A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A NEOANTIGEN DNA VACCINE STRATEGY IN PANCREATIC CANCER PATIENTS FOLLOWING SURGICAL RESECTION AND ADJUVANT CHEMOTHERAPY

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INVESTIGATORS

Principal Investigators:

Daniel A. Laheru, MD
Medical Oncologist
Johns Hopkins School of Medicine
401 N. Broadway
Baltimore, MD 21231
Telephone: 410-955-8974
E-mail: Laherda@jhmi.edu

William E. Gillanders, M.D.
IND Sponsor, Surgical Oncologist
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone: 314-747-0072
E-mail: gillandersw@wustl.edu

Co-Investigators:

Robert D. Schreiber, Ph.D.
Immunobiologist
Washington University School of Medicine
660 South Euclid, Box 8118
St. Louis, MO 63110
Telephone: 314-362-8747
E-mail: Schreiber@wustl.edu

Christopher A. Miller, Ph.D.
Genome Scientist
The Genome Center at WUSM
660 South Euclid, Box 8501
St. Louis, MO 63110
Telephone: 314-286-0263
E-mail: c.a.miller@wustl.edu

Elizabeth Jaffee, M.D.
Medical Oncologist
Johns Hopkins School of Medicine
1650 Orleans Street, Cancer Research Bldg #4M07
Baltimore, MD 21231
Telephone: 410-614-8216
E-mail: ejaffee@jhmi.edu

Feng Gao, M.D., Ph.D.
Biostatistician
Washington University School of Medicine
660 South Euclid, Box 8067
St. Louis, MO 63110
Telephone: 314-362-3682
E-mail: feng@wustl.edu

S. Peter Goedegebuure, Ph.D.
Immunologist
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone: 314-362-8612
E-mail: goedegep@wustl.edu

William Hawkins, M.D.
Surgical Oncologist
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone: 314-362-7046
E-mail: hawkinsw@wudosis.wustl.edu

Kian H. Lim, MD
Medical Oncologist
Washington University School of Medicine
660 South Euclid, Box 8089
St. Louis, MO 63110
Telephone: 314-747-9320
E-mail: klim@dom.wustl.edu

Marianna Ruzinova, M.D., Ph.D.
Pathologist
Washington University School of Medicine
BJCIH 03303
St. Louis, MO 63110
Telephone: 314-362-7704
E-mail: mruzinova@wustl.edu

SUMMARY

Protocol: **A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A NEOANTIGEN DNA VACCINE STRATEGY IN PANCREATIC CANCER PATIENTS FOLLOWING SURGICAL RESECTION AND ADJUVANT CHEMOTHERAPY**

Study Design: This is a phase 1 open-label study to evaluate the safety and immunogenicity of a neoantigen DNA vaccine strategy in pancreatic cancer patients following surgical resection and adjuvant chemotherapy. The neoantigen DNA vaccines will incorporate prioritized neoantigens and personalized mesothelin epitopes and will be administered with an electroporation device. The hypothesis of this study is that neoantigen DNA vaccines will be safe and capable of generating measurable neoantigen-specific CD4 and CD8 T cell responses. The primary objective of this study is to demonstrate the safety and feasibility of the neoantigen DNA vaccine strategy. The secondary objective is to evaluate the immunogenicity of the neoantigen DNA vaccine strategy as measured by ELISPOT analysis, a surrogates for T cell function.

Product Description: The neoantigen DNA vaccines are composed of closed circular DNA plasmids that are designed to express mutant tumor specific antigens identified by exome sequencing. The plasmids will be formulated as naked DNA plasmid vaccines. Vaccine vials will be supplied at a concentration of 2 mg/mL. DNA vaccines will be administered intramuscularly (in the deltoid or lateralis) using a TriGrid electroporation device (Ichor Medical Systems). Two injections (2 mg/injection, total dose 4 mg) will be administered at each time point.

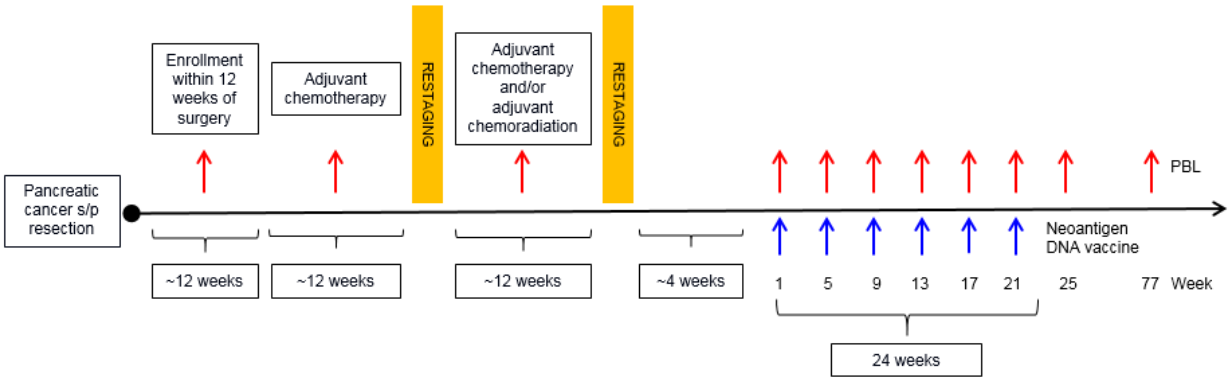
Subjects: Pancreatic cancer patients who have completed surgical resection and adjuvant chemotherapy without evidence of recurrent disease are eligible. Patients enrolled into the protocol must provide consent for exome sequencing and dbGAP-based data sharing and provide germline and tumor DNA samples of adequate quality for sequencing.

Study Plan: Fifteen pancreatic cancer patients will be enrolled. Subjects will be treated by electroporation with 4 mg of a neoantigen DNA vaccine at weeks 1, 5, 9, 13, 17, and 21 ± 7 days with at least 21 days between injection days. Each DNA vaccination will be 4 mg vaccine administered intramuscularly using a TriGrid electroporation device.

Study Duration: Each subject will be followed for 12 months following the last vaccination. Additional follow-up visits or telephone contact will be scheduled annually thereafter if the patient is alive and available for follow-up.

Study Endpoints: The primary endpoint is safety of the neoantigen DNA vaccine regimen. Safety will be closely monitored after injection with eight or more clinical and laboratory assessments in the first six months of the trial. Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. The secondary endpoint is immunogenicity of the vaccine regimen as measured by ELISPOT analyses. Exploratory analyses will include other measures of the immunogenicity of the vaccine regimen as measured by multiparametric flow cytometry and other measures of immune function, including CYTOF analysis.

SCHEMA


Enrollment within 12 weeks of surgery
Adjuvant chemotherapy
RESTAGING
Adjuvant chemotherapy and/or adjuvant chemoradiation
RESTAGING
PBL
Pancreatic cancer s/p resection
Neoantigen DNA vaccine
~12 weeks
~12 weeks
~12 weeks
~4 weeks
1
5
9
13
17
21
25
77
Week
24 weeks

PROTOCOL SIGNATURE PAGE

A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A NEOANTIGEN DNA VACCINE STRATEGY IN PANCREATIC CANCER PATIENTS FOLLOWING SURGICAL RESECTION AND ADJUVANT CHEMOTHERAPY

I have read the attached clinical protocol and agree to conduct this trial in accordance with all the stipulations of the protocol and in accordance with the Declaration of Helsinki/Tokyo/Venice on Experimentation in Humans as required by the United States Food and Drug Administration regulations, Code of Federal Regulations Title 21 parts 50, 56, 312, 800, Title 45 part 46 and all applicable guidelines.

Name of Investigator:

Signature Date

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1 OBJECTIVES

This is a phase 1 open-label study to evaluate the safety and immunogenicity of a neoantigen DNA vaccine strategy. The neoantigen DNA vaccine strategy is designed to target prioritized neoantigens that are present in an individual patient's pancreatic cancer but not in corresponding normal tissues. The vaccines will be formulated as naked plasmid DNA vaccines, and will be administered at a total dose of 4 mg intramuscularly using an integrated electroporation administration system (TDS-IM system, Ichor Medical Systems). The hypothesis of this study is that the neoantigen DNA vaccine strategy will be safe for human administration and capable of generating measurable T cell responses to the neoantigens.

1.1 Primary objective

The primary objective is to assess the safety and feasibility of the neoantigen DNA vaccine approach. This trial will be considered feasible if we are able to enroll 15 evaluable patients, successfully perform tumor/normal exome sequencing, identify at least 3 neoantigens per patient, and manufacture and administer neoantigen vaccines to more than 10 patients.

1.2 Secondary objective

The secondary objective and primary scientific endpoint is to assess the prevalence of antigen-specific T cells in the peripheral blood of patients pre- and post-vaccination as measured by ELISPOT.

1.3 Exploratory objectives

Exploratory objectives include measurement of immune response, including the phenotype and functional status of neoantigen-specific T cells as measured by CYTOF analysis, as well as assessment of the prevalence of antigen-specific T cells in the peripheral blood of patients pre- and post-vaccination as measured by flow cytometry.

An exploratory clinical endpoint is to assess clinical response as measured by disease-free survival and overall survival.

2 BACKGROUND

2.1 Pancreatic cancer

Pancreatic adenocarcinoma is currently the 4th leading cause of cancer related death with the incidence of disease expected to increase by 2030 [1, 2]. Despite recent advances in conventional chemotherapy regimens for pancreatic cancer there has been little improvement in outcomes for patients facing this recalcitrant disease [3, 4]. Currently, surgical resection remains the only curative treatment. However 5-year survival is achieved in only 20-25% of those with operative disease at time of diagnosis.

2.2 Mutational landscape in pancreatic cancer

Next generation sequencing technologies have been used to characterize the mutational landscape in pancreatic cancer. Exome sequencing studies have revealed an average of 63 genetic alterations affecting 12 core cellular pathways [5]. The majority of these alterations were point mutations with the most frequently mutated genes being KRAS, TP53, and SMAD4. Tumors demonstrated heterogeneity in regards to the genomic alterations present, indicating that a spectrum of mutations are present across individual patients.

2.3 Tumor microenvironment

Pancreatic cancer has a robust desmoplastic stroma with an abundant leukocyte infiltrate [6, 7]. This stroma is implicated in resistance to conventional therapies by limiting effective delivery of therapies to malignant cells [8]. The immune infiltrate is predominantly tumor promoting cells derived from the bone marrow, including tumor associated macrophages and myeloid derived suppressor cells (MDSC) [9, 10].

Furthermore, effector tumor infiltrating lymphocytes are scarce with the majority of tumor infiltrating lymphocytes being immunosuppressive FoxP3+ regulatory T-cells (Tregs).

2.4 Tumor antigens

Tumor antigens are often classified as shared tumor antigens and tumor-specific antigens (neoantigens). The majority of tumor-specific antigens are now believed to be the result of somatic mutations present in the tumor.

Shared tumor antigens are expressed in multiple cancers, and are often self-differentiation antigens that are expressed in a limited subset of normal tissues, but overexpressed in cancers. Examples of shared tumor antigens include MAGE (melanoma) [11], prostatic acid phosphatase (prostate cancer) [12], and HER2/neu (breast cancer) [13]. Mesothelin is a shared tumor antigen that is highly expressed in pancreatic adenocarcinoma [14]. A listeria modified to express mesothelin has been used in pancreatic cancer clinical trials and has demonstrated safety and efficacy [15]. Jaffee et al. have characterized the immunogenic epitopes for mesothelin for the HLA-A1, HLA-A2, HLA-A3, and HLA-A24 alleles [16, 17].

Tumor-specific antigens (or neoantigens) are uniquely expressed in individual cancers, and are typically the result of point mutations or other genetic changes that are present only in the tumor (reviewed in [18, 19]). As such, tumor-specific antigens represent the only antigens that are truly unique to the tumor and not expressed in normal tissues. The first human mutant tumor-specific antigen was described in 1995, resulting from a point mutation of cyclin-dependent kinase (CDK4) [20]. Since that time additional publications have described the expression of neoantigens in melanoma [21], non-small cell lung cancer [22] and other human cancers [23].

Cancer vaccine strategies targeting neoantigens have substantial theoretical advantages over strategies targeting shared tumor antigens. Theoretical advantages include: (1) Targeting neoantigens is potentially safer. Neoantigens are expressed only in the tumor, decreasing the risk of autoimmunity. (2) Targeting neoantigens is potentially more effective. T cell responses to neoantigens are high in affinity, and are not limited by central mechanisms of self-tolerance. (3) Targeting neoantigens potentially limits antigen-loss, a common tumor escape mechanism. One of the hallmarks of cancer is genome instability, and one clear weakness of cancer vaccines that target a single shared tumor antigen is antigen-loss. Targeting multiple neoantigens may preclude antigen loss. In addition, many neoantigens play a functional role in neoplastic transformation (driver mutations). (4) Targeting neoantigens is likely to be universally applicable in epithelial cancers, including pancreatic cancer. Epithelial cancers appear to have significant numbers of nonsynonymous mutations present, suggesting that a personalized vaccine approach could be used in most patients with pancreatic cancer, regardless of HLA type.

We have recently used next generation sequencing technologies to identify and study neoantigens in more detail as described in the sections below [24, 25].

2.5 Next generation sequencing

Robust next-generation sequencing strategies for the identification of neoantigens will be required for the successful clinical translation of personalized cancer vaccine strategies. As such, a major focus of our research studies has been the development of cost-effective and accurate next-generation sequencing strategies to identify neoantigens and validate the expression of these antigens at the mRNA level. Initially a cancer genome sequencing approach was used. While cancer whole genome sequencing is informative and provides comprehensive information about both the coding and noncoding regions of the genome, this level of information may not be necessary for identifying neoantigens, or prioritizing antigens for immune intervention. We have now confirmed that tumor/normal exome sequencing is a robust and accurate strategy for the identification of neoantigens [24, 25]. Of note, recent studies suggest that approximately 40% of mutations identified by cancer exome sequencing are not expressed at the mRNA level, so it is important to confirm expression of the mutant allele at the mRNA level. To evaluate mRNA expression, we have performed cDNA-capture sequencing analyses. We have confirmed that cDNA-capture sequencing can be used to successfully confirm expression of sequencing-identified neoantigens at the mRNA level. This analysis also provides an estimation of how highly expressed the mutated allele is expressed relative to other genes in the tumor. For the phase 1 clinical trial proposed, tumor/normal exome sequencing analysis will be used to identify mutations (single nucleotide variants, insertions and

deletions) present only in the tumor, and cDNA-capture sequencing will be used to confirm mutant allele expression and expression level in the tumor mRNA.

2.6 Personalized cancer vaccines

There are two conceptual strategies for creating personalized cancer vaccines targeting neoantigens: a candidate epitope strategy, and an unbiased strategy. The candidate epitope strategy uses computer algorithms [26, 27] and *in vitro* studies to predict immunodominant epitopes, which are then integrated into a personalized vaccine. In the unbiased strategy, no attempt is made to identify the immunodominant epitopes, and all candidate neoantigens are integrated into a personalized vaccine.

We have considered both the candidate epitope strategy and the unbiased strategy. We believe that the candidate epitope strategy is superior to the unbiased strategy for the following reasons. (1) Preliminary data from preclinical models and human correlative studies suggest that relatively few sequencing-identified neoantigens are processed, presented and effectively recognized by the immune system. (2) We have now developed and validated algorithms for the prediction and prioritization of sequencing-identified neoantigens [24, 25]. (3) Targeting a limited number of prioritized sequencing-identified neoantigens will facilitate vaccine design and manufacture, and streamline immune monitoring.

2.7 Prioritization of sequencing-identified neoantigens

Of note, we have now developed and validated an epitope prediction algorithm for the prioritization of sequencing-identified neoantigens. Once somatic mutations have been identified and mutant mRNA expression confirmed/quantified using the sequencing strategies outlined above, neoantigens will be prioritized using an epitope prediction algorithm that has been designed to select and prioritize the most promising sequencing-identified neoantigens. Currently, the most commonly used CD8 T cell epitope prediction algorithm is NetMHC. However, collaborative work conducted by Robert Schreiber, Elaine Mardis, Max Artyomov and William Gillanders has shown that a much more accurate prediction comes from calculating a median affinity for each sequencing-predicted mutant epitope using multiple available epitope prediction algorithms (i.e. NetMHC Pan; ANN; and SMM). We have significantly improved this epitope prediction algorithm by applying these filters to the initial prioritized output list: (a) elimination of hypothetical proteins; (b) use of an antigen processing algorithm to eliminate epitopes that are not likely to be proteolytically produced by constitutive proteasomes or immunoproteasomes; and (c) prioritization of “neo-epitopes” identified by a higher affinity binding of the mutant peptide sequence compared to the wildtype peptide sequence. The final output of these analyses is a rank-ordered list of the highest to lowest priority sequencing-identified neoantigens for each individual patient. In experiments performed using preclinical mouse sarcoma models, this refined prediction algorithm has successfully identified the major tumor rejection antigens in three out of three tumors tested to date [24, 25]. To our knowledge, this is the only algorithm that has been successfully applied to date to cancer vaccine development. Additional information about the preclinical validation of the epitope prediction algorithm is provided in Section 5.2 Nonclinical Studies. MHC class II epitopes can also be accurately predicted using a similar algorithm (Artimov, unpublished data).

Once a rank-ordered list of the highest to lowest priority sequencing-identified mutant tumor-specific peptide antigens is generated, we will generate tetramers corresponding to the top 5-10 class I neoantigens using a photo-activated peptide tetramer protocol. We will stain T cells from the peripheral blood of subjects using a state-of-the-art CyTOF processor. Mutant epitopes associated with positive tetramer staining will be prioritized for the generation of neoantigen DNA vaccines. Once the candidate mutant epitopes have been verified, multivalent DNA vaccine construction will commence.

2.8 Polyepitope DNA vaccines

The neoantigen DNA vaccine strategy is based on the DNA vaccine platform. The observation that direct administration of recombinant DNA can generate potent immune responses established the field of DNA vaccines in the early 1990s [28-33]. Since that time, DNA vaccines have remained an area of intense research interest, and vaccines targeting infectious disease agents and cancers have progressed into clinical trials. Advantages of the DNA vaccine platform include the remarkable safety profile of DNA vaccines, and the relative ease of manufacture relative to proteins and other biologics. Perhaps most important, however, is the molecular flexibility of the DNA vaccine platform, with the ability to genetically

manipulate encoded antigens, and/or incorporate other genes to amplify the immune response [34, 35]. The molecular flexibility of the DNA vaccine platform allows us to target multiple mutant tumor-specific antigens using a single polyepitope DNA vaccine. Polyepitope DNA vaccines integrate multiple epitopes in a single construct (Figure 1). We have optimized the polyepitope DNA vaccine platform to maximize antigen presentation of neoantigens by integrating a mutant ubiquitin molecule. Because MHC class I binding peptides are initially processed in the cytosol by the ubiquitin/proteasome pathway, we have integrated a mutant form of ubiquitin (UbG76V). Fusion of UbG76V to the N-terminus of the polyepitope construct promotes epitope generation and display.

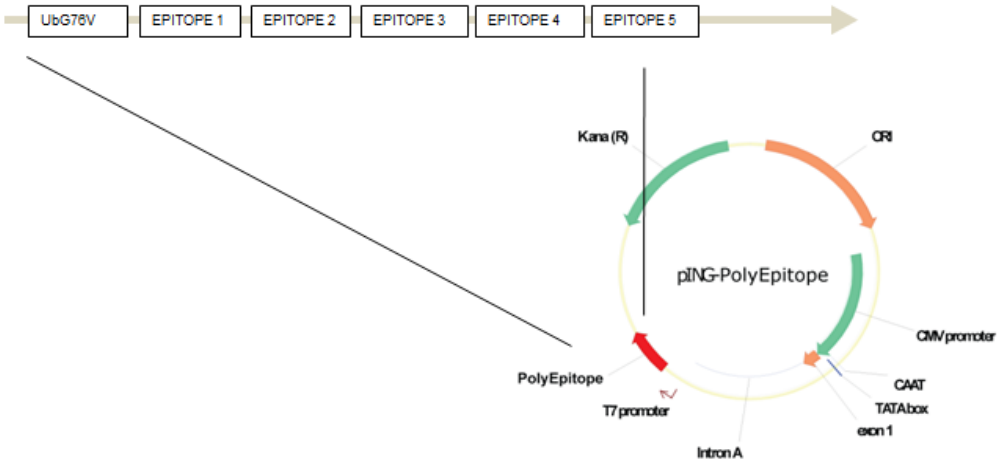


Figure 1: Polyepitope DNA vaccine design. Neoantigen DNA vaccines integrate multiple epitopes in a single construct. We have optimized the polyepitope DNA vaccine platform to maximize antigen presentation of the sequencing-identified neoantigens by integrating a mutant ubiquitin molecule.

2.9 Electroporation

Recent research provides valuable insights into why preclinical studies of DNA vaccines have been successful in rodents, but less successful in larger animals. One difficulty is scaling up DNA vaccine dose and injection volume [36]. In rodents, hydrostatic pressure from injecting DNA in a relatively large liquid volume significantly improves cellular uptake of DNA and antigen expression [37]. This effect is reduced in larger animals as the relative volume injected and hydrostatic pressure is reduced. Electroporation dramatically increases DNA uptake by muscle cells, antigen expression, and immunogenicity [38-41]. Of particular note, electroporation has now been used successfully in non-human primates, with responses at levels previously not observed with other DNA vaccine approaches and similar to or superior to responses induced by live vectors [42-49]. The importance of electroporation to the successful clinical translation of DNA vaccines was recently highlighted and emphasized in a high-profile review in *Nature Reviews* [50], and in the introduction to a special issue of *Vaccine* [51]. We have established a collaboration with Ichor Medical Systems and will use the TDS-IM electroporation device in the proposed phase 1 clinical trial.

2.10 Clinical trial design and dose considerations

The early clinical development paradigm for chemotherapeutic agents has significantly influenced the development of therapeutic cancer vaccines. However, there are major differences between these two classes of therapeutics that have important implications for early clinical development. Specifically, the phase 1 concept of dose escalation to find a maximum-tolerated dose does not apply to most therapeutic cancer vaccines. Most therapeutic cancer vaccines are associated with minimal toxicity at a range that is feasible to manufacture or administer, and there is little reason to believe that the maximum-tolerated dose is the most effective dose.

In a recent article from the biostatistics literature, Simon et al. write that "the initial clinical trial of many new vaccines will not be a toxicity or dose-ranging trial but rather will involve administration of a fixed dose of vaccine … in most cases the dose selected will be based on preclinical findings or practical considerations. Using several dose levels in the initial study to find the minimal active dose or to characterize the dose-activity relationship is generally not realistic" [52].

To date, the Ichor TriGrid™ Delivery System has been used as the means for delivery in nine DNA vaccine candidates. In the safety and toxicology studies, notable findings associated with electroporation-mediated delivery of DNA vaccines were limited to localized inflammatory responses of mild to moderate severity at the site of administration. Please see Sections 5.12.3 and 5.12.4 for a detailed description of the TDS-IM clinical experience to date.

We propose a vaccine dose of 4 mg based primarily on practical considerations and previous experience with the TDS-IM device. This dose is the within the range of DNA doses previously tested in the clinical setting with the TDS-IM device (0.2-8.0 mg) and, given the concentration of the neoantigen DNA vaccine (2.0 mg/mL), this dose is the maximal dose that can be administered feasibly by two administrations of electroporation.

2.11 Challenges of sequencing pancreatic cancers

The challenges of performing massively parallel sequencing (MPS) assays from clinical pancreatic cancer samples are considerable. At its essence, pancreatic adenocarcinoma has a low cellularity, with many stromal cells interspersed with the pancreatic cancer cells. A pathologist who is a specialist in gastrointestinal cancers will survey the tumor to identify islands of high tumor cellularity and take 1mm punches from formalin fixed paraffin embedded (FFPE) tissues. By selecting these areas for sequencing we are able to enrich the tumor purity to the level that allows neoantigen identification. Additionally, since the tissues have been previously subjected to FFPE, some degradation of the nucleic acids may result, further complicating the sample quality toward MPS.

Nevertheless, our group has worked extensively to optimize MPS library construction techniques that enable high diversity libraries to be constructed from as little as 10 ng of input DNA. In particular, we currently utilize the Swift Biosciences Accel-NGS 2S kit that is optimized for low input DNA from FFPE samples with an input range of 10-50 ng of isolated DNA. The resulting libraries have proven suitable for subsequent exome capture by hybridization such as the Integrated DNA Technologies (IDT) Rapid Exome reagent. This commercially available reagent has superior performance on FFPE-derived DNA libraries, and has a rapid hybridization time of only 4 hours, permitting a one-day turn around for library construction, quantitation, and hybrid capture prior to sequencing.

Another challenge of pancreatic adenocarcinoma is the rapid degradation of RNA that often occurs during surgery. Our surgical team has been evaluating whether the RNA stability can be influenced by changes to the surgical procedure that reduce ischemia time during surgery. In our early experience with several samples, this alteration in the surgical procedure leads to dramatic improvements to the RNA stability as gauged by RNA integrity (RIN) values obtained from our post-isolation QC procedures. In turn, the MPS libraries generated from these pancreatic RNAs are of high quality and diversity.

3 PATIENT SELECTION

3.1 Inclusion criteria

A patient will be eligible for the trial only if ALL of the following criteria apply:

1. Histologically or cytologically confirmed diagnosis of pancreatic adenocarcinoma; mixed histology will be included as long as the predominant histology is adenocarcinoma.
2. Completed an R0 or R1 surgical resection as determined by pathology
3. Pathology review demonstrates tumor cellularity no less than 30% in quantities sufficient to obtain 6-8 1mm biopsies from the original FFPE blocks.
4. At least 18 years of age.

5. Life expectancy of > 12 months.
6. ECOG performance status ≤ 2
7. Normal bone marrow and organ function as defined below:

a.	WBC	≥3,000/μL
b.	absolute neutrophil count	≥1,500/μL
c.	platelets	≥100,000/μL
d.	total bilirubin	≤2.5 X institutional upper limit of normal
e.	AST/ALT	≤2.5 X institutional upper limit of normal
f.	creatinine	≤1.5 X institutional upper limit of normal

8. International Normalized Ratio (INR) and activated partial thromboplastin time (PTT) < 1.5 x ULN provided the patient is not on anticoagulation therapy.
9. Patients who have had a stent placed for biliary obstruction can be included in the study provided serum bilirubin at time of enrollment is within protocol limits.
10. Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.
11. Able to understand and willing to sign an IRB approved written informed consent document.

Patients may be consented prior to receiving adjuvant therapy, or during the course of adjuvant therapy. Adjuvant therapy must meet the following criteria below for enrollment to the trial:

a. Initiation of adjuvant chemotherapy within 12 weeks of surgery
b. Completion of at least 4 months of adjuvant chemotherapy with gemcitabine/capecitabine or similar adjuvant chemotherapy at the discretion of the patient's medical oncologist.
c. Additional chemoradiation therapy as recommended by the patient's medical oncologist.
d. Reimaging within 4 weeks of last dose of chemotherapy demonstrates no evidence of recurrent disease and CA 19-9 is less than 92.5 u/mL.
e. Dose modifications and/or delays in adjuvant chemotherapy is at the discretion of the treating physician

2. There is a 1 week washout prior to Day 1 of vaccine for patients on daily systemic steroids at doses exceeding 10 mg prednisone.

3.2 Exclusion criteria

A patient will be ineligible for inclusion in this study if ANY of the following criteria apply:

1. Evidence of neuroendocrine tumor, duodenal adenocarcinoma, or ampullary adenocarcinoma.
2. Received neoadjuvant chemotherapy for their pancreatic adenocarcinoma.
3. Evidence of disease recurrence or metastasis following surgical resection at any time prior to the first vaccination administration. Most patients will undergo restaging midway through adjuvant chemotherapy and at the completion of therapy; however, timing of imaging is at the discretion of the patient's medical oncologist.
4. History of other malignancy ≤ 3 years previous with the exception of basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix.
5. Receiving any other investigational agents, or has received an investigational agent within the last 30 days.
6. Known allergy, or history of serious adverse reaction to vaccines such as anaphylaxis, hives, or respiratory difficulty.

7. Acute or chronic, clinically significant hematologic, pulmonary, cardiovascular, hepatic renal, and/or other functional abnormality that would jeopardize the health and safety of the participant as determined by the investigator based on medical history, physical examination, laboratory values, and/or diagnostic studies.
8. A psychiatric illness/social situations that would limit compliance with study requirements as determined by the investigator from the medical history, physical exam, and/or medical record
9. History of syncopal or vasovagal episode as determined by medical record and history in the 12 month period prior to first vaccination administration.
10. Individuals in whom a skinfold measurement of the cutaneous and subcutaneous tissue for eligible injection sites (left and right medial deltoid region) exceeds 40 mm.
11. Individuals in whom the ability to observe possible local reactions at the eligible injection sites (deltoid region) is, in the opinion of the investigator, unacceptably obscured due to a physical condition or permanent body art.
12. Therapeutic or traumatic metal implant in the skin or muscle of either deltoid region.
13. Any chronic or active neurologic disorder, including seizures and epilepsy, excluding a single febrile seizure as a child.
14. Current use of any electronic stimulation device, such as cardiac demand pacemakers, automatic implantable cardiac defibrillator, nerve stimulators, or deep brain stimulators.
15. Prior or currently active autoimmune disease requiring management with immunosuppression. This includes inflammatory bowel disease, ulcerative colitis, Crohn's disease, systemic vasculitis, scleroderma, psoriasis, multiple sclerosis, hemolytic anemia, immune-mediated thrombocytopenia, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, sarcoidosis, or other rheumatologic disease or any other medical condition or use of medication (e.g., corticosteroids) which might make it difficult for the patient to complete the full course of treatments or to generate an immune response to vaccines. In the case of asthma or chronic obstructive pulmonary disease taking inhaled corticosteroids that does not require daily systemic corticosteroids is acceptable. Additionally, local acting steroids (topical, inhaled, or intraarticular) will be allowed. Patients on intermittent or short course steroids will be allow if the dose does not exceed 4 mg of dexamethasone (or equivalent) per day for > 7 consecutive days. Any patients receiving steroids should be discussed with the PI to determine if eligible.
16. Pregnant and/or breastfeeding.
17. Known HIV-positive status. These patients are ineligible because of the potential inability to generate an immune response to vaccines.

3.3 Inclusion of women and minorities

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

4 REGISTRATION PROCEDURES

4.1 Prior to registration

4.1.1 Subject recruitment

The methods used for recruitment of subjects in the study will be devoid of any procedures that may be construed as coercive. The recruitment process will not involve any restrictions based on social or demographic factors including age, or ethnic characteristics of the subject population. However, the composition of the study subject population will depend on patient sources available to the investigators. Subjects will be identified and recruited for this study as follows:

Patients will be recruited from Washington University's Siteman Cancer Center and Johns Hopkins Sidney Kimmel Comprehensive Cancer Center outpatients or patient referrals by our community

oncologists to the principal investigator and co-investigators. Patients must be willing and able to give their written informed consent indicating that they are aware of the investigational nature of the study. After a patient is deemed eligible for study, the principal investigator (or co-investigators), or another delegated individual, will discuss the Washington University/Johns Hopkins IRB-approved informed consent with the patient. This written informed consent will be signed and dated by the patient and the principal investigator (or co-investigators), or another delegated individual. The original consent will be placed in the patient's permanent record and a copy will be given to the patient.

Washington University School of Medicine (WUSM)/Johns Hopkins Medicine (JHM) has an approved Multiple Project Assurance of Compliance with Department of Health and Human Services Regulations for the Protection of Human Research Subjects on file with the Office for Human Research Protection (OHRP). The Human Research Protection Office Policies and Procedures for Protection of Human Research Subjects details all policies and procedures for the protection of human research subjects and can be obtained upon request from the Human Research Protection Office.

4.1.2 Compliance and understanding

All patients who present with a diagnosis of pancreatic cancer will be screened for eligibility for entry into the study at their postoperative follow up with their surgeon or initial postoperative appointment with their medical oncologist, or during their adjuvant treatment with their medical oncologist. As in all trials, the goal is to achieve a high level of compliance with protocol requirements by assuring, during the eligibility assessment, that the potential subject is fully informed and agrees to the protocol requirements. In addition, subjects with a strong likelihood of non-adherence such as difficulties in adhering to follow-up schedule due to geographic distance from the Siteman Cancer Center/Sidney Kimmel Comprehensive Cancer Center, should not knowingly be registered. Adherence of the Siteman Cancer Center/Sidney Kimmel Comprehensive Cancer Center staff to careful assessment of the subject's understanding of the trial and a clinical center environment which supports the continued commitment of the subjects are essential for the trial to be successfully completed.

4.1.3 Presentation of informed consent

Consent will be obtained by either the principal investigator or by individuals approved by the principal investigator and whose names and copy of their *curriculum vitae* have been filed. The initial consent should be the IRB-approved version corresponding to the version of the protocol approved when the screening was initiated. Informed consent is to be obtained from the subject according to **Section 15.1 Informed Consent** of this protocol.

4.2 Registration procedures

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

Registration for this trial will be a two-step process. Step 0 will be screening and confirmation of eligibility for vaccine creation. Step 1 will be screening and confirmation of eligibility for vaccine administration. See Section 3: Patient Selection for eligibility criteria for each step and Section 8: Study Calendar for screening and procedures for each step.

Step 0 screening must occur within 16 weeks of surgical resection, and Step 0 enrollment can occur up to four weeks after initiation of adjuvant chemotherapy. Step 1 screening will occur when the patient's vaccine is ready or nearly ready, but must be within 14 days of the first vaccine administration.

The following steps must be taken before registering patients to this study:

(1) Confirmation of patient eligibility by Washington University (applies to both Step 0 and Step 1)

(2) Registration of patient in the OnCore Cancer Center database (This will be done by Washington University research coordinator. Step 0 enrollment will be "On Study" in the OnCore database, and Step 1 enrollment will be "On Treatment" in the OnCore database.)

(3) Assignment of unique patient number (UPN) (assigned as Step 0 only)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.3 Confirmation of patient eligibility

Confirm patient eligibility by collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least two business days prior to registering patient (if a quicker turnaround is needed, contact primary study coordinator at Siteman Cancer Center):

(1) Your name and contact information (telephone number, fax number, and email address)

(2) Your site PI's name, the registering MD's name, and your institution name

(3) Patient's race, sex, and DOB

(4) Three letters (or two letters and a dash) for the patient's initials

(5) Currently approved protocol version date

(6) Copy of signed consent form (patient name may be blacked out)

(7) Planned date of first injection

(8) Completed eligibility checklist, signed and dated by a member of the study team

(9) Copy of appropriate redacted source documentation confirming patient eligibility

4.4 Patient registration in the Siteman Cancer Center OnCore database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance, and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.5 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5 INVESTIGATIONAL AGENT

5.1 Chemical name and structure

Neoantigen DNA vaccines will be designed in the Gillanders laboratory and manufactured at the Siteman Cancer Center Biological Therapy Core Facility based on the following general steps:

(1) Pancreatic adenocarcinoma tissue and normal lymphocytes will be obtained from pancreatic cancer patients who are eligible for the phase 1 clinical trial.

(2) A pancreas specific pathologist will review the operative specimen slides to verify tumor cellularity and quantity sufficient for proceeding

(3) The corresponding FFPE blocks will be punched with a disposable 1mm biopsy punch. 6-8 full thickness punches will be taken from areas of high tumor cellularity and divided evenly into two DNA LoBind Eppendorf tubes (one tube for DNA and one for RNA).

(4) DNA and RNA are subsequently extracted in the Washington University Center for Human Immunology and immunotherapy Programs (CHIIPS) core.

(5) Tumor/normal exome sequencing and tumor cDNA-capture sequencing, if feasible, will be performed to identify candidate neoantigens. Candidate neoantigens will be prioritized based on epitope prediction algorithms as outlined previously.

(6) Mesothelin expression will be confirmed by RNA analysis, if patients are confirmed to express mesothelin and are HLA-A1, HLA-A2, HLA-A3, or HLA-24, the corresponding mesothelin epitopes will be added to the vaccine.

(7) Personalized polyepitope inserts integrating the prioritized neoantigens and mesothelin epitopes will be designed in the Gillanders laboratory and then synthesized and cloned into the pING parent vector by Blue Heron Biotech.

(8) Master cell banks will be established and validated at WUSM.

(9) The neoantigen DNA vaccines will be manufactured and vialed at WUSM.

(10) The neoantigen DNA vaccines will undergo product release tests at WUSM and other designated sites (See Table 3) prior to investigational use.

The neoantigen DNA vaccines will be manufactured in the SCC Biologic Therapy Core facility. Standard Operating Procedures for the GMP manufacture of the personalized vaccines have been established and are in accordance with “CGMP for Phase 1 Investigational Drugs 2008,” and “Considerations for Plasmid DNA Vaccines for Infectious Disease Indications 2007.”

Each polyepitope DNA vaccine drug product is composed of a deoxyribonucleic acid (DNA) plasmid purified from *E. Coli*. The pING parent vector was obtained from Alan Houghton, M.D. at Memorial Sloan Kettering Cancer Center (Figure 2). The pING vector has been used extensively in preclinical DNA vaccine studies [53, 54], and in human clinical trials, including a clinical trial at WUSM (ClinicalTrials.gov identifier: NCT00807781). This trial using a mammaglobin A DNA breast cancer vaccine is based on the pING vector as well and was manufactured at WUSM in the SCC Biologic Therapy Core Facility using manufacturing processes almost identical to the ones outlined here.

The pING vector contains the following elements: (1) a eukaryotic promoter and enhancer from the Towne strain of CMV; (2) a polylinker region to facilitate cloning of a variety of DNA fragments; (3) donor and acceptor splice sites and a poly adenylation signal sequence derived from the bovine growth hormone gene; (4) the ColE1 origin of replication and (5) a gene conferring kanamycin resistance. With the exception of the kanamycin resistance gene, which was cloned as a PstI fragment from the plasmid pUC4, all other gene segments were amplified by polymerase chain reaction (PCR).

The role of each element is as follows: (1) the CMV promoter/enhancer enables high-level expression of polyepitope insert in mammalian cells; (2) the polylinker region serves as a multiple cloning site for easy insertion of genes, in this case the polyepitope insert; (3) the polyadenylation signal sequence facilitates efficient transcription termination and polyadenylation of mRNA; (4) the origin of replication allows for high-copy number replication and growth in *E. coli*; (5) the kanamycin resistance gene provides for selection in *E. coli*; and (6) the prokaryotic T7 promoter of the pING plasmid produces high levels of polyepitope DNA transcripts in *E. coli* in the presence of bacterial T7 RNA polymerase. Of note, the kanamycin resistance gene is cloned in the opposite orientation from the polyepitope insert to limit transcription of kanamycin from the CMV promoter in human cells.

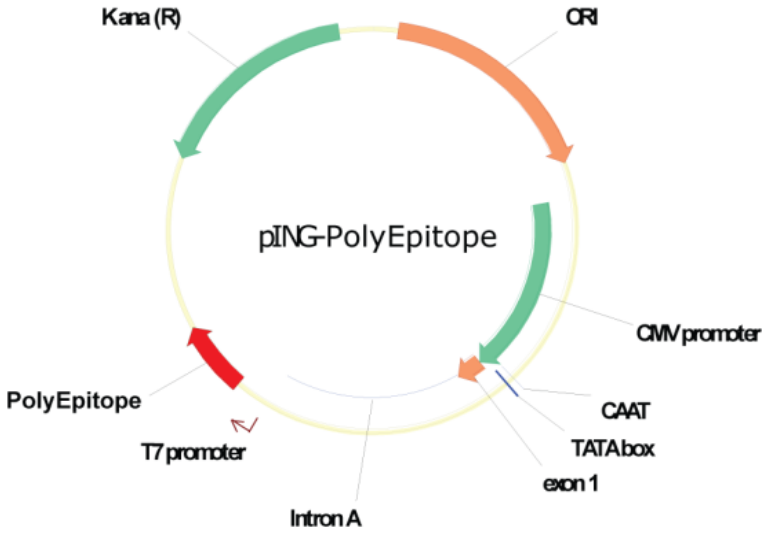


Figure 2: Polyepitope DNA vaccine design. Polyepitope DNA vaccines are based on the pING parent vector. The pING vector contains the following elements: (1) a eukaryotic promoter and enhancer from the Towne strain of CMV; (2) a polylinker region to facilitate cloning of a variety of DNA fragments; (3) donor and acceptor splice sites and a poly adenylation signal sequence derived from the bovine growth hormone gene; (4) the ColE1 origin of replication; (5) a gene conferring kanamycin resistance, and (6) a polyepitope insert integrating the prioritized neoantigens.

The personalized inserts will be designed in the Gillanders Laboratory based on the results of next-generation sequencing and epitope prediction algorithms from each individual patient. Personalized polyepitope inserts will incorporate up to 20 prioritized neoantigens. The personalized polyepitope inserts will be synthesized by Blue Heron Technologies and cloned into the pING parent vector as detailed below.

5.2 Nonclinical studies

5.2.1 Overview

We propose a phase 1 clinical trial of a neoantigen DNA vaccine strategy. The neoantigen DNA vaccine strategy is designed to target neoantigens present in the pancreatic cancer, but absent in normal tissues.

One of the reasons that we have pursued clinical development of a neoantigen DNA vaccine strategy targeting neoantigens is because we believe that this strategy has the potential to be safer than strategies targeting shared tumor antigens. Shared tumor antigens are typically expressed at high levels in the tumor, but are also typically expressed at lower levels in some normal tissues. Expression of shared tumor antigens in normal tissues may increase the risk of autoimmunity. Neoantigens are present only in the tumor. In addition, our next-generation sequencing-based epitope prediction algorithm prioritizes epitopes where the mutant epitope (but not the wildtype epitope) can bind to restricting HLA molecules. This decreases the potential that immune responses targeting neoantigens will be cross-reactive with wildtype antigens.

We do not think that GLP safety and toxicology studies will provide significant insight into the safety of the neoantigen DNA vaccine strategy. First, it is impossible to know *a priori* what mutations will be present and/or prioritized in individual patients. We estimate that there are as many as 7 million potential neoantigens that could be targeted by our approach. Only a limited number of mutations could be targeted in GLP safety and toxicology studies. Second, to our knowledge, no mammary tumor models exist that would be relevant for GLP safety and toxicology studies. Third, the pING parental vector has proven to be safe in phase 1 clinical trials.

We are not proposing to perform GLP biodistribution and integration studies at this stage in development. There is extensive GLP information available about the biodistribution and integration of DNA vaccines

following electroporation with the TriGrid device. This includes GLP information about biodistribution and integration of DNA vaccines using the pING parent vector. Additional information about the biodistribution and integration of DNA vaccines following electroporation with the TriGrid device is summarized in the IND application.

5.2.2 Nonclinical studies

It has long been known that there is a dynamic relationship between the immune system and cancer. This dynamic relationship has been studied in detail, ultimately resulting in the establishment of the cancer immunoediting concept [55-62].

We have recently focused on defining the antigens recognized by the immune system during the cancer immunoediting process. These studies, summarized below, demonstrate that neoantigens are important tumor rejection antigens, and provide strong support for our personalized pancreatic cancer vaccine strategy. Specifically, we have developed next-generation sequencing and epitope prediction algorithms to identify and prioritize neoantigens. We will use these algorithms in the proposed clinical trial. The preclinical data supporting the use of these algorithms are presented below.

In initial studies we used a combination of next-generation sequencing and epitope prediction algorithms to identify neoantigens in the d42m1 MCA sarcoma line. These algorithms identified one particular mutation (an R913L mutation of SPTBN2) as a top candidate, and subsequent analyses confirmed that this mutant tumor-specific antigen functioned as an immunodominant tumor rejection antigen. These studies were published in *Nature* [24].

The d42m1 MCA sarcoma is an unedited tumor, and would therefore be expected to express strong tumor rejection antigens. We have since turned our attention to examining the epitope landscape in edited MCA sarcomas that develop in immunocompetent wildtype mice. Specifically, we have asked the following questions: (1) Can the next-generation sequencing and epitope prediction algorithms be used more broadly to identify and prioritize important neoantigens in less immunogenic tumors? (2) Can the next-generation sequencing and epitope prediction algorithms be used to prioritize antigens for immune targeting and/or personalized vaccine therapy?

To address these questions we focused initial efforts on d42m1-T3. d42m1-T3 is a clone of d42m1 that lacks the immunodominant rejection antigen, mutant SPTBN2, and forms progressively growing tumors in wildtype mice. We specifically chose the d42m1-T3 clone because d42m1-T3 shares with naturally edited sarcomas the ability to form progressively growing tumors in wildtype mice and shows a similar sensitivity to checkpoint blockade.

To identify and prioritize mutant tumor specific antigens from the d42m1-T3 we used optimized next-generation sequencing and epitope prediction algorithms. Specifically, we pipelined the candidate mutant tumor-specific antigen sequences into four different MHC class I epitope prediction algorithms and calculated the median predicted affinity for binding to the relevant class I MHC alleles. We then applied filters that account for proteasomal processing of the antigen and differences in MHC class I binding affinity between mutant and native sequences to prioritize the neoantigens. We also deprioritized hypothetical Riken proteins.

Of the top 61 prioritized candidates, 20 were eliminated by the filtering process; including two of the top four candidates. Of those that remained, two [G1254V Laminin subunit α4 (mLama4) and A506T alpha-1,3 glucosyltransferase (mAlg8)] were clearly favored above the others based on predicted binding affinity.

To test whether these two “best” neoantigens were biologically relevant, we generated tumor-specific CD8 T cell lines from the spleens of three independent mice that had rejected d42m1-T3 cells after anti-PD-1 therapy and showed that each T cell line (CTL-62, CTL-73, CTL-74) displayed specificity for d42m1-T3 but not an unrelated sarcoma, F244. To determine if the “prioritized” neoantigens were recognized by anti-d42m1-T3 T cell lines, we incubated 8 amino acid synthetic peptides corresponding to each of the top 61 initially predicted H-2K^b neoantigens with irradiated splenocytes and CTL-74 T cells and monitored IFNγ production. The mLama4 and mAlg8 peptides strongly stimulated CTL-74 T cells, with mLama4 inducing ~10x more IFNγ than mAlg8. No other predicted mutant epitope induced significant levels of IFNγ production in this assay. Similar results were obtained with the other two d42m1-T3 specific CD8 T

cell lines. Subsequent dose response experiments showed that mLama4 stimulated the tumor-specific T cell lines to a greater extent than mAlg8 and that the T cells reacted specifically with mutant but not native peptides.

We then used four experimental systems to confirm that our optimized epitope prediction algorithms accurately prioritized neoantigens. *First***,** together with the groups of Hans-Georg Rammensee in Tübingen and Ruedi Abersold in Zurich we detected mLama4 and mAlg8 peptides bound to H-2K^b on d42m1-T3 tumor cells. To our knowledge this is the first time that mutant class I epitopes have been detected bound to tumor cell-associated MHC class I. *Second*, using PE-labeled H-2K^b tetramers carrying mLama4 or mAlg8 peptides, CD8 T cells with specificities for these two epitopes were found to accumulate in d42m1-T3 tumors in αPD-1 treated mice and reached peak values just prior to tumor rejection on day 12. Consistent with the results of the T cell stimulation experiments, mLama4-specific T cells were present in significantly higher numbers in the tumor than mAlg8-specific T cells. No mLama4- or mAlg8-specific T cells were observed in irrelevant, checkpoint blockade-sensitive F244 tumors. *Third*, vaccination of naïve WT mice with mutant-Lama4 or mutant-Alg8 short peptide vaccines (8mer) induced strong CD8 T cell responses that were specific for the mutant, but not the WT epitope (mLama4 = 1650 SFC/10^6 cells vs. wtLama4 = 75 SFC/10^6 cells; mAlg8 =606 SFC/10^6 cells vs. wtAlg8 = 50 SFC/10^6 cells). *Fourth*, prophylactic vaccination of mice with long peptides (~30mer) corresponding to either the mLama4 epitope alone, or both the mLama4 and mAlg8 epitopes induced protection against subsequent challenge with d42m1-T3 tumor cells. The combined peptide vaccine was more protective than the vaccine containing the mLama4 long peptide alone (Figure 5).

Of note, immune responses to both mLama4 and mAlg8 were also observed after vaccination with a polyepitope DNA vaccine encoding mLama4, mAlg8, and several additional epitopes. Mice were vaccinated three times and the immune response to mLama4, mAlg8 and control peptides was assessed by ELISPOT using splenocytes from vaccinated mice. Vaccination with polyepitope DNA vaccines induced responses that were similar in magnitude as synthetic long peptide vaccines encoding mLama4 or mAlg8 (data not shown).

5.2.3 Rationale for no GLP safety studies

We do not think that GLP safety and toxicology studies will provide significant insight into the safety of the neoantigen DNA vaccine strategy.

First, it is impossible to know *a priori* what mutations will be present and/or prioritized in patients. Next-generation sequencing of epithelial cancers has demonstrated that there are very few recurrent mutations present. Mutations can be present in any one of the approximately 20,000 protein-coding genes present in the human genome. Even more problematic is that the mutations targeted could be anywhere in the corresponding protein. It has been estimated that the average length of a protein in humans is 362 AA. Thus, there are potentially 7,240,000 potential neoantigens that could be targeted by our approach. This includes only point mutations and does not include mutations resulting from indels. If studies are performed in a preclinical model, only a limited number of mutations will be targeted. For example if we design a neoantigen DNA vaccine specific for a murine epithelial cancer targeting 5 genes, this would represent only 5 of 7,240,000 potential neoantigens. Even if 1000 GLP safety and toxicology studies were performed, each targeting 5 neoantigens, it would still provide information on < 0.07% of potential neoantigens.

Second, to our knowledge, no pancreatic cancer models exist that would be relevant for the proposed preclinical studies. In order to study neoantigens, sequencing analyses of paired tumor/normal tissues are required. As such, we would need to study spontaneous tumors in mice, as tumors propagated as cell lines do not have corresponding normal DNA to evaluate. There are very few models of spontaneous pancreatic cancer development in wildtype mice. Spontaneous tumors do develop in genetically engineered mice, but these oncogene-driven tumors in genetically engineered murine models of cancer typically have a very limited number of mutations. We have performed extensive studies in genetically engineered mice, and have found that there are very few mutations present in tumors derived from these mice. For example, we have performed extensive studies in the p53-null transplant mammary tumor model. p53 is a tumor suppressor gene and plays an important role in maintaining genome stability. As such one might expect that there would be a significant number of mutations present in p53-null transplant mammary tumors. However, we have sequenced > 10 p53-null transplant mammary tumors

and have found that there are only a limited number of mutations present in each tumor. This is not at all representative of human pancreatic cancers where significantly more mutations are present. Of note, a significant number of mutations must be present to reliably identify neoantigens that are immunogenic, as many candidate neoantigens are not processed and presented by the immune system, or cannot be recognized efficiently by T cells. If only a limited number of mutations is present, it is possible that no neoantigens will be identified that are immunogenic. For meaningful GLP safety and toxicology studies of a neoantigen DNA cancer vaccine, one of the key considerations is to assess any potential toxicity associated with immune responses to the vaccine. If no neoantigens are identified that are immunogenic, the GLP safety and toxicology studies will have only limited value as no immune response to the mutant tumor-specific antigen will be generated. As such, we are not aware of any pancreatic cancer tumor models in mice that would be appropriate for the GLP safety and toxicology studies.

5.2.4 Rationale for no GLP biodistribution studies

We are not proposing to perform GLP biodistribution and integration studies at this stage in development. There is extensive GLP information available about the biodistribution and integration of DNA vaccines following electroporation with the TriGrid device. This includes GLP information about biodistribution and integration of DNA vaccines using the pING parent vector. The information available about the biodistribution and integration of DNA vaccines following electroporation with the TriGrid device is detailed below.

5.3 Sequencing pipeline

Robust next-generation sequencing strategies for the identification of neoantigens will be required for the successful clinical translation of personalized cancer vaccine strategies. As such, a major focus of our research studies has been the development of cost-effective and accurate next-generation sequencing strategies to identify neoantigens and validate the expression of these antigens at the mRNA level.

The first step in the sequencing pipeline is exome sequencing of pancreatic cancer and normal DNA. Exome fragments are captured using Nimblegen's "VCRome" exome capture reagent. Background DNA is washed away while the bound exome DNA is eluted and sequenced. Separate libraries are made from the pancreatic cancer and normal DNA and processed independently. Exome sequencing is performed using the Illumina platform.

Exome sequences from pancreatic cancer and normal DNA are compared separately to the human reference sequence and then to one another to identify somatic variation. VarScan 2 software is used to detect misaligned sequences and identify structural variants in the pancreatic cancer DNA.

The second step in the sequencing pipeline is cDNA-capture sequencing. To validate the results of the exome sequencing, and confirm expression of the somatic mutations in the pancreatic cancer, cDNA-capture sequencing of the pancreatic cancer RNA will be performed. cDNA-capture sequencing is very similar to RNA sequencing, but cDNA is captured prior to sequencing to enrich for mRNA. cDNA-capture sequencing is a sensitive and accurate methodology to detect expression of somatic mutations at the mRNA level in pancreatic cancer. cDNA-capture sequencing is performed using the Illumina platform.

The third step in the sequencing pipeline is data analysis to identify the expressed somatic mutations. All somatic mutations are subjected to a set of filters to exclude “false-positive” calls. The filters include:

Exome data analysis:

(1) Normal Coverage $\geq$ 5x
(2) Normal Variant Allele Frequency (VAF) $\leq$ 2%
(3) Tumor Coverage $\geq$ 10x
(4) Tumor Variant Allele Frequency $\geq$ 20%

Tumor cDNA Capture Data
(5) Tumor Coverage $\geq$ 10x
(6) Tumor Variant Allele Frequency $\geq$ 20%
(7) FPKM > 1

Pancreatic cancer-specific mutations that meet these filters will be prioritized. We will further prioritize indels over missense mutations. The mutations will be further analyzed for epitopes using the epitope prediction algorithms detailed below (Please see Section 5.4).

Please see below for an explanation of the terms used above.

The term coverage is a general term to describe the fold oversampling of a DNA target by sequencing data. In covering a target region or genome, increasing depth of coverage leads to increased certainty of variant detection. Therefore 10x "coverage" implies that the given site was independently sequenced at least 10 times.

Variant allele frequency (VAF) is a metric that represents the sensitivity of identifying somatic variant over a range of sample purity and sequencing depths. The expected variant allele frequency acts as a surrogate for purity. For example, if a heterogeneous somatic variant is present in all tumor cells and the tumor cells represent 40% of the sample, then the observed VAF would be 20%.

FPKM stands for Fragments Per Kilobase of transcript per Million mapped reads. This is a way to estimate expression of a gene. In RNA sequencing (and cDNA-capture sequencing), the relative expression of a transcript is proportional to the number of cDNA fragments that originate from it. Paired-end RNA-Seq experiments produce two reads per fragment, but that doesn't necessarily mean that both reads will be mappable. For example, the second read is of poor quality. If we were to count reads rather than fragments, we might double-count some fragments but not others, leading to a skewed expression value. Thus, FPKM is calculated by counting fragments, not reads. If you have a gene with an FPKM of 0, you'll see a few reads that align to it. However, if there are 100 or more reads per sample, 1 or 2 reads is rather insignificant. So, while you can't really say the gene is not expressed with 100% certainty, you can say it was not detected.

5.4 Epitope prediction algorithm

We have developed and optimized an epitope prediction algorithm for the identification and prioritization of neoantigens. This optimized epitope prediction algorithm is described below and in the Gubin 2014 manuscript [25]. We have established this algorithm is collaboration with Dr. Robert Schreiber, an internationally-known expert in tumor immunology [24, 55-62]. Schreiber was one of the first to use next generation sequencing technologies to identify neoantigens, demonstrating that these antigens are important tumor rejection antigens [24]. Schreiber and Gillanders have now optimized the epitope prediction algorithm and have demonstrated that cancer vaccines targeting neoantigens are associated with antitumor immunity [25].

The goal of the optimized epitope prediction algorithm is to identify and prioritize up to 20 neoantigens. The algorithm uses a combination of binding algorithms, processing algorithms and *in vitro* binding assays.

Mutations that are expressed in the pancreatic cancer will be identified using the sequencing pipeline outlined in the document titled "Sequencing Pipeline." The predicted amino acid sequences corresponding to the expressed mutations will be pipelined through multiple class I and class II MHC epitope-binding algorithms provided by the Immune Epitope Database and Analysis Resource (http://www.immuneepitope.org) including but not limited to (i) Stabilized Matrix Method (SMM) [67], (ii) Artificial Neural Network (ANN) [68], and (iii) NetMHCpan [69].

A prioritized list of binding epitopes (i.e. IC_{50} < 500 nM) will be generated after calculating the median binding affinity value for each mutant sequence (affinity value expressed as $1/IC_{50}$ x 100).

Filters will be applied to the list to (a) eliminate epitopes that are not processed efficiently by the immunoproteosome based on the NetChop algorithm [70] (peptides with a NetChop score $\geq$ 0.6 will be prioritized), (b) deprioritize epitopes from hypothetical proteins or that form a weaker predicted binding epitope than that expressed by the corresponding wild type sequence, (c) prioritize mutant epitopes that have the highest difference in predicted binding affinity compared to their wild type counterpart, and (d) incorporate expression profiles of wild type genes that correspond to the mutant antigen candidates in normal vs tumor cells.

To confirm the ability of prioritized neoantigens to bind to the corresponding MHC class I allele, we may synthesize peptides corresponding to the prioritized list of candidate epitopes. Synthetic peptides (> 95% purity) will be tested for binding to the respective class I MHC molecule using the T2 cell line, or one of its derivatives expressing common class I MHC alleles. The T2 cell line is deficient in transporters for antigen presentation (TAP), and as such is commonly used to assess binding of exogenously added peptides [17, 71]. Expression of class I MHC alleles is measured by staining of T2 cells with antibody specific for the class I MHC allele followed by flow cytometric analysis to measure the fluorescence intensity of the class I MHC allele on the cell surface. Data are typically expressed as Mean Fluorescence Intensity (MFI) by subtracting the baseline fluorescence intensity of the class I MHC allele to that measured after addition of exogenous peptide. Neoantigens that do not bind to the corresponding class I MHC allele will be deprioritized. Similar algorithms will be used to predict class II neoantigens.

5.5 Manufacturing facility

The personalized polyepitope DNA vaccines will be prepared in the Siteman Cancer Center Biologic Therapy Core Facility at Washington University School of Medicine. The facility is located at 500 S. Kingshighway, Room 719 Southwest Tower, Saint Louis, MO 63110.

The Biologic Therapy Core Facility Operations Director is Johnnie Cartwright. She has extensive experience in writing, implementing, and strictly adhering to phase appropriate cGMP procedures & regulations to provide for the highest level of product quality.

The facility adheres to cGMP practices with regard to documentation, facility maintenance, and QC/QA review. Within the 2,615 sq ft GMP-facility on the 7th floor of the Southwest Tower, 6 manufacturing rooms are available for clinical grade manufacturing of cellular therapy products, recombinant DNA or gene therapy products (Floor plan below).

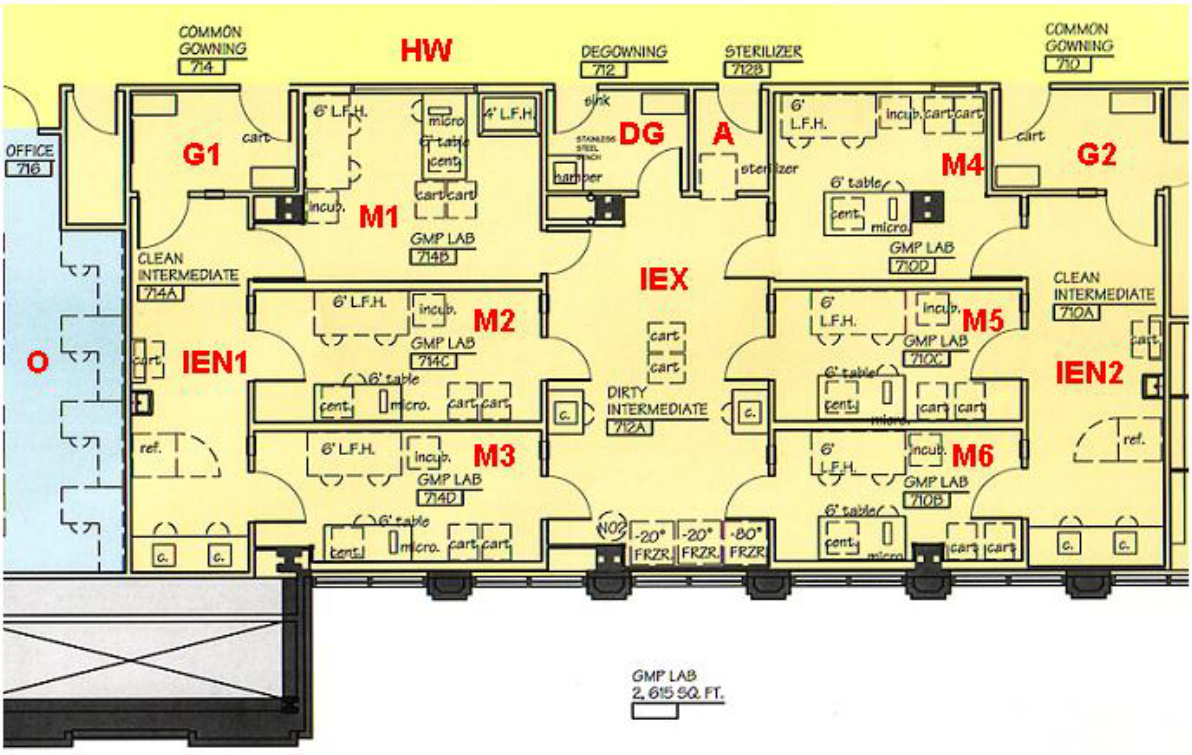


Figure 3: Siteman Cancer Center Biologic Therapy Core Facility floor plan. HW: Hallway, G1: Gowning Room 1; G2: Gowning Room 2; IEN 1: Intermediate Entry Room 1; IEN 2: Intermediate Entry Room 2; M1 to M3: Manufacturing Rooms 1 to 3; M4 to M6: Manufacturing Rooms 4 to 6; IEX: Intermediate Exit Room; DG: Degowning Room; O: Office; A: Autoclave Room.

In the Biologic Therapy Core facility, all manufacturing rooms are physically separated from each other with single pass air, and provide one-way personnel and product flow, which avoids cross-contamination of products. 6 different products can be worked on simultaneously without interfering with each other. Passthroughs are available from gowning rooms to intermediate rooms, and intermediate rooms to manufacturing rooms. All manufacturing rooms are completely isolated from each other by sealed walls and ceilings. A custom ceiling allows maintenance on the HVAC units, filters or lights without breeching the clean environment of the facility.

The manufacturing laboratories are only entered, one at a time, from an intermediate room. The laboratories are only exited, one at a time, into another intermediate room.

This provides easily manageable spatial and temporal segregation of products, which is a necessity to prevent product cross-contamination. Door interlocks, which allow for doors to be opened only one at a time, assure this unidirectional flow of personnel and products.

Manufacturing rooms, intermediate rooms and gowning/degowning rooms are isolated from each other by air pressure gradients. The manufacturing rooms have higher air pressure in respect to the intermediate rooms; gowning rooms have negative air pressure towards intermediate rooms and the hallway. This also assures protection of the outside environment from potentially biohazardous materials produced in the Biologic Therapy Core facility. Air pressure gradients are maintained by individual HVAC units for each room. The manufacturing rooms are certified and maintained as Class 10,000, the intermediate rooms are certified and maintained as Class 100,000.

Every manufacturing lab is equipped with a 6-foot biosafety cabinet and a dual chamber incubator. Sufficient room is available per manufacturing lab to accommodate specialized equipment (e.g. clinical grade magnetic bead cell separator) for different manufacturing processes. In the largest manufacturing room, besides the 6ft biosafety cabinet, a second 4ft biosafety cabinet is installed for work with infectious agents. Manufacturing room 1 can also be switched to negative air pressure and BSL-3 containment (vector manufacturing), and an IBC approved protocol to accomplish this task is in place.

Quality control and monitoring plans are in effect. Daily environmental cleaning is performed by dedicated personnel. The facility is electronically monitored continuously, daily monitoring by core personnel ensures adherence to standards and is prescribed by SOPs. Environmental monitoring includes non viable and viable particle enumeration with limits more stringent than the standards prescribed in the USP.

GMP regulations for even Phase 1 clinical trials are in place to assure a cellular or biological product to be safe and free of contaminants.

5.6 Manufacturing process

Overview of the polyepitope DNA vaccine manufacturing process. Schematic outline of product manufacture, in-process testing, and product release tests.

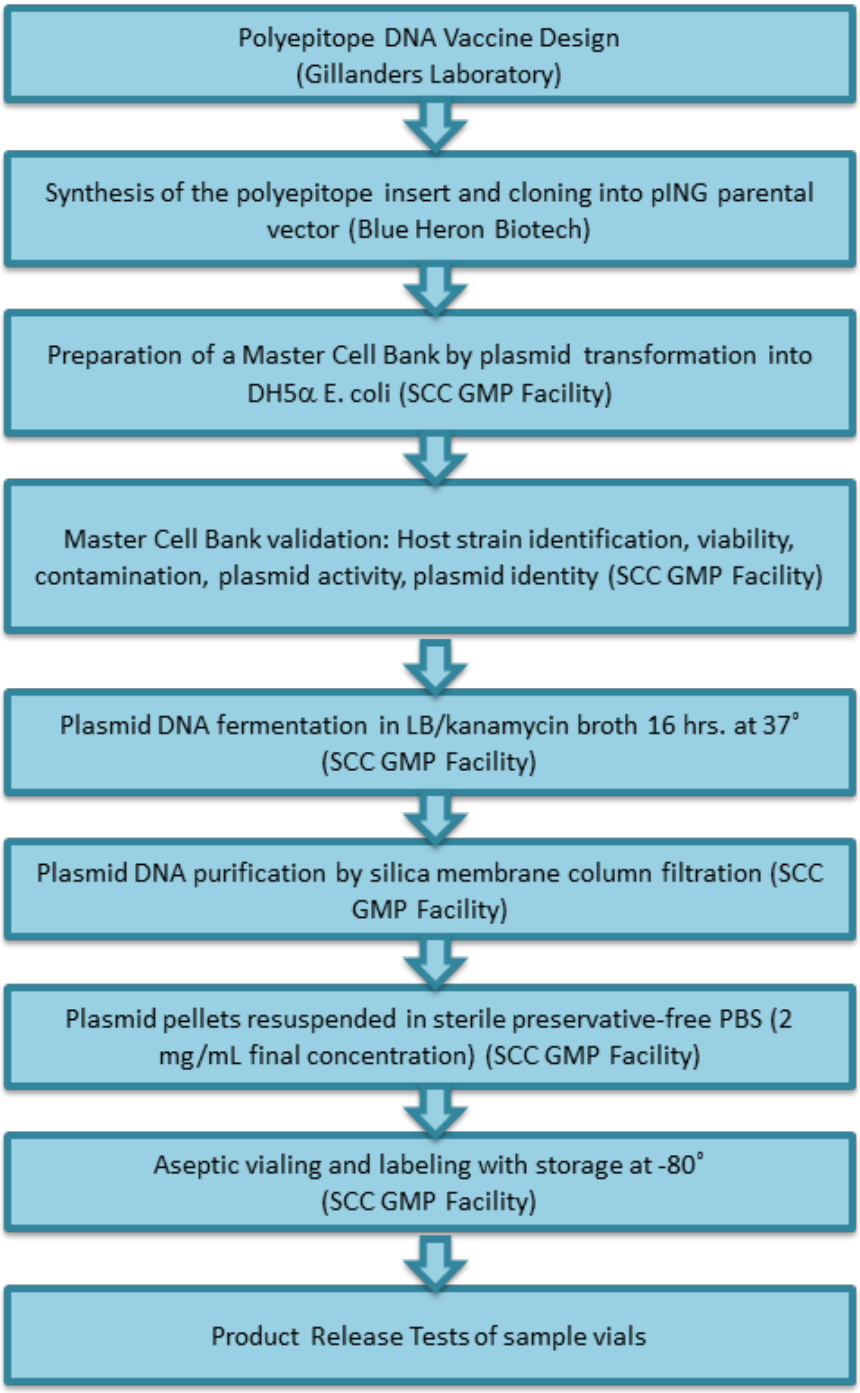


Figure 4: Overview of the polyepitope DNA vaccine manufacturing process. Schematic outline of product manufacture, in-process testing, and product release tests.

Design of the polyepitope DNA vaccine. Neoantigen DNA vaccines will be designed in the Gillanders laboratory at WUSM. Up to 20 candidate neoantigens and personalized mesothelin epitopes from each patient will be integrated into the polyepitope DNA vaccine. Neoantigens will be identified and prioritized based on the results of next-generation sequencing studies, epitope prediction algorithms, and *in vitro* studies.

Synthesis of the polyepitope insert. A polyepitope insert integrating up to 20 of the highest priority neoantigens and personalized mesothelin epitopes will be designed in the Gillanders laboratory. This insert will then be synthesized by Blue Heron Biotech. The insert will then be cloned into the pING parent vector by Blue Heron Biotech. The pING vector containing the polyepitope insert will be sequenced by Blue Heron Biotech. The plasmid will then be used to establish a master cell bank at WUSM as detailed below.

Creation of a master cell bank. Master cell banks for each individual polyepitope DNA vaccine will be constructed by transformation of *E. coli* strain DH5 alpha with each patient's unique pING-polyepitope plasmid. Briefly, 15 vials of transformed bacteria will be prepared and stored at -80°C in the Siteman Cancer Center Biologic Therapy Core Facility. The Johnson Controls monitoring system continuously monitor the freezer used for storage. The system triggers an alarm and electronically notifies staff when temperature values are out of specification. In addition, daily manual monitoring is applied. For each bacterial culture production, a vial of the glycerol stock will be taken from the master cell bank and used for culture inoculation.

Samples from each master cell bank will be characterized for host strain identity, contaminating organisms, viable cells, plasmid activity, and plasmid identity as detailed below in testing to be performed under GLP conditions at Barnes-Jewish Hospital and Washington University School of Medicine. Of note, in previous sequencing reactions we have identified three variations in pING vector sequence. These variations are in non-coding areas of the plasmid DNA, and are not expected to have any functional significance. They are also present in the pING parent vector obtained from MSKCC, suggesting that the published sequence for the pING vector may not be accurate. Please see Table 1 below.

TABLE 1: Master cell bank validation for polyepitope DNA vaccines

Criteria	Method	Specification	Test site
Host strain identity	Growth on selective media	Growth of *E. coli*	Barnes-Jewish Hospital Microbiology Laboratory
Contaminating organisms	Growth on selective media,	No growth	Barnes-Jewish Hospital Microbiology Laboratory
Viable cells	Growth in LB agar	Growth of *E. coli*	Barnes-Jewish Hospital Microbiology Laboratory
Plasmid activity	Transfect cells, RT-PCR	Detection of polyepitope construct mRNA	Gillanders Laboratory
Plasmid identity	DNA sequencing	Sequence as predicted	Gillanders Laboratory
Plasmid identity	Restriction analysis	As expected, no extraneous bands	Gillanders Laboratory

Plasmid DNA fermentation. The plasmid vectors will be grown in *E. Coli* strain DH5alpha. The host bacteria will be grown in 500 mL LB broth cultures in a bacterial shaker at 37°C for 16 hours. Antibiotic selection (kanamycin) will be applied to increase plasmid yield.

Plasmid DNA purification. The plasmid DNA will be purified using reagents obtained from Sigma Aldrich

(Saint Louis, MO). Distinct lots of the Genelute HP Select Plasmid Gigaprep Kit from Sigma-Aldrich (St. Louis, MO) will be used for the manufacture of the plasmids. The following narrative is a brief description of the protocol. The composition of buffers, solutions, and chromatography material is included in Table 2 below. (Please note that the precise composition of the resin and solutions are considered by Sigma Aldrich to be proprietary). The harvested bacteria will be recovered by centrifugation, and then resuspended in a cell suspension buffer containing RNase A. Lysis buffer will be added and a homogenous lysate will be obtained by gentle inversion. After neutralization of the bacterial lysate, a lysate clearing agent will be added and allowed to stand for 5 minutes for precipitates to form. The lysate will be cleared by filtration, and a binding solution optimized for endotoxin-free plasmid purification will be added. The lysate with binding solution will then run through an equilibrated column where the plasmid DNA is captured onto a silica membrane in the presence of high salt, while endotoxins are prevented from adsorbing to the membrane. The flow-through will be discarded, and the contaminants in the column will be removed in three wash steps. Finally, the bound plasmid DNA will be eluted in endotoxin-free water. The eluted DNA will be precipitated by adding 0.1 volume of 3 M sodium acetate and 0.7 volumes of isopropanol. The DNA will be recovered by centrifugation at 14,000 x g in a fixed angle rotor. A wash and pelleting of the final DNA with 70% ethanol will be performed and the resulting purified DNA will be air dried in a biosafety cabinet. All vessels and buffers used for DNA preparation will be sterile for single use, or will be autoclaved in a monitored and quality controlled autoclave in the Biological Therapy Core facility.

The only product of animal origin is the RNase solution. This is isolated from bovine pancreas and we have Certificates of Origin that clearly indicate that these were derived from a New Zealand herd. These animals passed ante and post mortem testing for viral pathogens; the samples were treated with low pH (1.7) for 12 hours to inactivate pathogenic viral agents; and no animal protein was fed to these animals.

Glycerol will be used in our manufacturing process. However, all Lot #s of the glycerol utilized will be of plant origin.

TABLE 2: Composition of buffers, solutions, and chromatography material	
Solution	Composition, Sigma #
Wash Solution 2	Tris-HCl (T3038), NaCl (S5150)
Neutralization Solution	Trizma-HCl (T5941)
Binding Column	Guanidine Thiocyanate (G9277), EDTA (E7889)
Column Preparation Solution	NaOH (S2770)
Resuspension/RNase A Solution	Tris-HCl (T3038), EDTA (E7889)
Lysis Solution	NaCl (S5150), Tris-HCl (T3038), EDTA (E7889), Ocytl-beta-Thioglucopyranoside (O6004)
Lysate Clearing Agent	Guanadine HCl (G3272), Tris-HCl (T2413), EDTA (E7889), Tween20 (P7949), Triton X-100 (T8787), Hydrochloric acid (H9892)
Binding Solution	Guanadine HCl (G3272), Tris Acetate (T1258), Isopropanol (I0398), NaOH (S2770)
Column Prep Solution	NaOH (S2770)
EndoCleaning Solution	NaOH (S2770), Tween20 (P7949)
Wash Solution 1	NaCl (S5150), Tris-HCl (T3038)
Wash Solution 2	Tris-HCl (T3038), NaCl (S5150)

5.7 Product formulation

The eluted plasmid preparations will be resuspended in sterile, preservative free-PBS. A small sample (100 µL) will be removed aseptically and the concentration tested using a certified NanoDrop spectrophotometer, Qubit Fluorometer or similar device. Typically, the eluted DNA is in the range of 3-4 mg/mL. The DNA product will be diluted to 2 mg/mL using sterile, preservative-free PBS, and aseptically added to 2.0 mL cryovials from Nalgene (Catalog# 5000-0020, Lot# 616412). The vials are made of polypropylene with a high-density polyethylene closure. They are externally-threaded for aseptic technique. They have a sealing ring in conjunction with specially designed threads. A white marking area, fill line, and graduations are printed on the vials with white paint. They are radiation-sterilized, non-cytotoxic, non-pyrogenic, and are RNase/DNase-free.

We plan to synthesize enough DNA for approximately 20 vials. We anticipate that 12 vials will be required for vaccination (2 vials per time point times 6 timepoints), and 5 vials will be required for product release tests. We are targeting 20 vials to have a reserve stock.

Following vialing, we anticipate that 4 vials (#1, 4, 10, 19) will be used for product release testing. Vials 1 and 19 will be used to test sterility and represent the beginning and end of the vialing procedure. Two additional vials will be used for the other product release tests listed in Table 5.

If any contamination would be detected in our plasmid samples, we would initiate an alcohol precipitation step. This procedure is basically the final stage of the gigaprep purification, where the soluble DNA is precipitated using 2x volumes of cold isopropanol and the DNA is pelleted by centrifugation. The pellet is

washed in 70% ethanol and air dried. This procedure is a standard method for eliminating any microbiologic contamination in DNA.

5.8 Product release tests

A key issue in the quality control of plasmid DNA for human use is the development of analytical methodologies capable of fully characterizing the product in its final form, ensuring the production of a consistent product. The following list of release criteria, analytical methodologies, and specifications has been adapted from [72]. Polyepitope DNA vaccines will be tested for the following specifications.

TABLE 3: Polyepitope DNA vaccine product release tests

Criteria	Method	Specification	Test Site
Plasmid activity	Transfect cells, RT-PCR	Polyepitope mRNA present	Gillanders Laboratory
Residual kanamycin	USP 29 <81>	< 8 mcg/mL	Alcami or equivalent
Plasmid homogeneity	Agarose gel electrophoresis	≥80% closed circular	Gillanders Laboratory
Residual RNA	Agarose gel electrophoresis	Less than 1%	Gillanders Laboratory
Residual bacterial chromosomal DNA	Agarose gel electrophoresis	Less than 1%	Gillanders Laboratory
Plasmid identity	Restriction analysis	As expected, no extraneous bands	Gillanders Laboratory
Appearance	Visual	Clear and colorless, free of particulates	Gillanders Laboratory
pH	Calibrated pH meter	Report pH	Biologic Therapy Core Facility
DNA concentration	Nano-drop spectrophotometer	Greater than 1 mg/mL	Gillanders Laboratory
Residual proteins	BCA reagent colorimetric	Less than 1% (< 21 µg/mL)	Gillanders Laboratory, Biologic Therapy Core Facility, or equivalent
Residual isopropanol, EtOH	USP <467>	Less than 5000 ppm	PACE Analytics or equivalent
Endotoxin	Chromogenic Limulus amebocyte lysate assay	Less than 175 EU/mL	Biologic Therapy Core Facility
Sterility	USP <71>	No growth	Biologic Therapy Core Facility

Alternatively, we will perform studies to demonstrate that the manufacturing process removes residual kanamycin, RNA, bacterial chromosomal DNA, proteins, isopropanol and EtOH below the specification levels, and not perform these residual studies on each lot. To date, testing on each lot has shown that residual kanamycin is undetectable and residual isopropanol and EtOH is consistently below the specification. This testing will no longer be performed. The criteria for sterility meets specification if no growth after 7 days. The sterility culture will be held and monitor for 14 days as per USP <71>.

5.9 Stability

The polyepitope DNA vaccines will be stored in the Biologic Therapy Core Facility in a -80°C freezer. The freezer used for storage is monitored 24/7 by Johnson Controls monitoring system with dedicated on-call personnel to address any aberrant occurrences. Overall, we anticipate no problems with plasmid stability as stability is considered to be one of the advantages of the DNA vaccine platform, and plasmid DNA formulated in PBS is considered to be very stable.

We are currently storing similar investigational DNA vaccines in the Biologic Therapy Core Facility in a -80°C freezer. These investigational DNA vaccines were manufactured in the Biologic Therapy Core Facility using an almost identical manufacturing process as the one proposed here, are based on the pING parental vector and are thus very similar in size and composition, and are formulated similarly in PBS at 2 mg/mL. These DNA vaccines have remained stable over a 3 year period.

We do not anticipate that long-term stability testing will be required. As each lot of vaccine is personalized to a specific patient, we anticipate that the vaccine will be used within 6 months of completion of the product release tests.

Vaccines to be administered at other participating institutions will be shipped on dry ice to the address provided by the institution.

5.10 Labels

Each polyepitope DNA vaccine product will be made as a single lot ensuring that the labeled product is unique and consistent for this trial. Please see the sample label below. Prior to administration a time out will be performed to confirm subject identity, and the investigational agent to be administered. This will be performed by the clinical research associate, the oncology nurse trained to administer the vaccine by electroporation and the experimental pharmacist.

SCC Biologic Therapy Core Facility
Saint Louis, MO
Store at -80°C
WUSM PPDV #1

Subject Study #, Subject Initials

LOT #	1
Vial #	003
Concentration	2mg/mL
Volume	1.2mL
Date	mm/dd/yyyy

CAUTION Investigational drug limited by federal law to investigational use

5.11 Investigational agent administration

Each DNA vaccination will be 1 mL vaccine administered intramuscularly using an integrated electroporation administration system (TDS-IM system, Ichor Medical Systems). At each vaccination time point, patients will receive two injections at separate sites.

5.11.1 Introduction to electroporation

Electroporation (EP) is a potent delivery technique based on the in vivo application of electrical fields that may improve immune responses following the administration of plasmid DNA vaccines. The EP effect is induced through the propagation of electrical fields of sufficient magnitude and duration within a target region of tissue. These electrical fields produce a transient increase in the membrane permeability of cells exposed to the electrical field, allowing enhanced intracellular uptake of agents distributed within the interstitium of the local tissue. Of note, agent delivery occurs only where the agent of interest is present contemporaneously with the propagation of threshold level electrical fields. Numerous non-clinical studies

have demonstrated that the application of EP can enhance gene expression and immune responses following plasmid DNA vaccine delivery.

Although DNA vaccines encoding antigens from a variety of cancers and infectious pathogens have exhibited considerable promise in non-clinical studies, clinical experience to date suggests that current administration methods may have insufficient potency to achieve the desired immune responses in humans. Poor delivery efficiency and low levels of antigen expression have been implicated as factors contributing to the sub-optimal responses observed to date [73, 74].

Electroporation (EP) is a potent physical delivery technique based on the *in vivo* application of electrical fields. The propagation of electrical fields of a sufficient magnitude within a target tissue induces a transient increase in cell membrane permeability, resulting in a significant improvement of intracellular uptake of exogenous substances. Shortly after pulse delivery, the cell membrane function stabilizes and cells within the affected tissue resume normal function. EP has been shown in non-clinical studies to be a potent method for delivery of DNA vaccines [47, 75-77]

EP has several important advantages that make it an appealing method for delivery of DNA vaccines. First, EP can reliably enhance delivery and expression of DNA antigens by 2-3 orders of magnitude. This improvement in delivery has been shown to enhance both cellular and humoral immune responses. Second, EP is a non-viral delivery method. As such, it has a favorable safety profile and does not elicit unwanted immune responses against the vector. Thus, the technique is particularly appropriate for indications likely to require multiple vaccine administrations to achieve response (e.g. prime / boost). It is also advantageous because the absence of immunogenic viral antigens minimizes potentially deleterious antigen competition, thereby ensuring that the immune response is concentrated on the antigen of interest. Lastly, unlike other non-viral approaches, EP is capable of inducing consistent responses from subject to subject, even at relatively low DNA doses.

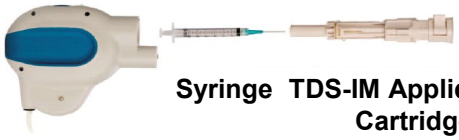
5.11.2 The TDS-IM Electrode Array System

The TDS-IM device is supplied by Ichor Medical Systems, Inc. The TDS-IM device utilizes the in vivo application of electrical fields to enhance the intracellular delivery of agents of interest in a targeted region of tissue (EP). The device complies with the applicable safety and electromagnetic compatibility requirements of International Electrotechnical Commission (IEC) 60601-1. During this study, the device will be operated according to the TDS-IM User Manual and applicable study-specific procedures to ensure consistent and safe utilization.

Each TDS device consists of 3 parts: A pulse stimulator, an integrated applicator, and a single-use application cartridge (see Figure below). The components are manufactured in ISO13485-compliant, FDA-registered facilities including BIT MedTech, LLC. (Pulse Stimulator) and Life Science Outsourcing, Inc. (Integrated Applicator and Application Cartridges.

TDS-IM Components

TDS Pulse Stimulator



Syringe **TDS-IM Application Cartridge**

TDS-IM Integrated Applicator

5.11.3 Summary of TDS-IM Clinical Experience to Date

To date, formal safety studies have been conducted to assess the TDS-IM as the means for delivery of nine DNA vaccine candidates. This includes studies of a melanoma DNA vaccine encoding a xenogeneic form of the tyrosinase antigen that uses the same vector backbone (pING) as the Mammaglobin-A DNA vaccine candidate (see Table 4 & Table 5). Safety studies for the various DNA vaccine candidates have included repeat dose studies in rabbits to assess safety and toxicology, and studies in rats to assess DNA vaccine biodistribution, persistence, and potential for integration into host DNA.

In the safety and toxicology studies, notable findings associated with electroporation-mediated delivery of DNA vaccines were limited to localized inflammatory responses of mild to moderate severity at the site of administration (for an example, see Dolter, K.E., *et al.* Immunogenicity, safety, biodistribution and persistence of ADVAX, a prophylactic DNA vaccine for HIV-1, delivered by in vivo electroporation. Vaccine, 2011. 29(4): p. 795-803). The injection site findings were most prominent in tissue samples obtained 48–72 hours after administration at that site. Tissue samples obtained from administration sites 14–43 days after administration indicate progressive resolution of the local inflammatory responses over time.

The results of the biodistribution studies indicated negligible systemic uptake of vaccine DNA following EP-based intramuscular delivery, with no significant differences observed among the different DNA vaccine candidates tested to date. Persistence analysis indicated that the presence of vaccine DNA 30 to 90 days after administration was confined to the tissues at the site of administration (muscle and skin), and only at very low levels (< 1,000 copies/µg host genomic DNA), suggesting minimal risk for potential integration of vaccine DNA into host DNA (for an example, see Dolter, 2011).

Reports describing the results of the tyrosinase GLP safety and toxicology studies are available in BB IND 13275; a letter of cross reference to this IND has been provided by Ichor.

5.11.4 Summary of TDS-IM clinical experience to date

The TDS-IM investigational device is currently being used as the means for DNA vaccine delivery in both the therapeutic and prophylactic setting. Six clinical trials of the TDS-IM device have been completed as detailed in the Table below.

TABLE 4: TDS-IM Based DNA Vaccine Administration: Completed Clinical Trials

ClinicalTrials.gov Study #	Vaccine Candidate	Subject Population
NCT00545987	Multigenic HIV-1 DNA vaccine candidate (ADVAX)	Healthy, HIV uninfected adult volunteers
NCT00471133	Xenogeneic tyrosinase DNA vaccine candidate (pINGmuTyr)	Patients with Stage IIB-IV melanoma
NCT01169077	Multi-epitope malaria DNA vaccine (EP-1300)	Healthy adult volunteers
NCT01502345	Multi-antigen hantavirus DNA vaccine (pWRG/HTN-M(x) and pWRG/PUUV-M(s2))	Healthy adult volunteers
NCT01641536	Multi-antigen HBV DNA vaccine administered with a DNA-based human IL-12 adjuvant (HB-110)	HBV infected adult volunteers
NCT01496989	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to or after administration of an adenovirus vector (Ad35GRIN)	Healthy, HIV uninfected adult volunteers

The TDS-IM device is currently being evaluated in seven ongoing clinical studies as detailed in the Table below.

TABLE 5: TDS-IM Based DNA Vaccine Administration: Ongoing Clinical Trials

ClinicalTrials.gov Study #	Vaccine Candidate	Subject Population
NCT01138410	Epitope-based TRP-2 melanoma vaccine (SCIB-1)	Patients that are HLA A1, A2, A24, or B35 positive with AJCC stage III-IV melanoma
NCT01859325	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to administration of an vesicular stomatitis virus vector (rVSVgag)	HIV infected adult volunteers

NCT01578889	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to administration of an vesicular stomatitis virus vector (rVSVgag)	Healthy adult volunteers
NCT01266616	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX)	HIV infected adult volunteers
NCT01634503	Multi-antigen HPV DNA vaccine administered with a DNA-based human IL-12 adjuvant (GX-188E)	Patients with Grade 3 Cervical Intraepithelial Neoplasia
NCT01493154	HPV DNA vaccine administered with a DNA-based calreticulin adjuvant (pNGVL-4a-CRT/E7 (detox))	HPV-associated squamous cell carcinoma of the head and neck
NCT01984983	VEEV DNA Vaccine Candidate	Healthy adult volunteers

To date, the 13 clinical trials that have been completed or are currently ongoing have enrolled over 350 subjects in the electroporation arms of the studies (including subjects receiving either the DNA vaccine candidate or placebo). The device has been used for administration of DNA injections of up to 1.0 ml volume and 4.0 mg DNA dose per injection site. Subjects have received the vaccine candidate either as a single injection in one muscle site (total DNA dose up to 4.0 mg per administration time point) or as 2 injections in 2 separate muscle sites (total DNA dose up to 8.0 mg per administration time point). Subjects given the DNA dose as a single injection have received up to 5 TDS-IM injections at up to 4.0 mg DNA, and subjects administered the DNA dose in 2 injections have received up to 5 administrations (i.e., 10 total TDS-IM injections).

Adverse responses reported in association with use of the device include localized muscle contractions and associated discomfort/pain during the application of EP, minor cutaneous bleeding at the site of injection, and transient injection site soreness of mild to moderate severity, typically resolving within 24–72 hours following administration. Several subjects in the 2 melanoma studies have reported lightheadedness immediately following procedure application which, in some cases, was accompanied by a decrease in blood pressure. In one subject, enrolled in the xenogeneic tyrosinase study, this was followed by a brief syncopal episode (~30 seconds duration) shortly after procedure application. The subject recovered without incident. At the time of enrollment, the subject indicated a life long history of sinus bradycardia of unknown origin, which was confirmed by electrocardiogram (EKG) during screening. Multiple EKGs performed after the syncopal episode indicated no changes from pre-procedure baseline. Based on the judgment of the investigator, the subject was withdrawn from the study and the study eligibility criteria were modified to exclude subjects with sinus bradycardia. No other serious or unanticipated adverse events attributed to the device or administration procedure have been observed.

The results of the completed HIV-1 ADVAX vaccine study in healthy subjects have been published (Vasan S *et al.* In vivo electroporation enhances the immunogenicity of an HIV-1 DNA vaccine candidate in healthy volunteers. PLoS One 2011;6(5):e19252). Briefly, results from this study indicate that EP-based delivery with the TDS-IM device at ADVAX DNA doses ranging from 0.2–4.0 mg was safe and effective in improving the magnitude, breadth and durability of cellular immune responses to a DNA vaccine candidate. Assessment of the tolerability of the EP procedure by questionnaire after each administration indicates that the procedure is generally acceptable for use in healthy subjects.

5.11.5 Preparation of the vaccine

Doses of the neoantigen DNA vaccines will be prepared in the Siteman Cancer Center Investigational Pharmacy/JHU equivalent pharmacy and delivered to the clinic on the day of vaccination. Vials will be thawed at room temperature on the day of study visits. After thawing the vaccine vial(s), the investigational pharmacist will mix the vial(s) completely by inverting the vial(s) at least 10 times (one inversion = one 180° turn of the wrist and back). The vaccine will then be withdrawn into 3.0 mL Becton Dickinson Model 309585 syringe under sterile conditions by the research pharmacist. For administration, a 22 gauge 1.5 inch injection needle will be affixed to the syringe. Once the dose is prepared, the research pharmacist will load the syringe into a TDS-IM Application Cartridge in a manner consistent with the instructions provided in the TDS-IM User's Guide.

5.11.6 Vaccine administration

Detailed instructions for procedure administration using the TDS-IM device are included in the TDS-IM User's Guide. Briefly, the TDS-IM Application Cartridge is loaded into the Integrated Applicator. The Pulse Stimulator is connected to an appropriate power source, turned on, and then connected to the Integrated Applicator through the supplied cable. Prior to administration, the skin at the site of administration is prepared according to the standard procedures for a conventional intramuscular injection. Once the skin has been disinfected, the skin around the injection site is held firmly while the Application Cartridge is placed against the injection site at a 90° angle. An indicator light on the device notifies the user that sufficient pressure has been applied. The activation button is then depressed, causing the electrodes and injection needle to be inserted into the target tissue. An automated safety check is performed, and, if passed, allows the injection of the study vaccine into the muscle. The procedure will conclude with a series of brief, localized muscle contractions at the administration site. The procedure will require approximately 7-10 seconds to complete, during which time the site is held firmly and with the device depressed against the skin. Once the indicator light on the device indicates that the procedure is complete, the device is withdrawn and site is covered with a sterile covering and pressure applied with 3 fingers for 1 minute.

5.12 Investigational agent accountability

5.12.1 Documentation

The investigational agent will be prepared by the study pharmacist at the Siteman Cancer Center Investigational Pharmacy/JHU equivalent pharmacy. The study pharmacist will be responsible for maintaining an accurate record of the codes, inventory, and an accountability record of vaccine supplies for this study. Electronic documentation as well as paper copies will be used.

5.12.2 Disposition

The empty vials and the unused portion of a vial will be discarded in a biohazard containment bag and incinerated or autoclaved. Any unopened vials that remain at the end of the study will be returned to the production facility or discarded at the discretion of the principal investigator in accordance with policies that apply to investigational agents. Partially used vials will not be administered to other subjects or used for *in vitro* experimental studies. They will be disposed of in accordance with institutional or pharmacy policy.

6 TREATMENT PLAN

6.1 Plasmid DNA administration

All subjects will be treated as outpatients in the Siteman Cancer Center/Sidney Kimmel Comprehensive Cancer Center.

Patients with pathologically verified R0/R1surgical resection and no evidence of recurrent and/or metastatic disease will be identified for tissue procurement and sequencing, and patients will be consented within the 16 weeks following surgical resection to allow time for vaccine creation. Patients will initiate gemcitabine/capecitabine, mFOLFIRINOX or comparable adjuvant chemotherapy no more than 12 weeks following resection. Adjuvant chemotherapy will be administered as per the NCCN recommendations for adjuvant chemotherapy following surgical resection. Additional chemoradiation, dose modifications, reductions, and supportive care will be performed at the discretion of the treating medical oncologist. After completion of adjuvant chemotherapy, patients will undergo repeat imaging by CT scan and/or MRI to evaluate for disease recurrence. Patients with no evidence of disease as determined by a board certified radiologist and without rising levels of tumor markers will be eligible for vaccination. If vaccine production is unable to be completed for a particular patient, that patient will discontinue participation in this study and will be replaced.

The schedule for vaccination will be weeks 1, 5, 9, 13, 17, and 21. All vaccines will occur within +/- 1 week with at least 3 weeks between vaccines. The first vaccine dose may be administered following confirmation of disease-free status. All study injections will be given intramuscularly using an integrated

electroporation device (TDS-IM system, Ichor Medical Systems) by a trained healthcare provider and per the manufacturer's instructions. At each vaccination time point, patients will receive two injections of the neoantigen DNA vaccine, one injection into each deltoid or lateralis. If both injections cannot be given on the same day, the patient will be asked to return to complete the second injection. Standard aseptic technique and precautions will be utilized in site preparation, vaccine administration, and medical waste disposal to ensure maximal safety of subjects and study personnel.

At the discretion of the treating physician, patients may be pre-medicated with lorazepam 1mg PO at least 30 minutes but no greater than 60 minutes prior to the first injection. Patients may also receive a second dose of lorazepam (1 mg PO) 10 minutes prior to injection. Patients may also receive acetaminophen 650mg PO at least 30 minutes but no greater than 60 minutes prior to the first injection.

The propagation of electroporation inducing electrical fields in the muscle will result in brief, localized muscle contractions at the site of administration, which are transiently painful. The neoantigen DNA vaccines will be administered by an experienced nurse who has completed a training seminar on the use of the TDS-IM device. The sites of immunization will be rotated for each of the immunizations.

Following study injections, subjects will be observed for a minimum of 30 minutes. Vital signs (temperature, blood pressure, pulse and respiratory rate) will be taken at 30-45 minutes post-immunization. The injection sites will be inspected for evidence of local reaction.

On each injection day (prior to injection), study subjects will be evaluated by clinical exam and laboratory tests. Post-vaccination follow-up visits are at Week 25 ± 7 days and Week 73 ± 14 days. Additional follow-up visits or telephone contact will be scheduled annually thereafter if the patient is alive and available for follow-up.

At intervals throughout the study (both before and after vaccination) subjects will have blood drawn for immunologic assays. Any cells, serum or plasma not used will be stored for future immunological assays.

Please see **Section 8 Study Calendar** for details on study visit procedures and monitoring.

6.2 Definitions of Evaluability

All enrolling patients are evaluable for the primary objective of feasibility, even patients whose tumor/normal exome sequencing is not completed or who are unable to have vaccine manufactured or administered.

A patient must have received at least one injection of vaccine in order to be evaluable for the primary objective of safety and the secondary objective (prevalence of antigen-specific T cells in peripheral blood).

6.3 Duration of therapy

In the absence of treatment delays due to adverse events, treatment may continue for 6 doses of neoantigen DNA pancreatic cancer vaccines. Under certain circumstances, a subject will be terminated from participating in further injections. Subjects who are discontinued from additional study injections will continue to be followed according to the schedule of safety and immunogenicity evaluations. Please see **Section 10 Removal of Patients from Protocol Therapy** for additional details.

6.4 Duration of follow up

Patients will examined at 4 and 52 weeks following the final vaccination (Weeks 25 and 73). Additional follow-up visits or telephone contact will be scheduled every year thereafter if the patient is alive and available for follow-up. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7 POTENTIAL TOXICITY AND DOSE MODIFICATIONS

7.1 Previous human experience

7.1.1 Experience with the pING parent vector

The neoantigen DNA vaccines have not been used in humans to date. Conventional DNA cancer vaccines based on the pING parent vector have been used extensively in phase 1 human clinical trials.

We have discussed this issue with Dr. Robert Jambou at the Office of Biotechnology Activities, National Institutes of Health. On January 12, 2009, we made a Freedom of Information Act (FOIA) request for copies of information on the study population, dosing regimen, and study results to include a detailed adverse events/safety information for six NIH OBA-registered clinical trials: 0005-394, 0105-474, 0105-474, 0303-573, 0312-617, 0412-684 and 0412-685. Dr. Jambou sent us the relevant clinical protocols, Appendix M documents, safety reports and annual reports.

In accordance with Appendix M-I-C-4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules posted April 1, 2002 in the Federal Register, investigators who have received authorization from the FDA to initiate a human gene transfer protocol must report in writing any unanticipated problems (serious adverse events or SAEs) to the NIH Office of Biotechnology Activities (OBA). These guidelines state that "Principal Investigators must submit, in accordance with Appendix M-I-C-4, a written report on any serious adverse event that is both unexpected and associated with the use of the gene transfer product. Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later 24 hours after the sponsor's initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information."

We have used this information to prepare Table 6 below. Of note, there was only one serious adverse event reported. One patient with metastatic prostate cancer developed urinary retention. It is not clear if this was related to the DNA vaccine. Review of the available annual reports confirms minimal toxicity associated with DNA vaccination in these trials. Over 90% of the toxicities related to treatment were grade 1 and included diarrhea, dizziness, edema, fatigue, injection site reaction, nausea, vomiting, and rigors.

Table 6: Clinical Experience with pING parent vector

Protocol number	Study population	Dosing regimen	Route of administration	Number and frequency	Safety
0005-394	Metastatic melanoma, 18 patients total	100, 500, or 1500 ug DNA	Intramuscularly using Biojector 2000	three vaccinations three weeks apart	No SAEs reported
0105-474	Prostate cancer, 36 patients	100, 500, or 1500 ug DNA	Intramuscularly using Biojector 2000	six vaccinations three weeks apart	One SAE reported, deemed to be unrelated to the vaccine
0303-573	Metastatic melanoma, 18 patients total	100, 500, or 1500 ug DNA	Intramuscularly using Biojector 2000	six vaccinations three weeks apart	No SAEs reported
0312-617	Renal Cell Carcinoma, 18 patients	500, 1500, or 3000 ug DNA	Intramuscularly using Biojector 2000	six vaccinations three weeks apart	No SAEs reported
0412-684	Breast cancer, 12 patients	500, 1000, 3000, or 6000 ug DNA	Intramuscularly using Biojector 2000	five vaccinations three weeks apart	No SAEs reported
0412-685	Metastatic melanoma, 18 patients	500, 2000, or 4000 ug DNA	Intramuscularly using Biojector 2000	six vaccinations three weeks apart	No SAEs reported

7.2 Potential toxicity

7.2.1 Potential toxicity related to the neoantigen DNA vaccine

The primary objective of this trial is to evaluate the safety of the neoantigen DNA vaccines. This is one of the first times that neoantigens identified by next generation sequencing have been targeted for immune therapy in humans. This is also the first time that neoantigen DNA vaccines have been administered to humans. However, clinical trials of similar investigational plasmid DNA vaccines suggest that these vaccines will be very safe. We expect that most of the toxicity to be limited to local grade 1 or 2 reactions at the vaccination site.

Please note that the risks detailed below are based on the risks of injections, the risks of vaccines in general, and the results of previous studies with investigational DNA vaccines.

Risks associated with intramuscular administration of a DNA plasmid include acute bleeding and/or bruising. Although highly unlikely, intramuscular injection can result in muscle damage, peripheral nerve damage, and/or injection site infection. Due to the insertion and activation of multiple electrodes, use of the TDS-IM electroporation device may increase these risks. The propagation of electroporation inducing electrical fields in the muscle will result in brief, localized muscle contractions at the site of administration, which are transiently painful. As with any immunization, discomfort or redness at the injection site in the days following DNA vaccine administration may be expected. Since intramuscular DNA delivery with electroporation results in increased intracellular uptake of plasmid at the site of injection and electric field application, the procedure may increase the frequency and/or severity of local site reactions compared to conventional intramuscular administration of DNA vaccines. Such symptoms should not last longer than several days.

Study subjects can receive medications such as acetaminophen, NSAIDs, or antihistamines as required. Steroids will not routinely be used in study subjects; if steroids are required the study subject will receive no further immunizations, but will continue to be monitored in follow-up visits. However, topical, inhaled, or locally injected steroids are acceptable on trial.

Subjects may exhibit general signs and symptoms associated with administration of a vaccine injection, including fever, chills, rash, aches and pains, nausea, headache, dizziness and fatigue. These side effects will be monitored, but are generally short term and do not require treatment.

The possibility of integration of the DNA plasmid vector into genomic DNA of transfected myocytes has been considered. Plasmid integration at a sufficiently high frequency carries the possibility of inducing deleterious mutations. Potential side effects could include an increased risk of malignancy arising from the cells harboring the mutation(s). However, current evidence from laboratory and animal studies indicates that the frequency of induced mutations following electroporation of DNA is conservatively estimated to be two to three orders of magnitude lower than that of naturally-occurring gene inactivating mutations in healthy humans.

The effect of this vaccine on a fetus or nursing baby is unknown, so female subjects of child bearing potential will be required to agree to use birth control for sexual intercourse beginning 21 days prior to enrollment and continuing through the last protocol visit. Women who are pregnant or nursing will be excluded from the study.

The potential discomforts of this study include having blood drawn, intramuscular injection of the vaccine, and possible reactions to the vaccine. Drawing blood causes transient discomfort and may cause fainting. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure for several minutes. Intramuscular injection also causes transient discomfort. Infection at the site of blood drawing or vaccination is extremely unlikely as alcohol swabbing and sterile equipment will be used.

The use of plasmid DNA has the potential to cause an allergic reaction due to the presence of bacterial endotoxin. However, each lot of DNA will be tested for endotoxin to ensure that endotoxin content does not exceed USP specifications. Antibodies to DNA may potentially develop in DNA vaccine recipients. However, the development of such antibodies in response to DNA vaccination is rare and has not been associated with disease in animals or humans to date.

7.2.2 Potential toxicity related to the TDS-IM device

The following are anticipated adverse reactions based on the previous clinical studies of TDS-IM based DNA vaccine delivery. All known risks and precautions described are explained in detail in the informed consent.

Local Reactions

Local site reactions associated with the use of the TDS-IM device in humans include acute pain associated with the localized muscle contractions during the application of electroporation in virtually all subjects. Mild, transient bleeding at the sites of electrode/needle penetration is commonly observed following removal of the device. Soreness, erythema, and/or induration of mild to moderate severity are commonly reported at the administration site. In one instance, local site soreness transiently graded as severe was reported in association with a device technical error related to needle deployment wherein the procedure was suspended prior to the application of electroporation. Other injection site findings of mild severity including mild bruising, hematoma, and paresthenia have been reported occasionally. All injection site reactions have typically resolved within 24–72 hours following administration, but, in rare instances, mild local tenderness has been reported to persist for up to one week.

For the TDS-IM device there is a theoretical risk that excessive energy could be delivered to the local tissues of the subject, resulting in more pronounced local reactions than have been observed to date. However, the TDS Pulse Stimulator incorporates multiple redundant mitigations to prevent this hazard, including performance of a pre-pulse safety check and the use of multiple circuits that monitor total energy delivered and will which will terminate energy delivery. There have been no reported occurrences of excessive energy delivery in any of the nonclinical and clinical studies conducted with the pulse stimulator to date.

Systemic Reactions

Systemic adverse events reported during the studies utilizing the TDS-IM and judged to be possibly related to the study product and/or delivery device have been generally mild to moderate in severity and include flu-like symptoms, headache, fever, dizziness, malaise, fatigue, arthralgia, myalgia, and aphthous stomatitis. Transient elevation in serum creatine phosphokinase of mild to moderate severity and judged to be associated with procedure administration have been reported in a small minority of subjects. Two subjects have reported the onset of severe fatigue within 24 hours of dosing that was resolved by the following day and was not observed following subsequent doses.

In approximately 1% of procedure administrations, one or more symptoms consistent with a vasovagal reaction (i.e., dizziness / lightheadedness, hypotension, diaphoresis, and/or skin pallor) have been observed immediately after procedure application. In rare instances, these reactions have progressed to brief syncope, particularly in subjects with pre-existing risk factors for syncope (e.g., pronounced sinus bradycardia). In all cases, the subjects have rapidly recovered from the syncope without incident.

A theoretical risk associated with the use of DNA vaccines is the possibility of uptake of the vaccine candidate into non-target tissues and/or integration of the vaccine DNA into the genomic DNA of the test subject. As described in Section 7.3 below, nonclinical studies have been performed to assess the potential for systemic biodistribution and genomic integration following TDS based delivery multiple DNA vaccine candidates, including a tyrosinase melanoma DNA vaccine candidate utilizing the same vector backbone (pING) as the Mammaglobin-A DNA vaccine candidate. To date, there has been no evidence of significant systemic uptake or genomic integration events following TDS based DNA vaccine administration.

7.3 Toxicity monitoring and management

Toxicity will be characterized according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE). Subjects who are immunized with the plasmid DNA vaccine will be evaluated at the time of each vaccination on weeks 1, 5, 9, 13, 17, and 21. Follow up on subject well-being will be performed by telephone on the first, second, or third day after each vaccination. All information will be recorded on case report forms. Adverse events will be reported to the Quality Assurance and Safety Monitoring Committee of the Siteman Cancer Center/Sidney Kimmel Comprehensive Cancer Center, the Institutional Review Board, the Institutional Biosafety Committee, the Office of Biotechnology Activities and the Food and Drug Administration as detailed in **Section 11 Adverse Event Reporting**.

Significant local inflammation will be treated with cold packs and oral analgesics as indicated. Skin ulceration at the vaccine site will be treated with local wound care and antibiotics as indicated. Development of signs and/or symptoms of autoimmune involvement will be initially treated conservatively with analgesics. More aggressive intervention (systemic corticosteroids) will be used as necessary and will result in termination of future vaccine inoculation.

7.4 Dose modifications

No dose modifications are planned. If a subject develops an adverse event that is classified as possibly, probably, or definitely associated with protocol therapy, this may result in removal of the subject from protocol therapy as outlined in **Section 10 Removal of Subjects from Protocol Therapy**. Protocol Stopping Criteria are outlined in **Section 12 Data and Safety Monitoring**.

8 STUDY CALENDAR

The window for Step 0 procedures is up to 28 days prior to enrollment. Enrollment must occur within 28 days of the patient starting on adjuvant chemotherapy. The window for Step 1 screening procedures is 14 days prior to first vaccine dose, with the exception of Immune monitoring labs and pathology review, which should be performed immediately after informed consent. Informed consent should be obtained within 16 weeks of surgical resection, but there is no window for informed consent prior to study enrollment in order to allow time for vaccine creation.

Skinfold measurements must be performed at site of vaccine injections (deltoids and thighs) during Step 0 screening, Step 1 screening, and prior to each vaccine dose.

	Enrollment	Eligibility Re-Check	Wk 1[f]	Wk 5[f]	Wk 9[f]	Wk 13[f]	Wk 17[f]	Wk 21[f]	Wk 25[f]	Wk 73[h]
Neoantigen DNA vaccine[a,i]			X	X	X	X	X	X		
Informed consent	X									
Demographics	X									
Medical history	X									
Physical exam	X	X	X	X	X	X	X	X	X	X
Concurrent Meds	X ---------- X									
Vital signs	X	X	X	X	X	X	X	X	X	X
Height	X									
Weight	X	X	X	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X	X	X	
CBC w/diff, plts	X	X		X	X	X	X	X	X	
CMP[b]	X	X		X	X	X	X	X	X	
INR	X	X								
EKG	X	X								
Radiologic evaluation	Radiologic measurements will be performed as clinically indicated. Documentation of tumor recurrence or metastatic disease must be provided for patients removed from study for progressive disease.									
Adverse event evaluation			X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X	
B-HCG	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]		
Immune monitoring[c]	X		X	X	X	X	X	X	X	X
Pathology Review[d]	X									

a: Personalized polyepitope pancreatic cancer DNA vaccine

b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

c: Immune monitoring labs to consist of 7 10mL green top heparin tubes for isolation of PBMCs. During adjuvant therapy, these should occur at a clinic visit associated with the restaging scan, as well as at each follow up visit. This will yield approximately 50-200 x106 PBMCs per collection.

d: Pathology review to confirm diagnosis of adenocarcinoma and evaluate tumor cellularity.

e: Serum or urine pregnancy test (women of childbearing potential).

f: +/- 7 days with at least 21 days between vaccinations

g: Adverse event evaluation on day of vaccine and by telephone contact on the first, second, or third day after vaccine.

h: ± 14 days; follow-up or telephone contact at week 73 and annually thereafter if the patient is alive and available for follow-up

i: patients may discontinue participation if vaccine production is unable to be completed

9 CRITERIA FOR RESPONSE

9.1 Primary objective: safety

Assessment of plasmid DNA safety will include both clinical observation and laboratory evaluation. Safety will be closely monitored after injection with eight or more clinical and laboratory assessments in the first 24 weeks of the trial. The following parameters will be assessed following vaccination:

(1) Local signs and symptoms

(2) Systemic signs and symptoms

(3) Laboratory evaluations, including blood counts and serum chemistries

(4) Adverse, and serious adverse events

Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0.

9.2 Secondary and exploratory objectives: evaluation of the immune response

9.2.1 Introduction to immune monitoring

The secondary endpoint is to evaluate the immunogenicity of the neoantigen DNA vaccine. Immunogenicity will be measured by ELISPOT analysis, a surrogate for CD4 and CD8 T cell function. Additionally, an exploratory endpoint is to evaluate the immunogenicity of the neoantigen DNA vaccine as measured by multiparametric flow cytometry. In both assays the quantity and quality of antigen-specific T cells is determined; the ELISPOT analysis is based on measuring the frequencies of IFN-γ producing T cells in response to polyepitope antigen, whereas the multiparametric flow cytometry assesses phenotypic as well as functional characteristics of epitope-specific T cells. In the proposed study, blood samples will be collected at multiple time points (n=8) and PBMC isolated and cryopreserved. Upon completion of the vaccination protocol, all samples will be analyzed simultaneously in order to minimize assay-to-assay variation.

9.2.2 Sample collection and processing

During Step 0 screening, tumor samples will be reviewed by a pathologist with expertise in pancreatic cancer who will review the operative specimen slides to verify tumor cellularity and quantity sufficient for proceeding. Once tissue availability is confirmed at the site, specimens from outside Washington University will be shipped to the Gillanders lab at the following address: Gillanders Lab, 425 S. Euclid Ave. CSRB 3344, St. Louis, MO 63110. The corresponding FFPE blocks will be punched with a disposable 1mm biopsy punch. 6-8 full thickness punches will be taken from areas of high tumor cellularity and divided evenly into two DNA LoBind Eppendorf tubes (one tube for DNA and one for RNA). To identify somatic mutations, DNA and RNA will be extracted from the FFPE preserved tissue by the Center for Human Immunology and Immunotherapy Programs (CHiiPS) core. All tissue selected for sequencing will be processed into a single-cell suspension by mechanical and enzymatic digestion, and used to extract nucleic acids. Tumor DNA + RNA will then undergo tumor exome and tumor cDNA-capture sequencing, respectively at the Genome Institute. Normal genomic DNA will be isolated from PBMCs by the CHiiPS core or Gillanders laboratory personnel for normal exome sequencing at The Genome Institute.

Patients will undergo their first blood draw during Step 0 screening to establish a baseline for immune monitoring and for creation of the vaccine. This first blood draw will be shipped ambient same day of draw for overnight delivery to the Gillanders lab at the following address: Gillanders Lab, 425 S. Euclid Ave. CSRB 3344, St. Louis, MO 63110. Additional optional pre-vaccine blood samples will be obtained midway through chemotherapy and at the end of chemotherapy coinciding as closely as possible to the restaging scans, and also shipped overnight same day of draw to the Gillanders lab. Immune monitoring labs will be draw prior to vaccination on weeks 1, 5, 9, 13, 17, and 21. Post- vaccine immune monitoring labs will be drawn on weeks 25, and 73. Seven collection tubes (BD Vacutainer® sodium heparin (green top), REF 367874, 10 mL each for a total of approximately 60 mL) are filled by venipuncture at each time point. Blood samples will be transported to Dr. Gillanders'/JHU equivalent laboratory (6th floor Clinical Sciences

Research Building, room 6664) within one hour of collection. PBMC will be obtained by Ficoll-Hypaque gradient centrifugation and cryopreserved in 10% DMSO according to standard procedures. Blood will be batch shipped after Week 9 collection and Week 21 collection to the Gillanders lab at the address listed above. Exome sequencing of PBMC will be performed to obtain germline sequences.

9.2.3 ELISPOT Analysis (Secondary Objective)

ELISPOT analysis, will be performed as previously described [78-82]. PBMC from subjects in the phase 1b clinical trial will be tested for secretion of IFN-γ by ELISPOT assay. PBMC will be plated at various concentrations starting at 300,000 cells per well, in triplicate, following the protocol previously described by Dr. Mohanakumar and colleagues. PBMC will be co-cultured with the mutant and mesothelin peptides encoded by the polyepitope construct. As negative controls, PBMC will be incubated in medium alone or stimulated with matching wild type peptides. As a positive control we will include a mix of viral peptides, CEF, containing immunodominant epitopes for multiple common HLA alleles from influenza virus, cytomegalovirus, and Epstein-Barr virus. After 24-48 hours, the plates will be developed and the spots counted in an ImmunoSpot Series I analyzer (Cellular Technology).

9.2.4 Multi-parametric flow cytometry (Exploratory Objective)

Patient-derived PBMC will be used for functional assays to characterize immunity to personalized pancreatic cancer DNA vaccines. Polyfunctional CD8 T cell responses will be determined after stimulation of cultured PBMC with polyepitope pulsed autologous PBMC using muti-parameter flow cytometry. Fluorescently labeled MHC class I tetramers expressing vaccine-encoded peptides will be used to gate on peptide-specific T cells. The choice of tetramers to be used in these analyses will be dictated by the neoantigens and mesothelin antigens used for a given patient's personalized vaccine, and the patient's HLA phenotype. We will also simultaneously stain tetramer-positive and tetramer-negative CD8 T cells for a variety of well-accepted markers that report the functional status of antigen specific CD8 T cells. These include the cell surface markers PD-1, CTLA-4, LAG-3, and TIM-3 (markers of T cell inactivation), the proliferation marker Ki-67, and the cellular activation markers ICOS, granzyme B, TNF-α and IFN-γ. We have already validated all of our staining reagents and demonstrated that specific combinations of mAb labeled with different fluorescent tags can be used together allowing for multi-color analysis in a single run. Analyses will be performed using either a BD Fortessa (6-color analysis) or BD LSR II cytometers (11-color analysis). A positive effect of immunotherapy is expected to show increased numbers of tetramer-positive cells that also display decreased expression of PD-1, CTLA-4, LAG3, and TIM3, and increased staining for Ki-67, ICOS, granzyme B, TNF-β and IFN-γ.

PBMC aliquots ($2x10^6$ cells/mL) will be stimulated with individual peptides encoded by the polyepitope vaccine and cultured in the presence of IL-2 (50U/mL). Ten days after activation, cells will be harvested, washed and restimulated with irradiated (10,000 rads) peptide-pulsed autologous PBMC for 16h (in the presence of Brefeldin A) and stained with antibodies for the various markers listed above. Cells will be analyzed by the Immune Monitoring Core, also known as the Center for Human Immunology and Immunotherapy Programs (CHiiPs), headed by Dr. Robert Schreiber. Cultured cells stimulated with unpulsed PBMC will be used as negative control, and cells stimulated with CEF-pulsed PBMC will be used as positive controls using an appropriate tetramer. One advantage of using 10 day activated/antigen-driven PBMC to measure polyfunctional responses is the expected relative high frequency of polyepitope-specific T cells providing a larger sample size for statistically significant data analysis.

9.3 Additional Exploratory objectives

We will also monitor the function and phenotype of antigen-specific T cells using time-of-flight mass spectrometry (CyTOF). The CyTOF is a next-generation technology based on inductively coupled plasma mass spectrometry (ICP-MS) that employs antibodies or peptide-MHC tetramers labeled with heavy metal isotopes instead of fluorophores to identify cell associated proteins of interest [83, 84]. This instrument permits investigators to probe up to 49 parameters simultaneously on individual cells thus allowing for cellular phenotyping at unprecedented depth. In addition, it facilitates the effective analysis of lymphocytes whose numbers may be limited such as those derived from human cancer patients. The Washington University Center for Human Immunology and Immunotherapy Programs has now obtained

and installed two CyTOF2 instruments that are being used successfully under the guidance of Dr. Stephen Oh, Assistant Professor of Medicine and Director of Mass Cytometry for CHiiPs.

Clinical responses and time to disease progression will be evaluated with physical examination and diagnostic imaging as clinically indicated.

10 REMOVAL OF SUBJECTS FROM PROTOCOL THERAPY

10.1 Removal of subjects from protocol therapy

Subjects may be removed from protocol therapy if any one or more of the following events occur:

(1) Development of recurrent or metastatic disease prior to the initial vaccination will result in stopping vaccine development at its current stage;

(2) Development of recurrent or metastatic disease during the vaccination period will result in stopping further vaccinations

(3) Intercurrent illness that prevents further administration of protocol therapy;

(4) Pregnancy;

(5) Type 1 hypersensitivity reaction associated with protocol therapy;

(6) Grade 2 systemic or injection site adverse event classified as possibly, probably, or definitely associated with protocol therapy that does not resolve to at least grade 1 prior to the next scheduled treatment;

(7) Grade 3 or 4 systemic or injection site adverse event classified as possibly, probably, or definitely associated with protocol therapy;

(8) Any significant autoimmune disease or phenomena presumed to be related to protocol therapy;

(9) Unable to complete vaccine production;

(10) Subject refusal to continue protocol therapy and/or observations;

(11) Significant protocol violation or noncompliance, either on the part of the subject or investigator(s);

(12) The principal investigator or study sponsor believes it is in the subject's best interest to discontinue participation in the study;

(13) Administrative reasons, e.g., study termination by the principal investigator, Siteman Cancer Center/Sidney Kimmel Comprehensive Cancer Center, HRPO, FDA, or other group.

Please note that even if a subject is removed from protocol therapy, they will continue to be followed for adverse events.

10.2 Voluntary subject withdrawal

The subject has the right to voluntarily withdraw from the study at any time for any reason without prejudice to her future medical care by the physician or at the institution.

For any subject who withdraws consent, the date and reason for consent withdrawal should be documented. Subject data will be included in the analysis up to the date of the consent withdrawal.

10.3 Procedure for discontinuation

The procedure to be followed at the time a subject either discontinues participation or is removed from the study is:

(1) Check for the development of adverse events.

(2) Complete the End-of-Study form and include an explanation of why the subject is withdrawing or withdrawn.

(3) Attempt to perform follow-up evaluations as outlined above.

11 ADVERSE EVENT REPORTING

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix A for definitions and Appendix B for a grid of reporting timelines.

Adverse events will be tracked from start of treatment through 30 days following the last day of study treatment. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF

Refer to the data submission schedule in Section 14 for instructions on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in section 11.1. Reporting requirements for secondary site study teams participating in Washington University-coordinated research may be found in Section 1.2.

11.1 Sponsor-Investigator Reporting Requirements

11.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

11.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Washington University Sponsor Investigator (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or BJH or SLCH institution that have been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC).

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

For events that occur at secondary sites, the Washington University Sponsor Investigator (or designee) is required to notify the QASMC within 10 days of Washington University notification via email to qasmc@wustl.edu. Submission to QASMC must include either the myIRB form and supporting documentation or (if not submitted to myIRB) the date of occurrence, description of the event, whether the event is described in the currently IRB approved materials, the event outcome, determination of relatedness, whether currently enrolled participants will be notified, and whether the informed consent document and/or any study procedures will be modified as a result of this event.

11.1.3 Reporting to Ichor Medical Systems

Since the TDS-IM device may be involved with the occurrence of adverse events, Ichor Medical Systems will be included in the Adverse Event reporting plan. Reports to Ichor for SAEs or for adverse events with possible relationship to the device should follow the timing for reporting to the WUSM Human Research Protection Office and should be directed to Drew Hannaman at dhannaman@ichorms.com. The Washington University PI will be responsible for submitting all MedWatch forms from secondary sites to Ichor Medical Systems.

11.1.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix A for definitions) no later than 7 **calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix A) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix A) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as
    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
    - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
    - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within 15 calendar days after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents (“IND Safety Report”) and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such (“Follow-up IND Safety Report”).

11.1.5 Reporting to the Institutional Biosafety Committee

In accordance with institutional policies and NIH guidelines, any unanticipated problems must be reported to the Institutional Biological and Chemical Safety Committee (IBC) at Washington University School of Medicine.

The Washington University Sponsor-Investigator (or designee) must report the following events to the Biosafety Officer at the time of submission to HRPO:

- Any overt personnel exposure to recombinant DNA-containing material, whether or not that exposure leads to illness
- Any significant spill of recombinant DNA-containing material outside of a biological safety cabinet, where a significant spill is:
    - A spill of recombinant risk group 1 agent-containing material which requires remediation by EH&S or other first responders
    - A spill of recombinant risk group 2 agent-containing material which is greater than 1 liter
    - Any size spill of risk group 3 agent-containing material or material the IBC has mandated to be handled using BSL2+ practices and procedures
    - Any incident which results in the release of recombinant DNA to the environment

In addition, the IBC must be informed of the following events:

- Any serious adverse event which is both unexpected and associated with the use of the gene transfer product

The Biosafety Officer may be contacted at ehsibc@wustl.edu.

When submitting reports to the IBC, the FDA MedWatch form will be used. The Washington University Sponsor-Investigator (or designee) will be responsible for submitting all MedWatch forms from secondary sites to the IBC.

WUSM IBC guidelines specify that any study modifications related to the investigational agent as well as the IRB renewal paperwork should be sent to the IBC for approval (if a modification) or acknowledgment (if an annual renewal). The IRB and IBC will review all submissions simultaneously.

11.1.6 Reporting to Secondary Sites

The Washington University Sponsor-Investigator (or designee) will notify the research team at each secondary site of all unanticipated problems involving risks to participants or others that have occurred at other sites within 10 working days of the occurrence of the event or notification of the Sponsor-Investigator (or designee) of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable. Refer to Section 16.0 (Multicenter Management) for more information.

11.2 Secondary Site Reporting Requirements

The research team at each secondary site is required to promptly notify the Washington University Sponsor-Investigator and designee of all serious adverse events (refer to Appendix A, Section D) within 1 working day of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using FDA Form 3500a (MedWatch) and Washington University's cover sheet (Appendix C). A formal written report must be sent to the Washington University Sponsor-Investigator and designee within 4 calendar days (for fatal or life-threatening suspected adverse reactions) or 11 calendar days (for serious unexpected suspected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA, IBC, and Ichor as needed.

Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

11.3 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 11.1 include:

- planned hospitalizations
- hospitalizations <24 hours
- respite care
- events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

12 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data. A DSMB will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. DSMB members must be employed by Washington University, Barnes-Jewish Hospital, or St. Louis Children's Hospital. Like investigators, DSMB members are subject to the Washington School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMB must also be disclosed.

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after study activation at Washington University (if at least one patient has been enrolled) or one year after study activation (if no patients have been enrolled at the six-month mark).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the QASM Committee. The DSMB must meet at least every 6 months beginning after study activation at Washington University, beginning six months after enrollment of the first patient at a secondary site, no more than one month prior to the due date of the DSM report to QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective

- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines (please refer to Section 11).

Refer to the Washington University Quality Assurance and Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC. This is located on the QASMC website at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

12.1 NIH Recombinant DNA Advisory Committee

12.1.1 Initiation of the clinical investigation

Appendix M-I-C-1 of the NIH Guidelines for Research Involving Recombinant DNA Molecules specify:

> *No later than 20 working days after enrollment (see definition of enrollment in Section I-E-7) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the FDA Investigational New Drug Application (IND) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.*

12.1.2 Annual reports

Appendix M-I-C-3 of the NIH Guidelines for Research Involving Recombinant DNA Molecules specify:

> *Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.*
>
> *(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.*
>
> *(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or*

considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

13 STATISTICAL CONSIDERATIONS

13.1 Experimental Design

This is a phase 1, open-label, trial of a neoantigen DNA vaccine strategy. Twenty patients who have had an R0/R1 surgical resection, and who have completed adjuvant chemotherapy will be enrolled. At our institution, pancreatic cancer patients undergo routine staging during (three months) and after adjuvant chemotherapy. If there is no evidence of disease, patients will be eligible for enrollment. After enrollment, exome sequencing of tumor/normal DNA and cDNA-capture sequencing of the tumor will be performed to identify somatic mutations present in the tumor and confirm expression of these mutations at the mRNA level. Epitope prediction algorithms will then be used to prioritize neoantigens for inclusion in neoantigen DNA vaccines, and the neoantigen DNA vaccines will be designed and manufactured. The neoantigen DNA vaccines will be administered following completion of adjuvant chemotherapy. The neoantigen DNA vaccines will be administered at six time points, weeks 1, 5, 9, 13, 17, and 21.

13.1.1 Sample size and accrual

Development of cancer vaccines is currently an area of intense research interest. The traditional paradigm for phase 1 clinical trials has been heavily influenced by phase 1 clinical trials of chemotherapeutic agents, where dose escalation designs are appropriate given the rather narrow dose versus safety concerns of these agents. However, there are major differences between these two classes of therapeutics that have important implications for early clinical development. Specifically, the phase 1 concept of dose escalation to find a maximum-tolerated dose does not apply to most therapeutic cancer vaccines. Most therapeutic cancer vaccines are associated with minimal toxicity at a range that is feasible to manufacture or administer, and there is little reason to believe that the maximum-tolerated dose is the most effective dose [52]. Consistent with recent recommendations published in the statistical literature, the general philosophy of this phase I clinical trial is to facilitate a prompt preliminary evaluation of the safety and immunogenicity of a personalized pancreatic cancer DNA vaccine strategy by testing a fixed dose of vaccine, instead of performing a dose escalation [52]. The sample size (n=15) is chosen to provide a reasonable ability to detect serious adverse events associated with vaccine administration. Based on extensive simulations regarding the sample size for pilot or translational studies, Piantadosi [83] recommended that a sample size of 10 to 20 patients would provide a reasonable precision for estimating preliminary information. We therefor expect that, as detailed below, the proposed sample size for this study will be adequate in estimating both safety and preliminary immune efficacy data. The sample size of n=15 will consist of EVALUABLE patients. Patients who screen fail due to sequencing issues such as poor sample quality will be replaced.

13.1.2 Sample Size Calculations for Safety

The primary objective of this study is to evaluate the safety of the neoantigen DNA vaccines. We have chosen to power the study to provide a reasonable ability to detect serious adverse events associated with vaccine administration.

Sample size calculations for safety are expressed in terms of the ability to detect serious adverse events. The ability of the study to identify serious adverse events is best expressed by the maximum true rate of events that would be unlikely to be observed and the minimum true rate of events that would very likely be observed. Table below shows the probabilities of observing 0, or 2 or more serious adverse events given variety of hypothesized "true" AE rates. If the true rate is at least 15%, for example, there is >90% chance of observing at least 1 serious adverse event among a sample size of n=15 evaluable patients.

Conversely, there is only 10% chance that we would observe 2 or more serious adverse event among n=15 evaluable patients if the true rate is less than 3.5%. Although we believe that the true event rate is likely to be quite low, this table presents a range of event rates in an attempt to illustrate the sensitivity of this study to identify potential safety problems with the neoantigen DNA vaccines.

Probability of detecting SAE for different safety scenarios

True Event rate	Pr(0/15)	Pr(2+/15)
0.010	0.860	0.010
0.035	0.586	0.100
0.050	0.463	0.171
0.100	0.206	0.451
0.150	0.087	0.681

13.1.3 Sample Size Calculations for Immunogenicity

The secondary objective and primary scientific endpoint is to assess the prevalence of antigen-specific T cells in the peripheral blood of patients pre- and post-vaccination as measured by ELISPOT.. A power analysis was performed using paired t-test for over time differences (e.g., changes in ELISPOT). Assuming a moderate correlation between measures taken from the same individual, the designed sample size (n=15 evaluable patients) allows us 80% power at 1-sided 0.05 alpha level to detect a minimum increase of 0.68*SD, where SD represents the standard deviation of measures for immunogenicity. Our preliminary data showed that antigen-specific T cells can be measured with a coefficient of variability (CV=SD/Mean) of approximately 20%. Therefore, if assuming similar variability in the proposed study, the designed sample size provides 80% power to detect a minimal of 15% change in the average antigen-specific T cells. We expect that more power could be achieved because multiple measurements will be taken from the same patient and this allows borrowing information across different time points.

13.2 Data Analysis

As a phase I trial to evaluate the preliminary data on the safety and immunogenicity of a neoantigen DNA vaccine strategy, the data analysis for this study is descriptive in nature. Demographic and clinical characteristics of the sample, toxicity by grade, as well as response and time to toxicity will be listed and summarized using descriptive statistics.

13.2.1 Primary objective: safety analysis

Toxicity evaluation is the primary objective of this trial. The data will be descriptive, and standard toxicity definitions and criteria will be used as outlined in the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Since enrollment will be concurrent with the first dose of the personalized pancreatic cancer DNA vaccines, all subjects will have received at least one vaccination, and all subjects will provide at least some safety data.

The number and percentage of subjects experiencing each type of adverse event will be tabulated by severity, and relationship to treatment. If appropriate, confidence intervals will be used to characterize the precision of the estimate. A complete listing of adverse events will also be tabulated, and will provide details including severity, relationship to treatment, onset, duration, and outcome.

Laboratory data measured on a continuous scale will be characterized by summary statistics (mean and standard deviation). Boxplots of laboratory data will be generated for baseline values and for values

measured during and after protocol therapy at each specific time point. Each boxplot will show the median, 1st and 3rd quartiles. Outliers will be individually plotted in a separate graph, as appropriate.

13.2.2 Secondary and Exploratory objectives: immune response as measured by ELISPOT analysis and multiparametric flow cytometry

Immune response as measured by ELISPOT analysis is the secondary objective of this trial. Immune response as measured by multiparametic flow cytometry is an exploratory objective of this trial. The frequency of antigen-specific T cells at each time will be summarized using means, standard deviations and medians, and the change over time will also be compared using two-ANOVA for repeated measurement data or Friedman rank-sum test as appropriate. The immunogenicity of the neoantigen DNA vaccine will also be analyzed qualitatively by summarizing the phenotypic and functional characteristics of antigen-specific T cells. Responses will be considered positive if the number of T cells after vaccination is greater than two standard deviations above the mean before vaccination [82]. The frequency of positive responses at each time point will be assessed and binomial response rates with 95% confidence interval estimates will be presented. In addition to presenting the binomial response rates, graphical and tabular summaries of the underlying distributions will be made.

13.2.3 Additional Exploratory objectives

Blood samples will be obtained at multiple time points (prior to vaccination, during, and post-vaccination) in order to procure PBMC for correlative studies. Functional studies will include ELISPOT, T cell poly-functionality by intra-cellular cytokine and degranulation analysis using multi-parametric flow cytometry, as detailed in Section 9.

Time to disease progression will be evaluated with physical examination and diagnostic imaging as clinically indicated. The time to disease progression will be described using Kaplan-Meier product limited method. The median progression-free survival (PFS), median overall survival (OS), and their 95% CIs will be estimated. The association between immunogenicity and PFS or OS will be explored by comparing the survival curves between immune responders versus non-responders. To determine whether the observed difference is larger than might be expected by chance, a permutation test will be used to compare the observed test statistic to the distribution of test statistics that would be seen if there were no difference between the two studies. Specifically, we will randomly shuffle the status of response and calculate the test statistic from the shuffled data. This procedure will be repeated 10,000 times and the resultant testing statistics will provide an accurate representation of the null distribution. The observed test statistics of between-study differences will be compared to the null distributions. For each outcome, the permuted p-value will be the fraction of permuted samples that resulted in a small statistic than the original sample [85].

14 DATA MANAGEMENT

Data collected will be collected using paper case report forms or OnCore electronic data capture forms. Forms must be completed within 28 days of time point.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Registration Form Eligibility Form Demographics Form On-Study Form Medical History Form	Prior to starting treatment
Physical Exam Form	Enrollment Mid chemo End of chemo Week 1 Week 5 Week 9 Week 13 Week 17 Week 21 Week 25 Week 73
Vaccine Administration Form	Week 1 Week 5 Week 9 Week 13 Week 17 Week 21
Immune Monitoring Form	Enrollment Mid chemo End of chemo Week 1 Week 5 Week 9 Week 13 Week 17 Week 21 Week 25 Week 73
Imaging Form	Mid chemo End of chemo
Toxicity Form	Week 1 Week 5 Week 9 Week 13 Week 17 Week 21 Week 25
Concomitant Medications Form	Continuous through Week 25
Follow Up Form	Week 25 Week 73 Year 2 and annually thereafter
MedWatch Form	See Section 11.0 for reporting requirements

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

14.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 11.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac disorders: cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

15 AUDITING

As coordinating center of this trial, Washington University (via the Quality Assurance and Safety Monitoring Committee (QASMC) will monitor each participating site to ensure that all protocol requirements are being met; that applicable federal regulations are being followed; and that best practices for patient safety and data collection are being followed per protocol. Participating sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Items to be evaluated include:

- Subject screening and enrollment
- Reporting of adverse events
- Maintenance of HIPAA compliance
- Completeness of regulatory documentation
- Completeness of participant documentation
- Acquisition of informed consent
- IRB documentation
- Issues of protocol adherence

Additional details regarding the auditing policies and procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/10/QASMC-Policies-and-Procedures-03.31.2015.pdf

16 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.

- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

17 REGULATORY AND ETHICAL OBLIGATIONS

17.1 Informed consent

In accordance with US FDA regulations (21 CFR 50) and guidelines (Federal Register, May 9, 1997, Vol. 62, Number 90 - ICH Good Clinical Practice Consolidated Guideline) it is the investigator's responsibility to ensure that witnessed informed consent is obtained from the subject before participating in an investigational study, after an adequate explanation of the purpose, methods, risks, potential benefits and subject responsibilities of the study. Procedures that are to be performed as part of the practice of medicine and which would be done whether or not study entry was contemplated, such as for diagnosis or treatment of a disease or medical condition, may be performed and the results subsequently used for determining study eligibility without first obtaining consent. On the other hand, informed consent must be obtained prior to initiation of any screening procedures that are performed solely for the purpose of determining eligibility for research.

Each subject must be given a copy of the informed consent. The original signed consent must be retained in the institution's records and is subject to review by the sponsor, the HRPO and any other applicable regulatory agencies responsible for the conduct of the institution. All elements listed in the ICH Good Clinical Practice guidelines must be included in the informed consent.

Informed consent will be obtained by either the principal investigator or by individuals approved by the principal investigator and whose names have been submitted to the IRB. Informed consent will be obtained from the subject after the details of the protocol have been reviewed. The individual responsible for obtaining consent will assure, prior to signing of the informed consent, that the subject has had all questions regarding therapy and the protocol answered.

17.2 Institutional Review Board

In accordance with US FDA regulations (21 CFR 56) and guidelines (Federal Register, May 9, 1997 Vol. 62 Number 90 - ICH Good Clinical Practice Consolidated Guideline) all research involving human subjects must be reviewed and approved by the local IRB. All modifications to the protocol, consent forms, or other study documents must be reviewed and approved by the local IRB. At Washington University School of Medicine, the Human Research Protection Office serves as the local IRB.

17.3 Subject confidentiality

In order to ensure subject confidentiality, each subject will be assigned a study number. Subject samples and medical information will be de-identified and labeled with the study number. The link between subject identification and study number will be safeguarded in a secure file in a locked room, and access will be restricted to the principal investigator, study coordinator, and other co-investigators as necessary.

Collected data will be recorded on case report forms. Case report forms will be safeguarded in a locked cabinet and/or a password-protected secure computer drive and access will be restricted to the principal investigator, study coordinator, and other co-investigators as necessary. Subject medical information related to, or obtained for the purposes of this trial are confidential, and disclosure to third parties is prohibited. The exception is regulatory authorities including the FDA, NIH/OBA, and the local IRB. Data from this study must be available for inspection on request of regulatory authorities including the FDA and the local IRB.

18 ADMINISTRATIVE AND LEGAL OBLIGATIONS

18.1 Study documentation and retention of records

18.1.1 Study documentation

Source documents are original documents, data, and records from which the subject's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, diagnostic imaging studies, and correspondence.

The principal investigator and staff are responsible for maintaining a comprehensive file of all study-related documents, suitable for inspection at any time by representatives from the PRMC, HRPO, FDA, and any other applicable regulatory agency.

Pertinent documents in the study file include:

(1) The original protocol with all amendments

(2) Curriculum vitae of principal investigator and co-investigators

(3) Approval notification and any other correspondence with the PRMC, HRPO, NIH RAC and FDA

Pertinent documents in each individual subject file include:

(1) Informed consent forms

(2) Case report forms

(3) Supporting copies of source documentation

All original source documentation must be readily available.

18.1.2 Retention of records

The principal investigator must retain records related to this study including protocols; amendments; IRB/IBC approvals; FDA IND records and other correspondence; completed, signed and dated consent forms; patient medical records; case report forms; drug accountability records and any other correspondence related to the conduct of the study.

U.S. FDA regulations (21 CFR 312.62[c]) require that all records pertaining to the conduct of this study, must be retained by the responsible investigator for a minimum of 2 years after marketing application approval. If no application is filed, these records must be kept 3 years after the investigation is discontinued and the U.S. FDA and the applicable local health authorities are notified.

18.2 Policy regarding research-related injuries

Washington University School of Medicine investigators and their staffs will try to reduce, control, and treat any complications from this research.

Any subjects who believe that they have been injured as a result of participation in this study will be instructed to contact the principal investigator, William E. Gillanders, M.D. at (314) 747-0072. Alternatively, they can contact Dr. Jonathan Green, Chairman of the Human Research Protection Office, at (800) 438-0445.

Decisions about payment for medical treatment for research-related injuries will be made by Washington University School of Medicine.

In general, Washington University School of Medicine will provide no long-term medical care or financial compensation for research-related injuries.

18.3 Study termination

The principal investigator and the Siteman Cancer Center reserve the right to terminate the study. The principal investigator will notify the PRMC and HRPO in writing of the study's completion or early termination.

19 REFERENCES

1. Siegel, R., et al., *Cancer statistics, 2014.* CA Cancer J Clin, 2014. **64**(1): p. 9-29.
2. Rahib, L., et al., *Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States.* Cancer Res, 2014. **74**(11): p. 2913-21.
3. Conroy, T., et al., *FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer.* N Engl J Med, 2011. **364**(19): p. 1817-25.
4. Von Hoff, D.D., et al., *Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine.* N Engl J Med, 2013. **369**(18): p. 1691-703.
5. Jones, S., et al., *Core signaling pathways in human pancreatic cancers revealed by global genomic analyses.* Science, 2008. **321**(5897): p. 1801-6.
6. Clark, C.E., G.L. Beatty, and R.H. Vonderheide, *Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer.* Cancer Lett, 2009. **279**(1): p. 1-7.
7. Clark, C.E., et al., *Dynamics of the immune reaction to pancreatic cancer from inception to invasion.* Cancer Res, 2007. **67**(19): p. 9518-27.
8. Mahadevan, D. and D.D. Von Hoff, *Tumor-stroma interactions in pancreatic ductal adenocarcinoma.* Mol Cancer Ther, 2007. **6**(4): p. 1186-97.
9. Porembka, M.R., et al., *Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth.* Cancer Immunol Immunother, 2012. **61**(9): p. 1373-85.
10. Sanford, D.E., et al., *Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis.* Clin Cancer Res, 2013. **19**(13): p. 3404-15.
11. van der Bruggen, P., et al., *A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma.* Science, 1991. **254**(5038): p. 1643-7.
12. Peshwa, M.V., et al., *Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide.* Prostate, 1998. **36**(2): p. 129-38.
13. Peoples, G.E., et al., *Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide.* Proc Natl Acad Sci U S A, 1995. **92**(2): p. 432-6.
14. Johnston, F.M., et al., *Circulating mesothelin protein and cellular antimesothelin immunity in patients with pancreatic cancer.* Clin Cancer Res, 2009. **15**(21): p. 6511-8.
15. Le, D.T., et al., *Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer.* J Clin Oncol, 2015. **33**(12): p. 1325-33.
16. Lutz, E., et al., *A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation.* Ann Surg, 2011. **253**(2): p. 328-35.
17. Thomas, A.M., et al., *Mesothelin-specific CD8(+) T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients.* J Exp Med, 2004. **200**(3): p. 297-306.
18. Parmiani, G., et al., *Unique human tumor antigens: immunobiology and use in clinical trials.* J Immunol, 2007. **178**(4): p. 1975-9.
19. Sensi, M. and A. Anichini, *Unique tumor antigens: evidence for immune control of genome integrity and immunogenic targets for T cell-mediated patient-specific immunotherapy.* Clin Cancer Res, 2006. **12**(17): p. 5023-32.
20. Wolfel, T., et al., *A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma.* Science, 1995. **269**(5228): p. 1281-4.
21. Wang, R.F., et al., *Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen.* Science, 1999. **284**(5418): p. 1351-4.
22. Echchakir, H., et al., *A point mutation in the alpha-actinin-4 gene generates an antigenic peptide recognized by autologous cytolytic T lymphocytes on a human lung carcinoma.* Cancer Res, 2001. **61**(10): p. 4078-83.
23. Brandle, D., et al., *A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma.* J Exp Med, 1996. **183**(6): p. 2501-8.

24. Matsushita, H., et al., *Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting.* Nature, 2012. **482**(7385): p. 400-4.
25. Gubin, M.M., et al., *Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens.* Nature, 2014. **515**(7528): p. 577-81.
26. Parker, K.C., M.A. Bednarek, and J.E. Coligan, *Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains.* J Immunol, 1994. **152**(1): p. 163-75.
27. Rammensee, H., et al., *SYFPEITHI: database for MHC ligands and peptide motifs.* Immunogenetics, 1999. **50**(3-4): p. 213-9.
28. Cox, G.J., T.J. Zamb, and L.A. Babiuk, *Bovine herpesvirus 1: immune responses in mice and cattle injected with plasmid DNA.* J Virol, 1993. **67**(9): p. 5664-7.
29. Davis, H.L., M.L. Michel, and R.G. Whalen, *DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody.* Hum Mol Genet, 1993. **2**(11): p. 1847-51.
30. Fynan, E.F., et al., *DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations.* Proc Natl Acad Sci U S A, 1993. **90**(24): p. 11478-82.
31. Tang, D.C., M. DeVit, and S.A. Johnston, *Genetic immunization is a simple method for eliciting an immune response.* Nature, 1992. **356**(6365): p. 152-4.
32. Ulmer, J.B., et al., *Heterologous protection against influenza by injection of DNA encoding a viral protein.* Science, 1993. **259**(5102): p. 1745-9.
33. Wang, B., et al., *Gene inoculation generates immune responses against human immunodeficiency virus type 1.* Proc Natl Acad Sci U S A, 1993. **90**(9): p. 4156-60.
34. Donnelly, J.J., et al., *DNA vaccines.* Annu Rev Immunol, 1997. **15**: p. 617-48.
35. Gurunathan, S., D.M. Klinman, and R.A. Seder, *DNA vaccines: immunology, application, and optimization*.* Annu Rev Immunol, 2000. **18**: p. 927-74.
36. Stevenson, F.K., et al., *DNA fusion gene vaccines against cancer: from the laboratory to the clinic.* Immunol Rev, 2004. **199**: p. 156-80.
37. Dupuis, M., et al., *Distribution of DNA vaccines determines their immunogenicity after intramuscular injection in mice.* J Immunol, 2000. **165**(5): p. 2850-8.
38. Aihara, H. and J. Miyazaki, *Gene transfer into muscle by electroporation in vivo.* Nat Biotechnol, 1998. **16**(9): p. 867-70.
39. Mathiesen, I., *Electropermeabilization of skeletal muscle enhances gene transfer in vivo.* Gene Ther, 1999. **6**(4): p. 508-14.
40. Mir, L.M., et al., *High-efficiency gene transfer into skeletal muscle mediated by electric pulses.* Proc Natl Acad Sci U S A, 1999. **96**(8): p. 4262-7.
41. Widera, G., et al., *Increased DNA vaccine delivery and immunogenicity by electroporation in vivo.* J Immunol, 2000. **164**(9): p. 4635-40.
42. Boyer, J.D., et al., *SIV DNA vaccine co-administered with IL-12 expression plasmid enhances CD8 SIV cellular immune responses in cynomolgus macaques.* J Med Primatol, 2005. **34**(5-6): p. 262-70.
43. Chong, S.Y., et al., *Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques.* Vaccine, 2007. **25**(26): p. 4967-82.
44. Hirao, L.A., et al., *Combined effects of IL-12 and electroporation enhances the potency of DNA vaccination in macaques.* Vaccine, 2008. **26**(25): p. 3112-20.
45. Hirao, L.A., et al., *Intradermal/subcutaneous immunization by electroporation improves plasmid vaccine delivery and potency in pigs and rhesus macaques.* Vaccine, 2008. **26**(3): p. 440-8.
46. Luckay, A., et al., *Effect of plasmid DNA vaccine design and in vivo electroporation on the resulting vaccine-specific immune responses in rhesus macaques.* J Virol, 2007. **81**(10): p. 5257-69.
47. Otten, G., et al., *Enhancement of DNA vaccine potency in rhesus macaques by electroporation.* Vaccine, 2004. **22**(19): p. 2489-93.
48. Otten, G.R., et al., *Potent immunogenicity of an HIV-1 gag-pol fusion DNA vaccine delivered by in vivo electroporation.* Vaccine, 2006. **24**(21): p. 4503-9.

49. Schadeck, E.B., et al., *A dose sparing effect by plasmid encoded IL-12 adjuvant on a SIVgag-plasmid DNA vaccine in rhesus macaques.* Vaccine, 2006. **24**(21): p. 4677-87.
50. Rice, J., C.H. Ottensmeier, and F.K. Stevenson, *DNA vaccines: precision tools for activating effective immunity against cancer.* Nat Rev Cancer, 2008. **8**(2): p. 108-20.
51. Weiner, D.B., *DNA vaccines: Crossing a line in the sand Introduction to special issue.* Vaccine, 2008.
52. Simon, R.M., et al., *Clinical trial designs for the early clinical development of therapeutic cancer vaccines.* J Clin Oncol, 2001. **19**(6): p. 1848-54.
53. Bowne, W.B., et al., *Coupling and uncoupling of tumor immunity and autoimmunity.* J Exp Med, 1999. **190**(11): p. 1717-22.
54. Hawkins, W.G., et al., *Immunization with DNA coding for gp100 results in CD4 T-cell independent antitumor immunity.* Surgery, 2000. **128**(2): p. 273-80.
55. Dunn, G.P., et al., *A critical function for type I interferons in cancer immunoediting.* Nat Immunol, 2005. **6**(7): p. 722-9.
56. Dunn, G.P., C.M. Koebel, and R.D. Schreiber, *Interferons, immunity and cancer immunoediting.* Nat Rev Immunol, 2006. **6**(11): p. 836-48.
57. Dunn, G.P., L.J. Old, and R.D. Schreiber, *The three Es of cancer immunoediting.* Annu Rev Immunol, 2004. **22**: p. 329-60.
58. Shankaran, V., et al., *IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity.* Nature, 2001. **410**(6832): p. 1107-11.
59. Diamond, M.S., et al., *Type I interferon is selectively required by dendritic cells for immune rejection of tumors.* J Exp Med, 2011. **208**(10): p. 1989-2003.
60. Dunn, G.P., et al., *Cancer immunoediting: from immunosurveillance to tumor escape.* Nat Immunol, 2002. **3**(11): p. 991-8.
61. Vesely, M.D., et al., *Natural innate and adaptive immunity to cancer.* Annu Rev Immunol, 2011. **29**: p. 235-71.
62. Vesely, M.D. and R.D. Schreiber, *Cancer immunoediting: antigens, mechanisms, and implications to cancer immunotherapy.* Ann N Y Acad Sci, 2013. **1284**: p. 1-5.
63. Klco, J.M., et al., *Functional heterogeneity of genetically defined subclones in acute myeloid leukemia.* Cancer Cell, 2014. **25**(3): p. 379-92.
64. Welch, J.S., et al., *The origin and evolution of mutations in acute myeloid leukemia.* Cell, 2012. **150**(2): p. 264-78.
65. Ding, L., et al., *Genome remodelling in a basal-like breast cancer metastasis and xenograft.* Nature, 2010. **464**(7291): p. 999-1005.
66. Ellis, M.J., et al., *Whole-genome analysis informs breast cancer response to aromatase inhibition.* Nature, 2012. **486**(7403): p. 353-60.
67. Peters, B. and A. Sette, *Generating quantitative models describing the sequence specificity of biological processes with the stabilized matrix method.* BMC Bioinformatics, 2005. **6**: p. 132.
68. Lundegaard, C., et al., *NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11.* Nucleic Acids Res, 2008. **36**(Web Server issue): p. W509-12.
69. Hoof, I., et al., *NetMHCpan, a method for MHC class I binding prediction beyond humans.* Immunogenetics, 2009. **61**(1): p. 1-13.
70. Nielsen, M., et al., *The role of the proteasome in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage.* Immunogenetics, 2005. **57**(1-2): p. 33-41.
71. Dannull, J., et al., *Prostate stem cell antigen is a promising candidate for immunotherapy of advanced prostate cancer.* Cancer Res, 2000. **60**(19): p. 5522-8.
72. Prazeres, D.M., et al., *Purification of plasmids for gene therapy and DNA vaccination.* Biotechnol Annu Rev, 2001. **7**: p. 1-30.
73. Manoj, S., L.A. Babiuk, and S. van Drunen Littel-van den Hurk, *Approaches to enhance the efficacy of DNA vaccines.* Crit Rev Clin Lab Sci, 2004. **41**(1): p. 1-39.
74. Donnelly, J., K. Berry, and J.B. Ulmer, *Technical and regulatory hurdles for DNA vaccines.* Int J Parasitol, 2003. **33**(5-6): p. 457-67.
75. Scheerlinck, J.P., et al., *In vivo electroporation improves immune responses to DNA vaccination in sheep.* Vaccine, 2004. **22**(13-14): p. 1820-5.

76. Babiuk, S., et al., *Electroporation improves the efficacy of DNA vaccines in large animals.* Vaccine, 2002. **20**(27-28): p. 3399-3408.
77. Luxembourg, A., et al., *Enhancement of immune responses to an HBV DNA vaccine by electroporation.* Vaccine, 2006. **24**(21): p. 4490-3.
78. Jaramillo, A., et al., *Identification of HLA-A3-restricted CD8+ T cell epitopes derived from mammaglobin-A, a tumor-associated antigen of human breast cancer.* Int.J.Cancer, 2002. **102**(5): p. 499.
79. Jaramillo, A., et al., *Recognition of HLA-A2-restricted mammaglobin-A-derived epitopes by CD8+ cytotoxic T lymphocytes from breast cancer patients.* Breast Cancer Res.Treat., 2004. **88**(1): p. 29.
80. Manna, P.P., et al., *Generation of CD8+ cytotoxic T lymphocytes against breast cancer cells by stimulation with mammaglobin-A-pulsed dendritic cells.* Breast Cancer Res.Treat., 2003. **79**(1): p. 133.
81. Narayanan, K., et al., *Response of established human breast tumors to vaccination with mammaglobin-A cDNA.* J.Natl.Cancer Inst., 2004. **96**(18): p. 1388.
82. Hobeika, A.C., et al., *Enumerating antigen-specific T-cell responses in peripheral blood: a comparison of peptide MHC Tetramer, ELISpot, and intracellular cytokine analysis.* J Immunother, 2005. **28**(1): p. 63-72.
83. Andersen, R.S., et al., *Parallel detection of antigen-specific T cell responses by combinatorial encoding of MHC multimers.* Nat Protoc, 2012. **7**(5): p. 891-902.
84. Cheung, R.K. and P.J. Utz, *Screening: CyTOF-the next generation of cell detection.* Nat Rev Rheumatol, 2011. **7**(9): p. 502-3.
85. Westfall, P.H. and S.S. Young, *Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment*. 1993, New York: John Wiley & Sons, Inc.

20 APPENDICES

20.1 Abbreviations

AE	Adverse Event
CBER	Center for Biologics Evaluation and Research
CFR	Code of Federal Regulations
CPA	Cooperative Project Assurance
CR	Complete response
CRA	Clinical Research Associate
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DNA	Deoxyribonucleic acid
DCTD	Division of Cancer Treatment and Diagnosis
ELISPOT	Enzyme-linked immunospot assay
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FWA	Federal-wide Assurance
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GPC	Gel permeation chromatography
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human leukocyte antigen
HRPO	Human Research Protection Office (Institutional Review Board at WUSM)
IBC	Institutional Biosafety Committee
ICH	International Conference of Harmonization
IDB	Investigational Drug Branch
IRB	Institutional Review Board
LD	Longest diameter
MPA	Multiple Project Assurance
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities
OHRP	Office for Human Research Protection
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PHI	Protected Health Information
PR	Partial response
RAC	Recombinant DNA Advisory Committee
RECIST	Response Evaluation Criteria in Solid Tumors
QASMC	Quality Assurance and Safety Monitoring Committee
SAE	Serious Adverse Event
SAS	Statistical Analysis System; Analytical software from the SAS Institute, Cary, NC
SCC	Siteman Cancer Center
SCIP	Siteman Cancer Information Portal
SD	Stable disease
SD	Standard Deviation
THF	Tetrahydrofuran
UPN	Universal Product Number
WUSM	Washington University School of Medicine
WUSTL	Washington University in Saint Louis

20.2 ECOG/Zubrod performance status scale

ECOG/Zubrod Score	Performance Status
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed < 50% of the day
3	Symptomatic, in bed > 50% of the day but not bedridden
4	Bedridden
5	Dead

20.3 National Cancer Institute Common Terminology Criteria for Adverse Events

This study will collect adverse events using the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE), if applicable. The CTCAE provides a descriptive terminology that is to be used for adverse event reporting. A grading (severity) scale is also provided in the CTCAE for each adverse event term. An electronic version of the CTCAE may be accessed through the web at http://ctep.cancer.gov. Alternatively, a full copy is available from the principal investigator.

20.4 APPENDIX A: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:

http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed

protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

20.5 APPENDIX B: Reporting Timelines

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Ichor Medical Systems**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	Report at time of HRPO submission; IBC will retrieve information from myIRB and will follow up with study team if needed	Since the TDS-IM device may be involved with the occurrence of adverse events, Ichor Medical Systems will be included in the Adverse Event reporting plan. Reports to Ichor for SAEs or for adverse events with possible relationship to the device should follow the timing for reporting to the WUSM Human Research Protection Office and should be directed to Drew Hannaman at dhannaman@ichorms.com
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information		
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment			
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.				
A series of minor	Report within 10 working days.				

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Ichor Medical Systems**
deviations that are being reported as a continuing noncompliance					
Protocol exception	Approval must be obtained prior to implementing the change				
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting		
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.				
Breach of confidentiality	Within 10 working days.				
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be				

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Ichor Medical Systems**
	withdrawn, report at continuing review.				

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Ichor Medical Systems**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.	
Minor deviation	Report summary information at the time of continuing review.			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Expedited Reporting Timelines for Secondary Sites				
Event	**WU (Coordinating Center)**	**Local IRB**	**FDA**	**Ichor Medical Systems**
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.	The research team at Washington University is responsible for reporting all applicable events to Ichor as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.			
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.			
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations			
Protocol exception	Approval must be obtained prior to implementing the change.			

20.6 APPENDIX C: Washington University Unanticipated Problem Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Admission Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**