Short Title: Docetaxel ± Vaccine for metastatic breast cancer.

NCI Protocol #: 05-C-0229
OBA #:0505-709
CTEP #: 6977
IBC #: RD-05-V-11
Version Date: 8/19/2014
Amendment: T

Title: Randomized Pilot Phase II Study of Docetaxel alone or in Combination with PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) in Adults with Metastatic Breast Cancer

NCI Principal Investigator:	James L. Gulley, MD, Ph.D.[1]
Protocol Chair :	James L. Gulley, MD, Ph.D.[1]
Participating Site (MD Anderson) Principal Investigator:	Nuhad K. Ibrahim, M.D.[5]
Statistician:	*Seth Steinberg, Ph.D.[3]
Associate Investigators:	Philip M. Arlen, M.D.[4] Ravi Madan, M.D.[1] Chris Heery, M.D.[2] Myrna Rauckhorst, R.N.[6] Sheri McMahon, R.N.[6] Jennifer Marte, M.D.[2] (non-Clinical)
Primary Research Nurse:	Myrna Rauckhorst, R.N.[6] Sheri McMahon, R.N.[6]
Study Coordinator:	Jennifer Marte, M.D.[2] (non-Clinical)

CCR Branches / Laboratories:

[1]Genitourinary Malignancies Branch, CCR, NCI
10 Center Drive
Building 10, Rm. 12N226
Bethesda, MD 20892
(301) 496-4916

[2]Laboratory of Tumor Immunology and Biology, CCR, NCI
10 Center Drive
Building 10, Rm. 12N226
Bethesda, MD 20892
(301) 496-4916

[3]Biostatistics and Data Management Section
Office of the Director, CCR, NCI
9609 Medical Center Drive, Room 2W334
Rockville, MD 20850
(240) 276-5563

[4]NCI Special Volunteer
Genitourinary Malignancies
Branch, CCR, NCI
10 Center Drive
Building 10, Rm. 12N226
Bethesda, MD 20892
(301) 496-4916

[5]Participating Institution:
U.T. M. D. Anderson Cancer Center
515 Holcombe, Unit 1354
Houston, TX 77030
(713) 792-2817

[6]Office of the Clinical Director,
CCR, NCI

Multi-Institutional Center:

NAME OF PI: Nuhad K. Ibrahim, M.D.
INSTITUTION: U.T. M. D. Anderson Cancer Center (MDACC)
FWA NUMBER: FWA00000363
ADDRESS: 1515 Holcombe, Unit 1354,
Houston, TX 77030
PHONE NUMBER: 713/792-2817
E-MAIL: nibrahim@mdanderson.org

U.T.M.D. Anderson Cancer Center
IRB CONTACT: Martha Matza
COMPLETE ADDRESS: 1515 Holcombe, Unit 574,
Houston, TX 77030
PHONE NUMBER: 713 563-5444
E-MAIL: mmatza@mdanderson.org

PHARMACY CONTACT: Kim McConnell, PharmD
Charge Pharmacist
Investigational Pharmacy Services
MD Anderson Cancer Center
1515 Holcombe Blvd, Room B1.4533
Houston, TX 77030
PHONE NUMBER: 713 792-2848
Email: kmcconnell@mdanderson.org

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)

Commercially Available Agents:

Drug Name: Sargramostim (Leukine®) ** only to be given at the NCI site
Manufactured by Berlex Laboratories, Inc., Richmond CA

Drug Name: Docetaxel (Taxotere)
Manufactured by Aventis Pharmaceuticals; Bridgewater, NJ

Table of Contents

PRÉCIS 6

SCHEMA 7

1 INTRODUCTION 8
1.1 Study Objectives 8
1.2 Background and Rationale 8

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT 19
2.1 Eligibility Criteria 19
2.2 Research Eligibility Evaluation 22
2.3 Patient Registration and Treatment Randomization 22

3 STUDY IMPLEMENTATION 23
3.1 Study Design 23
3.2 Drug Administration 25
3.3 Treatment Modifications 26
3.4 Pharmacokinetic Studies: N/A 28
3.5 Protocol Evaluation: (See Appendix C) 29
3.6 Concurrent Therapies 32
3.7 Surgical Guidelines: N/A 32
3.8 Radiation Therapy Guidelines: N/A 32
3.9 Off Study Criteria: 33
3.10 Post Treatment Evaluation (see Appendix C) 33

4 SUPPORTIVE CARE 34
4.1 Treatment of Vaccinia Vaccination Complications 34

5 DATA COLLECTION AND EVALUATION 35
5.1 Data Collection 36
5.2 Response Criteria 36
5.3 Toxicity Criteria 39
5.4 Statistical Considerations 39
5.5 MULTI-INSTITUTIONAL GUIDELINES 41
5.6 Data and Safety Monitoring Plan 42

6 HUMAN SUBJECTS PROTECTIONS 44
6.1 Rationale for Subject Selection 44
6.2 Participation of Children 44
6.3 Evaluation of Benefits and Risks/Discomforts 44
6.4 Risks/Benefits Analysis 45
6.5 Consent and Assent Process and Documentation 46

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS 46
7.1 Expedited Adverse Event Reporting to CTEP 46
7.2 NCI-IRB Reporting 46
7.3 Routine Adverse Event Reporting 48
7.4 Secondary AML/MDS 48
7.5 NCI Guidance for Reporting Expedited Adverse Events for Multi-Center Trials 49
7.6 Expedited Reporting Guidelines 49
7.7 Comprehensive Adverse Events and Potential Risks list (CAEPR) 51
7.8 Record Keeping 52
7.9 Regulatory Issues 53

8 PHARMACEUTICAL INFORMATION 55
8.1 PANVAC™-V (NSC 727026) 55
8.2 PANVAC™-F (NSC 727027) 67
8.3 Sargramostim (GM-CSF, LEUKINE®) 71
8.4 Docetaxel 72
8.5 Dexamethasone 74
8.6 Agent Procurement 75

REFERENCES 77

APPENDIX A: Performance Status Criteria 82

APPENDIX B: Instructions for Care of the Vaccine Site 83

APPENDIX C: Protocol Evaluation 86

APPENDIX D: Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference: ... 88

APPENDIX E: ELISPOT ASSAY 94

APPENDIX F: Instructions for pre-study and follow-up blood tests 95

APPENDIX G: NCI-IRB SERIOUS UNEXPECTED ADVERSE EVENT REPORT FORM .. 96

APPENDIX H: Instructions for Collection and Shipping of Research Blood Samples 98

APPENDIX I: CTEP MULTICENTER GUIDELINES 100

PRÉCIS

Background:

- Weekly docetaxel therapy is currently used as a standard treatment for patients with metastatic breast cancer.
- Although many patients initially respond to this form of therapy, the majority will eventually develop disease progression and die from their disease.
- We have explored the use of combining pox vector vaccines with docetaxel. In a recent clinical trial, men with prostate cancer were given both the vaccine and docetaxel without any significant toxicity and the docetaxel did not diminish immune responses to the vaccine.

Objectives:

- To evaluate progression free survival comparing PANVAC + docetaxel vs. docetaxel alone in patients with metastatic breast cancer.
- To evaluate overall survival comparing PANVAC + docetaxel vs. docetaxel alone in patients with metastatic breast cancer.
- To evaluate in both arms CD8+ T cell responses directed against CEA and MUC-1 in HLA-A2, A3, and A24 positive patients by interferon-gamma ELISPOT assay.

Eligibility:

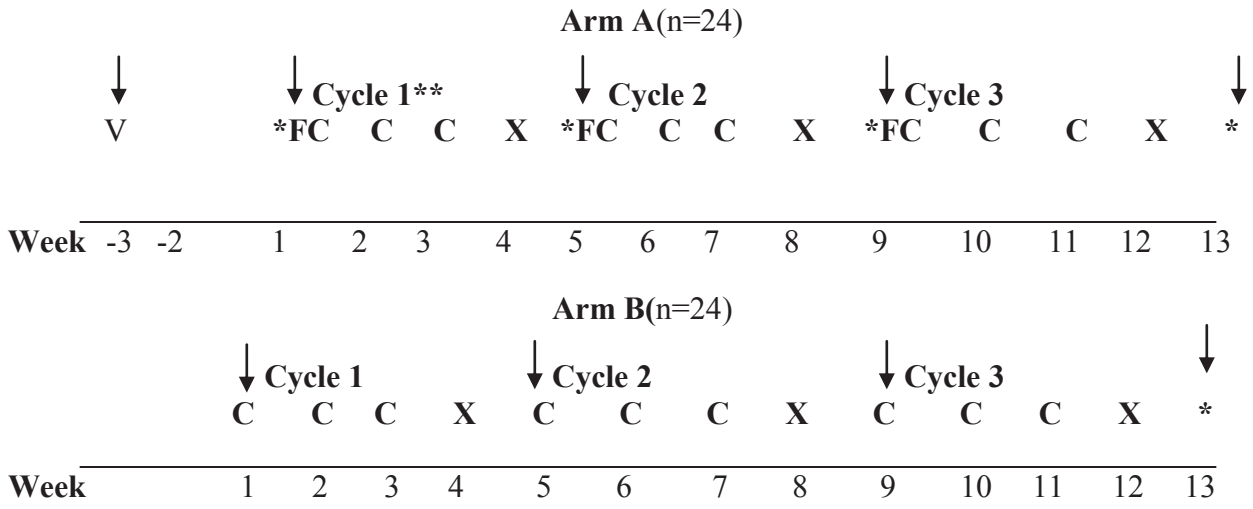
- Metastatic breast cancer (either male or female) with evidence of metastatic disease (must have radiographic evidence of disease) and life expectancy of at least 4 months
- Patients may have received unlimited prior hormonal therapy or chemotherapy, but no prior docetaxel for metastatic disease
- Hematological eligibility parameters within 16 days of starting therapy:
  Granulocyte count ≥1,500/mm3, Platelet count ≥100,000/mm3, Hgb ≥ 8 Gm/dL

Design:

- We propose a randomized Phase II study evaluating the role of combining docetaxel with PANVAC™-V (recombinant vaccinia containing the genes encoding for CEA, MUC-1, LFA-3, ICAM-1 and B7.1) and PANVAC™–F (recombinant fowlpox containing the genes encoding for CEA, MUC-1, LFA-3, ICAM-1 and B7.1) vs. docetaxel alone to determine if the addition of the vaccine can prolong the time to disease progression as well as overall survival in patients with metastatic breast cancer.
- Patients randomized to docetaxel alone may receive sequential therapy with PANVAC™-V and PANVAC™-F at time of disease progression.
- In patients randomized to the concurrent therapy, PANVAC-V will be administered to patients 3 weeks prior to the start of chemotherapy as the initial vaccine.
- The immune responses to CEA and MUC-1 will then be boosted by administration of PANVAC-F
- GM-CSF will be administered with each vaccine inoculation for four consecutive days only for patients at the NCI

SCHEMA

This pilot study proposes to evaluate in a population of patients with metastatic breast cancer, a potential clinical benefit using an initial vaccination with 2 x 10^8 pfu PANVAC-V (vaccinia) followed by monthly boosting vaccinations with 1 x 10^9 pfu PANVAC-F (fowlpox) in combination with weekly docetaxel vs. docetaxel alone. Sargramostim (100 microgram) will be given at the site of the vaccination on each vaccination day and for three consecutive days thereafter, only for patients at the NCI. After week 12, patients who demonstrate disease progression on Arm A, will be taken off study. Patients who do not progress will continue with docetaxel weekly for three consecutive weeks out of every four and PANVAC-F (fowlpox) vaccinations every 4 weeks. After week 12, patients who demonstrate progression on Arm B will have the docetaxel stopped and may commence therapy with PANVAC vaccines (prime with PANVAC-V (vaccinia) and every 4 week boosts with PANVAC-F (fowlpox) if clinically judged to be in the best interest of the patient (see section **3.1.1**) until evidence of progressive disease. Patients who do not progress will continue weekly docetaxel therapy until disease progression. HER-2 + patients will be stratified based on whether they continue to receive Trastuzumab (Herceptin) therapy along with the arm they are randomized to above. Trastuzumab, if continued, will be provided by the referring physician (not at the clinical center) at either the standard maintenance dose of 6mg/kg IV every 3 weeks or 2mg/kg IV q week as determined by the physician providing the drug.



C= Docetaxel 35 mg/m^2 IV over 30 min (Dexamethasone 4 mg po 12 hrs before, 1 hour before and 12 hours post Docetaxel)

V= PANVAC-V (vaccinia) 2 x 10^8 pfu subcutaneously x **1** day (week -2) + **Sargramostim** **100 mcg subcutaneously x 4 days.

***F** = **PANVAC-F (fowlpox)** 1 x 10^9 pfu subcutaneously x 1 dose + **Sargramostim** **100 mcg subcutaneously daily x 4 days [first fowlpox dose to begin 3 weeks after PANVAC-V (vaccinia)]. Each fowlpox dose is to be administered 24-48 hours prior to the first docetaxel dose in each 4 week cycle.

X= no treatment on that day

**** Sargramostim will be given only to patients enrolled at the NCI.**

↓ Blood draw for immunologic assays-

***Radiologic studies consisting of Bone Scan and CT chest/abdomen/pelvis will be performed on week 13. Scans will be repeated every 8 weeks (after each additional 2 cycles) until disease progression. Patients whose disease has not progressed on scans for a period of one year on study, will have the interval for CT scans and bone scan increased to every 3 months (12 weeks) while on study.**

** If there are two or more patients within one arm who experience grade 4 toxicity related to the regimen, we will halt further accrual to this study until we review the protocol with CTEP and IRB.

1 INTRODUCTION

1.1 Study Objectives

Primary

1.1.1 To evaluate progression free survival comparing PANVAC + docetaxel vs. docetaxel alone in patients with metastatic breast cancer.

Secondary

1.1.2 To evaluate overall survival comparing PANVAC + docetaxel vs. docetaxel alone in patients with metastatic breast cancer.

1.1.3 To evaluate in both arms CD8+ T cell responses directed against CEAor MUC-1 in HLA-A2, A3 or A24 positive patients by interferon-gamma ELISPOT assay. A minimum of 12 HLA-A2 positive patients will be required in each arm for evaluation.

1.1.4 To descriptively compare the progression free survival and overall survival in patients who did or did not receive GM-CSF.

1.2 Background and Rationale

1.2.1 Preclinical Support Data

In 2003, 211,000 American women will be diagnosed with breast cancer, and 40,000 will die from this disease (Error! Reference source not found.). Breast cancer is the most common female cancer in the United States, the second most common cause of cancer death in women, and the main cause of death in women ages 45 to 55. Patients with metastatic breast cancer are unlikely to be cured of their disease by any means. A complete remission (CR) from chemotherapy, which is a prerequisite for cure, is uncommon, and only a fraction of those remain progression-free for a prolonged period.

In light of the fact that current therapeutic strategies have had very limited success in the metastatic disease setting, vaccine strategies represent an alternative approach to therapeutic intervention. Preclinical studies in experimental murine models have demonstrated that therapeutic intervention using vaccines can be achieved by the activation of T cells directed against tumor-associated antigens (TAAs). Tumor-associated antigens are by definition, however, either very weakly immunogenic or functionally nonimmunogenic in the tumor-bearing host. Thus, vaccine strategies must be developed in which the presentation of a given tumor-associated antigen or antigens to the immune system results in far greater activation of T cells than is being achieved naturally in the host.

This immunotherapeutic approach to the treatment of cancer is based on the observation that human tumor cells express a variety of 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 a 180,000-dalton glycoprotein that is overexpressed on most adenocarcinomas of the colon, rectum, stomach, and pancreas, and non-small-cell lung cancers. It is also present on most adenocarcinomas of the breast (approximately 60%) (**16**). 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 granulocyte.((1))

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.(**2**) In addition, a number of clinical trials using recombinant vaccinia and/or avipox viruses expressing CEA have been conducted. 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.(**(3)-(7)**)

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.(**8**) MUC-1 is expressed in 90% of all breast cancers (**15**) A few studies, in colorectal neoplasia (**(9)**;**(10)**) as well as in other human malignancies (**(11)**;**(12)**), have suggested that increased expression of the core peptide of MUC-1 is associated with poor prognosis.

Finally, immunization with a MUC-1 peptide or a recombinant vaccinia virus expressing MUC-1 has been shown to induce MUC-1-specific immune responses in pancreatic and breast cancer patients.(**(13)**;**(14)**) Thus, immunization of breast cancer patients with pox viruses expressing MUC-1 may boost the antitumor immunity against their cancers.

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

1.2.2 Experimental Studies

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 very similar to that of humans. Experimental studies have demonstrated that no immune responses can be generated in these mice when CEA protein in adjuvant is used as an immunogen. However, when either recombinant vaccinia CEA (rV-CEA) vector or recombinant avipox CEA (fowlpox (rF-CEA)) vector is used to vaccinate CEA transgenic mice, a vigorous CEA-specific T-cell response can be generated.(**(15)**) 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 anti-tumor immunity of established tumors.(**(16)**-**(18)**) Preclinical studies have also demonstrated that vaccination with CEA-TRICOM vectors can eliminate spontaneous tumor development.(**(19)**) The above studies also demonstrated the absence of long-term toxicity, including autoimmunity, in mice that have eliminated established tumors. Recent preclinical studies have also shown that these vectors can be used in combination with radiation or drugs such as COX-2 inhibitors, or can be administered intratumorally, to enhance anti-tumor responses.

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.(**(20)**) Preclinical studies in double transgenic mice containing MUC-1 as a transgene, and developing spontaneous pancreatic cancer, have demonstrated that adoptively transferred MUC-1–specific CTL can eliminate pancreatic tumors.(**(21)**)

Primate studies. 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.

In vitro human studies. Several CEA peptides have now been identified that are capable of being used to generate CEA-specific human cytolytic T-cell lines (CTLs), which in turn are capable of lysing human tumors expressing CEA.(**(22)**-**(24)**) An agonist CEA epitope designated CAP1-6D has been shown to enhance the generation of CEA-specific T-cell lines as compared with the native epitope. These T-cell lines, in turn, have been shown to lyse human tumors expressing CEA.(**(24)**;**(25)**) Several MUC-1 peptides have also now been identified that are capable of generating MUC-1–specific human CTLs. An agonist epitope of a MUC-1 peptide has been shown to enhance the generation of MUC-1–specific human T-cell lines as compared to the native epitope. These T-cell lines, in turn, have been shown to efficiently lyse human tumors expressing MUC-1 in an MHC-restricted manner.

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™-V (vaccinia) and PANVAC™-F (fowlpox) vectors were as efficient as CEA/TRICOM or MUC/TRICOM vectors in activating CEA-and MUC-1-specific human T cells, respectively. Most importantly, these studies also demonstrate that when human antigen-presenting cells are infected with PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) vectors there is no interference in the expression of both CEA and MUC-1 peptide MCH complexes on the cell surface that would inhibit the activation of both CEA-and MUC-1-specific human T cells, i.e., both MUC-1-and CEA-specific T cells were activated to the same level as when using antigen presenting cells infected with either MUC-1-or CEA-specific vectors. In a recent study performed by Tsang et al.(14), T-cell lines generated from peripheral blood of normal volunteers and two pancreatic cancer patients, using DCs infected with rV-CEA/MUC/TRICOM or rF-CEA/MUC/TRICOM vectors had the ability to lyse the HLA-A2 positive MCF-7 human breast carcinoma line, which is positive for MUC-1 and negative for CEA. The SK-Mel-24 human melanoma line, also an HLA-A2+ cell line, which is negative for both MUC-1 and CEA expression did not lyse in the presence of these specific T-cell lines.

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.(**(26)**;**(27)**)

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 avipox-TRICOM.(**(28)**)

Preclinical studies performed in the LTIB using these TRICOM constructs have shown them to be superior to those constructs that do not contain the costimulatory molecules.(**(28)-(30)**) In CEA transgenic mice, which contain CEA as a self-antigen, much greater anti-tumor activity against established CEA-expressing tumors was seen using CEA/TRICOM vectors as opposed to the use of CEA 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.((31)) Recent studies have also shown that infection of antigen-presenting cells with TRICOM vectors leads to the generation of higher avidity T cells.((32))

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.((3);(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.

As described above for CEA, a selected epitope within the MUC-1 protein sequence was modified to increase its binding to the major histocompatibility complex (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. P93L was also able to induce CD8+ CTLs from peripheral blood mononuclear cells (PBMC) 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.

Protein antigens are presented to cytotoxic T lymphocytes (CTLs) as small peptides (approximately 9-10 amino acids long) bound to class I molecules of the major histocompatibility 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).((2)) 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 CTLs. In contrast to the native peptide, CAP-1(6D) was able to induce CD8+ CTLs from normal PBMC that were able to recognize both the modified and native peptides. In addition, these CTLs recognized and lysed tumor cell lines expressing CEA. These studies indicate that CEA 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.((34)-(36))

Using murine tumor models, several researchers have now shown that modification of tumor cells to enhance GM-CSF expression, using retroviral vectors (**(35)**) or vaccinia virus vectors (**(37)**;**(38)**), 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.

However, we have recently analyzed data from a randomized phase II trial designed to evaluate the role of GM-CSF with the PSA-TRICOM vaccines (NCI 5911) in patients with metastatic castration dependent prostate cancer. While this was a small study (n=32), to our surprise it appeared that the proportion of patients responding immunologically and the magnitude of their response was similar regardless of whether they received GM-CSF or not. In this trial, overall survival was also analyzed. There was no suggestion that patients who did not receive GM-CSF had worse survival. In fact, the cohort receiving no GM-CSF had the best median overall survival of patients treated in this small study.

Also, GM-CSF is associated with well known toxicities. While these are generally low grade, the absence of supporting data from NCI 5911 limits the enthusiasm for its use. So we will be giving sargramostim (GM-CSF) only to patients enrolled at the NCI.

Pox Virus Vectors. Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile. The 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.(**(39)**;**(40)**)

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.(**(41)**;**(42)**) Fowlpox cannot replicate in non-avian species, making systemic infections unlikely and making it potentially safer than a replicative virus. Fowlpox virus can be given multiple times and immune responses to vaccinia are not cross-reactive with fowlpox. Results from NCI-sponsored Phase 1 studies of other fowlpox-based vaccines support the safety of this vector.
Immunization with 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 killer T cells that specifically destroy the selected tumor with little incremental toxicity.

Role of Taxane Therapy in Metastatic Breast Cancer

Docetaxel, approved by the FDA for anthracycline-resistant locally advanced or metastatic breast cancer, has demonstrated overall response rates of 41% in doxorubicin-resistant disease. It has been shown to be superior to mitomycin/vinblastine in patients whose disease progressed after an anthracycline-based chemotherapy regimen. Although 100mg/m^2 is the dose of docetaxel approved by the FDA, weekly dose schedules of docetaxel have been reported to produce high response rates and lower toxicity than the 3-week schedules. The weekly dose schedules have become a community standard.

In the publication by O'Shaughnessy J et al. Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results- (J Clin Oncol. 2002 Jun 15;20(12):2812-23.)- single agent docetaxel was used as the control arm in anthracycline-pretreated patients with metastatic breast cancer. In this study 256 patients were randomized to docetaxel alone. The median PFS was 4.2 months with the overall survival at 11.5 months.

It is important to note that a significant drawback to the combination therapy with Capecitabine/docetaxel is the toxicity profile. The percentage of grade 3 adverse events was 71% in the combination arm. Metastatic breast cancer is often treated as a chronic disease and studies have now examined sequential therapy with single agent therapy compared to combined therapy. A recent phase III study by Sledge et al (**(11)**) looked at single agent doxorubicin and paclitaxel and compared them to the combination therapy. Patients on the single agent therapy were allowed to cross over to the other agent at time of progression. There were no differences in the response rates and quality-of-life measurements in the combination vs cross over groups. In the O'Shaughnessey trial there was no comparison using a sequential regimen of docetaxel and capecitabine. However, it is standard of care in the community to use single agent chemotherapy, followed sequentially by a different chemotherapy agent at time of progression. In addition, Tabernero et al (**(12)**) reported the use of weekly vs 3-weekly docetaxel as single agent therapy for metastatic breast cancer in a randomized multicenter trial. The weekly regimen had a comparable efficacy and a favorable toxicity profile compared to the q 3 weekly regimen and is currently accepted as a standard of care treatment for metastatic breast cancer.

When looking at combining biologics with chemotherapy, Slamon et al. (10) published the efficacy of adding Herceptin + Taxol (n=92) vs. Taxol (n=96). The median TTP in months was 6.9 with the combination arm vs 3.0 using Taxol alone in metastatic breast cancer patients ($p<.001$). Thus, there is precedent to attempt such a goal using the combination of chemotherapy with biologics.

Preclinical studies performed in the Laboratory of Tumor Immunology and Biology, CCR, NCI were designed to look at the effect of the addition of Herceptin 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 both HLA-A2 + 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 our target cells. T-1101 cells were used as CTL in this assay to determine the % lysis of this breast cancer cell line with and without the addition of Herceptin (at a concentration of 100µg/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 Herceptin. This suggests that the use of Herceptin may augment the ability of cytotoxic T-cells to kill tumor cells.

Rational for combining chemotherapy and immunotherapy

The use of chemotherapeutic agents for the treatment of human malignancies has been based on the well-characterized cytotoxic effects on malignant cells. It has been felt that due to this effect, chemotherapy would have an adverse effect on the immune system. However recent experimental studies in vitro support the concept that certain anti-cancer agents may actually exhibit immune modulatory activities, resulting in the upregulation of (a) cell surface expression of major histocompatibility complex (MHC) molecules, (b) tumor associated antigens (TAA), or (c) Fas expression on malignant cells rendering them more sensitive to immune destruction. Detailed studies involving 5FU, cisplatin and cyclophosphamide have shown these agents to have the ability to upregulate components of the immune system. (**(48)-(51)**) Recently, newer agents such as Irinotecan have been shown in vitro to upregulate immunologic properties of both primary and metastatic colon carcinoma cell lines.(**(52)**) Clinical studies using Her-2/neu targeted antibody in combination with chemotherapy have revealed the synergistic effect of passive immunity with chemotherapy (**(53)**,**(54)**). Finally as part of a planned subanalysis of a recent randomized Phase III clinical trial in patients with stage II and III breast cancer, there was higher T-cell blastogenesis and NK cell lytic activity in patients treated with taxane-based adjuvant chemotherapy compared to those not receiving taxanes at long-term follow-up (**(60)**).

Investigators at Johns Hopkins recently published preclinical data addressing the issue of chemotherapy and cancer vaccines. This study suggests that taxane therapy may in fact augment and increase T cell precursors rather than depleting them. The data presented in this study support the following two conclusions: (*a*) Taxane chemotherapy when given in a defined sequence with a murine GM-CSF secreting neu-expressing whole-cell vaccine, enhance the potential of the vaccine to delay tumor growth in tolerized *neu* transgenic mice. The optimal immune-modulating dose for each chemotherapeutic agent appears to be just above doses that begin to induce cytopenias; and (*b*) the enhanced antitumor response appears to be mediated, at least in part, by an increase in number and function of antigen-specific T cells in particular the Th1 response. These findings suggest that combined treatment with immune-modulating doses of chemotherapy and the GM-CSF-secreting vaccine can overcome immune tolerance and induce a more potent antigen-specific antitumor immune response than vaccine alone (**(55)**). This preclinical data suggests that administering taxane therapy one day prior to a single vaccine can enhance the immunologic and tumor response.

Preclinical studies were conducted in the LTIB, CCR, NCI to determine if the presence of docetaxel would inhibit the infection of cells with recombinant fowlpox and the subsequent expression of the encoded transgene(s). Murine tumor cells were treated with a docetaxel dose

equivalent to 30 mg/m^2. We found that if the cells were treated with docetaxel 1 day prior to the fowlpox infection, expression of the transgene was reduced by 45-63%. If the cells were first incubated with recombinant fowlpox followed by treatment with docetaxel, however, there was no inhibition of infection/expression observed. Optimal expression of the transgene(s) was observed when infection occurred at least 1 hour prior to the administration of docetaxel. These studies were carried out using both recombinant vaccinia and recombinant fowlpox viruses with similar results.

Additional studies have looked at administering docetaxel following vaccine therapy. Preclinical data indicates that the dose of docetaxel and the scheduling of the docetaxel administration in relation to the vaccine are important. Preclinical data indicates that optimal results are obtained when docetaxel is administered following vaccination. These studies used mice that were transplanted with subcutaneous tumors engineered to express human CEA. Mice vaccinated with rV-CEA/TRICOM on day 4, and boosted with rF-CEA/TRICOM on day 11 demonstrated no significant anti-tumor effects. Similarly, mice administered docetaxel on days 15, 17, and 19, demonstrated no antitumor effects. However, when mice were first vaccinated with rV-CEA/TRICOM on day 4, boosted with rF-CEA/TRICOM on day 11 and then administered docetaxel on days 15, 17, and 19, significant antitumor activity was noted (p=0.03 vs. vaccine alone, p=0.02 vs. docetaxel alone, at day 28 post tumor transplant). In addition, 43% of mice receiving the sequential therapy of vaccine and docetaxel demonstrated complete resolution of the tumor mass.

1.2.3 Clinical Studies

Phase I studies using rV-CEA (**(43)**) or avipox-CEA (**(44)**;**(45)**) 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 the clinical benefit of priming with rV-CEA and giving multiple boosts with avipox-CEA vaccine.(**(46)**) 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.(**(47)**) Patients 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 6–12 months. 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.(**(48)**)

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.(**(3)**)

A Phase I clinical study has recently been completed in collaboration with Georgetown University (John Marshall, PI) employing rV-CEA/TRICOM (V), avipox-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+GM-CSF, 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, and 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.(**(6)**) Three other trials employing TRICOM vectors are ongoing at Fox Chase Cancer Center, Duke University Cancer Center, and Columbia Presbyterian.

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.(**(14)**) 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.(**(49)**) 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 TRICOM (not even one costimulatory molecule), an agonist MUC-1 epitope, or are being used in diversified prime and boost regimens.

In a recent clinical trial at the NCI, men with androgen insensitive metastatic prostate cancer were initially administered vaccinia-PSA vaccine alone followed 3 weeks later with a fowlpox-PSA vaccine combined with docetaxel and decadron premedication without any significant toxicity. The docetaxel was administered weekly, 3 of every 4 weeks and the fowlpox-PSA vaccine was administered monthly, 1 day following the first weekly dose of docetaxel per cycle. Immune assays were performed prior to the start of therapy and after completing 3 months of therapy (11 patients in each arm). The median fold increase in PSA-specific T-cell precursors in both arms was 3.33 (P2=0.92 by the Wilcoxon rank sum test). Thus, immunologic responses to the vaccine were not diminished with the combination of therapy.

Furthermore, the role of short term steroids on the immune system has been examined in other clinical situations. Although it is accepted that chronic use of steroids are immunosuppressive, it is not clear that short bursts of steroids would have a negative impact on the immune responses to the vaccine in these patients. A recent study by Farchok, et al.(Arch Pediatr Adolesc Med. 152(12), 1101-5,1998.) evaluated the immunogenicity of the influenza virus vaccine in children receiving short-course predinisone for acute asthmatic exacerbations. Children were randomized to receive influenza virus vaccine with or without steroids. Those randomized to steroids received prednisone (2mg/kg per day for 5 days). The results showed no diminished responses in the steroid group. Furthermore the data from our previous prostate cancer trial (see paragraph above) that used a similar vaccine regimen with the same chemotherapy and steroids did not reveal any diminished immune responses to the combination therapy when compared to a vaccine control arm.

In addition to the safety and immunologic data from the prostate trial, preliminary data has demonstrated clinical responses with the above regimen. Historical data for median time to disease progression for similar patients receiving weekly docetaxel as a single agent is approximately 111 days. On the combination arm of vaccine and docetaxel the median time to disease progression on study was 134 days. Furthermore, 2 patients had significant PSA >90% declines (168.4 ng/dl to 4.0ng/dl and 19.7ng/dl to 1.8 ng/dl respectively).

There has only been one serious toxicity thought to be possibly due to the vaccine, described below.

One patient treated with rF-PSA(L155)-TRICOM developed grade 4 thrombotic thrombocytopenic purpura (TTP) thought to be possibly related to study drug, approximately 3.5 weeks after receiving the last dose of his vaccine. The patient had a history of hypertension, hyperlipidemia and atrial fibrillation. The patient presented with chest pain and was found to have elevated cardiac enzymes, acute renal failure and thrombocytopenia with evidence of intravascular hemolysis. He was treated for an MI and with serial plasmapheresis and hemodialysis and his myocardial infarction has resolved without sequelae and his TTP has resolved, although one month after diagnosis with TTP he continued to require hemodialysis.

Recently, we have enrolled 14 patients with metastatic CEA and MUC-1 cancers on the NCI Phase I PANVAC 04C-0246 study. One patient had a questionable syncopal epispode and it is not clear if this had any relationship to the vaccine. However, no other grade 3 toxicities have been reported and the therapy has been otherwise very well tolerated.

Immune Assays

Our studies in the LTIB have demonstrated the ELISPOT assay for IFN-gamma production to be quantitative and reproducible as a measure of human T-cell responses to vaccination.(**(50)**-**(52)**) 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.

1.2.4 Summary

This study examines the combination of weekly docetaxel with PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) to observe if there is a clinical advantage compared to docetaxel alone.

- Based on the available preliminary data, rV-CEA(6D)/TRICOM, rV-MUC-1, and rF-CEA(6D)/TRICOM in combination with GM-CSF appear to be well tolerated. There have been no dose limiting toxicities (DLTs) or serious adverse events causally related to the vaccine regimen and no subject has withdrawn due to a DLT, inoculation-related event, or other adverse event related to the treatment.
- Adverse reactions causally related to the vaccine are limited to grade 1 and 2 events associated with local cutaneous injection site reactions and systemic events of fatigue, chills and fever.
- A similar vaccine regimen combining weekly docetaxel and pox vector vaccines has completed accrual of 28 patients at the NCI CC. The combination was well tolerated and the docetaxel did not suppress specific T-cell responses to the vaccine.
- Although docetaxel is a standard therapy for treatment of metastatic breast cancer, most patients will eventually progress and succumb to their disease. In this pilot Phase II study, we will see if the addition of PANVAC™-V (vaccinia) and PANVAC™-F (fowlpox) to weekly docetaxel can prolong progression free survival (PFS).

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 **Inclusion Criteria**

A. Metastatic Breast Cancer (either male or female) with evidence of metastatic disease (must have radiographic evidence of measurable disease on CT scan or X-ray, or evidence of evaluable disease on bone scan that is consistent with metastasis) and a life expectancy of at least 4 months. Patients may have received unlimited prior hormonal therapy and chemotherapy.
B. Histologically confirmed adenocarcinoma of the breast cancer confirmed in the Pathology Clinical Center at NCI, (or NNMC) or MD Anderson Pathology Department prior to starting this study. Note: However, if no pathologic specimen is available, patients may enroll with a clinical course consistent with breast cancer and a pathological documentation of the disease.
C. 18 years of age or greater.
D. May have received docetaxel in the adjuvant setting at least 12 months prior to study entry.

E. Able to understand and give informed consent.

F. Able to avoid close household contact (close household contacts are those who share housing or have close physical contact) for at least two 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. (see **APPENDIX B**). We have vaccinated over 700 cancer patients and have reported no cases of either self inoculation or person to person transmission of the virus.

G. ECOG performance status of 0 – 1.

H. Serum creatinine < 1.5 x ULN OR creatinine clearance on a 24 hour urine collection of ≥ 60 mL/min, standard LFT limitations for patients receiving docetaxel therapy include bilirubin within ULN and SGOT/SGPT < 1.5 x ULN. If transaminases are > 1.5 x ULN up to 2 x ULN (as currently indicated), then alk phos should be < 2.5 x ULN. (Patients with renal abnormalities should be evaluated for CrCl and interstitial abnormalities. A Cr Cl of > 60ml/min measured or calculated and proteinuria less than 1000mg per 24 hours are eligible unless explained by non-renal causes.)

I. Recovered completely from any grade 3 or 4 reversible hematologic and non hematologic toxicity associated with recent therapy. Typically this is 3-4 weeks for patients who most recently received cytotoxic therapy. Patients previously treated with mitomycin c or carboplatin will require a minimum of 6 weeks.

J. Hematological eligibility parameters (within 16 days of starting therapy):

- Granulocyte count ≥1,500/mm3
- Platelet count ≥100,000/mm3
- Hgb ≥ 8 Gm/dL

K. Must agree to use effective birth control or abstinence during and for a period of 4 months after the last vaccination therapy.

L. Patients whose tumors are ER positive should have failed primary hormone therapy, unless clinically indicated, i.e. in patients with visceral disease or symptomatic bone disease where upfront chemotherapy is warranted. Patients who progressed or recurred following Trastuzumab (Herceptin) therapy if a patient is FISH positive or IHC 3+ positive for Her-2 neu. Those patients who have progressed on trastuzumab may continue to receive the drug by their referring physician. However, if trastuzumab has been discontinued at the time of enrolling on study, it cannot be resumed while a patient remains on study.

M. Patients randomized to docetaxel alone (arm B) may at time of progression go on to receive vaccine alone if their ECOG performance status remains 0-1, and they do not have any uncontrolled pain or organ dysfunction that would require another intervention such as radiation or chemotherapy. Furthermore, patients initially randomized to arm B that would like to cross over and continue vaccine therapy must meet on-study eligibility and exclusion criteria with the exception of liver transaminase requirement indicated in 2.1.1 H. Patients with liver transaminase

levels within Grade 1 by CTCAE v4.0 (up to 3 x ULN) will be allowed to crossover to vaccine.

N. 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.
O. No other active malignancies within the past 12 months (with the exception of non-melanoma skin cancers or carcinoma in situ of the bladder) or life threatening illnesses.
P. Patients with cardiovascular symptoms should be fully evaluated for signs and symptoms of cardiovascular disease and other standard evaluations including EKG, chest X-ray, cardiac enzymes, and echocardiogram as clinically indicated.

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
   - Concurrent use of systemic steroids, except for local (topical, nasal, or inhaled) steroid use

B. History of allergy or untoward reaction to prior vaccination with vaccinia virus.
C. Pregnant or breast-feeding women
D. 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)
E. 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
F. Clinically active brain metastasis, or a history of seizures that have been active within one year
G. Medical conditions, which, in the opinion of the investigators would jeopardize the patient or the integrity of the data obtained
H. Prior docetaxel chemotherapy for metastatic disease
I. Serious hypersensitivity reaction to egg products
J. Clinically significant cardiomyopathy requiring treatment
K. Chronic hepatitis infection, including B and C, because of potential immune impairment
L. Although topical steroids are allowed, steroid eye-drops are contraindicated
M. Patients who have received prior PANVAC vaccine therapy

N. Patients with a prior history of allergy to eggs or egg products should not receive the vaccine
O. Patients with cardiac disease that have fatigue, palpitation, dyspnea or angina with ordinary physical activity (New York Heart Association class 2 or greater) are not eligible.
P. Prior splenectomy
Q. 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)
- Serum CEA, CA 27-29
- CD4:CD8 * **only** done for patients enrolled at the NCI
- Urinalysis
- Beta-HCG for women of child-bearing potential (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

C. HIV, Hepatitis B and C test within the past 8 weeks
D. Electrocardiogram (EKG) within the past 28 days
E. Computerized Tomography (CT) of the chest/abdomen/pelvis, a CT or MRI of the brain to rule out CNS metastasis, and Technetium-99 Bone Scintigraphy within the past 28 days
F. Class 1 MHC profile labs to be drawn at baseline; however, results need not be available at patient enrollment.
G. Echocardiogram within 28 days prior to enrollment on study. Patients who are HER-2 + and continue to receive Trastuzumab by their referring physician while on study will require a follow-up Echocardiogram at week 13 of study.

2.3 Patient Registration and Treatment Randomization

All patients must have completed an eligibility checklist. HER-2 + patients will be stratified based on whether they continue to receive Herceptin therapy along with the arm they are randomized to above. Authorized staff must register patients by faxing the eligibility checklist from the web site to the Central Registration Information Services at (301) 480-0757 (telephone

301 402-1732) Monday through Friday between the hours of 8:30 am and 5:00 pm Eastern time. A recorder is available during nonworking hours. After confirming eligibility, Central Registration will provide the randomization assignment.

For participating site Registration:

All patients must be registered through the NCI Central Registration Office (CRO). The CRO is open from 8:30am to 5:30pm EST Monday through Friday, excluding federal holidays. A protocol registration form and cover memo will be supplied by the Coordinating Center, NCI CCR and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To register a subject, fax the completed registration checklist and cover memo to the CRO at 301-480-0757. Please indicate on the protocol registration form whether the patient is screening or is eligible to start treatment. The CRO will notify you either by e-mail or fax that the protocol registration form has been received. The CRO will assign a unique patient/subject ID number for each subject that will be used to enter data into the C3D data base. Questions about eligibility should be directed to the Coordinating Center's Research Nurse, Sheri McMahon, 301-496-9812, smcmahon@mail.nih.gov.

Technical questions about the form should be directed to the Central Registration Office (301-402-1732)

A copy of the registration checklist form should be faxed to Coordinating Center Research Team at 301-480-1779.

After confirmation of eligibility at Central Registration Office, registration staff will notify the enrolling site as well as the Coordinating Center of each patient registration simultaneously through each site's coordinating research nurse. NCI coordinating research nurse can be reached at 301-480-1779 or 301-480-5094. The registration staff will also call the respective site's Pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents.

3 STUDY IMPLEMENTATION

3.1 Study Design

This randomized pilot study proposes to evaluate in a population of patients with metastatic breast cancer, a potential clinical benefit using an initial priming vaccination with 2×10^8 pfu PANVAC-V (vaccinia) followed by monthly boosting vaccinations with 1×10^9 pfu PANVAC-F (fowlpox) in conjunction with docetaxel therapy administered weekly for 3 weeks in each 4-week cycle (arm A), versus docetaxel monotherapy administered once weekly for three consecutive weeks in each 4-week cycle (Arm B). Sargramostim (100 mcg) will be given at the site of the vaccination on each vaccination day and for three consecutive days thereafter. Patients will receive education for self-injection or for family member to provide GM-CSF for 3 days which is necessary and essential. **NOTE**: Only patients at the NCI will receive Sargramostim.

At time of disease progression on scans, patients on arm A will be taken off trial. Those patients on arm B will have their docetaxel discontinued, but may have the option to remain on study (after discussion of additional treatment options) to receive the same vaccine regimen described above priming with PANVAC-V (vaccinia) followed by PANVAC-F (fowlpox) boost 3 weeks later. Then, PANVAC-F boosting vaccines will be given every 4 weeks along with sargramostim. After the initial radiographic assessment at week 13, CT scans and bone scan will be performed every eight weeks to evaluate for disease progression and clinical responses (patients with measurable disease) to therapy for patients on study. Patients, whose disease has not progressed on scans for a period of one year on study, will have the interval for CT scans and bone scan increased to every 3 months while on study. Forty-eight patients will be enrolled on this study.

3.1.1 **Trial Outline**

*Arm A (n = 24)

Week -3	PANVAC-V (vaccinia) 2 x 10^8 pfu subcutaneously x 1 dose Sargramostim 100 mcg subcutaneously daily x 4 days at the vaccination site (only for patients at the NCI).
Weeks 1, 5, 9	*PANVAC-F (fowlpox)1 x 10^9 pfu subcutaneously x 1 dose Sargramostim 100 mcg subcutaneously daily x 4 days at the vaccination site (only for patients at the NCI).
Weeks 1, 2, 3 5, 6, 7 9, 10, 11	Docetaxel 35 mg/m^2 IV over 30 minutes x 1 dose (Dexamethasone 4 mg PO 12 hours before, 1 hour before and 12 hours post each docetaxel dose)

**Arm B (n = 24)

Weeks 1, 2, 3 5, 6, 7 9, 10, 11	Docetaxel 35 mg/m^2 IV over 30 minutes x 1 dose (Dexamethasone 4 mg PO 12 hours before, 1 hour before and 12 hours post each docetaxel dose)

* Each PANVAC-F (fowlpox) dose is to be administered 24-48 hours prior to the first docetaxel dose in each 4 week cycle.

ARM A – After week 12 (at week 13 visit), patients who demonstrate disease progression will be taken off study. Patients who do not progress will continue with docetaxel weekly for three consecutive weeks out of every four and PANVAC-F (fowlpox) + sargramostim vaccination every 4 weeks until disease progression. Sargramostim will be given only to those patients

enrolled at NCI.

** ARM B – After week 12, (at week 13 visit), patients who demonstrate disease progression will have docetaxel therapy stopped and may commence therapy with PANVAC vaccination as described for arm A [PANVAC-V (vaccinia) + sargramostim priming vaccination x 1 dose, followed 3 weeks later with PANVAC-F (fowlpox) + sargramostim boosting vaccinations every 4 weeks] until further evidence of disease progression. Sargramostim will be given only to those patients enrolled at NCI.

Vaccine will be optional and offered only if the experimental vaccine is clinically judged to be in the best interest of the patient at the time of crossover. If it is in the patient's best interest to receive an additional chemotherapy regimen, i.e. rapidly progressing disease where a patient would not meet the eligibility criteria at crossover (Section **2.1**), we will not offer the patient the vaccine at crossover. Patients who do not progress will continue with docetaxel weekly for three consecutive weeks out of every four until disease progression.

HER-2 + patients will be stratified based on whether they continue to receive Trastuzumab (Herceptin) therapy along with the arm they are randomized to above, as well as according to whether they will be receiving Sargramostim or not. Trastuzumab, if continued, will be provided by the referring physician at the standard maintenance dose of 6mg/kg IV every 3 weeks or 2mg/kg IV weekly as determined by the referring physician providing the drug. Participating institutions (NIH Clinical Center and MD Anderson Cancer Center) will not be responsible for providing nor administering the trastuzumab.

Note: If there are two or more patients within one arm who experience grade 4 toxicity related to the regimen, we will halt further accrual to this study until we review the protocol with CTEP and IRB.(see section 5.4).

3.2 Drug Administration

3.2.1 Study drugs

Study drugs will be prepared and placed in syringes by the NIH Clinical Center pharmacy personnel or MD Anderson pharmacy staff.

This pilot randomized Phase II study will be conducted by delivering in Arm A an initial priming vaccination with 2 x 10^8 pfu PANVAC-V (vaccinia) (see section **8.1**) followed by monthly boosting vaccinations with 1 x 10^9 pfu PANVAC- F (fowlpox) (see section **8.2**) in conjunction with weekly docetaxel therapy (see section **8.5**). Sargramostim 100 mcg will be given at the site of the vaccination on each vaccination day and for three consecutive days thereafter (see section **8.4**). Sargramostim will be given only to those patients enrolled at NCI. At week 13, patients with stable scans (CT and BS) will continue weekly docetaxel (3 out of 4 weeks) and PANVAC-F (fowlpox) vaccine with Sargramostim every 4 weeks with scans repeated every 8 weeks (2 months). Patients randomized to arm B will receive monotherapy with weekly docetaxel (3 of 4

weeks).). At week 13, patients with stable scans (CT and BS) will continue weekly docetaxel (3 out of 4 weeks) Those patients on arm B who experience disease progression on scans will have their docetaxel discontinued, but have the option to remain on study to receive the same vaccine regimen described above priming with PANVAC-V (vaccinia) followed by monthly PANVAC- F (fowlpox) boosts along with sargramostim.

Forty-eight patients will be enrolled on this study. Patients will be seen at each vaccination visit for physical examination and collection of laboratory data and adverse event information. Patients will have the option to receive the docetaxel chemotherapy at the NCI or MD Anderson Cancer Center or it can be administered by a local oncologist, but will continue to be assessed at a minimum of every 4 weeks at the NCI Clinical Center or MD Anderson Cancer Center while on-study.

If the patient elects to receive docetaxel by their local oncologist, study drug will be provided by either the NIH pharmacy for patients enrolled at the NCI or by their local oncologist. Any toxicity observed or dose modifications made by the local oncologist must be communicated with the research team at the site where the patient is enrolled, including copies of the medical records reflecting this. A preliminary assessment of immune response in HLA-A2, A3, and A24 patients will also be performed at the end of the study (see Trial Outline section **3.1.1**).

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 **8**, "Pharmaceutical Information").
- The proper procedure for disposing the live vaccine is a critical part of drug administration (see the disposal sections of section **8**, "Pharmaceutical Information").

3.3 Treatment Modifications

3.3.1 PANVAC™-V (vaccinia) and PANVAC-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. In addition, patients who develop ≥ grade 2 allergic disease to the vaccine or ≥ grade 2 autoimmune disease that may threaten vital organ function or any grade 3 or greater autoimmunity will be removed from the protocol. Follow-up will be performed during each vaccination and blood draw interval for immunologic testing as described in section **3.5.3**.and **3.5.4**. Vaccination will be held for > grade 2 urinalysis or > 20 RBC/HPF.

A medical workup will be performed to determine if the cause of this is related to any renal

abnormality prior to resuming vaccine therapy if the urinalysis recovers to < Grade 2 toxicity.

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.

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 PANVAC-F (fowlpox).

Vaccine Dose Modification: None

3.3.2 Sargramostim (GM-CSF) - Only for patients enrolled at the NCI

If reversible grade ≥ 3 toxicity attributable to sargramostim administration is encountered, sargramostim will be reduced by 50% for the following cycle. If similar reversible grade ≥ 3 toxicity attributable to sargramostim recurs in more than two cycles and/or in the rare event that a patient cannot tolerate GM-CSF, the GM-CSF will be discontinued. However, the patient will still be able to receive vaccine.
In addition, patients who develop ≥ grade 2 allergic disease to the vaccine or ≥ 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.

3.3.3 Docetaxel

Dose Modifications for docetaxel: Weekly administration of docetaxel will be withheld for the occurrence of an ANC < 1500 cells/mm^3, a platelet count < 100,000 cells/mm^3 or grade 3 non-hematologic toxicity. Patients with grade 2 neurotoxicity should have docetaxel withheld until it improves to grade 1 or less. In addition patients with grade three or worse peripheral neuropathy should have docetaxel discontinued. Patients with a severe hypersensitivity reaction to docetaxel should have the drug discontinued. For those patients on both Arm A and Arm B who have toxicity requiring docetaxel to be discontinued, they will be taken off study. Patients should have an ANC ≥1500 cells/mm^3, a platelet count ≥ 100,000 cells/mm^3, and total bilirubin in the normal range for the treating lab for retreatment. In addition if SGOT and/or SGPT are > 1.5 times the upper limit of normal and Alk Phos (from liver) is > 2.5 times the upper limit of normal docetaxel should be held. Finally, one must have the resolution of any other non-hematologic toxicity to grade 2 or baseline in order to initiate another treatment cycle of docetaxel. The occurrence of grade ≥ 3 toxicity (with the exception of lymphopenia) or a delay of > 2 weeks in initiating a new treatment cycle for the recovery of toxicities would result in a docetaxel dose reduction of 25%. In addition, patients with documented Grade 3 febrile neutropenia (as defined by ANC <1000/mm3 with a single temperature of >38.3 degrees C (101 degrees F) or a

sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour, CTC 4.0), ≥ Grade 2 mucositis, ≥ Grade 3 cutaneous toxicity upon recovery of toxicity will have a docetaxel dose reduction of 25%. Patients should be allowed to continue to be treated on successively reduced doses of docetaxel as long as they do not have progressive disease regardless of the time to recovery. For dose-limiting toxicities related to docetaxel, vaccine should not be interrupted. All dose modifications will be coordinated between the outside physician and the NIH based on the modifications outlined above. This will be communicated between the outside oncologist and clinical trials investigator at the time of enrollment on study.

3.3.4 Premedication

Dexamethasone p.o. 4mg will be given Q 12 hours before, then 1 hour before docetaxel dose, then 12 hours post docetaxel dose. If patient fails to take their premedication dexamethasone, give 10mg dexamethasone IV from 60 minutes to immediately before starting docetaxel.

3.3.5 Trastuzumab (Herceptin)

For those patients who are HER-2 + and will continue to receive Trastuzmab therapy by their referring physician, we will continue to monitor patients at the NIH or MD Anderson for the toxicities (discussed below) related to Trastuzumab. The following treatment modifications will be discussed with the referring M.D if they occur:

a. Infusion Related Toxicity

Trastuzumab is associated with infusion reactions. This is best documented where in 40% of cases during the first infusion, a symptom-complex consisting of chills and fever is observed. Meperidine may be used to treat chills and rigors, acetaminophen may be used to treat fever or headaches and an anti-histamine, such as diphenhydramine may be used to treat rash. Since patients will have already been receiving trastuzumab prior to coming on to study, we expect the likelihood of such reactions to be much less frequent.

b. Cardiac dysfunction

Cardiac dysfunction can occur when trastuzumab is combined with chemotherapy. All patients continuing with trastuzumab therapy will undergo an echocardiogram (section **2.2**) prior to enrolling on study and at week 13. Patients with cardiac disease that have fatigue, palpitation, dyspnea or angina with ordinary physical activity (New York Heart Association class 2 or greater) are not eligible to enroll on study (see exclusion criteria section **2.1.2**). Patients who are found to have a ≥ 15 percentage decline in left ventricular ejection fraction (LVEF) will have trastuzumab discontinued. Any patient who develops symptoms of cardiac dysfunction will undergo an echocardiogram to determine if these symptoms are cardiac in origin. If these symptoms are determined to be secondary to cardiac dysfunction, trastuzumab will be discontinued.

3.4 Pharmacokinetic Studies: N/A

3.5 Protocol Evaluation: (See APPENDIX C)

3.5.1 All patients who are deemed eligible and who sign the informed consent form will be enrolled onto this trial.

3.5.2 History and Physical Exam, performance status will be completed within 16 days prior to enrollment.

3.5.3 Laboratory studies:

Baseline only:

- Hepatitis B, C and HIV panel (within 8 weeks prior to day 1)
- HLA-A2 profile (blood should be drawn at baseline, but testing may be performed after patient is enrolled)
- Beta-HCG for women of child-bearing potential (within 48 hours prior to day 1). In addition, patients should be willing to practice effective birth control during the study and four months following the last study treatment

Baseline (within 16 days prior to on study date) and during study:

- CBC/differential, with platelet count.
- Serum chemistries (Na+, K+, Cl-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH)
- Serum CEA, CA 27-29(baseline and monthly prior to vaccination)
- CD4:CD8 (baseline and monthly prior to vaccination) This will be done **only** for patients at NCI.
- Urinalysis prior to enrollment and prior to each vaccination.

These laboratory tests will be done at baseline, and about weeks 1, 5, 9, 13 and every 4 weeks while on-study prior to each subsequent vaccination. Additional CBC/differential as well as bilirubin, AST, ALT and alk phosphatase will be obtained prior to each treatment with docetaxel. 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.

3.5.4 Collection of immunologic blood samples

Patients who are HLA-A2 positive will be requested to undergo apheresis at baseline, around week 13, and then every 4 months while on study. Blood samples will be collected at these time points via apheresis as described in Section 5.2. **NOTE:** Apheresis will **only** be performed on patients at NCI. In addition, baseline and prior to each vaccine, 6 green top tubes and 2 red SST tubes will be obtained.

For patients at MD Anderson, research samples consisting of **7** green tops tubes and 2 red SST, will be drawn at baseline. At the first re-staging appointment (approximately 12 weeks), 7 green top tubes and 2 red SST will be drawn. The green top tubes will be sent via overnight FED-EX to the NCI Frederick Repository at the address below: See APPENDIX H.

Attn: Bill Kopp, Ph.D.
Bldg 560/Room 11-40
SAIC-Frederick
Bldg 1050 Boyles Street
Frederick, MD 21702
Phone No. 301-846-5125, or 301-846-1707

The red SST will be spun down and stored in -80º C freezer at MD Anderson. These serum samples will be batched and then shipped to the NCI Frederick Repository (address above) at intervals.

Immunologic testing will include:
IFN-gamma ELISPOT assays for CEA-specific T lymphocytes and MUC-1-specific T lymphocytes.

Antibodies to CEA, vaccinia, fowlpox, MUC-1 . (Samples from baseline and about around week 13.)

Only patients enrolled at the NCI will have CD3, CD4, and CD8 drawn at baseline and monthly prior to vaccination while the patient remains on trial. 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.

3.5.5 For all patients, appropriate imaging with computerized tomography (CT) of the chest/abdomen/pelvis and bone scan to evaluate for disease progression and to determine tumor measurements (in patients with measurable disease) will be done at baseline, week 13 and every 8 weeks thereafter. Patients whose disease has not progressed on scans for a period of one year on study, will have the interval for CT scans and bone scan increased to every 3 months (12 weeks) while on study.

3.5.6 Monitoring after the initial phase of vaccinations
All patients will to have clinical / immunologic monitoring as outlined in **APPENDIX C**.

3.5.7 The samples will be processed at

Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick MD 21702

301-846-1000

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

For research samples drawn at MD Anderson, the green top tubes will be sent via Fed-Ex overnight to the NCI Frederick central repository. The SST will be processed at MD Anderson and stored in -80º C freezer. Samples will be batched and then shipped to the NCI Frederick central repository. Fed-Ex shipments can be billed to James Gulley's Fed-Ex account # 281312103. See **APPENDIX H**.

The weekly NCI patient list of samples drawn will be emailed to Sandra Doren at dorens@mail.nih.gov and Jen Bangh at jb478s@nih.gov.

3.5.8 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 SAIC 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.

Fisher BioServices manages the NCI-Frederick Central Repositories under subcontract to SAIC, Frederick, Inc. NCI-Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance will applicable HHS and FDA Protection of Human subjects Regulations in accordance with Fisher BioServices Federal-wide Assurance. Fisher BioServices role is limited to clinical research databases and repositories containing patient specimens. Fisher BioServices does not conduct nor has any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects. The Fisher BioServices IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

It is the intent and purpose of Fisher BioServices to accept only de-identified samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking

system is used to manage the storage and retrieval of specimens as well as 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.

3.5.9 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.

3.6 Concurrent Therapies

Concurrent anticancer treatment with chemotherapy, hormonal therapy, systemic glucocorticoids (topical, nasal, and inhaled steroids allowed), major surgical procedures or radiation therapy or immunotherapy is not permitted during the initial vaccine treatment. An exception to this would be to allow HER-2+ patients who have previously progressed on a Herceptin-containing regimen to remain on Herceptin therapy provided by their referring physician.

3.7 Surgical Guidelines: N/A

3.8 Radiation Therapy Guidelines: N/A

3.9 Off Study Criteria:

- Progression of disease as described in section **5.2**. Patients who demonstrate disease progression on docetaxel therapy alone will have docetaxel discontinued. They may elect to receive vaccine therapy alone and remain on study if they have undergone prior standard chemotherapy and are aware of alternative treatment options including other chemotherapy agents as well as other clinical studies. For those patients who choose to receive vaccine alone, they will be taken off study at time of disease progression. For patients randomized to the combination therapy arm of the study, patients determined to have progression of disease they will come off treatment (see section **3.10**)
- Patients who have non-symptomatic disease progression while docetaxel is being held for toxicity (e.g., pleural effusion), may continue to receive treatment if it is felt that the disease may be responsive to docetaxel (no progression within 2 weeks of docetaxel treatment) and the toxicities requiring holding the docetaxel have resolved as described in Section **3.3**.
- Patients that progress on docetaxel and remain on study will be restaged after 2 cycles of re-treatment with docetaxel. Restaging may be performed sooner if clinically indicated. The most recent radiologic studies prior to restarting docetaxel will serve as the new baseline radiologic studies.
- Unacceptable treatment-related toxicity (dose limiting toxicity) as described in sections **3.3**, **5.3**, and **5.4**
- 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 >1g per 24 hours
- If two or more patients within one arm experience grade 4 toxicity related to the regimen, we will review the protocol with CTEP and IRB.

An Off Study form must be completed and faxed to Central Registration at (301) 480-0757 to officially take a patient off study.

3.10 Post Treatment Evaluation (see APPENDIX C)

The Biologic Response Modifiers Advisory Committee has recommended that long-term follow-up extend over a period of 15 years. Patients may undergo annual follow-up examinations at the NIH Clinical Center or MD Anderson Cancer Center. The research nurse may obtain the information from their local physician for the first 5 years following examination. Additional data will be obtained annually for years six through fifteen via telephone contacts from the research nurse. 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. Patients will be enrolled on to the "Follow-Up Study of Subjects Previously Enrolled in Poxviral Vector Gene Transfer Studies" once off treatment.

4 SUPPORTIVE CARE

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

4.1 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 vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. Upon receipt of a call from a patient or upon direct observation of a patient or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible: 1) to initiate review of the clinical case, 2) to seek consultation on the appropriateness of VIG therapy, 3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and 4) to determine how to access and have the appropriate doses of VIG delivered. Early institution of VIG therapy is advised following recognition of clinical symptoms compatible with some vaccinia complications (eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of inadvertent inoculation). The effectiveness of VIG therapy appears to be time dependent. VIG has not proven to be of benefit in the treatment of post-vaccinial encephalitis, and is contraindicated for treatment of isolated vaccinial keratitis due to the increased risk of corneal scarring. A new 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 11, 2003. Available at URL: http://www.bt.cdc.gov/agent/smallpox/vaccination/mgmt-adv-reactions.asp]. The CDC has informed the NCI/CTEP that cidofovir will not be supplied through Strategic National Pharmaceutical Stockpile to investigators involved in CTEP-sponsored protocols utilizing recombinant vaccinia-based vaccines. 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 (except when used for docetaxel administration) 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/mm^3. 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/mm^3.

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 Central Registration.

5.1.2 The Principal Investigator, protocol chairperson and the research nurse will meet weekly at each clinic to review all adverse events for each subject in this trial. Unexpected adverse events and/or serious adverse events will be reported to the NCI's Institutional Review Board (IRB), the Cancer Therapy Evaluation Program (CTEP), (see section **7.1**) and MD Anderson's IRB, if applicable. If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent will be modified accordingly. The Safety Monitoring Committee (SMC) will monitor the study at its meetings, which are held yearly. All monitoring will take place by the principal investigator, study chairman, and others they wish to include in their discussions. Unexpected and/or serious adverse events must also be reported to each institution's OBA and IRB for gene therapy trials. As well, the NCI IRB will review new adverse events monthly.

5.1.3 The study will be monitored by Clinical Data Update System (CDUS). Data will be secured in the Cancer Central Clinical Database (C3D) based on software produced by Oracle Corporation. Data will be collected using protocol-specific case report forms, verified for accuracy and completeness, and submitted to CDUS quarterly by electronic means. Paper reports will not be accepted. The instructions for the CDUS and the CDUS Smart Loader can be downloaded from the CTEP Home page http://ctep.cancer.gov. If you are unable to access the CTEP Home page please contact CDUS by telephone (301) 840-8202, fax (301) 948-2242 or email ncictephelp@CTEP.nci.nih.gov. Reports are due January 31, April 30, July 31, and October 31.

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 will be evaluated for disease progression and those patients with measurable disease 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: http://ctep.cancer.gov/guide. A copy of this quick reference is found in **APPENDIX D**.

The minimum restaging evaluation a patient who has measurable disease to be considered for response assessment will be about 85 days. Patients who experience disease progression on the combined vaccine and docetaxel will be removed from study, except as defined in Section **3.9**, Off-Study Criteria. If they have had a response or remain stable they can continue to receive the combined therapy and will have restaging every 2 months at this point. Patients on the docetaxel alone arm will also be able to continue on docetaxel if they do not progress on scans. Patients, who progress on a docetaxel holiday, may be able to receive subsequent docetaxel as defined in Section **3.9**. At time of progression they will have their docetaxel discontinued and may be offered vaccine therapy. The same imaging studies used to define the extent of tumor at baseline upon study entry will be used for restaging. In those patients who have progressed on docetaxel and have commenced therapy with vaccine, the time to disease progression will be defined as from the first date that docetaxel therapy is-discontinued and vaccine is initiated until the first notation of clinical progression.

5.2.1 Criteria for Response Assessment

Measurable disease (by RECIST Criteria) 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.

For those patients with evaluable only disease, any new lesion on bone scan that is consistent with metastatic disease would be considered disease progression.

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 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. 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. Briefly, 96-well milliliter HA plates (Millipore Corporation, Bedford, MA) are coated with 100μl/well of capture MoAb against human γ-IFN at a concentration of 10 μg/ml for 12h at RT. Plates are blocked for 30 min with RPMI 1640 plus 10% human Ab serum. 1 x 10^5 PBMC are added to each well. PSA-3 pulsed C1R-A2 cells we added into each well as antigen presenting cells (APC) at an effector:APC ratio of 1:1. Unpulsed C1R-A2 cells were used as a negative control. HLA-A2 binding Flu Matrix peptide 59-66 is used as a positive peptide control. Cells are incubated for 24h and lysed with phosphate buffered saline (PBS)-Tween (.05%). Biotinylated anti γ-IFN antibody diluted to 2 μg/ml in PBS-Tween containing 1%bovine serum albumin (BSA) is added and incubated overnight in 5% CO_2 at 37°C. Plates are washed 3 times and developed with avidin alkaline phosphatase (GIBCO/BRL, Grand Island, NY) for 2h. After washing the plates 3 times, each well is examined for positive dots. The number of dots in each well is counted by two separate investigators in a blinded manner, and the frequency of responding cells was determined for a total ranging from 0.6- 1.2 x 10^6 effector cells plated. This calculation represents the number of PSA specific CTL present at

different time points during therapy.

ELISPOT assays employing the CEA agonist peptide and the MUC-1 agonist peptide have already been developed. (See **APPENDIX E** for details).

For patients who are HLA-A2 positive, we will be 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 HLA-A2 positive patients undergoing apheresis, 5 x 10^8 to 2 x 10^9 mononuclear cells will be obtained by a single-access (single venipunture) "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)
This study will utilize the CTCAE version 4.0 for toxicity and adverse drug experience reporting beginning January 1, 2011. A copy of the CTCAE version 4.0 will be available from the CTEP webpage http://ctep.info.nih.gov/reporting/ctc.html. All appropriate treatment areas will have access to a copy of the CTCAE version 4.0.

Toxicity Grading for Vaccinia Toxicity (49)
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 objective of this trial is to demonstrate in a pilot fashion whether use of PANVAC vaccine in conjunction with docetaxel is able to potentially improve the progression free survival of patients with metastatic breast cancer compared to patients who receive docetaxel alone. The trial will be conducted as a two arm randomized single multi-institution pilot trial of PANVAC + Docetaxel vs. Docetaxel alone. Any suggestion of positive findings will be evaluated for the potential to conduct a subsequent multi-institutional definitive trial through an established cooperative group mechanism. Patients, who are HER-2+ and have progressed on prior

Trastuzumab (Herceptin) therapy, may continue to receive Herceptin therapy along with the above arm they are randomized to. HER-2+ patients will be stratified for receiving or not receiving Herceptin, as well as according to whether they will be receiving Sargramostim or not. It is not anticipated that use of Sargramostim or not will have a substantial impact on progression free survival; however, an exploratory analysis will be performed to determine the degree to which this is true.

Trastuzumab, if continued, will be provided by the referring physician (not at the NIH Clinical Center or MD Anderson) at the standard maintenance dose of 6mg/kg IV every 3 weeks.

Since this is intended to be a small randomized pilot trial, there will be no stratification for potential prognostic factors.

A total of 48 evaluable patients will be randomized in a 1:1 ratio between the two arms (24 evaluable patients per arm). Using standard formulae as implemented in nQuery Advisor version 5, this number was selected to provide 80% power to detect a difference between 4.2 month median progression free survival on the docetaxel alone arm and 8 month median progression free survival on the arm receiving PANVAC plus docetaxel, with a one-tailed alpha=0.10, assuming 36 months accrual and an additional 12 months of follow-up after the last patient has been enrolled.

The Safety Monitoring Committee (SMC) will monitor the study at its meetings, which are held yearly. This trial will also be monitored by the principal investigator, study chairman, and associate investigators who wish to include in their discussions. As well, the NCI IRB will review new adverse events monthly.

An early stopping rule will be applied to the combination therapy arm to ensure that use of vaccine is not adversely impacting clinical responses to docetaxel. Data from the literature suggests that the overall response rate (PR +CR) to docetaxel alone in this population should be 30%. An early evaluation will take place after the initial 12 patients have enrolled and evaluated on the combination arm. Should 2 or more of these 12 patients have a PR or CR, then accrual will continue as described above, since the upper 90% one-sided confidence interval about 2/12 is 38.6%, which would show results consistent with 30%. On the other hand, if 1/12 or fewer patients respond, then this arm should stop accrual, and accrual to the trial as a whole will terminate, since the 90% upper one-sided confidence bound on 1/12 is 28.7%, which is inconsistent with an anticipated 30% response rate.

Furthermore, an early stopping rule will be applied to patients who have progressed on docetaxel alone (arm B) who go on to receive vaccine therapy alone. Among the first 10 patients who receive vaccine alone following progression on chemo, if 0 of 10 either have a PR or disease stabilization for at least 3 months while on vaccine alone, that no further patients be allowed to go onto vaccine after progression. 0/10 has an associated upper 90% one sided exact confidence interval of 20.6%; thus, if 0/10 have any clinical benefit there is 90% confidence that the true probability of clinical benefit is approximately 20% or less and thus is not worth further evaluation for this purpose in this population. Thus if no patient has either a PR or stable disease

we will not accrue further patients to vaccine alone. Finally, we will perform an evaluation of the risk of Grade 4 cytopenia with docetaxel alone and use this as the basis for determining the number of patients with Grade 4 toxicity that would warrant study discontinuation. (See section **3.1.1**)

If there are two or more patients within one arm who experience grade 4 toxicity related to the regimen, we will halt further accrual to this study until we review the protocol with CTEP and IRB.

The primary analysis will be performed using a log rank test with a one-sided p-value, with all eligible, randomized patients on whom follow-up is available included in the analysis. The patients will be analyzed as randomized. A Kaplan-Meier curve will be constructed starting at the date of randomization.

It is anticipated that approximately 18-20 patients per year will be entered onto this trial; an accrual period of up to 36 months is anticipated in order to enroll 48 patients.

5.5 MULTI-INSTITUTIONAL GUIDELINES

IRB Approvals:
The NCI IRB must approve the addition of each participating site to the protocol and will require a copy of the local IRB approval from each participating site before NCI IRB approval will be granted.

Participating institutions will provide the NCI PI with copies of the initial local IRB approvals and semi-annual or annual continuing review approvals. The NCI PI will then furnish these to the NCI IRB. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NCI IRB. Only one version of the protocol will be the correct version; amendments must be initiated through the coordinating center; amendments will be submitted to the NCI IRB and to the IRBs of participating institutions. Each center will be responsible for its own IRB submissions.

Amendments and Consents:
The NCI PI will provide the NCI IRB with copies of all amendments, consents and approvals.

Patient Registration:
Participating institutions must register patients with the CCR Central Registration as specified under section **2.3**. Such patients will be treated, monitored, and managed according to the guidelines of this protocol, after signing a current version of the informed consent. A copy of the registration checklist and off-study forms should be faxed to Coordinating Center Research Team at 301-480-1779 or 301-480-5094.

Drug Distribution:
Site PI's or their designees will order vaccines from the CTEP Pharmaceutical Management

Branch by completing and sending by fax the Clinical Drug Request (NIH - 986) Form. The drug will be shipped to the pharmacy for each institution. Contact information: Pharmaceutical Management Branch (http://ctep.cancer.gov) 6130 Executive Blvd, Rm 7419, Rockville, MD 20852, 301-496-5725, FAX 301-480-4612

Data Collection and Toxicity Reporting:
Participating institutions must submit research and clinical data to the Coordinating Center, NCI, on a monthly basis. Data will be entered into the C3D database at participating facilities, training of designated data entry staff will be provided. Required data to be entered into C3D and/or maintained at the study site include, not exclusively: prior disease related therapies, with dates, disease type, stage, disease sites, lab results, RECIST measurements, ECG, Scan reports and images on CD, and adverse events. Supporting eligibility source documentations should be reviewed and the eligibility checklist for registration needs to be sent within 3 business days to the Coordinating Center for eligibility review. Concurrent medications need to be entered into the database. All > grade 2 adverse events at least possibly attributed to research and not in the consent must be submitted as soon as possible so that they may be reported to the NCI IRB within 7 days of receipt (see section **7**). Reporting to local IRB should be done as stipulated by local IRB guidelines. All data collection forms and records may be audited on-site at participating institutions and should be made available at the time of the study audit. CTEP will perform a site visit every 3 years at participating facilities.

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

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

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

5.6 Data and Safety Monitoring Plan

5.6.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead

associate investigator. Adverse events will be reported as required in section **7**. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations and violations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

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

The members of the site specific 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), MD Anderson's IRB and Cancer Therapy Evaluation Program (CTEP) (see section **7.1.1**). The study PI will review all safety data. 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 SMC will monitor the study at its meetings, which are held yearly. Unexpected and/or serious adverse events must also be reported to OBA for gene therapy trials

5.6.2 Safety Monitoring Committee (SMC)

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

5.6.3 Confidentiality of Trial Documents and Subject Records

Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released with outpatient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the Food and Drug Administration (FDA) or other authorized user, only under guidelines established by the Federal Privacy Act. The Coordinating Center is responsible for establishing conference calls between participating sites to discuss protocol issues. The Coordinating Center will conduct an "on-site" monitoring visit at least annually for source document verification and regulatory document review.

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 Studies Support Center (CSSC), 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 Evaluation of Benefits and Risks/Discomforts

The investigation nature and objective of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits and potential alternative therapies will be carefully explained to the patient and a signed informed consent document will be obtained. The response rates for various chemotherapy regimens will be discussed based on whether patients have had prior chemotherapy for metastatic disease. 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.

6.3.1 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.

6.3.2 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 Protocol Evaluation 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 NIH Clinical Center, Bethesda, Maryland. Patients at MD Anderson will have their care managed by the care guidelines at their participating institution. 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.

6.3.3 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 Cancer Institute 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.

6.4 Risks/Benefits Analysis

The patients we will be enrolling will have metastatic breast 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 breast cancer. Patients will undergo multiple vaccinations. Alternative treatments include chemotherapy, other clinical trials, or supportive care. 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.

6.5 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. 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 HLA-A2 expression. In such cases, a protocol investigator will review the Screening Sample Consent form by telephone. The consent/assent signatures will be witnessed and a copy will be faxed and the original sent by mail to the PI. Prospective subjects who consent to send such samples for outside testing will NOT be registered with Orkand 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 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Expedited Adverse Event Reporting to CTEP

This protocol will follow CTEP, DCTD, NCI's expedited reporting requirements for Investigational New Drugs sponsored by NCI. An expedited adverse event report requires submission to CTEP via the AdEERS web-based electronic reporting system available on the CTEP home page: http://ctep.cancer.gov.

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 AdEERS by the original submitter at the site.

7.1.1 All expedited adverse events reports will also be sent to the local Institutional Review Board (IRB) at NCI as well as the Office of Biotechnology Activities (OBA) at the NIH.

7.1.2 MD Anderson will be required to send all serious adverse events (SAEs) and AdEERs reports in accordance with the guidelines below, and the NCI Adverse Event form (see **APPENDIX G**) to the NCI either electronically or via fax (301-480-5094).

7.2 NCI-IRB Reporting

7.2.1 NCI-IRB Expedited Reporting of Adverse **Events, Unanticipated Problems,** and Deaths

The Protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research
- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

For reporting of adverse events at time of continuing review, the NCI-IRB requires a summary report of adverse events that have occurred on the protocol **since the previous continuing review**. The method of presentation should provide the NCI-IRB with the information necessary to clearly identify risks to participants and to make a risk:benefit determination. The summary report is based on the following guidance:

Any unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk:benefit of study participants in the narrative.

The protocol PI will report to the NCI-IRB:

1. All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
2. All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
3. All Grade 5 events regardless of attribution;
4. All Serious Events regardless of attribution.

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

NCI IRB Contact Information:
E-mail- nciirbadmin@mail.nih.gov
Mail- Building 82/Room 115, 9030 Old Georgetown Road, Bethesda, MD, 20814
FAX- 301-480-0106

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that require a sponsor recommended change to the protocol or the

consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.

7.2.4 DEFINITIONS

7.2.4.1 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved study procedures in a research protocol that **does not** have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

7.2.4.2 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the IRB-approved study procedures in a research protocol that **does** have a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

7.2.4.3 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

- Places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine (CTMS or CDUS) study data submissions. **AEs reported through AdEERS must <u>also</u> be reported in routine study data submissions.**

7.4 Secondary AML/MDS

AML/MDS events must be reported via AdEERS (in addition to routine AE reporting mechanisms). In CTCAE v 4, the event(s) may be reported as either: 1) Leukemia secondary to oncology chemotherapy, 2) Myelodysplastic syndrome, or 3) Treatment-related secondary malignancy.

7.5 NCI Guidance for Reporting Expedited Adverse Events for Multi-Center Trials

The site PI must immediately report to the coordinating center PI any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event within 48 hours of PI awareness of the event. The Site PI must also report any protocol deviations or violations to the coordinating center PI within 7 days of PI awareness. Participating centers must also submit the report to their IRB in accordance with their institutional policies.

7.6 Expedited Reporting Guidelines

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: AdEERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**

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

- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS 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 AdEERS 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 via AdEERS.http://ctep.cancer.gov

- All serious adverse events must be electronically reported to the NCI IRB within 24-hours at: nciirbadmin@mail.nih.gov. The NCI AE form should be used for AE reporting.

- A 24-hour notification is to be made to CTEP by telephone at 301-897-7497 only when internet connectivity is disrupted. Once Internet connectivity is restored, an AE report submitted by phone must be entered electronically into AdEERS by the original submitter at the site.

- Include IRB reporting information—use the NCI IRB electronic mailbox for 24-hour serious adverse events reporting (nciirbadmin@mail.nih.gov) and the NCI AE form for AE reporting.

- In addition, all serious adverse events (whether or not they are thought to be related to the gene intervention) should be reported to OBA at NIH per their requirements. The Serious Adverse Event Reporting Form can be found on the OBA web site (oba@od.nih.gov.)

- All AEs reported via AdEERS must also be reported via the routine AEs reporting defined by the protocol.

- 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 8B09) immediately by telephone 301-435-2956 or 301-496-1211 (after hours).

7.7 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 Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for PANVAC-VF/TRICOM.

Version 1.1, February 3, 2010[1]

Adverse Events with Possible Relationship to PANVAC-VF/TRICOM (CTCAE 4.0 Term)		**EXPECTED AEs FOR ADEERS REPORTING Agent Specific Adverse Event List (ASAEL)**
GASTROINTESTINAL DISORDERS		***Expected***
	Constipation	
	Dry mouth	
	Nausea	
	Vomiting	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	Chills	
	Fatigue	
	Fever	
	Injection site reaction	
METABOLISM AND NUTRITION DISORDERS		
	Anorexia	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Myalgia	
NERVOUS SYSTEM DISORDERS		
	Headache	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Pruritus	
	Rash maculo-papular	

[1]This 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.

Also reported on PANVAC-VF/TRICOM trials but with the relationship to PANVAC-VF/TRICOM still undetermined:

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Flu like symptoms;

Neck edema
INVESTIGATIONS - Weight loss

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 (vaccinial 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-vaccinial 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].

7.8 Record Keeping

Data will be secured in the NCI database named C3D. Data will be CDUS monitored. The NCI/DCT Case Report Form 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.

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. Records will be requested on a monthly basis from outside physicians

treating protocol patients with either docetaxel and/or trastuzumab. Any report of adverse events received by the referring physician should be reported to the principal investigator or a clinical associate investigator listed on this study. 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.

7.9 Regulatory Issues

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http:// ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):

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

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

    c. Any Collaborator having the right to use the Multi-Party Data from these trials

must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order.. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

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

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

   Regulatory Affairs Branch, CTEP, DCTD, NCI
   Executive Plaza North, Suite 7111
   Bethesda, Maryland 20892
   FAX 301-402-1584
   Email: anshers@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.

7.9.1 The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 and a CV.

7.9.2 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.)

Data will be submitted to CTEP as CDUS reporting with auditing to be scheduled by CTMB.

8 PHARMACEUTICAL INFORMATION

8.1 PANVAC™-V (NSC 727026)

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

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

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 geonome 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).

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.

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.**

Preparation Instruction for:
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 x 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 x 10^8 pfu/mL. Vortex vigorously at high power for at least ten seconds. Withdraw 0.32 mL (2 x 10^8 pfu) for subcutaneous administration.

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

Stability: Shelf-life studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8°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 2-8°C for up to 4 hours following preparation.

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

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 ≤ 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 included 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 and will be evaluated for cardiovascular risk factors and recent or symptomatic events as indicated in section **2.1.1**. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction for according to standard medical practice. At this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare event. If not otherwise excluded, patients with known symptomatic CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility at this time.

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 vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. Upon receipt of a call from a patient or upon direct observation of a patient or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible: 1) to initiate review of the clinical case, 2) to seek consultation on the appropriateness of VIG therapy, 3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and 4) to determine how to access and have the appropriate doses of VIG delivered. Early institution of VIG therapy is advised following recognition of clinical symptoms compatible with some vaccinia complications (eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of inadvertent inoculation). The effectiveness of VIG therapy appears to be time dependent. VIG has not proven to be of benefit in the treatment of post-vaccinial encephalitis, and is contraindicated for treatment of isolated vaccinial keratitis due to the increased risk of corneal scarring. A new 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 11, 2003. Available at URL: http://www.bt.cdc.gov/agent/smallpox/vaccination/mgmt-adv-reactions.asp]. The CDC has informed the NCI/CTEP that cidofovir will not be supplied through Strategic National Pharmaceutical Stockpile to investigators involved in CTEP-sponsored protocols utilizing recombinant vaccinia-based vaccines. 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.

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.

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, 2002. Available at URL: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html 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 eye ware, 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^{o}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 eye ware, 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^{o}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.

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 symptomatic 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 and will be evaluated for cardiovascular risk factors and recent or symptomatic events as indicated in section **2.1.1**. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction for according to standard medical practice. However, 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.

8.2 PANVAC™-F (NSC 727027)

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

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

Product Description: PANVAC™-F is a recombinant fowlpox virus vector vaccine containing genes for human CEA, MUC-1 and three co-stimulatory molecules: 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. 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 for insertion of the sequences into the fowlpox viral geonome to generate the final recombinant vaccine. 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, and thus, makes systemic infections unlikely. PANVAC™-F is manufactured by plasmid transfection of primary chicken embryo dermal cells infected with the recombinant parental vaccinia virus.

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.

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 10^9 pfu/mL, 0.3mL vial)
Thaw one vial completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least 10 seconds. Withdraw 0.16mL (1 x 10^9 pfu) from the thawed vial for subcutaneous injection.

Preparation Instructions for PANVAC–F, Lot 3-052203 (5.8 x 10^9 pfu/mL, 0.3mL vial)
Thaw one vial completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least 10 seconds. Withdraw 0.18mL (1 x 10^9 pfu) from the thawed vial for subcutaneous injection.

Storage: Intact vials of PANVAC™-F should be stored at –70°C or colder.

Stability: Shelf-life studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8°C. Thawed vials should not be re-frozen. Vials of PANVAC™-F 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 2-8°C for up to 4 hours following preparation.

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

Special Handling
Fowlpox virus is classified as a Biosafety Level 1 organism. 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. They can be handled safely in the laboratory without special apparatus or equipment, using techniques generally acceptable for nonpathogenic material. The recombinant product is a preparation of a live virus (infectious for birds) and contains DNA sequences derived from the human genome. It is recommended that the product be handled as a hazardous biological substance and waste materials should be disposed of as hazardous biological waste and incinerated according to local institutional policy and according to local, state, and federal regulations. Healthcare workers involved in handling the fowlpox product should avoid direct contact with pet birds for at least 72 hours after working with the agent. For more information regarding biohazard risk group classification and biohazard safety levels see NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines): April, 2002. Available at URL: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html 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 1 General Requirements Related Recombinant Fowlpox Virus Vaccines

At a minimum, the following procedures should be adhered to:

1. Special containment apparatus is generally not required for manipulation of Biosafety Level 1 agents. They can be handled safely in the laboratory without special apparatus or equipment, using techniques generally acceptable for nonpathogenic material.
2. Work surfaces are decontaminated prior to and following dose preparation.
3. Access to preparation areas should be limited or restricted while dose preparation is in progress.
4. Appropriate infection control measures (*e.g.*, thorough hand washing) should be utilized after handling any materials.
5. Appropriate personal protective apparel should be utilized (see item #3 in the following section "*Preparation and Disposal Recommendations*.")
6. All procedures are performed carefully to minimize creation of aerosols.
7. Policies for the safe handling of sharps are instituted.
8. No eating, drinking or applying cosmetics in the work area is permitted.

Preparation and Disposal Recommendations

The recombinant product is a preparation of a live virus. It is recommended that the product be handled as a hazardous biological substance. The following are recommendations with respect to preparation, handling, and disposal of fowlpox-based agents:

1. 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.
2. Agent for dose preparation should be transported from the -70^{o}C freezer to the work area in leak proof bag.
3. 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 [*e.g.*, gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eye ware, hair cover] utilized for preparation of antineoplastic agents should be utilized during preparation of recombinant fowlpox products for patient administration.
4. 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, rinsed, 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.
5. 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.

6. 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 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.
7. The final prepared dose container should be sprayed or wiped 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.
8. All waste should be placed into the 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, rinsed, then followed by sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
9. 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.
10. Accidental spills should be handled similarly to antineoplastic spills and/or according to institutional policy:
    a. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    b. Use protective clothing, eyewear, mask, and gloves.
    c. Spill should be covered with absorbent towels.
    d. Area should be decontaminated with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
    e. All waste should be disposed of as biohazardous waste and incinerated according to institutional policy and according to any local, state, or federal regulations.

Patient Care Implications and Contraindications

It is recommended that a sterile dry dressing (*e.g.*, Telfa pad) should cover vaccination sites. Once the injection site is healed, no further barrier is necessary. As a precaution, it is recommended that injection sites exhibiting evidence of weeping, oozing or ulceration be protected and patients be instructed to avoid direct 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, patients should avoid becoming pregnant, fathering a child, or breast-feeding for at least 4 months following completion of therapy with the recombinant vaccine. 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.

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.

8.3 Sargramostim (GM-CSF, LEUKINE®)

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.

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- mcg multidose liquid vial.

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.

Stability and Storage: LEUKINE Liquid and Lyophilized LEUKINE should be stored refrigerated at 2-8^{o}C. Each vial bears an expiration date. LEUKINE Liquid may be stored for up to 20 days at 2-8^{o}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^{o}C once the vial has been reconstituted. Any remaining solution should be discarded after 20 days.

Administration procedures
Route of administration: Sargramostim will be administered subcutaneously in a dose of 100 mcg 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.

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, 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.

8.4 Docetaxel

Please refer to package insert for complete information

Synonyms

RP56976
Trademark (manufacturer): Taxotere® (Rhône-Poulenc Rorer)

Chemical Names

(2R,3S)-N-carboxy-3-phenylisoserine, N-*tert*-butyl ester, 13-ester with 5□-20-epoxy-1,2□,4,7□,10□,13□-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate

Chemical Identification

Empiric formula $C_{43}H_{53}NO_{14}• 3H_2O$
Molecular weight 861.9 daltons

Source: Docetaxel will be purchased from commercial sources by the NIH Clinical Center Pharmacy Department.

Formulation: Docetaxel is supplied in a single-dose vial as a sterile, non-pyrogenic, non-aqueous, clear yellow to brownish-yellow viscous solution in a single–dose vials with an accompanying vial that contains a sterile, nono-pyrogenic, diluent (13 % ethanol in Water for Injection). The commercial drug product is available in packages containing either:

1. Docetaxel 80 mg (anydrous) in 2 mL polysorbate 80 (Fill: 94.4 mg docetaxel in 2.36 mL polysorbate 80) plus 13 % (w/w) ethanol in Water for Injection (Fill: 7.33 mL) diluent.
2. Docetaxel 20 mg (anydrous) in 0.5 mL polysorbate 80 (Fill: 23.6 mg docetaxel in 0.59 mL polysorbate 80) plus 13 % (w/w) ethanol in Water for Injection (Fill: 1.83 mL) diluent.
   Each milliliter contains 40 mg docetaxel (anhydrous) and 1040 mg polysorbate 80.

Preparation

Preparing the Premix Solution (10 mg docetaxel/mL)

1. Before proceeding, the solution should be inspected for clarity.
2. Aseptically withdraw the entire contents of the diluent vial into a syringe and transfer it to the vial containing docetaxel.

3. Gently rotate each premix solution vial for approximately 15 second to assure full mixture of the concentrate and diluent.
4. Docetaxel premix solution should be clear; however, there may be some foam on top of the solution. Allow the premix solution to stand for a few minutes to allow any foam to dissipate. It is not required that all foam dissipates prior to continuing the preparation.

Preparing the Infusion Solution

1. Aseptically withdraw the required amount of docetaxel Premix Solution (10 mg docetaxel/mL) and inject the solution into a bag containing 5% Dextrose solution to produce a final concentration between 0.3-0.74 mg docetaxel/mL.
2. Thoroughly mix the Infusion Solution by repeatedly inverting the drug container.
3. Visually inspect the Infusion Solution for particulate matter or discoloration. If the solution is not clear or appears to have precipitation, it should be discarded.

Docetaxel must not come into contact with polyvinyl chloride (PVC) equipment or devices during solution preparation or administration. Docetaxel should be prepared in polypropylene- or polyolefin-lined drug product containers and administered using polyethylene-lined administration sets; e.g., Gemini® PUMP ET #2264 (non-PVC, polyethylene-lined fluid pathway, with integral 0.2 μm polysulfone filter [Alaris Medical Systems; San Diego, CA]).

Stability: Each vial bears an expiration date. Freezing does not adversely affect the product.

The Premix Solution and Infusion Solution should be used as soon as possible after preparation. The Premix Solution is stable for 8 hours between 2°-25°C (36°-77°F). When diluted to concentrations from 0.3-0.74 mg/mL in 5% Dextrose Injection, USP (D_5W) and 0.9% Sodium Chloride Injection, USP docetaxel is stable for up to 4 hours.

Storage: Store between 2 and 25 °C (36°-77°F). Retain in the original package to protect from bright light.

Administration: Docetaxel will be administered as an intravenous infusion over 30 minutes every 7 days for 3 consecutive weeks, followed by a docetaxel free week.

Toxicities:

Cardiac:	Arrhythmias, pericardial effusion
Gastrointestinal:	Nausea and vomiting, diarrhea, oral mucositis
Neurologic:	Reversible dysesthesias or paresthesias, peripheral neuropathy, mild or moderate lethargy or somnolence, headaches
Skin:	Desquamation, macular-papular eruption or skin erythema with edema.
Hepatic:	Increased transaminase, increased alkaline phosphatase, increased bilirubin, hepatic failure, hepatic drug reaction.

Pulmonary:	Dyspnea with restrictive pulmonary syndrome, pleural effusions.
Other:	Asthenia, alopecia, anorexia, nail changes, conjunctivitis, extravasation reaction, reversible peripheral phlebitis, arthralgia, muscle aches, myopathy, peripheral edema, low back pain-possibly a manifestation of hypersensitivity reaction.
Adverse Effects:	Myelosuppression, alopecia, rash, nail disorders, hyperpigmentation, inflammation, redness, dryness, nausea and vomiting, diarrhea, stomatitis, fluid retention/weight gain, pleural effusion, cardiac tamonade, elevated liver function tests, paresthesia, weakness, myocardial infarction, gastrointestinal perforation, neutropenic enterocolitis

Drug Interactions:
There have been no formal clinical studies to evaluate the drug interactions of docetaxel with other medications.

In vitro studies suggest that concurrent administration of drugs that interact with or are metabolized by—cytochrome P450-3A may alter the metabolism of docetaxel. Those include:

Ketoconazole
Midazolam
Erythromycin
Testosterone
Orphenadrine
Troleandomycin
Cyclosporine

Caution should be exercised with these drugs when treating patients receiving docetaxel, as there is a potential for a significant interaction.

8.5 Dexamethasone

Please refer to the package insert for complete drug information

Synonyms
Trademark (manufacturer): Decadron® (Merck Sharpe & Dohme, West Point, PA) And several others

Chemical Names
9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione

Chemical Identification
Empiric formula $C_{22}H_{29}FO_5$
Molecular weight 392.47 daltons

Product Description: Dexamethasone is used as an anti-inflammatory agent to suppress the inflammatory reactions associated with docetaxel.

Source: Dexamethasone will be purchased from commercial sources by the NIH Clinical Center Pharmacy Department.

Formulation: Tablets Dexamethasone(Decadron) are compressed, pentagonal-shaped tablets, colored to distinguish potency. They are scored and coded on one side and embossed with Dexamethasone on the other. They are available in 4 mg (white), 0.75 mg (bluish-green), 0.5 mg (yellow)

Stability: Commercially packaged dexamethasone tablets bear the manufacturer's expiration dating.

Storage: Store between 15 and 30 °C; store in well-closed container.

Administration: Dexamethasone 4 mg po 12 hrs before, 1 hour before and 12 hours post Docetaxel

Adverse Effects: Can cause high blood pressure, increase in stomach acid, high blood sugars, fluid retention, increase in blood sodium levels, decrease in both blood potassium and calcium, pancreatitis, psychological disturbances (mood swings), proximal muscle weakness, osteoporosis, easy bruisability, posterior subcapsular cataracts with long-term use.

Drug Interactions: Aminoglutethimide, barbiturates, estrogens, ephedrine, hydantoins, ketoconazole, rifampin, anticholinesterases, oral anticoagulants, cyclosporine, digoxin, isoniazid, nondepolarizing muscle relaxants, salicylates, protropin, theophylline. Patients taking any of these medications will be counseled with regard to the specific drug interactions that may occur.

8.6 Agent Procurement

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

Agent Ordering and Agent Accountability:
NCI-supplied PANVAC™-V (NSC 727026) and PANVAC™-F (NSC 727027) are investigational agent supplied by Therion Biologics Corporation to CTEP. It 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).

REFERENCES

(1) Jemal A. Cancer statistics, 2003. CA Cancer J Clin 2003 Jan-Feb;53(1):5-26.

(2) Hodge JW. Carcinoembryonic antigen as a target for cancer vaccines. Cancer Immunology Immunotherapy 1996; 43(3):127-134.

(3) 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.

(4) Fong L, Hou YF, Rivas A, Benike C, Yuen A, Fisher GA et al. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proceedings of the National Academy of Sciences of the United States of America 2001; 98(15):8809-8814.

(5) 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.

(6) 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.

(7) Marshall J, Odogwu L, Hwang J, Fox E, Arlen P, Gulley J et al. A Phase I Study of Sequential Vaccinations with Fowlpox-CEA (6D)-TRICOM (B7/ICAM/LFA3) alone, and in Combination with Vaccinia-CEA (6D)-TRICOM and GM-CSF in Patients with CEA Expressing Carcinomas. Proceedings Am Soc Clin Onc . 2003. Ref Type: Abstract

(8) 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.

(9) 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.

(10) Slamon et al. (NEJM 2001;344:783) published the efficacy of adding Herceptin + Taxol (n=92) vs. Taxol (n=96)

(11) Sledge et al (JCO 2003:21(4):588-92) single agent doxorubicin and paclitaxel compared to combination therapy.

(12) Tabernero et al (Ann Oncol 2004: 15(9):1358-65) reported the use of weekly vs 3-weekly docetaxel as single agent therapy for metastatic breast cancer in a randomized multicenter trial.

(13) Arlen PM, Gulley JL, Novik L, Rucker S, Bastian A, Marte J, Panicali D, Palena C, Tsang KY, Schlom J, Dahut W. " A Randomized Phase II Trial of Either Vaccine Therapy (Recombinant Pox Viruses Expressing PSA and the B7.1 Costimulatory Molecule) Versus Hormone Therapy (Nilutamide) in Patients with Hormone Refractory Prostate Cancer and No Radiographic Evidence of Disease." Journal of Urology, American Urologic Association (AUA) 2003, 169(4): 941a

(14) Tsang KY, Palena C, Yokokawa J, Arlen PM, Gulley JL, Yafal AG, Manson K, Mazzara GP, Panicali D, Schlom J. Analysis of Recombinant Vaccinia and Fowlpox Vaccine Vectors Expressing Transgenes for Two Human Tumor Antigens and Three Human Costimulatory Molecules. Clinical Cancer Research (in press).

(15) (Hadden JW Int J Immunopharmacol. 1999 Feb;21(2):79-101.) MUC-1 is expressed in 90% of all breast cancers

(16) Steward AM, Nixon D, Zamcheck N, et al: Carcinoembryonic antigen in breast cancer patients: Serum levels and disease progress. Cancer 33:1246-1252, 1974.)

(17) 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.

(18) 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.

(19) 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.

(20) Yonezawa S, Sato E. Expression of mucin antigens in human cancers and its relationship with malignancy potential. Pathology International 1997; 47(12):813-830.

(21) 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.

(22) 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.

(23) 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.

(24) 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.

(25) 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.

(26) 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.

(27) 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.

(28) 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.

(29) 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.

(30) 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.

(31) 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.

(32) 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.

(33) 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.

(34) Hodge JW, Sabzevari H, Yafal AG, Gritz L, Lorenz MGO, Schlom J. A triad of costimulatory molecules synergize to amplify T-cell activation. Cancer Research 1999; 59(22):5800-5807.

(35) 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.

(36) 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.

(37) 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.

(38) Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nature Medicine 1998; 4(3):321-327.

(39) 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.

(40) 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.

(41) 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.

(42) Moss B. Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. Proceedings of the National Academy of Sciences of the United States of America 1996; 93(21):11341-11348.

(43) 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.

(44) Mastrangelo MJ, Maguire HC Jr, Eisenlohr LC, Laughlin CE, Monken CE, McCue PA, Kovatich AJ, Lattime EC. Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneousmelanoma. Cancer Gene Ther 1999; 6:409-22.

(45) Moss B. Replicating and host-restricted non-replicating vaccinia virus vectors for vaccine development. Developments in Biological Standardization 1994; 82:55-63.

(46) Taylor P, Weinberb R, Languet B. Fowlpox virus based recombinant vaccines. Advances in Vaccine Development 1988; 33:321-324.

(47) 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.

(48) 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.

(49) Maas IW, Boven E, Pinedo HM, et al; The effects of gamma-interferon combined with 5-fluorouracil or 5-fluoro-2-deoxyuridine on proliferation and antigen expression in a panel of human colorectal cancer cell lines. Int J. Cancer 1991; 48:749-756.

(50) Abdalla EE, Blair GE, Jones RA, et al: Mechanism of synergy of levamisole and fluorouracil: induction of human leukocyte antigen class I in a colorectal cancer cell line. H Natl Cancer Inst 1995; 87:489-496.

(51) Aquino A, Prete SP, Greiner JW, et al: Effect of the combined treatment of 5-fluoruracil, gamma-interferon or folinic acid on carcinoembryonic antigen expression in colon cancer cells. Clin Cancer Res 1998; 4;2473-2481.

(52) Mizutani y, Wu XX, Yoshida O, et al: Chemoimmunosensitization of the T24 human bladder cancer line to Fas-mediated cytotoxicity and apoptosis by cisplatin and 5-flourouracil. Oncol. Rep 1999; 6:979-982.

(53) Bergmann-Leitner ES, and Abrams SI. Treatment of Human Colon Carcinoma Cells Enhances Their Lytic Sensitivity to Antigen-Specific CD8+ Cytotoxic T Lymphocytes. Cancer Immunol Immunother. 2001 Nov;50(9):445-55.

(54) Pegram MD, Lipton A, Hayes DF, et al. Phase II Study of Receptor-Enhanced Chemosensitivity Using Recombinant Humanized Anti-p185HER2/neu monoclonal antibody plus Cisplatin in Patients with HER2/neu-overexpressing Metastatic Breast Cancer Refractory to Chemotherapy Treatment. J. Clin Oncol 1998; 16: 2659-2671.

(55) Pegram M, Hsu S, Lewis G. et al. Inhibitory Effects of Combinations of HER-2/neu Antibody and Chemotherapeutic Agents Used for Treatment of Human Breast Cancers. Oncogene 1999; 18:2241-2251.

(56) Slack R, Ley L, Chang P, Arlen P, Tsang K, Schlom J et al. Association Between CEA-Specific T Cell Responses (TCR) Following Treatment with Vaccinia-CEA (V) and Alvac-CEA (A) and Survival in Patients (Pts) with CEA-Bearing Cancers. Proceedings Am Soc Clin Onc . 2001.
Ref Type: Abstract

(57) Miles DW. Phase II clinical trial using VV-MUC1-IL2 in women with metastatic breast cancer. 22nd Annual San Antonio Breast Cancer Symposium . 12-10-1999.

Ref Type: Abstract

(58) 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.

(59) Eder JP, Kantoff PW, Roper K, Xu GX, Bubley GJ, Boyden J et al. A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. Clin Cancer Res 2000; 6(5):1632-1638.

(60) Carson WE 3rd, Shapiro CL, Crespin TR, Thornton LM, Andersen BL**.** Cellular immunity in breast cancer patients completing taxane treatment. Clin Cancer Res. 2004;10(10):3401-3409.

(61) 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.

(62) 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.

(63) Moss B. Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. Proceedings of the National Academy of Sciences of the United States of America 1996; 93(21):11341-11348

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.

Center for Cancer Research

Medical Oncology Branch
MOB

U.S. Department of Health
and Human Services
Public Health Service
National Institutes of Health
Clinical Center
9000 Rockville Pike
Bethesda, MD 20892
(301) 496-0684

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. 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. You may develop a blister at the vaccine site which will have a scab form and fall off within this 2 week period. 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. You may have a blister form during this time which will develop a scab and virus may shed 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. You should avoid close contact with susceptible individuals for a period of 2 weeks. 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. We have vaccinated over 700 cancer patients and have reported no cases of either self inoculation or person to person transmission of the virus.

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 Southeast Outpatient Cancer Center	(301) 451-1152
On-Call physician	(301) 496-1211

APPENDIX C: Protocol Evaluation

	Baseline[1]	Week 1[a]	Week 5	Week 9	Week 13	Every 4 Weeks on Study
History and Physical[1]	X					
Medical Assessments[2]		X	X	X	X	X
CT Scan / BS[3]	X				Every 2 months on study	
CT Brain[3B]	X					
Serum CEA, Serum CA 27-29	X	X	X	X	X	X
Urinalysis[4]	X	X	X	X	X	X
Serum HIV antibody[5]	X					
Serum Hepatitis B & C[5]	X					
CBC with differential[6]	X	X	X	X	X	X
Serum Beta-HCG[7]	X					
24-hour urine collection[8]	X					
Serum Chem [6,9]	X	X	X	X	X	X
EKG	X					
Echocardiogram[13]	X				X	
Immunology (blood)[10**]	X	X	X	X	X	X
HLA typing	X					
Apheresis[12] (NCI only)	X				X	

[1a]Patients randomized to Arm A will have these labs initially drawn at week - 3 in addition to the other timepoints noted above

[1]Baseline: refer to section **2.2**, Research Eligibility Evaluation for dates of tests to be obtained prior to initiation of treatment. 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.

[3] Radiologic studies consisting of CT chest/abdomen/pelvis or BS will be performed within 4 weeks prior to initiating treatment. Repeat scans on or around week 13, then every 2 months thereafter while on-study. For patients whose disease has not progressed on scans for a period of one year on study, will have the interval for CT scans and bone scan increased to every 3 months while on study.

[3B] Radiologic studies consisting of CT (or MRI) brain will be performed within 4 weeks prior to initiating treatment (Baseline only).
[4]Urinalysis prior to enrollment and prior to each vaccination,.
[5] Serum HIV, Hepatitis B and C antibody should be completed within 8 weeks of initiating treatment.
[6] CBC/differential, with platelet count, Bilirubin, AST, ALT and alk phosphatase should be obtained prior to administering each dose of weekly docetaxel.
[7]Beta-HCG for women of child-bearing potential (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.
[8]A 24-hour urine collection for protein CrCL should be performed if baseline serum Cr is above normal institutional level of normal
[9] CBC/differential, with platelet count, serum chemistry panel: Na+, K+, Cl-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, uric acid and total protein. [10]Blood will be obtained for immunologic assays including ELISPOT assay.
CD3, 4, 8 subsets and CD4:CD8 ratio will be done **only** on patients enrolled at NCI. Research blood will be drawn prior to each vaccination. For those patients on Arm B, research bloods will be drawn at each scheduled clinic visit. Research blood should consist of 6 green top tubes and 2 red SST.
For patients at MD Anderson, research samples will be collected at baseline and first restaging. At baseline, collect, 7 green top tubes and 2 red SST. At first restaging, collect 7 green top tubes and 2 red SST. See **APPENDIX H.
[11] Post Treatment Evaluation: Long term follow up (LTFU): See section **3.10**
The Biologic Response Modifiers Advisory Committee has recommended that long-term follow-up extend over a period of 15 years. Patients may undergo annual follow-up examinations at the NIH Clinical Center or our research nurse will obtain the information from their local physician for the first 5 years following examination. Additional data will be obtained annually for years six through fifteen via telephone contacts from the research nurse. 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. Patients will be enrolled on to the "Follow-Up Study of Subjects Previously Enrolled in Poxviral Vector Gene Transfer Studies" once off treatment.
[12]Apheresis for NCI HLA-$A2^+$ patients only.
[13] All patients will undergo a baseline Echocardiogram. Those patients who are HER-2 + and continue with Trastuzumab therapy will have a repeat Echocardiogram at week 13 (if cardiac symptoms develop, an Echocardiogram will be performed prior to this on any patient).

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. The primary endpoint of this study is to evaluate progression free survival; thus, we have also included patients whose metastatic disease is evaluable (not measurable per RECIST criteria).

  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.

APPENDIX E: ELISPOT ASSAY

We plan to examine the immune response in selected patients (HLA-A2 positive). 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).(58) Briefly, 96-well milliliter HA plates (Millipore Corporation, Bedford, MA) are coated with 100μl/well of capture MAb against human γ-IFN at a concentration of 10 μg/ml for 12h at RT. Plates are blocked for 30 min with RPMI 1640 plus 10% human Ab serum. 2×10^5 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. (58) 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 μg/ml in PBS-Tween containing 1% bovine serum albumin (BSA) is added and incubated overnight in 5% CO_2 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. Two separate investigators will count the number of dots 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.

APPENDIX F: Instructions for pre-study and follow-up blood tests

Blood Studies:	**Blood tube/Comments:**	**Destination**:
CBC, WBC with differential	1 purple top	Hem/Onc Lab Bldg. 10
BUN, Creatinine SGOT, Alk. Phos, Bilirubin, Albumin	1 gold top	Hem/Onc Lab Bldg. 10
CD4:CD8	1 purple top	Hem/Onc Lab Bldg. 10
Hepatitis B and C	1 brown & black tiger top	Hem/Onc Lab Bldg. 10
Serum for HIV antibody	HIV Consent "Protocol Pt. Please run in house"	Hem/Onc Lab Bldg. 10
Serum for PSA	1 gold top	Hem/Onc Lab Bldg. 10
Lymphocyte Subsets	1 purple top	Hem/Onc Lab Bldg. 10
HLA-A2	1 yellow top ACD solution A	NIH Clinical Center Bldg. 10 - HLA Lab
Immunology Assays	6 10cc green tops 2 SST tubes Apheresis product	NCI-Frederick (SAIC) (1-301-846-5893)
Urinalysis	specimen container	Hem/Onc Lab Bldg. 10

APPENDIX G: NCI-IRB SERIOUS UNEXPECTED ADVERSE EVENT REPORT FORM

Definition of a serious unexpected adverse event: For the purposes of this form, a serious adverse event is any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others. A serious adverse event is considered unexpected if it is not described in the Package Insert or in the Investigator's Brochure (for FDA investigational agents), in the protocol, or in the informed consent document.

Please complete the information requested below and forward it to the NCI-IRB with a copy to Office of the Clinical Director, as soon as possible, but no later than 10 working days. In addition, continue to follow FDA and the NIH Office of Biotechnology Activities (OBA) reporting requirements and procedures if your research involves an IND/IDE or gene transfer.

Protocol number: _____ Protocol Title:
_____
Principal Investigator:
_____
Preferred Contact Information:
_____
Date of Event: _____
Is this a multi-center trial? Yes [ ] No [ ]. If so, is NCI the Coordinating Center? Yes [ ] No [ ]
Location of the event: NIH [ ] Elsewhere [ ] Name of Institution: _____

CTCAE Category: _____ Grade: _____ Toxicity: _____ Severity (If more explanation is required):
_____
Is the Adverse Event Attributable to the research? Yes [ ] No [ ]
Was this unexpected? Yes [ ] No [ ]
Was this in the Consent Form? Yes [ ] No [ ]
Have similar unexpected adverse events occurred on this protocol? Yes [ ] No [ ]
If "Yes", how many? ____ Describe:
_____
_____
What steps do you plan to take as a result of the adverse event reported above? Provide documentation to the IRB for review and approval of any of the steps checked below.

[ ] no action required
[ ] amend protocol
[ ] amend consent document
[ ] inform current subjects
[ ] terminate or suspend protocol
[ ] other (describe)

Is this a sponsored trial? Yes [ ] No [ ]. If yes then you may sign below and attach the required sponsor AE reporting form to the NCI-IRB Adverse Event Report Form. If no, please continue on this form.

Is NCI the Coordinating Center for a Multi-institutional trial? Yes [ ] No [ ]. If yes and this event

occurred at an outside facility, you may attach a copy of the AE report. If No, continue on this form.

Signature of the Principal Investigator: _____ Date: _____
Or PI Designated Responsible MD

NCI-IRB SERIOUS UNEXPECTED ADVERSE EVENT REPORT FORM – Continuation Sheet

Brief description of subject(s) (do not include identifiers such as names or SS#s)
Sex: Age: Diagnosis:
MRN:
Brief description of the nature of the serious unexpected adverse event to include: drug, cycle, start date, last dose, narrative of event in relation to time, and a list of the other serious unexpected related events within the study (attach description separately if more space is needed):

_____
_____
_____
_____
_____
_____
_____FDA

Category for this serious unexpected adverse event:

[ ] death
[ ] disability / incapacity
[ ] life-threatening
[ ] congenital anomaly / birth defect
[ ] hospitalization-initial or prolonged
[ ] required intervention to prevent permanent impairment
[ ] other:

Relationship of Serious Unexpected Adverse Event to research:

[ ] 1 = Unrelated (clearly not related to the research)
[ ] 2 = Unlikely (doubtfully related to the research)
[ ] 3 = Possible (may be related to the research)
[ ] 4 = Probable (likely related to the research)
[ ] 5 = Definite (clearly related to the research)

Signature of the Principal Investigator: _____ Date: _____
Or PI Designated Responsible MD

Version Date: 8/19/2014

APPENDIX H: Instructions for Collection and Shipping of Research Blood Samples

Collection:
7 green top (Na heparin, 10 mL) tubes (Please collect **7** green top tubes at baseline)
2 red serum separator (10 mL) tubes

* Blood is collected at **2 time points**: baseline and first restaging (approximately 12 weeks).

Shipping:
1. Materials

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

Contents of the kit:
EXAKT-PAK for Vials Category B D-pak MD8702V06 (Accommodates 6 vials)

Includes Inner pack (Ambient) and Insulated Cooler
With 2 cool packs per cooler, part # CP1003, slightly cooled

2. Address

- For Research Blood Samples, please FED-EX Overnight to the shipping address below. USE NIH / NCI/ LTIB FedEx Account Number: (281312103)

Attn: Bill Kopp, Ph.D.
Bldg 560/Room 11-40
SAIC-Frederick
Bldg 1050 Boyles Street
Frederick, MD 21702
Phone No. 301-846-5125, or 301-846-1707

- Please notify the SAIC Frederick laboratory when specimens are being shipped. Please email SAIC Frederick prior to shipping to notify the lab.

- Emails should be sent to the following individuals:
  Bill Kopp, koppw@mail.nih.gov
  Theresa Burks, burkst@mail.nih.gov
  Helen Rager, ragerh@mail.nih.gov
  Bernie Thompson, thompsonb3@mail.nih.gov

  Sheri McMahon, sheri.mcmahon@nih.gov
  Sandra Doren dorens@mail.nih.gov
  James Gulley gulleyj@mail.nih.gov

- Additionally, **NO** specimens should be shipped on Fridays or the day before a Federal holiday. Please check the web address link provided for the list of Federal Holidays and

make sure to click on the appropriate year.
http://www.opm.gov/Operating_Status_Schedules/fedhol/2009.asp

3. Labeling of Blood Samples

- Please label research tubes with a coding mechanism. The following information should be contained on the label:
  - site – list MD Anderson
  - patient enrollment number (provided by the Central Registration Office upon registration/randomization)
  - arm – list whether patient is randomized to Arm A or Arm B
  - NCI protocol # 05-C-0229
  - Date Drawn
  - Time point – list sample as either baseline or 1st restaging

Example:

MDA – 01 — Arm A
Protocol # NCI 05-C-0229
Date Drawn: August 1, 2010
Time point: baseline

4. Supply for shipping kits

SHIPPING PAKS to be ordered by the NCI and shipped to participating cancer center in multiples of 10.
Please contact the NCI team for requesting additional shipping kits.
Sheri McMahon 301-496-9812 sheri.mcmahon@nih.gov

EXAKT Technologies, Inc.
Mid-Atlantic Region – Account Executive
410-313-9700 Baltimore
Home office: 7002 N. Broadway Extension
Oklahoma City, OK 73116-9006
800-866-7172

APPENDIX I: CTEP MULTICENTER GUIDELINES

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

Responsibility of the Protocol Chair

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

Responsibilities of the Coordinating Center

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