

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.

5. What about contact with other people?

Because you may "shed" live virus for several days after vaccination, you must be able to avoid close contact with certain individuals for at least two weeks after vaccination. These individuals include children <3 years of age; women who are pregnant or breast-feeding; individuals with eczema, a history of eczema or other skin conditions such as active cases of extensive psoriasis, exfoliative skin diseases, 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 HIV positive blood test, or those receiving immunosuppressive treatment. "Close contact" means that these people share your house, you have repeated bodily contact with them, and/or you take care of them and touch them with your hands.

6. Whom 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.

PHONE NUMBERS FOR NIH PATIENTS

3 South East Outpatient Cancer Center	(301) 451-1152
On-Call physician	(301) 496-1211

13 APPENDIX C: TREATMENT AND MONITORING SCHEDULE: PRE, DURING AND POST THERAPY

	Baseline ¹	Day 15	Day 29	Day 43	Day 71	Every 28 days on Extension Phase	Every 3 months on Maintenance Phase
History and Physical ¹	X						
NIH Advanced Directives Form ¹²	X						
Medical Assessments ²	X	X	X	X	X	X	X ¹⁰
Imaging studies ³	X				X	Every 2 months	Every 3 months ¹¹
Serum CEA & CA-125	X		X		X	X	X ¹⁰
Urinalysis	X	X	X	X	X	X	X ¹⁰
Serum HIV antibody ⁴	X						
Serum Hepatitis B & C ⁵	X						
CBC with differential	X	X	X	X	X	X	X ¹⁰
Chemistry ⁵	X	X	X	X	X	X	X ¹⁰
PT, PTT	X						
EKG	X				X		
Apheresis ⁶	X				X		
Immunology (blood) ⁷	X	X	X	X	X	X	X ¹⁰
ANA titer, CD3, 4, 8 subsets and CD4:CD8 ratio ⁷	X				X		
HLA typing	X						
Serum Beta-HCG ⁸	X						

¹ Baseline: H & P and laboratory studies should be completed within 16 days of initiating treatment. Baseline radiographic and immunologic studies should be obtained within 28 days of initiating treatment.

² Medical assessments: interim history (since last visit), vital signs, physical examination and ECOG performance status.

Abbreviated Title: Vaccine for metastatic cancer

Version Date: 05/04/2017

CTEP #: 6536

³ Radiologic studies consisting of CT chest/abdomen/pelvis or MRI will be performed within 28 days prior to initiating treatment.

⁴ Serum HIV antibody should be completed within 8 weeks of initiating treatment.

⁵ Serum Hepatitis B & C antibody should be completed within 8 weeks of initiating treatment

⁶ Chemistry panel: Na⁺, K⁺, Cl⁻, CO₂, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, and LDH.

⁷ Apheresis will be requested for immunologic testing from patients at baseline and approximately day 71.

⁸ Blood will be obtained for immunologic assays including ELISPOT assay. Research blood will be drawn prior to each vaccination (at baseline and about days 15, 29, 43, 71) then every 28 days on the extension phase. ANA titer, CD3, 4, 8 subsets and CD4:CD8 ratio will be drawn at baseline and about day 71.

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

¹⁰ Medical assessment, clinical labs, and research labs will be performed monthly. For patients on the Maintenance phase, these assessments will occur at least with every 3-month vaccination visit.

¹¹ Imaging studies will be performed for patients on the Maintenance phase who show no radiographic evidence of disease, at the clinical discretion of the PI with patient input, at least every 12 months, if the patient is clinically stable

¹² As indicated in section **6.3**, all subjects \geq age 18 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.

14 APPENDIX D: RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST) QUICK REFERENCE

Eligibility

- Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter ≥ 20 mm using conventional techniques or ≥ 10 mm with spiral CT scan.

Non-measurable lesions - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

- All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.
- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.
- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.
- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline documentation of “Target” and “Non-Target” lesions

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs should be identified as target lesions and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).
- A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.
 - All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of target lesions

- * Complete Response (CR): Disappearance of all target lesions
- * Partial Response (PR): At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
- * Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
- * Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of non-target lesions

- * Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
- * Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
- * Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1)

- (1) Although a clear progression of “non target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of best overall response

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

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

Duration of overall response

- The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.
- The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response review

- For trials where the response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

Reporting of results

- 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. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).
- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.
- 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 violations, 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 be provided.

15 APPENDIX E: ELISPOT ASSAY

We plan to examine the immune response in selected patients (HLA-A2 positive) from each arm. Lymphocytes will be separated from heparinized blood using density gradient centrifugation. The lymphocytes will then be placed in human AB serum with 10% DMSO and stored in liquid nitrogen. When samples are available from pre and post-treatment, the ELISOT assay will be performed. The ELISPOT assay, measuring γ -IFN production, is used for determination of CTL precursor frequency to CAP-1 6D peptide (ALWGQDVTSV) and an HLA-A2 restricted MUC-1 peptide (ALWGQDVTSV) in both pre and post-vaccination peripheral blood mononuclear cells (PBMC).⁽⁵⁵⁾ Briefly, 96-well milliliter HA plates (Millipore Corporation, Bedford, MA) are coated with 100 μ l/well of capture MAb against human γ -IFN at a concentration of 10 μ g/ml for 12h at RT. Plates are blocked for 30 min with RPMI 1640 plus 10% human Ab serum. 2 x 10⁵ PBMC are added to each well. CAP-1 6D or MUC-1 peptide pulsed C1R-A2 cells are added into each well as antigen presenting cells (APC) at an effector:APC ratio of 1:1. Unpulsed C1R-A2 cells are used as a negative control. HLA-A2 binding Flu matrix peptide 59-66 is used as a positive peptide control.⁽⁵⁶⁾ We also perform each sample with six replicates to control for variability. In addition, each sample is run with a flu peptide control (pre and post vaccine) as well as samples from a “normal” control HLA-A2+ individual with previously determined levels of flu-specific T cell precursors. Cells are incubated for 24h and lysed with phosphate buffered saline (PBS)-Tween (.05%). Biotinylated anti γ -IFN antibody diluted to 2 μ g/ml in PBS-Tween containing 1% bovine serum albumin (BSA) is added and incubated overnight in 5% CO₂ at 37°C. Plates are washed 3 times and developed with avidin alkaline phosphatase (GIBCO/BRL, Grand Island, NY) for 45 min. After washing the plates 3 times, each well is examined for positive dots. This assay will be performed in the Laboratory of Tumor Immunology and Biology, NCI, NIH. The number of dots in each well will be counted by two separate investigators in a blinded manner, and the frequency of responding cells is determined. It is planned that all patients will undergo exploratory analysis of the ability to detect CD4 positive responses using a whole protein CEA assay.

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient
-----------------------	---

INSTITUTE: National Cancer Institute

STUDY NUMBER: 04-C-0246 PRINCIPAL INVESTIGATOR: James Gulley, M.D., PhD.

STUDY TITLE: An Open Label Pilot Study to Evaluate the Safety and Tolerability of PANVAC™-V (Vaccinia) and PANVAC™-F (Fowlpox) in Combination with Sargramostim in Adults with Metastatic Carcinoma

Continuing Review Approved by the IRB on 02/06/17

Amendment Approved by the IRB on 06/29/17 (U)

Standard

Date Posted to Web: 07/11/17

Introduction

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled.

However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Why is this study being done?

The purpose of this study is to see the effects of specific vaccines aimed at your cancer. In this study, the safety and side effects of using specific vaccines will be evaluated. This research is being done because studies in the laboratory using human tumor cells grown in culture dishes and studies on animals have shown that vaccines may be effective in killing cancer cells.

PATIENT IDENTIFICATION	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient NIH-2514-1 (4-97) P.A.: 09-25-0099 File in Section 4: Protocol Consent (1)
------------------------	---

STUDY NUMBER: 04-C-0246

CONTINUATION: page 2 of 18 pages

One way in which your body can fight disease is with its own immune system. Your immune system can recognize certain proteins, e.g. bacterial or viruses as foreign and eliminate them from your body. For unknown reasons however, the immune system fails to fully recognize proteins made by cancer cells. This may be one reason why cancer may grow or spread. Two proteins in particular are usually produced by many cancers and may be used as a target for your immune system to attack the cancer. These proteins are carcinoembryonic antigen (commonly known by the initials CEA) and mucin-1 (known as MUC-1). We have developed an experimental vaccine in which we place the genes for CEA and MUC-1 inside a virus vaccine in order for your body to recognize these proteins as "foreign" invaders. If your immune system responds to this invasion, your tumor may become susceptible to your body's immune system. In this study, we are attempting to stimulate your body's immune system to recognize and destroy the tumor cells that produce CEA or MUC-1. This is a vaccination, in concept, like other vaccinations you have probably received, except that it is attempting to increase your body's ability to specifically reject cancer cells. In this study, you will be vaccinated against the CEA and MUC-1 proteins that your tumor contains. These proteins are commonly present in your type of tumor. Your tumor will be tested to confirm the presence of one or both of these proteins. All of this is an attempt to help your immune system fight the cancer.

The vaccine consists of 3 parts. Part 1 is a modified vaccinia virus, termed PANVAC-V. Vaccinia is the same virus that has been used for many years to vaccinate against smallpox. We will refer to PANVAC-V as the "priming vaccination." This priming vaccine contains three kinds of human genetic material (DNA) that have been put inside of the vaccinia virus. The first two types of DNA produce the CEA and MUC-1 proteins and are designed to focus the body's immune response on CEA and MUC-1 found in your tumor. The next type of DNA produces three other proteins (B7-1, ICAM-1, LFA-3), which help increase an immune cells ability to destroy its target. Part 2 is a similar virus, called the fowlpox virus. This virus also contains the same DNA genes as the vaccinia virus and is termed PANVAC-F. We will refer to this as the "boosting vaccination." These 2 vaccines are experimental agents. Part 3 is a protein that boosts the immune system, called granulocyte-macrophage colony stimulating factor (known as sargramostim). Sargramostim will be used to try to increase the usefulness of the vaccine by increasing the number of immune cells at the vaccination site.

This study is therefore undertaken to gain further knowledge regarding the interaction of vaccines in patients that have CEA or MUC-1 producing metastatic cancer. The main focus of this study will be on the safety and tolerance of the vaccines.

Why are you being asked to take part in this study?

You have been asked to take part in this research study because you have metastatic cancer that has not been controlled by standard treatments.

MEDICAL RECORD

CONTINUATION SHEET for either:
NIH 2514-1, Consent to Participate in A Clinical Research Study
NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: 04-C-0246

CONTINUATION: page 3 of 18 pages

How many people will take part in this study?

Up to 51 patients will be enrolled in this study.

Description of research study

All patients in this study have CEA or MUC-1 producing metastatic cancers or have tumors (breast cancer or ovarian cancer) known to express MUC-1 or CEA in more than 90% of cases. The study is divided into 2 phases; the core phase and the extension phase. The core phase is the first part of the study. All patients in the study will start in the core phase where they will receive vaccine and sargramostim injections at a set schedule. Sargramostim is also known as GM-CSF for “granulocyte-macrophage stimulating factor. Patients will be monitored closely during this phase for any side effects from their treatment as well as for determining the status of their cancer. At the completion of the core phase, patients who have not had major side effects from the vaccine and who have not had worsening of their cancer will have the option to continue on an extension phase of the trial. The extension phase will be your choice if you are eligible. In the extension phase, patients will receive vaccine and sargramostim injections monthly for a year. Details of the study are provided below.

What will happen if you take part in this research study?**Before you begin the study**

Before starting this study you will be checked into the outpatient clinic to see if you qualify. The overall evaluation time may take several days or several weeks, depending on your situation. To find the extent of your cancer, scans will be done within 4 weeks of the start of the study. The scans include computerized tomographic (CT) scans or magnetic resonance imaging (MRI) of the chest, abdomen and pelvis. To qualify for this study and to receive the vaccine, you must not have a history of allergy to eggs or to egg products. You must not have had your spleen removed for any reason. Blood tests will be done to be sure that your immune system is normal. The immune system is made up of certain blood cells, lymph nodes (glands), the spleen and other parts of the body and protects the body against foreign substances like bacteria and possibly certain tumor cells. One of the tests of your immune system will be a blood test for the human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS). For the HIV blood test, you will need to sign a separate consent form, which explains how you will be notified if the HIV test is positive. Another test that will be performed is to look for possible infection with Hepatitis B and C. If either of these tests are positive you will not be eligible to participate in this study because of the potential harm the vaccine may cause in patients who test positive for HIV and Hepatitis B and C. Additional blood tests will check to see if your liver, kidneys, and other organs are working well enough to be safe to start the treatment. Women of childbearing potential will have a pregnancy test that must be negative before entering the study. In addition, your blood will also be tested for the CEA marker that is produced by most colorectal cancers. Altogether these initial tests will require about 100 cc (6-7 tablespoonfuls) of

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:

NIH-2514-1 (10-84)

NIH-2514-2 (10-84)

P.A.: 09-25-0099

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 4 of 18 pages

blood. In order to qualify for the study your blood should be positive for the CEA marker or the tumor should show evidence of CEA or MUC-1.

You will have blood drawn from a vein at almost every clinic visit. The amount of blood drawn may vary, but will usually be approximately 4-7 tablespoons. The blood tests will be drawn approximately every 2 weeks to monitor your liver, kidneys, and other organs (as previously mentioned above).

During the study

Core Phase: On day 1 of the study you will receive the priming vaccination. This vaccination will be given as an injection under your skin. You will return in 2 weeks to receive a different vaccine, the boosting vaccine. This vaccine will also be given as an injection under the skin. You will return every 2 weeks 2 more times to receive the boosting vaccinations. Overall, the boosting vaccinations will be given on or about days 15, 29 and 43 from the start of the study.

With each vaccination (priming vaccination and boosting vaccination), you will also receive injections of sargramostim. Sargramostim is designed to increase the effectiveness of the vaccine by increasing the number of immune cells at the vaccination site. It will be given as an injection under your skin at the same site as the vaccine injection. The sargramostim injections will be given on the day of vaccination and daily for the next 3 days after each vaccination. Therefore, sargramostim injections will be given on days 1-4, 15-18, 29-32 and 43-46 from the start of the study.

While you are receiving the experimental treatments you will be monitored closely for any signs that might signal the earliest stage of toxicity so that appropriate intervention can be done. This will include blood and urine testing as well as clinic visits every 2 weeks. You will also be seen 4 weeks after the final boosting vaccination for physical examination and collection of laboratory data and any information regarding any side effects from the treatment. You may also undergo repeat imaging scans to assess the status of your cancer.

Extension Phase: If your cancer has not gotten worse and there have been no serious side effects from the treatment, you will be offered to continue on the study with an optional extension phase. This phase of the trial offers continued monthly boosting vaccinations along with sargramostim injections. Sargramostim will be given on the day of the boosting vaccination as well as the next 3 days after the vaccination. The extension phase will continue for 12 months.

Maintenance Phase: If you have completed the extension phase with no serious side effects and have not experienced progression of your disease, you may continue on the study beyond the extension phase. In this part of the trial, you will receive vaccines every 3 months, while also getting sargramostim on the same day as the boosting vaccine and for the next 3 days following the vaccination. If your tumor(s) starts to grow on the maintenance phase yet your doctor feels you are clinically stable, you may have the option to revert back to monthly vaccines.

PATIENT IDENTIFICATION

**CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099**

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 5 of 18 pages

Apheresis

In order to measure your immune response, sufficient amount of immune cells called lymphocytes will be needed. You will be asked to undergo a procedure called apheresis in order to obtain the quantity of lymphocytes needed to measure the immune response. This testing will provide no benefit to you and is part of the experimental portion of this research therapy. You will undergo this procedure 2 times, on day 1 and again on day 71 of the study. Apheresis is a procedure in which blood will be taken from you by a machine that will take out lymphocytes and then return the rest of your blood to you. Blood will be withdrawn via a needle placed in one arm and channeled into a cell separator machine. The machine will separate the lymphocytes from the remaining blood elements. The lymphocytes will be taken for processing and the rest of the components will be returned to you through the same needle. The procedure will take from 2 to 4 hours during which time you will have to remain in a bed or a reclining chair. Individuals with bleeding disorders can be harmed by this procedure. You will be evaluated for such a condition before apheresis is done. All attempts will be made to protect you from any complications. Patients do not need to be hospitalized for the procedure. The apheresis procedure will be done at the Department of Transfusion Medicine (Blood Bank) in the NIH Clinical Center and is supervised by Blood Bank physicians.

PANVAC-V (Priming Vaccination)

PANVAC-V is an investigational drug. This vaccine will be given as injection (shot) under the skin, subcutaneously (usually in the thigh). Your vaccination treatment will be given to you in the outpatient clinic. The injections (shots), themselves, take less than a minute to give. You will need to have blood tests prior to each vaccine. After each vaccine, you will be observed in the clinic for 1 hour. If you do experience bad side effects (see below), modifications, which may include withholding additional treatments, may be made to your vaccine therapy depending upon the side effects that may be experienced. If side effects are severe enough to withhold the vaccine for a period of more than 6 weeks, you will be taken off the study.

PANVAC-F (Boosting Vaccination)

PANVAC-F is an investigational drug. Previous CEA vaccine studies have shown increases in immune responses directed against CEA when patients were given the similar vaccine strategy (priming vaccination followed by boosting vaccinations). This vaccine will be given as injection (shot) under the skin, subcutaneously (usually of the thigh).

Your vaccination treatment will be given to you in the outpatient clinic. The injections take less than a minute to give. You will need to have blood tests prior to each vaccine. After each vaccine, you will be observed in the clinic for 1 hour. If you do experience bad side effects (see below), modifications, which may include withholding additional treatments, may be made to

PATIENT IDENTIFICATION

**CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099**

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 6 of 18 pages

your vaccine therapy depending upon the side effects that may be experienced. If side effects are severe enough to withhold the vaccine for a period of more than 6 weeks, you will be taken off the study.

Sargramostim (GM-CSF)

Sargramostim is an FDA approved drug that is usually used to increase a patient's blood counts or to stimulate the immune system. However its use in this protocol is investigational. Sargramostim will be used to try to increase the usefulness of the vaccine by increasing the number of immune cells at the vaccination site. This drug will be given as injection (shot) under the skin. It will be given at the site as the vaccine injection. Sargramostim is given as a separate injection for 4 days beginning on the same day as either vaccine. This administration is usually in the thigh. After the first vaccine cycle, you will be observed in the clinic for 1 hour after the first two doses (days) of sargramostim in order to detect and treat any side effects (described in detail below) should they occur. The site of the injection will be marked with a marker since the other doses of sargramostim and vaccine will be administered at the same site. Either someone in your family or you may be taught to give the sargramostim shot so that you do not need to come to the clinic for every injection.

Risk or Discomforts of Participation

If you choose to take part in this study, there is a risk that:

- You may lose time at work or home and spend more time in the hospital or doctor's office than usual
- You may be asked sensitive or private questions which you normally do not discuss

The PANVAC-VF used in this study may affect how different parts of your body work such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects from the study drug(s)/study approach.

Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

PATIENT IDENTIFICATION

**CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099**

MEDICAL RECORD

CONTINUATION SHEET for either:
NIH 2514-1, Consent to Participate in A Clinical Research Study
NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: 04-C-0246

CONTINUATION: page 7 of 18 pages

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.
- The study doctor may adjust the study drugs to try to reduce side effects.

The tables below show the most common and the most serious side effects that researchers know about. There might be other side effects that researchers do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Possible Side Effects of PANVAC

POSSIBLE, SOME MAY BE SERIOUS
<ul style="list-style-type: none">• Anemia which may require blood transfusion• Diarrhea, nausea, vomiting• Pain• Chills, tiredness, fever• Flu-like symptoms including body aches• Swelling and redness at the site of the medication injection• Loss of appetite• Muscle weakness• Headache, fainting• Cough• Increased sweating• Itching, skin changes
REPORTED BUT UNDETERMINED
<ul style="list-style-type: none">• Rash

Vaccinia (Priming Vaccination)

Many of the potential side effects from the vaccination are related to allergic responses to vaccinia or to an abnormal immune system. If you previously have had a smallpox (vaccinia) vaccination, you must have never had an allergic or severe reaction to such a vaccination. You must not have an allergy to eggs or egg products. You must have no skin diseases or open wounds. You must not have any other history of altered immune function, such as HIV. You must not have eczema or a history of eczema or other eczematoid skin disorders. You must not be pregnant or nursing. You must also not be immunosuppressed (by disease or therapy), including HIV infection, atopic dermatitis, or autoimmune disease (autoimmune neutropenia, thrombocytopenia, or hemolytic anemia; systemic lupus erythematosus, Sjogren syndrome, or scleroderma; myasthenia gravis; Goodpasture syndrome; Addison's disease, Hashimoto's

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 8 of 18 pages

thyroiditis, or active Graves' disease). There is a good chance that if you had any of these diagnoses in your medical history, you would know it and you would recognize these medical terms. You must not have a history of seizures, encephalitis (brain infection) or multiple sclerosis ("MS").

Because you may "shed" live virus through your lesion for several days after vaccination, you must be able to avoid close contact with certain individuals for at least two weeks after each vaccination. These individuals include children under 3 years of age; women who are pregnant or breast-feeding; individuals with active or a history of eczema or other eczematoid skin disorders such as active cases of extensive psoriasis, exfoliative skin diseases, 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 HIV positive blood test, or those receiving immunosuppressive treatment. "Close contact" means that these people share your house, you have repeated bodily contact with them, and/or you take care of them and touch them with your hands. You must not start treatment if you have any healing scars or skin rashes (for example, a burn or poison ivy), until the skin condition has healed. If you have any questions about this list of precautions or any of these medical terms and diagnoses, you should ask about them before starting treatment. It is very important that you tell us if you have any concerns about these precautions for your own safety and the safety of those you may come in contact with. Furthermore, due to the unknown risk to the fetus, you are advised to avoid pregnancy and avoid fathering a child by practicing effective birth control during and four months following the last injection.

Vaccinia vaccinations have been given to over a billion people to immunize against smallpox. These vaccinations rarely have resulted in serious or fatal (deadly) complications (widespread infection of the virus in the skin, or infections of the eyes, or the brain).

On average, vaccinia stays active in your body for approximately 13-14 days. Therefore, prior to receiving your next vaccine, you will be evaluated for evidence of pyoderma, vesicles (lesions seen on your skin at or around your vaccine site), or evidence of persistent vaccinia infection. Physical evidence of persistent viral replication (which would be evidenced by the skin lesions, swelling of lymph nodes, and or fever) would require an evaluation prior to next vaccine administration that might include a skin or lymph node biopsy and may delay the next vaccine.

When vaccinia is given to protect against smallpox, it is usually scratched into the outer layers of the skin (scarification) with a two-pronged needle. You will be receiving your vaccine under the skin. A normal reaction after the "scarification" administration in a person who has been previously vaccinated with vaccinia includes appearance of a small bump (papule) in 3 days, a small blister or cluster of blisters in 5-7 days, and healing with little scarring within 2 to 3 weeks. Swollen lymph nodes ("swollen glands") and/or fever are infrequent. In individuals who have not previously received vaccinia, a red bump appearing on the third to fifth day is followed by a blister on day 5 to 6 and then by a pustule or "boil" 1-2 inches in diameter on day 9 to 11. A

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:

NIH-2514-1 (10-84)

NIH-2514-2 (10-84)

P.A.: 09-25-0099

MEDICAL RECORD

CONTINUATION SHEET for either:
NIH 2514-1, Consent to Participate in A Clinical Research Study
NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: 04-C-0246

CONTINUATION: page 9 of 18 pages

large area of redness may surround the blister and boil. A crusted scab usually forms by the second week and falls off by the third week leaving a vaccination scar roughly one inch in diameter. Fever and feeling like the flu (malaise) may occur during the blister and boil phases. Enlarged lymph nodes may develop and persist for months. Because you will receive your vaccine by a shot under the skin rather than by scratching it onto the skin the way a smallpox vaccination is given, you may have less of a skin reaction than is described above.

Side effects from the vaccinia vaccine are most common in young children, patients with disorders of the immune system, and individuals with skin disorders. That is why precautions are taken to exclude such individuals from exposure. It is important that you not touch the vaccination site and then touch other parts of your body. This is because the vaccinia virus may be transferred to other sites including the eye, the mucus membrane of the nose or mouth, or other area by rubbing the vaccination site and subsequently rubbing the eye or an open skin area. Spreading the virus in this way is known as autoinoculation. Healing usually occurs in 5-7 days. Blindness can result if vaccinia gets into the eye. A dressing will be placed over the vaccination site to reduce this risk. Generalized vaccinia may be characterized by several small blisters around the vaccination site or by widely distributed lesions developing 7-12 days after immunization. This is also known as a disseminated vaccinia infection. These lesions tend to follow a course of healing similar to that of the inoculation site. An allergic reaction to the vaccine with a rash or hives may occur within 7-10 days of vaccination and usually goes away within 2-4 days. Rarely, a serious allergic reaction requiring hospitalization may occur. The most serious reactions include post-vaccinia encephalomyelitis ("brain infection"), which can lead to coma and death and vaccinia gangrenosum which leads to a large non-healing sore and may lead to death. They occur almost exclusively in very young children who are exposed to vaccinia for the first time or in patients with impaired immunity; such individuals are not eligible for this study and those that must be avoided after vaccinia vaccination. The death rate for people receiving revaccination with vaccinia for smallpox is about 0.1 per million. Vaccinia Immune Globulin (VIG) has been successful as a therapy for some but not all of these complications. VIG is an injectable antibody preparation made from the plasma of people vaccinated with the vaccinia vaccine. VIG not a commercially available drug and is considered an experimental agent. If symptoms develop suggestive of one of the previously described vaccinia complications, or a close contact occurs between a recently vaccinia-vaccinated patient and a susceptible person with one of the pre-existing medical conditions described above, the patient should report the findings immediately to the protocol investigator or other established contact, for consideration for VIG therapy, since VIG may work better if given early. There is currently no other known effective treatment for these complications.

During the reintroduction of smallpox vaccination, several individuals thought to be at risk for heart disease experience heart attacks or angina. It is not known if the vaccinations had any connection to these events. A few individuals who had not been previously vaccinated developed inflammation in or around the heart. This was seen in <0.5% and generally peaked 2 weeks after getting vaccinia. If you notice symptoms such as chest pain, shortness of breath, cough, irregular

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:

NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099

MEDICAL RECORD

CONTINUATION SHEET for either:
NIH 2514-1, Consent to Participate in A Clinical Research Study
NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: 04-C-0246

CONTINUATION: page 10 of 18 pages

heart beat, or leg swelling after the first vaccine (vaccinia) you should seek prompt medical advice.

Fowlpox (Boosting Vaccination)

The virus does not grow (replicate) in human cells, thus it does not have some of the safety concerns listed above with vaccinia. However, with any experimental compound, there is the risk of unexpected and serious or deadly complications even if they have not been seen previously. The most common side effect from the boosting vaccination is cutaneous (skin) reactions. You may also experience fatigue, fever, anemia (low red blood cell count), and leukopenia (low white blood cell count). Other side effects possibly related to vaccine include itchy skin, anorexia, vomiting, constipation, dry mouth, and muscle pain. An additional side effect could be caused by an immune response to the CEA or MUC-1 protein that may be induced by either the priming or boosting vaccinations. If the vaccine causes an immune reaction against normal cells, you could develop inflammation of these tissues. This may cause no symptoms or it could cause pain. If these symptoms are caused by an immune (or "allergic") reaction to your own normal tissues, the effect could be prolonged and difficult to reverse. It is also possible that if you develop a very active antibody (immune) reaction to CEA or MUC-1 after the vaccination, you could develop an immune-complex disease (or serum sickness) which can cause fevers, rashes, joint pains, and, less commonly, kidney failure and severe allergic reaction inside blood vessels (vasculitis) of any part of your body. Care will be taken to minimize the side effects but other unknown or unanticipated side effects that could be severe or fatal are possible. While on this study you will be monitored for side effects and will be treated with appropriate medical care if they occur. Patients receiving fowlpox vaccines should avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site.

Sargramostim (GM-CSF)

Some of the side effects with sargramostim depend on the dose. The dose of sargramostim that will be used in this trial is relatively low. You will be receiving 100 micrograms per day for 4 days as a shot under the skin. In contrast, when sargramostim is given to help the blood counts in patients who are receiving chemotherapy, the doses are usually 350-500 micrograms per day intravenously (into a vein) for 10-14 days. Giving sargramostim by injection under the skin (as is planned in this trial) is better tolerated than when given by vein. Common side effects of sargramostim include fever, chills, muscle aching, fatigue, nausea and facial flushing. Some patients have complained of headaches. These side effects have usually responded to medication such as acetaminophen (Tylenol®). Bone pain has occurred with sargramostim, especially at doses higher than will be used in this study. The bone pain has generally responded to pain medication and quickly goes away upon stopping the sargramostim. Temporary weight gain from fluid retention may occur. Small numbers of patients have had arthritis (painful joints), pleuritis (inflammation of the lining of the lung) and pericarditis (inflammation around the heart),

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:

NIH-2514-1 (10-84)

NIH-2514-2 (10-84)

P.A.: 09-25-0099

MEDICAL RECORD

CONTINUATION SHEET for either:
NIH 2514-1, Consent to Participate in A Clinical Research Study
NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: 04-C-0246

CONTINUATION: page 11 of 18 pages

fluid around the lung in the chest cavity, and fluid around the heart. Irregular heart beats have occurred in a few patients. In general, these symptoms have gotten better when the dose of sargramostim is lowered or stopped, and when other medicines are given (anti-inflammatory and pain medication). Blood clots have occurred in some patients. At very high doses of sargramostim, a small number of patients have developed temporary difficulty in breathing, related to fluid collection in the lungs. This has improved with oxygen therapy and medicines. When the dose of sargramostim is lowered or stopped, the breathing problems have gotten better and have not come back. Sudden lowering of the blood pressure has occurred, but this is an uncommon side effect. In rare cases, patients have experienced chest pain (angina) following administration of sargramostim. Elderly patients, particularly those with a history of heart disease, may be at increased risk for these side effects. Since sargramostim is a protein, there is a risk that you might develop a serious allergic reaction to it. "Anaphylactic shock," a very severe allergic reaction (as seen, for example, when an individual highly allergic to bee stings is stung by a bee), has been observed rarely in patients treated with sargramostim. Mild skin reactions near the site of drug injection have occurred. Appropriate precautions will be used during therapy as mentioned above.

Although we feel it would be unlikely for kidney damage to be seen in humans, you will have blood and urine tests to look for any signs of kidney damage. If you develop any signs of kidney damage now or in the future, your doctor should contact the Principal Investigator of this study for further information.

Apheresis

There will be minimal discomfort due to the needle stick. Insertion of the intravenous lines may cause temporary discomfort, pain, or bruising at the site of insertion and there is the remote possibility of fainting and/or bleeding. Numbness or tingling may be experienced during apheresis but would be expected to go away after the procedure. Blood infections from contamination of the apheresis machine are a remote possibility, but this has not occurred at the NIH. There is a very small chance of introducing infection at the site of the needle. The procedure is generally very safe for individuals without anemia, bleeding problems, or heart problems. A small amount of anti-coagulant ("blood thinning" medication) is in the blood that is returned to you, but your body rapidly eliminates this. This medication may cause chills, nausea, heartburn, nasal stuffiness, or a tingling feeling when the blood is returned to your body. These symptoms are brief and may often be stopped by slowing the procedure. TUMS will be administered for symptoms of hypocalcemia.

Duration of Treatment

Your participation in this research project may continue as long as your cancer has not progressed (gotten worse) or there have been no serious side effects from the treatment, and as long as the vaccine is available. In general, the core phase is expected to take approximately 71 days to complete. An additional 12 months on the study can take place if you qualify and wish to

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 12 of 18 pages

continue onto the extension phase of the study. Furthermore, you can continue on the study into the maintenance phase and receive vaccine/sargramostim every 3 months. Your participation in this project may be ended by the investigator, the National Cancer Institute, or by the Food and Drug Administration (FDA) for reasons that would be explained to you. For example, the investigator may stop your protocol therapy for medical or safety reasons. New information developed during the course of this study that may affect your willingness to continue in this research project will be given to you as it becomes available.

The FDA requires patients that receive gene therapy be monitored even after the completion of the study. Once you have completed this study, you will be monitored on long-term basis (for up to 15 years) to see how well you're doing. At a minimum, you will be contacted yearly and asked questions about your health. This will include questions such as whether you have developed any new cancers or have developed problems with your blood or immune system. You will also be asked about unexpected hospitalizations as well as the medications you are taking.

Additional data will be obtained annually for years six through fifteen years following your last vaccination via telephone contacts. Information from these findings will be reported to the FDA. It is important for you to provide a current address and telephone number.

Potential Benefit for Participation

For patients with metastatic cancer, second line chemotherapy is available and may be of clinical benefit. However, for patients who have progressed on second line treatment and for those who have no remaining standard treatment options, the proposed protocol therapy represents a reasonable alternative. Theoretically, this vaccine could stimulate your own immune system to fight the cancer, although there is no guarantee that the therapy will stimulate the body to fight the cancer. The information we obtain from both the laboratory studies and the vaccination will also allow us to design more effective treatments in the future. This is an investigational study; therefore, no benefit is known or guaranteed. You will be monitored closely while you are receiving this experimental treatment for any signs that might signal the earliest stage of toxicity so that appropriate intervention can be done.

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

You may or may not benefit from participation in this study. Participation in this study is purely voluntary. Choosing not to participate (or withdrawing at any point) from this study will in no way penalize your medical care or your relationship with your physicians. If you choose not to participate in this study or have disease progression on the maintenance phase of study (vaccine every 3 months) but are told you may be eligible to revert to monthly vaccines, there are several alternative treatments/therapy for your cancer. They include:

PATIENT IDENTIFICATION

**CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099**

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 13 of 18 pages

- Treatment with standard chemotherapy or hormonal therapy
- Other Clinical Trials
- Surgery
- Radiation therapy
- Supportive care
- No further therapy at all

Treatments with standard chemotherapy for colorectal cancer include 5-fluorouracil and leucovorin either alone, with oxaliplatin or with irinotecan. These newer combination therapies may offer a survival benefit depending on which chemotherapy you have previously received. Each of these alternative therapies has a unique set of benefits and risks. Your physician will discuss these options with you. Furthermore, other centers are performing clinical trials with investigational agents as well as standard chemotherapy that you may consider as an alternative to this proposed trial.

Research Subject's Rights

Your participation in this study is entirely voluntary, and you may refuse to participate, or withdraw from this protocol at any time and receive care from a physician of your choice. Your participation in this study may be ended by the Principal Investigator or an Associate Investigator without your consent if they feel termination is medically indicated.

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

PATIENT IDENTIFICATION

**CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099**

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study
-----------------------	--

STUDY NUMBER: 04-C-0246

CONTINUATION: page 14 of 18 pages

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board
- The study Sponsor, NCI CTEP or their agent(s)
- Qualified representatives from BN Immuno Therapeutics (BNIT), the pharmaceutical company with a commercial interest in PANVAC-V and PANVAC-F.

A description of this clinical trial will be available on [://www.Clinicaltrials.gov](http://www.Clinicaltrials.gov), as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Completion of Study

Upon completing this study, you may be given the choice of taking part in other research protocols that may be appropriate for you. If you are not eligible for this study, we will use these tests (CEA, MUC-1 / HLA) to assess eligibility for other research studies. Otherwise, you will be returned to the care of your referring physician. It is important to stress that your participation in this study does not constitute a promise of long term care at the NIH Clinical Center. If there is no research study that can help you, you will be returned to the care of your private doctor. It is important to remember that if you do take part in this study, it is possible that you may be ineligible for certain future clinical trials because you would have received a form of gene or viral therapy. You may decide now not to receive treatment in this protocol, or you may choose at any time to stop the drug and withdraw from the protocol. In either case, you would be returned to the care of your referring physician.

Your signature on this form indicates that you agree to participate in this medical research study under the direction of the principal investigator as listed above.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest
- if your disease comes back during treatment
- if you have side effects from the treatment that your doctor thinks are too severe

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099

MEDICAL RECORD

CONTINUATION SHEET for either:
NIH 2514-1, Consent to Participate in A Clinical Research Study
NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study

STUDY NUMBER: 04-C-0246

CONTINUATION: page 15 of 18 pages

- if new information shows that another treatment would be better for you

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to NCI CTEP and BN Immuno Therapeutics or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases **cannot** be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to \$15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study are using a vaccine provided by BN Immuno Therapeutics (BNIT) through a joint study with your researchers and the company. The company also provides financial support for this study.

The National Institute of Health and members of the research team have developed a drug which is being used in this research. This means that it is possible that the results of this study could lead to payments to NIH scientists and to the National Institute of Health. By law, government scientists are required to receive such payment for their inventions. You will not receive any money from development of the drug/device/product.

Use of Specimens and Data for Future Research

To advance science, it is helpful for researchers to share information they get from studying human samples. They do this by putting it into one or more scientific databases, where it is stored along with information from other studies. A researcher who wants to study the

PATIENT IDENTIFICATION

CONTINUATION SHEET for either:
NIH-2514-1 (10-84)
NIH-2514-2 (10-84)
P.A.: 09-25-0099

STUDY NUMBER: 04-C-0246

CONTINUATION: 16 of 18 pages

information must apply to the database and be approved. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease.

We plan to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of these future uses. These specimens and data will be stripped of identifiers such as name, address or account number, so that they may be used for future research on any topic and shared broadly for research purposes. Your specimens and data will be used for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you do not want your stored specimens and data used for future research, please contact us in writing and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research. However, it may not be possible to withdraw or delete materials or data once they have been shared with other researchers.

STUDY NUMBER: 04-C-0246

CONTINUATION: 17 of 18 pages

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or other authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, James Gulley, M.D., Ph.D.; Building 10, Room 13N208, Telephone: 301-480-7164. You may also call the Clinical Center Patient Representative at 301-496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 240-760-6070.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

STUDY NUMBER: 04-C-0246

CONTINUATION: 18 of 18 pages

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/
Legal Representative

Date

Print Name

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/ Guardian

Date

Print Name

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian

Date

Print Name

**THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE
FROM FEBRUARY 6, 2017 THROUGH FEBRUARY 5, 2018.**

Signature of Investigator

Date

Signature of Witness

Date

Print Name

Print Name

PATIENT IDENTIFICATION

CONSENT TO PARTICIPATE IN A CLINICAL
RESEARCH STUDY (Continuation Sheet)

• Adult Patient or • Parent, for Minor Patient

NIH-2514-1 (5-98)

P.A.: 09-25-0099

File in Section 4: Protocol Consent