A Phase II Trial of PANVAC Vaccine with GM-CSF boosting for Resectable Non-small-cell Carcinoma of the Lung

Hilary Yegen

A. Study Purpose and Rationale

Lung cancer is the leading cause of cancer deaths in the United States, accounting for approximately 160,000 deaths per year. Cases of non-small cell lung cancer (NSCLC) compose approximately 75% of lung cancer diagnoses and have a poor prognosis, with five year survival rates under 15% for all stages combined. Treatment of early stage NSCLC involves surgical resection, frequently with adjuvant dual-agent chemotherapy. The poor prognosis despite therapy is due to locally recurrent and metastatic disease, presumably arising from micro metastasis present at the time of surgical resection. Alternatives to traditional oncologic treatments, such as immunotherapy, aim to harness the innate immune response for the purpose of eradicating existing disease. Ideally, such treatments would be tumor specific, systemic in action, and less toxic than radiation or chemotherapy.

Cancer vaccines are a prominent example of tumor immunotherapy, proposed in the 1950s, whereby the host immune system is activated to generate a tumor-specific response to malignant cells (Mocellin, Lancet 2004). Vaccines harness the basic immune response to an antigen, whereby antigenic peptides are presented by an antigen presenting cell in the groove of an MHC class I or II receptor to the T cell receptor on a CD8+ or CD4+ cell, respectively. A second, antigen-independent, or co-stimulatory signal is also required. Following binding of both signals, CD8+ and CD4+ cells are stimulated to produce cytokines, including IFN-gamma for CD8 cells, and activated to attack their targets.

In the setting of cancer, the goal of a vaccine is to create an immune response directed at an antigen differentially expressed in tumor cells, called a tumor-associated antigen (TAA). Carcinoembryonic antigen (CEA) is an example of a TAA over expressed in most adenocarcinomas, including the lung, GI tract, and breast; it is also expressed in fetal development and in small amounts on normal colonic mucosa (Hodge, 1996). Multiple vehicles have been utilized to deliver CEA to immune effector cells, including dendritic cells, peptide vaccines, and recombinant viral vectors (Huang 2002). The latter take advantage of the innate immunogenicity of a live (inactivated) virus, and are less cumbersome than autologous dendritic cells. Several viral vectors in the poxvirus family have been used to date, including vaccinia virus, the virus from which the smallpox vaccine is made, and fowlpox and canaryviruses. Poxvirus vaccine constructs containing CEA have been used in early clinical trials since 1993 with continued improvements in design; they have now been tested in over 600 patients. Frequently, immune stimulation is augmented by the use of GM-CSF (Samanci, 1998, Disis, 1996), which will be used in this study as well.

The vaccine to be used in this study is PANVAC, an experimental vaccine engineered to contain CEA as well as several co-stimulatory molecules. It and other poxvirus constructs have been shown in pre-clinical and Phase I/II clinical studies to be well tolerated with side effects limited to local skin reactions and constitutional symptoms (McAneny, 1996, Marshall 1999, 2000, 2005, Conry 1999, von Mehren, 2000, Horig 2000). The vaccine has also proven capable of eliciting a CEA-specific immune response, as measured by humoral and cell-mediated responses, in 75-100 percent of patients. One measurement of CD8+ T cell response and production of IFN-gamma, the ELISPOT, can only be performed in patients who are HLA-A2 positive (approximately 50% of the Caucasian population and 34% of African-Americans) (Huang, 2002); therefore this study will be restricted to HLA-A2 positive patients in order to best assess an immunologic response.

Although elicitation of immune response is frequent, clinical responses to the vaccines have been primarily anecdotal (Marshall 2000, 2005, Horig 2000). Potential explanations include limitations of both the vaccine and the patient population. Initial studies of CEA vaccines utilized vaccinia virus, to which many patients developed a robust immune response due to prior smallpox vaccination, limiting further doses of the vaccine. To circumvent this issue, replication-defective viruses are now used in a prime-and-boost fashion, as will be detailed below. Other issues have included the advanced stage and extensive tumor burden in many patients, potentially limiting the ability of immune cells to lyse malignant cells. Also, immune function was potentially compromised in these patients who had received at least one, and often multiple chemotherapy regimens (von Meheren, 2000), as well as by the immunosuppressive factors produced by tumor cells. These considerations have bred an interest in utilizing a vaccine early in the malignant process when the burden of disease is lower and the immune system more robust (Mocellin 2002).

The limited clinical responses to date also suggest that more information is needed as to how immune effector cells function following vaccination with regard to tumor lysis. To date, most studies have used peripheral blood immunologic endpoints as evidence of vaccine efficacy, though these endpoints tell us little about immune cell function at the tumor site. Neoadjuvant vaccination has been performed to quantitatively evaluate immune response in biopsy tumor specimens, demonstrating a humoral response to an anti-idiotype vaccine in colorectal cancer patients (Durrant, 2000). Quantification of the immune response at the tumor site could be assessed by evaluating CD8 positive T cells in pathology specimens from tumor resection after vaccine administration.

A candidate functional assay at the tumor site would be DNA microarray technology, which has the power to determine the expression level of thousands of genes at one point in time. This technology has the potential to inform our knowledge of differential gene expression of activated versus naïve T cells as well as of the tumor microenvironment. We expect that activated CD8 cells will express a profile including upregulation of CD8 specific cytokines, including IFN-gamma and IL-2, adhesion molecules, including integrin VLA-4 and downregulation of L selectin, among others. Currently, DNA microarray technology is being utilized to evaluate these and other questions. For example, Mocellin, et al, showed that different molecular signatures exist in melanoma tissue when treated with a peptide-IL2 vaccine (Mocellin S, 2004).

We therefore propose a phase II study to evaluate HLA-A2 patients with stage I-IIIA lung cancer in a neoadjuvant study of the PANVAC vaccine with GM-CSF boosting. The study will serve to evaluate peripheral immunologic response to the vaccine, and correlate this with histological and functional data regarding immune function at the site of the tumor.

B. Study Design and Statistical Analysis

This Phase II clinical trial is a single center, single arm open label trial to evaluate the safety and efficacy of the PANVAC-VF vaccine. Patients will receive 2 x 10^8 pfu of PANVAC-V (vaccinia prime) delivered subcutaneously on Day 0, followed by vaccination with 1x 10^9 pfu of PANVAC-F (fowlpox boost) on Days 28 and 56. 100 micrograms of GM-CSF will be administered subcutaneously at the injection site on the day of each vaccination and for three consecutive days thereafter. Surgery will take place on or about days 14-21.

Extension phase: An optional provision of up to 12 additional monthly boosting immunizations with the same dose of PANVAC-F in combination with GM-CSF will be offered to subjects who have completed the core phase and who have not experienced disease progression or unacceptable toxicity, and who, in the opinion of the investigator, may benefit from continuing treatment. The vaccine will be held during time periods at which the subjects may be receiving chemotherapy or radiation, and may be re-started 3-6 weeks after the completion of those therapies.

Safety will be assessed by examining the incidence of treatment-emergent adverse events, changes from baseline in physical exam finding, vital sign measurements, and laboratory results (hematology, CBC, and urinalysis). Safety assessments will occur on Day 0, day of surgery, day 28, and

day 58. Stopping rules will be determined in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. One occurrence of grade 5 toxicity attributable to the treatment regimen will result in study termination. In the first ten subjects, if there are two occurrences of grade 4 toxicity that are attributed to the treatment regimen the study will be terminated. If More than one of the first three subjects, two of the first six subjects, or three of the first nine subjects experience a dose limiting toxicity the study must be terminated.

Efficacy will be assessed by an interferon-gamma ELISPOT assay to evaluate CD8+ T cell response. These measures will be tested before and one month after vaccination. A positive response is defined as a two-fold increase in the number of antigen-specific CD8+ T cells comparing pre- and post-vaccination levels. An additional ELISPOT assay to influenza will be performed as a negative control at each time point.

Pathology samples will be evaluated at two time points, by the same pathologist who is blinded to the patient's clinical information. The first will be the initial pathologic sample at diagnosis and the second will be the pathologic specimen from the time of resection. The samples will be stained for CD8 T cell positivity and three separate fields will be evaluated for the maximum number of cells per high powered field (HPF). The mean number of cells per HPF will be compared pre- and post-vaccination, and will be compared with an unpaired T-test. Post-vaccination specimens will then be subject to laser capture microdissection, in order to analyze specifically those cells which are CD8 positive. DNA will be extracted from these CD8 + cells for microarray analysis, looking for genes which show a two-fold difference in expression using a program called Significance Analysis in Micrarrays (SAM) with a false positive discovery rate of 1.5%. Genes which show differential expression are then evaluated for clinical significance.

The study will aim to enroll forty-six patients, all of who will receive the vaccine, to determine whether or not an immunologic response was generated. With this sample size, we have more than an 80% power to detect an expected response of 80%, assuming that the smallest difference of clinical significance is 60%, with a $p=0.05$.

C. Study Procedure

Once subjects have been properly consented and all inclusion/exclusion criteria have been documented as met, subjects will be registered for the trial. There will be no randomization, as this is a single arm trial. Subjects should be registered 7-14 days prior to their anticipated treatment start date (Day 0).

At screening (7-14 days prior to start date), patients will undergo evaluations including informed consent, demographic information, medical history, Karnofsky performance status, urine pregnancy test for women of child-bearing potential, complete physical exam, vital sign measurements, laboratory testing including chemistries, electrolytes, hematology, coagulation, HLA-A2 typing, and urinalysis.

On day 0, the patients will undergo physical exam, vital signs measurements, and laboratory tests as noted above with the exception of HLA-A2. In addition, peripheral blood will be drawn for a pre-vaccination ELISPOT assay to both CEA and influenza.

Surgery will occur approximately between days 14-21.

These evaluations are repeated on day 28 when the second vaccination is given, and on repeated visits for subsequent vaccinations. A post-vaccination ELISPOT assay is drawn after the third vaccination,

Many of these studies are likely to be performed in routine clinical care, with the exception of HLA-A2 typing and ELISPOT assays.

As noted previously, patients who continue to be followed in the extension phase of the trial will have the vaccine held during periods which they are receiving chemotherapy. Vaccine use may be re-started 3-6 weeks following the termination of chemotherapy.

D. Study Drugs

Drug: PANVAC-VF is the name of an investigational agent comprised of two recombinant poxvirus vectors, the first called vaccinia (PANVAC-V) and the second fowlpox virus (PANVAC-F). These viruses have been engineered to express the tumor- associated antigen CEA as well as co-stimulatory molecules B7.1, leukocyte function-associated antigen-3 (LFA-3), and intracellular adhesion molecule-1 (ICAM-1). The vaccine can safely be administered either subcutaneously (SC) or intradermally (ID) (Conry, 1999), and in this study will be given subcutaneously. The safety of doses up to 1.2x 10^8 pfu of rV-CEA and 4x 10^8 pfu of rF-CEA have been established without significant side effects (Marshall 2005) and these doses will be used in this study. GM-CSF will be used at doses of 100 micrograms, as used in previous studies (Marshall 2005).

Vaccinia virus has been used clinically for decades as the vector for the smallpox vaccine, with a well-established safety profile. It actively replicates in human cells, creating a specific immune response to the virus, which is then cleared. The second vector, fowlpox virus, is another poxvirus capable of infecting mammalian cells but cannot replicate in humans. It is therefore less likely to cause systemic infection. It can, however, be given on multiple occasions without the induction of neutralizing antibodies (Berzofsky, JCI 2004). The combination of viruses in a so-called prime and boost regimen has been shown to be efficacious in preclinical (Hodge 1995) and clinical data (Marshall 2000, 2001, Grosenbach 2001).

The tumor associated antigen used in this vaccine is CEA. Recombinant CEA was shown to protect mice against a challenge with CEA-expressing tumor cells, and to decrease the growth of established tumors (Kantor, 1992). In humans, Phase I trials in the mid-1990s also established the safety of this approach, for example in patients with advanced colorectal cancer (McAneny, 1996).

The addition of co-stimulatory molecules serves to provide the second, antigen-independent, signal for T cell proliferation to viral challenge. Several molecules present in the normal antigen presenting cell are capable of providing this signal, including B7.1, the ligand for T cell receptor CD28; ICAM-1 which binds to LFA-1 on lymphocytes; and LFA-3 which binds to CD2 on B and T cells. Recombinant vectors containing this co-stimulatory signal have been shown to increase immunogenicity of the vaccine in preclinical and clinical trials. In tumor-bearing mice, administration of vaccinia vectors containing CEA and B7.1 increased T cell lymphoproliferative assays and in vitro cytotoxicity assays compared to animals given rV-CEA alone (Hodge, 1995). Several pilot studies showed this approach to be valid in humans as well (von Mehren 2000, 2001, Horig 2000). More recently, three co-stimulatory molecules in combination have been developed (called TRICOM), showing superior amplification of the immune response (Hodge, 1999).

Human GM-CSF will be administered to the vaccination site on the day of vaccination and daily for three subsequent days. GM-CSF supplements the immune response by increasing antigen processing and presentation by dendritic cells. Improved immune response seen in GM-CSF patients (Samanci, 1998, Disis, 1996)

Side effects of the study drug have generally been tolerable, and none have required the termination of Phase I studies. These side effects have included grade I local skin reactions at the vaccine site, regional lymphadenopathy, fatigue, and mild flu-like symptoms lasting for a few days following vaccination. The manufacturers note other vaccinia self-limited reactions, including autoinoculation, erythematous or urticarial rash, and generalized vaccinia, as well as more serious complications, including progressive vaccinia, excema vaccinatum, and post-viral encephalitis. With the exception of CNS involvement, vaccinia immune globulin (VIG) has been used to treat vaccination complications. No additional toxicities are noted for fowlpox. GM-CSF has been noted by the manufacturers to have multiple toxicities including: fever, chills, diaphoresis, myalgia, fatigue, headache, dizziness, dyspnea, bronchospasm, pleural effusion, anorexia, indigestion, nausea, vomiting, diarrhea, injection site tenderness, urticaria, puritis, hypersensitivity reaction, bone pain, thromboembolic events, phlebitis, hypotension, peripheral edema, leukocytosis, thrombocytosis or thrombocytopenia, hepatic enzyme abnormalities, and bilirubin elevation. All listed GM-CSF toxicities were seen at a much higher dose that was given intravenously (250 micrograms).

E. Medical Device

Not applicable

F. Study Questionnaires

Not applicable

G. Study Subjects

Inclusion Criteria

- Subjects > 18 years of age who have been vaccinated against smallpox as evidenced by a scar at the vaccination site and/or verbalization by subject
- Histologically confirmed diagnosis of non-small cell carcinoma of the lung with expression of CEA demonstrated in serum sample or on immunohistochemistry staining of tumor pathology
- Clinical stage I-IIIA
- Candidate for surgical resection
- Patients may have received neo-adjuvant chemotherapy or radiation if indicated but no post-operative adjuvant chemotherapy or radiation
- HLA-A2 positive

Exclusion Criteria

- Evidence of being immunocompromised, as defined by: known HIV positive, other diagnosis of severely compromised immune function; present diagnosis of severe skin disease including active cases or history of extensive excema, psoriasis, severe acneiform rash, impetigo, varicella zoster, burns, or other traumatic or puritic skin conditions (e.g. atopic dermatitis).
- Past or present diagnosis of autoimmune disease (e.g. thyroiditis or lupus)
- Concurrent steroid use
- Unable to avoid close contact with children five years of age or younger, pregnant women, individuals with excema or other skin conditions, and/or immunosuppressed individuals for three weeks after the first vaccination with the investigational product
- Known allergy to eggs or egg products
- Known positive for hepatitis B or C
- Compromised hematopoetic function as defined by: Hb <8 g/dl, ANC < 1500 cells/mm^3, platelet count < 100, 000 cells/mm^3
- Severe hepatic dysfunction as defined by bilirubin value >2x ULN, AST/ALT greater than 2x ULN
- Severe renal dysfunction as defined by serum Creatinine value > 2 mg/dl
- Significant cardiovascular abnormalities or diseases including congestive heart failure (NYHA class 3), myocardial infarction within the past six months, unstable angina, coronary angioplasty within the last six months, uncontrolled atrial or ventricular arrhythmias
- Diseases or conditions that are uncontrolled despite current therapy other than lung cancer
- Concurrent malignancy except non-melanoma cancer of the skin or in situ carcinoma of the cervix, or prior malignancy where subjects have been curatively treated and disease free for < 2 years
- Evidence of active uncontrolled infection
- Currently enrolled in another clinical study or have completed participation in another clinical study or have received investigational drug within 28 days preceding the first dose of study drug

- Failure to use medically acceptable contraceptive methods such as surgical sterilization, hormonal contraception, barrier methods or intrauterine devices so as to prevent pregnancy for the duration of the study and for three months after the final scheduled study visit (it is not known if the study treatment may be harmful to an embryo/fetus)
- Prior chemotherapy completed less than 28 days prior to the first vaccination
- Concurrent immunotherapy, chemotherapy, or biotherapy
- Women who are pregnant or nursing

H. Recruitment of Subjects

Study patients will be recruited from outpatients referred to private offices or clinics in the departments of Oncology, Pulmonary, or Surgery at Columbia Presbyterian Medical Center. They will also be recruited from patients hospitalized at CPMC. Informed consent must be obtained from study patients.

I. Confidentiality of Study Data

Study data will be confidential, and available only to the responsible physicians, the vaccine sponsor, and the FDA. For publication purposes, the subjects may be identified by their assigned subject numbers. All subject information and medical records will be handled in compliance with HIPPA regulations.

J. Potential Conflict of Interest

None

K. Location of Study

CPMC

L. Potential Risks

The potential risks involved in the study are those enumerated as the adverse events. The most serious, but rarest, adverse events are progressive vaccinia, excema vaccinatum, and post-viral encephalitis. With the exception of CNS involvement, vaccinia immune globulin (VIG) has been used to treat vaccination complications. Most commonly, patients experienced local injection site reactions and constitutional symptoms such as fatigue and myalgias.

M. Potential Benefits

The individuals in this study may or may not benefit from treatment. Previous trials have suggested that certain individuals may experience a complete response to vaccine therapy that is stable over time. However, many patients will experience only partial response, and other patients no change in disease progression. Although some patients will experience no benefit themselves from vaccine therapy, society as a whole and future cancer patients may benefit from advances in tumor immunology,

N. Compensation to Subjects

None

O. Costs to Subjects

None

P. Minors as Research Subjects

Not applicable

Q. Radiation or Radioactive Substances

Not applicable

R. References

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