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

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SUMMARY

Protocol: **A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A NEOANTIGEN PEPTIDE 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 peptide vaccine strategy in pancreatic cancer patients following surgical resection and adjuvant chemotherapy. The neoantigen peptide vaccines will incorporate prioritized neoantigens and personalized mesothelin epitopes and will be co-administered with poly-ICLC. The hypothesis of this study is that neoantigen peptide 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 peptide vaccine strategy. The secondary objective is to evaluate the immunogenicity of the neoantigen peptide vaccine strategy as measured by ELISPOT analysis and multi-parametric flow cytometry, surrogates for T cell function.

Product Description: The neoantigen peptide vaccines are composed of synthetic long peptides which are 25-30 amino acids in length and designed to express mutant tumor specific antigens identified by exome sequencing. These are processed by the immune system differently than minimal peptide vaccines, prolonging the duration of peptide presentation, and T cell activation. Peptide vaccines will be administered subcutaneously and will be co-administered with poly-ICLC.

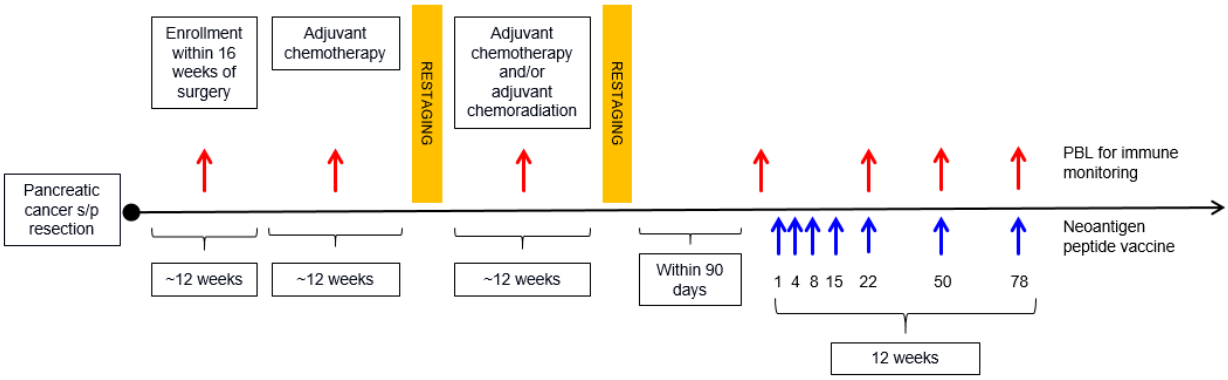
Subjects: Pancreatic cancer patients who have completed surgical resection and adjuvant chemotherapy without evidence of recurrent disease are eligible. Patients enrolled into the protocol must provide consent for exome sequencing and dbGAP-based data sharing and provide germline and tumor DNA samples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

Study Plan: Fifteen pancreatic cancer patients will be enrolled. Subjects will be treated with synthetic long peptides corresponding to prioritized neoantigens in combination with poly-ICLC, a synthetic toll-like receptor ligand. The peptides and poly-ICLC will be mixed prior to injection. Peptide and poly-ICLC will be administered subcutaneously at days 1, 4, 8, 15, 22, 50, and 78 with at least 24 hours between injection days.

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 peptide 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 5.0. The secondary endpoint is immunogenicity of the vaccine regimen as measured by ELISPOT analyses and multiparametric flow cytometry. Exploratory analyses will include other measures of immune function, including CYTOF analysis.

SCHEMA


Enrollment within 16 weeks of surgery
Adjuvant chemotherapy
RESTAGING
Adjuvant chemotherapy and/or adjuvant chemoradiation
RESTAGING
Pancreatic cancer s/p resection
PBL for immune monitoring
Neoantigen peptide vaccine
~12 weeks
~12 weeks
~12 weeks
Within 90 days
1 4 8 15 22 50 78
12 weeks

PROTOCOL SIGNATURE PAGE

A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A NEOANTIGEN PEPTIDE 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 peptide vaccine strategy. The neoantigen peptide 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 synthetic long peptides and will be administered subcutaneously in conjunction with poly-ICLC. The hypothesis of this study is that the neoantigen peptide 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 peptide vaccine approach. This trial will be considered feasible if we are able to successfully perform tumor/normal exome sequencing, identify at least 3 neoantigens per patient, and manufacture and administer neoantigen vaccines to more than 50% of enrolled 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 flow cytometry and 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.

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 regard 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 peptide vaccines. Once the candidate mutant epitopes have been verified, multivalent peptide vaccine construction will commence.

2.8 Synthetic long peptide vaccines

The neoantigen peptide vaccine strategy is based on the synthetic long peptide vaccine platform. We have focused on this platform because of the established safety and documented efficacy of this platform. First generation peptide-based cancer vaccines have typically included "minimal epitope" peptides, i.e. peptides of 9-10 amino acids in length known to bind to specific MHC class I alleles. First generation peptide-based cancer vaccines have an excellent safety profile and have been successful in inducing peptide-specific T cell responses in the peripheral blood of cancer patients, as measured by ELISPOT or tetramer analysis.

These initially promising results with first-generation peptide-based cancer vaccines prompted investigators to explore improvements to the platform. These investigations have resulted in "next-generation" peptide-based cancer vaccines [28] including synthetic long peptide vaccines. Synthetic long peptide vaccines are 25-30 amino acids in length and are processed by the immune system differently than minimal epitope peptide vaccines. This prolongs the duration of peptide presentation and T cell activation [29, 30]. Synthetic long peptide vaccines have proven to be both safe and remarkably effective in clinical translation [31]. In our previous experience, we have been able to successfully design and manufacture neoantigen synthetic long peptide vaccines in > 85% of cases. Processing of synthetic long peptides and T cell activation can be further enhanced through the activation of toll-like receptors on the surface of APC. In particular, the TLR3 agonist, poly-ICLC is commonly used as an adjuvant to enhance the efficacy of peptide-based cancer vaccines, including synthetic long peptide-based vaccines [31].

2.9 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" [32].

The peptide vaccine will integrate multiple peptides corresponding to prioritized neoantigens in combination with the molecular adjuvant poly IC:LC. We propose to test a fixed dose of peptide consistent with recent reports [33]. The final peptide concentration is 300 µg/mL of each peptide after mixture with poly-ICLC

2.10 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 evaluation and sequencing of tissue for vaccine development (Step 0) and vaccine administration (Step 1) 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	≤1.5 X institutional upper limit of normal (Subjects with Gilbert's syndrome may be enrolled despite a total bilirubin level >1.5 mg/dL if their conjugated bilirubin is <1.5 x ULN)
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.

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, carcinoma *in situ* of the cervix, or LCIS/DCIS of the breast.
5. Known allergy, or history of serious adverse reaction to vaccines or TLR agonists such as anaphylaxis, hives, or respiratory difficulty.

6. 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.
7. 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
8. 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.
9. Pregnant and/or breastfeeding.
10. Known HIV-positive status. These patients are ineligible because of the potential inability to generate an immune response to vaccines.

3.3 Step 1 eligibility

At Step 1 eligibility confirmation prior to vaccination, the above criteria must be met plus:

1. Completed adjuvant chemotherapy:
    a. Initiation of adjuvant chemotherapy within 16 weeks of surgery
    b. Completion of at least 4 months of adjuvant chemotherapy with gemcitabine/capecitabine FOLFIRINOX, 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 post-completion 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. Receiving any other investigational agents, or has received an investigational agent within the last 30 days.

3.4 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) 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). 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. 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 17.1 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 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 Siteman 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)

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 for both Step 0 and Step 1 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, please contact primary study coordinator at Washington University):

(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 enrollment

(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 two business days. 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 Neoantigen Peptide Vaccine

5.1.1 Physical, chemical and biological characteristics

The personalized synthetic long peptide vaccine strategy is based on the synthetic long peptide vaccine platform. Synthetic long peptides will be 16-35 amino acids in length. The peptides will be designed to generate an immune response to neoantigens found in an individual patient's tumor. The amino acid sequence of the synthetic long peptides will correspond to the amino acid sequence of the prioritized candidate neoantigens. Synthetic long peptide vaccines will be designed in the Gillanders laboratory 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.

(6) Candidate neoantigens will be prioritized based on epitope prediction algorithms and in vitro studies as outlined previously.

(7) 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.

(8) Synthetic long peptides corresponding to the prioritized neoantigens will be designed. For single nucleotide variants, the mutant amino acid will be at or near the center of the synthetic long peptide and the entire minimal epitope will be included. The exact length and sequence of the synthetic long peptide will also integrate manufacturing considerations in consultation with peptide chemists at CSBio.

(9) The neoantigen peptide vaccines will be manufactured and vialed at CSBio.

5.1.2 Manufacturing facility

Synthetic long peptides supplied by CSBio will be manufactured according to the process described below. The sequencing pipeline to identify candidate neoantigens and the epitope prediction algorithms to prioritize candidate neoantigens are described in the IND.

Synthetic long peptides supplied from CSBio will be provided to WUSM with a Certificate of Analysis of product specifications to confirm product quality prior to release. Release specification criteria will include: 1) Appearance; 2) Identity by Mass Spectral Analysis (MW); 3) Peptide Content by Nitrogen Elemental Analysis; 4) Residual Solvents By Gas Chromatography; 5) Peptide Purity by Analytical HPLC; 6) Trifuoracetic Acid Content by analytical HPLC. The pooled peptides will also be tested with the Bacterial Endotoxins and Microbial Limit Test and the results provided to WUSM.

5.1.2.1 Manufacturing Process for CSBio Peptides

The CSBio solid phase Fmoc peptide synthesis is based on sequential addition of alpha-amino and side chain protected amino acid residues to an insoluble polymeric support (see Production Scheme below for detailed method). Synthesis is carried out in a batchwise manner using a CS Bio automated peptide synthesizer. Resin is contained in a filter reaction vessel and reagents added and removed under

computer control. Synthesis is performed by the stepwise addition of activated amino acids to the solid support starting from the carboxy terminus to the amino terminus. The activation and coupling of amino acids is performed by HBTU/HOBT chemistry and dimethylformamide is used as the main wash solvent.

At the end of synthesis, the peptide is cleaved off the resin by reagent K (TFA + Scavengers) for 2-5 hours at room temperature, and subjected to multiple ether extractions. Crude peptides are subjected to vacuum filtration followed by RP-HPLC purification using a C18 column. HPLC purification is performed on a system that is qualified by a trained specialist to comply with FDA standards. The purity and identity of the peptide is confirmed by running analytical HPLC and mass spectrum analysis. The final purified peptides will be shell frozen in glass jars using liquid nitrogen, lyophilized, and packaged at the GMP facility as a dry lyophilized powder under environmentally controlled conditions.

Solid Phase Fmoc Peptide Production Scheme:

A. Synthesis:

Resin: MBHA Resin, Wang Resin, ClTrt Resin or AM Resin
Resin Scale: mmol
AA: Arg(Pbf), Asn/Gln/Cys/His (Trt), Asp/Glu (OtBu), Lys/Trp (Boc), Ser/Thr/Tyr (tBu), Met/Gly/Ile/Leu/Phe/Pro/Val/Ala
Coupling Method (Fmoc Chemistry)
Coupling Reagent:
Rx/DIC/HOBt/AA, in DMF (First Coupling)
Rx/DIC/HOBt/AA and/or DIEA/HBTU/AA, in DMF (Optional, Double Coupling)
Capping with Ac_2O and DIEA in DMF (Optional)
Coupling time (water-jacketed, with heat):
46 minutes for each AA attachment (First Coupling)
46 minutes (HOBt/DIC) or 20 to 46 minutes (HBTU/DIEA) for each AA attachment (Optional, Double Coupling)
2 minutes (Optional, Capping)
Coupling time (no heat):
2 to 6 hours for each AA attachment (First Coupling)
2 to 6 hours (HOBt/DIC) or 1 to 2 hours (HBTU/DIEA) for each AA attachment (Optional, Double Coupling)
5 minutes (Optional, Capping)

B. Cleavage Method:

Reagent K (TFA/EDT/TIS/H_2O, 94/2/2/2), 2 ~ 5 hours at room temperature

C. Purification:

Column: C18, 4.1-10 cm x 22.5-27.5 cm
Column Equilibration: 2-10% Buffer B for NLT 1.5 Column Volumes
Sample Loading: Dilute with purified water or Buffer A as necessary to drop Acetonitrile level below elution percentage. Load to column maintaining a system pressure ≤ 1500 PSI.
Preparative HPLC Run Gradient:
The starting %B at or above equilibration %B but lower than peptide elution percentage
The ending %B above elution but below wash method percentage
Typical gradient has a change of 0.3-1.0 %B change per minute over a 50 to 80 minute period.
Mobile Phase: Aqueous Buffer A (0.1% TFA or 1% TEAP or 0.1% NH_4OH, or 50-100mM NH_4OAc or 0.5-1% AcOH) and Organic Buffer B (Acetonitrile).

D. Ion-exchange:

Column: C18, 4.1-10 cm x 22.5-27.5 cm
Column Equilibration: 2-10% Buffer B for NLT 1.5 Column Volumes
Mobile Phase (pre-gradient):
1.) Buffer A, 1 % TEAP; Buffer B, ACN
2.) Buffer A, 50-100mM NH_4OAc; Buffer B, ACN

3.) Buffer A, 0.5-1% AcOH; Buffer B, ACN

Preparative HPLC Run Gradient:
The starting %B at or above equilibration %B but lower than peptide elution percentage
The ending %B above elution but below wash method percentage
Typical gradient has a change of 0.3-1.5 %B change per minute over a 40 to 70 minute period.
Mobile Phase: Aqueous Buffer A (0.5-1% AcOH) and Organic Buffer B (Acetonitrile).

E. Lyophilization:
Shell frozen in 1 L glass jars using liquid nitrogen and lyophilized on Virtis Freezemobile 35 L.
Condenser Temperature: < -55 °C
Vacuum: ≤ 1000 mT after attaching jar

5.1.2.2 Product Formulation for CSBio Peptides

Peptides will be reconstituted at CSBio immediately prior to product release tests. Random vials will be selected for product release testing. The recommended methods for identity, purity and contents for testing of peptides produced as Active Pharmaceutical Ingredients (APIs) under cGMP, will be performed as outlined in Table 1:

TABLE 1: Synthetic long peptide product release tests

Criteria	Method	Specification	Test Site/SOP #
Appearance	Visual inspection	White to off-white powder	CS Bio / SOP 08-16
Molecular Weight Determination	Identification by Mass Spectral Analysis	MW+/- 1.0 up to 2000d	CS Bio / SOP 08-08
Peptide content by CHN Analysis	Nitrogen Elemental Analysis	Report result	CS Bio / SOP 08-10
Primary Counter Ion	Analytical HPLC	Report result	CS Bio / SOP 08-13 or SOP 08-111
Secondary Counter Ion	Analytical HPLC	Report result	CS Bio / SOP 08-13 or SOP 08-111
Residual Organic Solvents	Gas chromatography	Within ICH guidelines	CS Bio / SOP 08-12
Peptide Purity	Analytical HPLC	≥ 95%	CS Bio / SOP 08-06

Visual Inspection of Appearance: Physical appearance testing can be the most subjective but important test performed on drug substances that can give valuable information about the color and solid state of the peptide. Typical testing involves viewing the material against a white background under good lighting and reporting the color and solid state. Thus, ensuring that the sample products have the appropriate visual characteristics based on established specifications.

Identification by Mass Spectral Analysis: Mass spectrometry provides key tools for the analysis of peptides and a powerful technique for the identification and elucidation the structure of peptides. Molecular weight analysis is determined by flow injection analysis – mass spectrometry (FIA-MS) using a single quadrupole detection. The peptide sample is dissolved in a mixture of MeOH/Water and Acetonitrile (ACN) which is further injected into the MS system.

Peptide Content by CHN Nitrogen Elemental Analysis: The content of the peptide can be easily determined by Nitrogen determination by CHN elemental analysis. Briefly, Carbon, Hydrogen, and Nitrogen are determined using a 2400 Perkin-Elmer CHN Analyzer. The analyzer uses combustion to convert the sample elements to simple gasses, i.e. CO_2, H_2O, and N_2. Upon entering the analyzer,

the sample is combusted in pure oxygen environment at high temperatures of ~ 1000°C. Peptide content is calculated from the determined content of nitrogen in the sample compared to the theoretical nitrogen content of the specified peptide.

Primary and Secondary Counter ion by RP-HPLC: The quantitation of counter ions of trifluoroacetate (TFA) and acetate can be determined by RP-HPLC. The primary counter ion TFA is exchanged under acidic conditions and separated by gradient Reverse-Phase HPLC using a C18 column and a mobile phase. The mobile phase normally consists of Phosphoric acid (H_3PO_4), Methanol (MeOH) and deionized water. UV detection at 210nm is normally used for this measurement.

Residual Organic Solvents by Gas Chromatography: Residual organic solvents of Acetonitrile (ACN), and N,N-Dimethylformamide (DMF) are extracted using DMSO and separated by headspace has chromatography (HS-GC) using a Zebron capillary column. The mobile phase or gas carrier is normally Helium. Measurement is by Flame Ionization Detector (FID). Quantitation of residual solvents in sample peptides is by determined by comparing to reference standards for each residual solvent. Other solvents can be easily added to residual solvents testing if they were used in the manufacturing process.

Peptide Purity by RP-HPLC: The peptide purity is normally determined using RP-HPLC, which is the most common, widely used and well-established tool universally used in quality control. The separation of sample peptides by RP-HPLC involve continuous partitioning of the molecules between the mobile phase and the hydrophobic stationary phase. The mobile phase consists of various amounts of Trifluoroacetic acid, Acetonitrile and Water and normally measurements is by UV absorption at ~ 214nm.

The above described attributes are considered essential components of a peptide specification.

Stability assays are not likely to be appropriate for this project, as peptides synthesized for use in the phase 1 clinical trial will be used within weeks of their synthesis.

5.1.2.3 Labels

CSBio pooled peptides will be provided with the following labels:

Peptide Synthesis Facility
Product Name: Study # - PPI # - Peptide Pool (A, B, C, etc)
Component:
[1]PP-XXXX, SLP name, PP-XXXX, SLP name ….
[2]Process Lot #: PLYY-XXX
Manufacture Date: mm/yyyy
Quantity: 25.0 +/- 5.0 mL
Concentration: 0.4 mg/mL
Store ≤ -75°C ± 10°C
Not for human use without sterile processing

Note:

[1]Part Numbers will include the number for each of the peptides in the pool that will be reflected on the corresponding peptide sequence located in the shipping manifest.

[2]Process Lot # reflects the batch production record for all pools in a batch order

5.1.3 Product Formulation

Synthetic long peptides manufactured by CSBio will be supplied to WUSM as low bioburden pooled peptide solutions. The low bioburden, pooled peptides will be supplied in a 50 mL conical tube at a final

concentration of 0.4 mg/mL in 26.7% DMSO, and shipped to the Biologic Therapy Core Facility (BTCF) at Washington University School of Medicine on dry ice and will be stored at -75°C +/- 10°C. The peptide pools will then be sterile filtered and aliquoted into sterile cryovials (**Step 1**), representative cryovials will be selected for sterility and endotoxin testing (**Step 2**), and then transferred or shipped to the appropriate investigational pharmacy (**Step 3**).

Steps 1-3 are summarized and then described in detail below.

(1) The peptide pools will be sterile filtered and aliquoted into sterile cryovials

(2) Representative cryovials will be selected for sterility and endotoxin testing

(3) Cryovials will be transferred to the investigational pharmacy at WUSM (or shipped to the appropriate investigational pharmacy at participating sites). The vialed product will be stored at -75°C +/- 10°C prior to use.

Specifically:

Step 1. The peptide pools will be sterile filtered and aliquoted into sterile cryovials.

Each diluted peptide pool will then be sterile filtered with a 0.2 micron DMSO-compatible filter (for example, a 0.2 micron DSMO-safe Acrodisc syringe filter; Pall Corporation: Product# 4433 or equivalent). Each peptide pool will then be aliquoted into 12 x 1.8 mL sterile cryovials (1.5 mL per 1.8 mL sterile cryovial). 7 cryovials will be designated for injection, 3 cryovials will be designated for product release tests, and 2 cryovials will be held in reserve. Cryovials will be stored at -75°C +/- 5°C.

Each patient will have 12-48 cryovials depending on the number of peptides that were successfully manufactured (Table 1). For example, if 16 peptides are successfully manufactured, there will be 48 cryovials (4 peptide pools x 12 cryovials per peptide pool).

Step 2. Representative cryovials will be selected for sterility and endotoxin testing.

Representative cryovials from each peptide pool will be tested for sterility and endotoxin (two cryovials will be tested for sterility and one cryovial will be tested for endotoxin). Sterility testing will be performed using the USP <71> sterility test method. The DMSO concentration has been tested to ensure it will not interfere with the sterility test. The release criteria for the sterility test is no growth at 14 days. The release criteria for endotoxin is < 5 EU/mL. If the peptide pools pass these product release tests, the cryovials will be transferred to the investigational pharmacy at WUSM, or shipped to the appropriate investigational pharmacy at participating sites.

Step 3. Cryovials will be transferred to the investigational pharmacy at WUSM (or shipped to the appropriate investigational pharmacy at participating sites).

Following sterility and endotoxin testing, the BTCF will transfer cryovials on dry ice to the WUSM investigational pharmacy, or the BTCF will ship cryovials on dry ice to the appropriate investigational pharmacy at participating sites. Cryovials will be packaged and shipped in temperature monitoring containers.

Step 4. At each vaccination timepoint, peptide pools will be mixed with poly ICLC immediately prior to administration.

Step 4 is the final step in product formulation and will be performed on the day of vaccine administration by an investigational pharmacist. Additional details are provided in the Investigational Agent Administration section, below.

At each vaccination timepoint, one cryovial from each peptide pool will be mixed with poly ICLC.

Each peptide pool will be drawn up into a syringe to facilitate mixture with poly ICLC and administration. Up to four syringes will be prepared at each vaccination time point corresponding to each peptide pool.

Each prepared syringe will contain both the synthetic long peptides and poly-ICLC. 0.75 mL of the peptide pool will be mixed with 0.25 mL of poly ICLC.

The adjuvant, poly-ICLC is formulated with clinical grade poly I; poly C stabilized with carboxymethylcellulose and poly-L-Lysine.

Poly-ICLC is composed of the following:

- 0.6 to 3% DMSO
- 3.6 to 3.7% dextrose in water
- 3.6 to 3.7 mM sodium succinate
- 0.5 mg/mL polyL:polyC
- 0.375 mg/mL poly-L-Lysine
- 1.25 mg/mL sodium carboxymethylcellulose
- 0.225% sodium chloride solution

The final peptide concentration is 300 µg/mL of each peptide after mixture with poly ICLC.

5.1.4 Packaging and Labeling

Up to four peptide pools (A, B, C, D) are prepared for each patient.

Seven cryovials per peptide pool (up to 28 cryovials total) will be transferred to the investigational pharmacy at WUSM or shipped to the investigational pharmacy at the appropriate clinical site.

Each peptide pool will be in a separate labeled case. Each cryovial contains a single peptide pool containing up to four peptides (with each individual peptide at a concentration of 400 mcg/mL, 1.5 mL/vial in 26.7% DMSO). Each cryovial will be labeled.

An example of the label for the pooled peptides is presented below.

Patient ID: Study #, INT designates the appropriate subject study ID number and initials.

Vial #XX designates the appropriate vial number.

Peptide Pool Y designates the appropriate peptide pool (A, B, C, D).

For CTEP studies, the appropriate NCI Protocol Number will be inserted onto the label as shown.

WUSM Neoantigen Vaccine
NCI Protocol Number (if applicable)
Patient ID: Study#, INT Lot # 00X, Vial #XX
Date of Manufacture: MM/DD/YY
Peptide Pool: Y
Concentration 0.4 mg/mL, 1.5 mL/vial Store at -75 ºC ± 10 ºC
NSC 804336
CAUTION: Investigational drug - Limited
by Federal Law to investigational use

An example of the case label is presented below.

Patient ID: Study #, INT designates the appropriate subject study ID number and initials.

Peptide Pool Y designates the appropriate peptide pool (A, B, C, D).

X Total vials indicates the number of vials/case

For CTEP studies, the appropriate NCI Protocol Number will be inserted onto the label as shown.

WUSM Neoantigen Vaccine
Contents: Synthetic long peptides for injection
Patient ID: Study#, INT
Date of Manufacture: MM/DD/YY
NCI Protocol Number (if applicable)
Peptide Pool: Y, Lot 00X
Concentration 0.4 mg/mL, 1.5 mL/vial X Total vials
NSC 804336
Directions: Administer as directed in the protocol
Store at -75°C +/- 10°C
Store vials in carton until time of use
CAUTION: Investigational drug - Limited
by Federal Law to investigational use

5.1.5 Storage conditions

The low bioburden, lyophilized synthetic long peptides provided by CSBio will be received by designated personnel in the Biologic Therapy Core Facility at Washington University School of Medicine, handled and stored safely and properly, and kept in a secure, limited-access location. Once formulated into a drug product, the synthetic long peptides may be dispensed only by the Investigator or designated personnel specifically authorized by the Investigator.

Upon receipt, the low bioburden, lyophilized synthetic long peptides will be stored in a controlled access location according to the instructions specified on the package labels. Similarly, once formulated into a drug product, the synthetic long peptides will be stored in a controlled access location according to the instructions specified on the package labels.

- The synthetic long peptides will be stored frozen at -75°C +/- 5°C
- The poly-ICLC vials (Hiltonol) are to be stored refrigerated between 2°C to 8°C.

The investigator will be notified of any expiry or retest date extension of clinical study material during the study conduct. It is not anticipated that the expiration date of the synthetic long peptides will change as no stability tests are planned.

Changes to the retest date of the poly-ICLC will be communicated via memo from Oncovir, or a designee. The retest dates of the poly-ICLC may be updated throughout the study. On expiry date notification site personnel must complete all instructions outlined in the notification, including segregation of expired clinical study material for return to the sponsor or its designee for destruction.

All study drugs are for investigational use only and are to be used only within the context of this study.

5.1.6 Dispensation and Accountability

The investigator and study pharmacist must maintain 100% up-to-date written records of the vaccine study drug dispensing activities for each patient. Accountability for all study medication received and dispensed during the entire study are recorded for each patient using Patient Drug Accountability Logs. If any dispensing errors or discrepancies are discovered you should notify the Clinical Research Associate (CRA) monitor or Principal Investigator immediately.

The Patient Drug Accountability Logs must accurately reflect the drug accountability of the study medication at all times. The following information will be recorded as a minimum:

- Protocol number and title
- Name of the investigator and site identifier/number
- Description of study medication
- Expiry and/or retest date (if not recorded elsewhere) and kit number
- The date and amount dispensed, including the initials of the person dispensing study medication
- The log should include all required information as a separate entry for each patient for whom study medication is dispensed

5.1.7 Safety and Handling

The Material Safety Data Sheets (MSDS) for Hiltonol are provided in Section 5.2. Refer to the appropriate MSDS for safety and handling guidelines.

Due to the personalized nature of the vaccine study drug (WU-PVAX), the potential toxicity has not been evaluated. Universal precautions should be used when handling a patient's vaccine. All preparation should be performed within a biosafety cabinet.

5.1.8 Vaccine Preparation

Each vaccination consists of up to 4 separate injections, with each syringe containing peptides from one of the up to four peptide pools combined with adjuvant.

- The vaccine should be prepared in a biosafety cabinet and using aseptic handling procedures to prevent contamination. Place the peptide pool vials, poly-ICLC vial, mixing syringes, needles, and dosing syringe into the biosafety cabinet.
- Remove one cryovial of pooled peptides for each of the up to four peptide pools (i.e. Pool A, Pool B, Pool C, Pool D) from the box and allow to thaw and warm to ambient conditions (15°C to 30°C) by placing them at room temperature for 1 to 4 hours. Accelerating aids such as a water bath should not be used. Each 1.5 mL pooled peptide vial contains 400 µg/mL of each peptide in the pool in a solution of 26.7% DMSO/sterile water.
- Remove one vial of the poly-ICLC (adjuvant) from the refrigerated storage and allow it to warm to room temperature for at least 1 hour and a maximum of 4 hours prior to dose preparation. One vial of poly-ICLC will provide enough adjuvant for each of the four peptide pools.
- The peptide pools and poly-ICLC will be mixed by using two connected syringes and passing the suspension back and forth between the syringes for a minimum of 5 full passes (1 pass = back and forth). The suspension will have an opaque white appearance.
    - Gently swirl each thawed peptide pool vial. Using a syringe with a luer-lock dispensing needle withdraw 0.75 mL from the peptide pool vial.
    - Gently swirl the poly-ICLC vial. DO NOT INVERT the poly-ICLC vial. The vial must remain upright and tipped to an approximately 30-degree angle to remove enough adjuvant for each of the four pools. One vial of poly-ICLC will provide enough adjuvant for each of the four peptide pools. Insert a new syringe with a luer-lock 21gauge 1½ inch needle into the vial placing the tip of the needle near the bottom of the vial. Withdraw the poly-ICLC to the 0.25 mL mark on the syringe. An air bubble may be in place between the stopper of the plunger and the 0.25mL mark on the syringe. Please DO NOT attempt to remove the air bubble. This air bubble will be removed during the next mixing step. Removing the air bubble at this step will limit the amount of poly-ICLC which can be removed from the vial.
    - The contents of the two syringes (peptide and poly-ICLC) are connected to a Braun Medical (Bethlehem, PA) Fluid Dispensing Connector, Product code FDC 1000, REF 415080 or FDC

2000, REF 4150810. The peptides and poly-ICLC are mixed by gently transferring the contents back and forth between the syringes, thru the Fluid Dispensing Connector.

- Draw the entire 1 mL into one of the syringes, remove the syringe from the device and attach a 27 gauge 1/2-inch or 5/8-inch stainless steel needle for SC administration.

- Label the syringe to correspond with the peptide pool of the vaccine along with the protocol number, patient name, and ID number.
- Repeat steps for the remaining peptide pool vials and adjuvant. There will be up to four peptide pools as identified on the pooled peptides label. Prepared dosing syringes may be stored at room temperature for up to 6 hours prior to administration.

5.1.9 Route of Administration

Each pool of peptides + poly IC:LC will be administered to one of the four limbs (Pool A – Right Arm, Pool B - Left Arm, Pool C – Right Leg, Pool D – Left Leg) by subcutaneous (SC) injection. In the setting where a limb is unavailable for injection, the injection should be administered to the nearest flank. The same pool should be administered to the same limb across doses.

5.2 Poly-ICLC (Hiltonol)

5.2.1 Poly-ICLC Description

Polyinosinic-Polycytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose (Poly-ICLC, Hiltonol ®) is a synthetic double-stranded ribonucleic acid (dsRNA) 'host-targeted' therapeutic vial-mimic and Pathogen-associated molecular pattern (PAMP) with broad innate and adaptive immune enhancing, vaccine adjuvant, antiviral and antiproliferative effects

5.2.2 Clinical Pharmacology

Poly-ICLC stimulates at least 4 interrelated systems, any of which (alone or in combination) might be responsible for its possible antitumor and antiviral activity; induction of IFN, a broad immune-enhancing effect, direct antiviral/antineoplastic effect, and modulation of gene expression.

Poly-ICLC induces a 'natural mix' of Interferons, cytokines and chemokines, including IFN production. As expected, the amount of these measurable in serum is dose dependent, although biologically significant induction likely occurs locally. In previous studies, the minimal serum IFN levels induced by the currently recommended low doses of Poly-ICLC have not been associated with antitumor or antiviral action. A study of the immunomodulatory effect of Poly-ICLC in cancer patients showed no detectable serum IFN in patients receiving 1 mg/m2 Poly-ICLC by IM injection. In contrast, IFN was detectable in the serum of patients receiving 4 mg/m2 IV Poly-ICLC.

Low-dose Poly-ICLC directly stimulates the immune system through activation of NK cells, myeloid dendritic cells via TLR3 and MDA5, T-cells, macrophages and by inducing a 'natural mix of interferons, cytokines and chemokines. Some of these actions are related to the potent PAMP-adjuvant actions of Poly-ICLC with various vaccine platforms, as well as to the broad antiviral state induced by the drug.

The third action of Poly-ICLC is a more direct broad host-targeted antiviral and perhaps antineoplastic effect mediated by the two IFN-inducible nuclear enzyme systems, the 2'5'-OAS and the P1/eIF2a kinase, also known as the PKR, as well as RIG-I helicase and MDA5. The concentration of the 2'5'-OAS was elevated in the peripheral blood mononuclear cells of all Poly-ICLC-treated patients. Intramuscular administration of low-dose Poly-ICLC increased NK cells and growth inhibitory activities and 2'5'-OAS levels to a greater extent than high-dose IV infusion. Clinically, a maximal OAS response was observed at an IM dose of 30 µg/kg Poly-ICLC, and was greatly decreased at greater than 100 µg/kg in normal volunteers. The hypothesis that OAS and/or PKR may be involved in the antineoplastic effect of Poly-ICLC may thus explain the relative ineffectiveness of high dose Poly-ICLC in early cancer trials. However, further studies are needed to confirm this.

A fourth aspect of the action of Poly-ICLC involves modulation of the expression of a broad range of innate immune and other genes in a pattern closely paralleling that of a live virus vaccine. Some of these genes play critical roles in the body's natural defenses against a variety of neoplasms and microbial

infections, and in controlling other cell functions, including protein synthesis, programmed (apoptotic) cell death, cell metabolism, cellular growth, the cytoskeleton and the extracellular matrix. For example, preliminary studies have confirmed marked clinical elevation of the p56 gene in white blood cells some 24 hours after administration of 20 mcg/kg poly-ICLC in glioma patients. The clinical significance of these findings is not known, although there was no evidence of significant clinical toxicity in these patients

5.2.3 Pharmacokinetics and Drug Metabolism

Poly-ICLC is normally metabolized by a serum endonuclease.

Four monkeys were injected with 1 mg/kg of poly-ICLC and 4 others at 3 mg/kg IV. Serum samples were obtained at 1, 5, 10, and 30 minutes, and at 1, 2, 3, 4, 8, 12, and 24 hours. Poly-ICLC was assayed in loRK cells. Serum poly-ICLC levels had decreased to 5% of peak values 4 hours after injection. There was no change in clearance rate after six additional injections over a two-week period.

Preclinical data on nasal use of poly-ICLC or poly-IC in various murine viral challenge studies is reported in several publications. These indicate a dose-dependent increase in lung and bronchial lavage interferon and TLR3 expression in response to drug alone, independent of viral challenge. Preliminary data from very recent unpublished studies of poly-ICLC applied rectally or nasally to macaque monkeys also confirm induction of various cytokines and chemokines.

Poly-ICLC is not detectable in serum 24 hours after administration. However, the clinical half-life of the OAS response to 30 µg/kg IM poly-ICLC in healthy human volunteers is about 2.5 days, suggesting an optimum dose schedule of two or three times per week.

Twenty-four of 29 patients treated with 10 to 20 µg/kg poly-ICLC by IM injection showed at least a 300% increase in serum OAS. A significant association of serum OAS with tumor response has been observed in patients with malignant glioma participating in an open pilot trial.

5.2.4 Supplier

Poly-ICLC will be supplied by Oncovir, Inc.

5.2.5 Dosage Form and Preparation

Poly-ICLC is supplied by Oncovir in single-dose vials containing 1 mL of 2 mg/mL opalescent white suspension. Each milliliter of poly-ICLC for injection contains 2 mg/mL poly-IC, 1.5 mg/mL poly-L-lysine, and 5 mg/mL sodium carboxymethylcellulose in 0.9% sodium chloride solution and adjusted to pH 7.6-7.8 with sodium hydroxide.

Poly-ICLC is withdrawn from the vial under sterile conditions as detailed above.

5.2.6 Storage and Stability

Poly-ICLC is stable at room temperature for brief periods (days). It is normally refrigerated at about 40ºF (2-8°C) but should not be frozen

5.2.7 Administration

Poly-ICLC will be mixed with the synthetic long peptide vaccines as detailed above.

5.3 Nonclinical studies

5.3.1 Overview

We propose a phase 1 clinical trial of a neoantigen peptide vaccine strategy. The neoantigen peptide 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 peptide 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 peptide 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.

5.3.2 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 [34-41].

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*, <u>prophylactic</u> 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).

5.3.3 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 peptide vaccine strategy.

<u>First, it is impossible to know *a priori* what mutations will be present and/or prioritized in patients.</u> 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 peptide 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.

<u>Second, to our knowledge, no pancreatic cancer models exist that would be relevant for the proposed preclinical studies.</u> 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 peptide 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.

5.4 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.

Additional details are included in the “Sequencing Pipeline” section of IND 16509.

5.5 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, 34-41]. 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) [42], (ii) Artificial Neural Network (ANN) [43], and (iii) NetMHCpan [44].

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 [45] (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.

Additional details are included in the “Epitope Prediction Algorithm” section of IND 16509.

6 TREATMENT PLAN

6.1 Peptide Vaccine 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. Patients will initiate gemcitabine/capecitabine or comparable adjuvant chemotherapy no more than 16 weeks following resection. Adjuvant chemotherapy will be administered for a total of six cycles 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 Days 1, 4, 8, 15, and 22 (delays of up to 96 hours are allowed for each dose based on the AEs experienced). Additional vaccinations will be given on Days 50 and 78 (+/- 2 weeks). The first vaccine dose may be administered following confirmation of disease-free status and within 90 days following date of repeat imaging. All study injections will be given subcutaneously and co-administered with poly-ICLC by a trained healthcare provider. At each vaccination time point, patients will receive up to four injections of the neoantigen peptide vaccine + adjuvant. The peptides and poly-ICLC will be mixed prior to 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. Please refer to Section 5.10 for Vaccine preparation instructions.

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.

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 4 weeks following last injection (± 7 days) and 1 year following last injection (± 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 Concomitant Medication Guidelines

Patients may receive other vaccinations while on study, including influenza and pneumococcal.

Patients will be treated for toxicities at the discretion of the physician. Growth factor support with either filgrastim or pegfilgrastim is at the discretion of the investigator.

There are no prohibited medications.

6.3 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).

6.4 Duration of therapy

In the absence of treatment delays due to adverse events, treatment may continue for 7 doses of neoantigen peptide 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.

6.5 Duration of follow up

Patients will examined at 4 and 52 weeks following the final vaccination. 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 Potential toxicity

7.1.1 Potential toxicity related to the neoantigen peptide vaccine

This is the one of the first times that mutant tumor-specific antigens identified by next generation sequencing have been targeted for immune therapy in humans, and one of the first times that personalized synthetic long peptide vaccines have been administered to humans. However, clinical trials of similar investigational peptide 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 site of vaccination.

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 subcutaneous injections include acute bleeding and/or bruising. Although highly unlikely, intradermal injection can result in injection site infection. As with any immunization, discomfort or redness at the injection site in the days following vaccine administration may be expected.

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.

7.2 Toxicity monitoring and management

Toxicity will be characterized according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (CTCAE). Subjects who are immunized with the peptide vaccine will be evaluated at the time of each vaccination on days 1, 4, 8, 15, 22, 50 and 78. 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.

7.3 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 screening procedures is up to 28 days prior to enrollment. Enrollment must occur within 28 days of the patient starting on adjuvant therapy. The window for Step 1 screening procedures is 14 days prior to first vaccine dose.

	Step 0 Screening	Step 1 Screening	Day 1	Day 4[f]	Day8[f]	Day15[f]	Day 22[f]	Day 50 (+/-2wk)	Day 78 (+/-2wk)	EOT	F/U[h]
Neoantigen peptide vaccine[a,i]			X	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	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		
CMP[b]	X	X	X				X	X	X		
INR, PTT	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	X	
B-HCG	X[e]	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[j]
Pathology Review[d]	X										
Eligibility Confirmation	X	X									

a: Personalized synthetic long peptide pancreatic cancer 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 seven 10mL green top heparin tubes for isolation of PBMCs. During adjuvant therapy, the mid and end of chemo draws are optional. Generally, 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: +/- 1 days with at least 24 hours between vaccinations

g: Adverse event evaluation on day of vaccine

h: ± 28 days; follow-up or telephone contact at 4 weeks and 1 year after last injection and annually thereafter if the patient is alive and available for follow-up. F/U visits may be done at the participant's local MD office.

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

j: optional based on patient availability

9 CRITERIA FOR RESPONSE

9.1 Primary objective: safety

Assessment of synthetic long peptide 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 v5.0.

9.2 Secondary objectives: evaluation of the immune response

9.2.1 Introduction to immune monitoring

The secondary endpoint is to evaluate the immunogenicity of the neoantigen peptide vaccine. Immunogenicity will be measured by ELISPOT analysis, a surrogate for CD4 and CD8 T cell function, and 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 enrollment, 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 viability is confirmed, specimens from outside Washington University will be shipped to the Gillanders lab at the following address: Gillanders Lab, 425 S. Euclid Ave. CSRB 3339, 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 to the Gillanders lab at the following address: Gillanders Lab, 425 S. Euclid Ave. CSRB 3339, St. Louis, MO 63110. Additional optional pre-vaccine blood samples will be obtained midway through chemotherapy and at the end of chemotherapy. Immune monitoring labs will be draw prior to vaccination on Days 1, 22, 50, and 78. Post- vaccine immune monitoring labs will be drawn at the first two follow-up time points (4 weeks after last injection and 1 year after last injection). Seven collection tubes (BD Vacutainer® sodium heparin (green top), REF 367874, 10 mL each for a total of approximately 70 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 3339) 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 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

ELISPOT analysis, will be performed as previously described [46-50]. 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

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 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 [51, 52]. 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. Due to the high recurrence rate associated with pancreas cancer, it is expected that some percentage of patients will recur during the period of vaccine production and administration. Due to the poor response to traditional chemotherapeutics, patients who recur or develop metastatic disease may still benefit from vaccine administration, vaccine administration in combination with chemotherapy, and/or checkpoint blockade. The decision to continue or discontinue vaccine administration will be left to the treating physician.

(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 excluding priming vaccine on Days 1, 4, 8, and 15, where dose delays are permitted per Section 6);

(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 for 30 days after last injection, and thereafter only report treatment related SAEs or AEs.

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 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Section 20.4 for definitions and Section 20.6 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 form

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 11.2

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 any 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 the FDA

The conduct of the study will comply with all FDA safety 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 Section 20.4 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 Section 20.4) no later than 15 calendar days after it is determined that the information qualifies for reporting. Report an adverse event (refer to Section 20.4) 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”).

11.1.4 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 Section 20.4, 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, CSBio, and Oncovir 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 11.1 include:

- planned hospitalizations
- hospitalization <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 University 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 six months beginning six months 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
- Power analysis and/or interim analysis
- 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 DSMB. This is located on the QASMC website at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

12.1 Developmental therapeutics

Given the nature of this human gene transfer protocol, the principal investigator will monitor and analyze study data as they become available and will review this data on a monthly basis with the Developmental Therapeutics Group at the Siteman Cancer Center. This is an independent group that will provide more rigorous oversight than is routinely provided by the QASMC and the HRPO. The director of the Developmental Therapeutics group will advise Dr. Gillanders in the proper conduct of this study and will review patient treatment and all toxicities in her weekly Phase 1 meeting. In addition, the director of the Developmental Therapeutics group will assist in the oversight of the regulatory and data management personnel involved in this clinical trial.

12.2 Protocol stopping criteria

The principal investigator will closely monitor and analyze study data as they become available and will make determinations regarding the presence and severity of adverse events. The administration of study injections and new enrollments will be halted and the QASMC promptly notified if any of the following events occurs:

(1) **One** (or more) subject(s) experiences a Grade 3 or 4 adverse event that is classified as probably or definitely related to vaccination;

(2) **One** (or more) subject(s) experiences a vaccine-related SAE;

(3) **One** (or more) subject(s) experiences death (other than death related to progressive disease) that occurs within 30 days of neoantigen vaccine administration;

(4) **Two** (or more) subjects experience the **same** Grade 2 or higher adverse event that is classified as probably or definitely related to vaccination: this criterion applies to fever, vomiting, laboratory abnormalities or other clinical adverse experiences, but does not apply to the subjective local or systemic symptoms of pain/tenderness, malaise, fatigue, headache, chills, nausea, myalgia, or arthralgia.

(5) Any other observation occurs that in the opinion of the PI results in a recommendation to halt enrollment.

If one of these events does occur, study injections and study enrollments would only resume if review of the adverse events that caused the halt resulted in a recommendation to permit further study injections and study enrollments.

The QASMC, in consultation with the principal investigator, will conduct any review and make the decision to resume or close the study for any Grade 2 or 3 events leading to a halt in the study.

The QASMC, with participation by the principal investigator, will consult with the FDA to conduct the review and make the decision to resume or close the study for all Grade 4 adverse events leading to a halt in the study.

13 STATISTICAL CONSIDERATIONS

13.1 Experimental Design

This is a phase 1, open-label, trial of a neoantigen peptide 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 peptide vaccines, and the neoantigen peptide vaccines will be designed and manufactured. The neoantigen peptide vaccines will be administered starting two to four weeks after the completion of adjuvant chemotherapy. The neoantigen peptide vaccines will be administered at seven time points, Days 1, 4, 8, 15, 22, 50, and 78.

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 [32]. 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 [32]. 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

One of the primary objectives of this study is to evaluate the safety of the neoantigen peptide 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 peptide vaccines.

Probability of detecting SAE for different safety scenarios if 15 evaluable patients are enrolled

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 flow cytometry and ELISPOT. A power analysis was performed using paired t-test for over time differences (e.g., changes in ELISPOT and multi-parameter flow cytometry). 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 peptide 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 5.0.

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 objective: immune response as measured by ELISPOT analysis and multiparametric flow cytometry

Immune response as measured by ELISPOT analysis and multiparametric flow cytometry is the secondary 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 peptide 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 [50]. 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 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 [53].

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 forms with appropriate source documentation will be completed according to the schedule listed in this section.

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 Day 1 Day 4 Day 8 Day 15 Day 22 Day 50 Day 78 4 weeks after last injection 1 year after last injection
Vaccine Administration Form	Day 1 Day 4 Day 8 Day 15 Day 22 Day 50 Day 78
Immune Monitoring Form	Enrollment Mid chemo End of chemo Day 1 Day 22 Day 50 Day 78
Imaging Form	Mid chemo End of chemo
Toxicity Form	Day 1 Day 4 Day 8 Day 15 Day 22 Day 50 Day 78
Concomitant Medications Form	Continuous through Week 25
Follow Up Form	4 weeks after last injection 1 year after last injection Year 2 and annually thereafter
Death Form	Time of death
Progression Form	Time of disease progression
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 captures 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 DSMB (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. Amanda Cashen, Chair 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.

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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 v5.0 (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 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 Reporting Timelines

Expedited Reporting Timelines			
Event	**HRPO**	**QASMC**	**FDA**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting
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 deviations that are being reported as a continuing noncompliance	Report within 10 working days.		
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 withdrawn, report at continuing review.		

Routine Reporting Timelines			
Event	**HRPO**	**QASMC**	**FDA**
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**
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.
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 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**