NEOANTIGEN VACCINES IN PANCREATIC CANCER IN THE WINDOW PRIOR TO SURGERY

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SUMMARY

Protocol: **NEOANTIGEN VACCINES IN PANCREATIC CANCER IN THE WINDOW PRIOR TO SURGERY**

Study Design: This is a randomized phase 1 clinical trial to evaluate the safety of optimized neoantigen synthetic long peptide (SLP) vaccines in pancreatic cancer patients following neoadjuvant chemotherapy. The neoantigen SLP vaccines will incorporate prioritized neoantigens and will be co-administered with poly-ICLC. Patients will be randomized to one of two arms: Arm 1 (neoantigen vaccine following neoadjuvant chemotherapy and surgery) or Arm 2 (neoantigen vaccine following neoadjuvant chemotherapy in the window prior to surgery). The primary objective of this study is to demonstrate the safety of the optimized neoantigen SLP vaccine strategy in the neoadjuvant/adjuvant setting. The secondary objective is to assess the number and phenotype of neoantigen-specific T cells in the peripheral blood of patients pre- and post-vaccination, as measured by ELISpot. Exploratory objectives are focused on the phenotype and function of neoantigen-specific T cells in the peripheral blood as measured by CyTOF analysis and tetramer analysis, and the phenotype and function of neoantigen-specific T cells in the tumor as measured by coupled scRNA-seq/TCR-seq and CODEX.

Product Description: The neoantigen SLP vaccines are composed of synthetic long peptides which are 25-30 amino acids in length and designed to express cancer neoantigens identified by tumor/normal exome sequencing. Synthetic long peptides are processed by the immune system differently than minimal peptide vaccines, enhancing T cell activation. Peptide vaccines will be administered intramuscularly and will be co-administered with poly-ICLC.

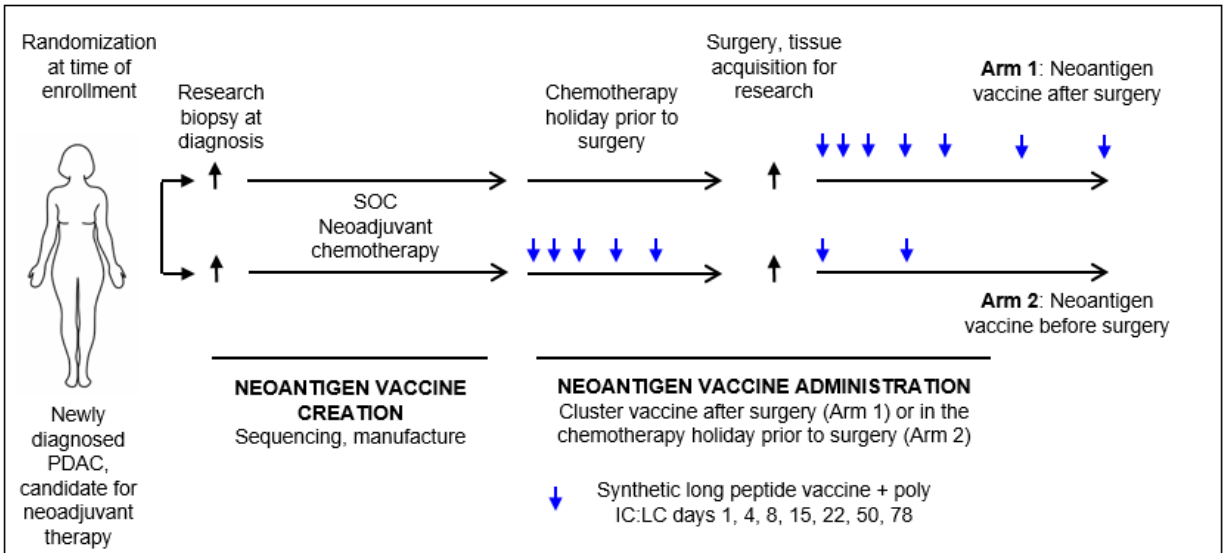
Subjects: Newly diagnosed pancreatic cancer patients who have localized disease and are candidates for neoadjuvant chemotherapy are eligible. Patients enrolled into the protocol must provide consent for exome sequencing and dbGAP-based data sharing and provide germline and tumor DNA samples of adequate quality for sequencing.

Study Plan: 36 patients will be enrolled. Subjects will be consented and enrolled around the time the patients start of standard of care chemotherapy in order to begin the vaccine creation process. Subjects will be randomized to either Arm 1 (n=18, vaccine given after neoadjuvant chemotherapy and surgery) or Arm 2 (n=18, vaccine given after neoadjuvant chemotherapy but before surgery). Subjects randomized to both arms will have a neoantigen peptide vaccine manufactured during standard of care neoadjuvant chemotherapy (given outside of this protocol). Once the vaccine is ready, subjects will receive 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 intramuscularly on Days 1, 4, 8, 15, and 22, 50 and 78. For patients in Arm 1, Day 1 will be 1 month after surgery. For patients in Arm 2, Day 1 will be 1 week after end of chemotherapy. Patients whose disease progresses or recurs during neoadjuvant chemotherapy and patients who are otherwise unable or unwilling to complete a surgical resection after sufficient progress has been made on the vaccine may still receive treatment with the peptide vaccine at the discretion of the treating physician and study PI. The patients will be considered evaluable for the primary endpoint of safety.

Study Endpoints: The primary endpoint is safety of the neoantigen SLP vaccine. Safety will be closely monitored throughout the trial. Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events version 5.0. The

secondary endpoint is the impact of neoantigen vaccine on the number and phenotype of neoantigen-specific T cells as measured by ELISPOT.

SCHEMA



Schematic of phase 1 clinical trial design. Patients with newly diagnosed PDAC who are candidates for neoadjuvant chemotherapy are eligible for enrollment. Subjects will be randomized at the time of enrollment, to either neoantigen vaccine following surgery (Arm 1), or neoantigen vaccine in the chemotherapy holiday prior to surgery (Arm 2). Subjects will complete standard of care neoadjuvant chemotherapy during vaccine manufacture. Following neoadjuvant chemotherapy, subjects will be treated with a neoantigen vaccine following surgery (Arm 1), or neoantigen vaccine in the chemotherapy holiday prior to surgery (Arm 2). Subjects in Arm 2 will also receive additional vaccines following surgery.

A total of 36 subjects will be enrolled in this study. We anticipate that there will be 18 evaluable subjects. The randomization will be performed upfront at the initiation of neoadjuvant chemotherapy, and subjects will be randomized to either Arm 1 (n=18 randomized, anticipate 9 evaluable subjects) or Arm 2 (n=18 randomized, anticipate 9 evaluable subjects). Patients whose disease progresses or recurs during neoadjuvant chemotherapy or who are otherwise unable to complete a surgical resection after sufficient progress has been made on the vaccine production may still receive treatment with the peptide vaccine at the discretion of the treating physician and study PI.

PROTOCOL SIGNATURE PAGE

NEOANTIGEN VACCINES IN PANCREATIC CANCER IN THE WINDOW PRIOR TO SURGERY

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

1.1 Primary objective

The primary objective of the phase 1 clinical trial is to assess the safety of optimized neoantigen SLP vaccines in patients with pancreatic cancer following neoadjuvant chemotherapy. Safety assessment will include both clinical observation and laboratory evaluation.

1.2 Secondary objective

The secondary objective of the trial is to assess the number and phenotype of neoantigen-specific T cells in the peripheral blood of patients pre- and post-vaccination, as measured by ELISPOT.

1.3 Exploratory objectives

Exploratory objectives are focused on the phenotype and function of neoantigen-specific T cells in the peripheral blood as measured by CyTOF analysis and tetramer analysis, and the phenotype and function of neoantigen-specific T cells in the tumor as measured by coupled scRNA-seq/TCR-seq and CODEX.

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 Introduction to cancer neoantigens

Cancer 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 [11, 12]). As such, cancer neoantigens are unique to the tumor and not expressed in normal tissues.

Next-generation sequencing has revolutionized our ability to identify cancer neoantigens. In initial studies, we demonstrated that (1) cancer neoantigens are important targets of cancer immunoediting (*Nature*, 2012; cited > 1000 times) [13], and (2) the success of immune checkpoint inhibition (ICI) is related to the ability of ICI to activate neoantigen-specific T cell responses (*Nature*, 2014; cited > 1500 times) [14]. Human translational studies confirm that cancer neoantigens are important targets of ICI. A strong correlation between tumor mutational burden (TMB) and clinical benefit has been established in melanoma [15, 16], NSCLC [17], and colorectal cancer [18].

Using preclinical models, we and others demonstrated that cancer neoantigens can be successfully targeted with neoantigen vaccine strategies [14, 19-23].

In human translational studies, the first report of a neoantigen vaccine demonstrated that dendritic cell (DC) vaccines are capable of generating neoantigen-specific T cell responses in human melanoma patients [24]. Two additional papers co-published in *Nature* by Ott et al. [25] and Sahin et al. [26] confirmed the potential of neoantigen vaccines. These initial studies employed strategies similar to ours to identify cancer neoantigens using tumor/normal exome sequencing.

We recently developed novel algorithms for identification of class II neoantigens, demonstrating the critical importance of class II neoantigens to the success of neoantigen vaccines (*Nature*, 2019; cited > 250 times) [20]. We have now integrated these novel class II prediction algorithms into the pVACtools software suite [27-31]. This clinical trial represents the first-in-human study of optimized neoantigen vaccines integrating both class I and II neoantigens.

2.5 Neoantigen 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 [32] 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 [33, 34]. Synthetic long peptide vaccines have proven to be both safe and remarkably effective in clinical translation [35]. 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 [35].

Neoantigen synthetic long peptide vaccines are capable of inducing neoantigen-specific immune responses [25, 36-38]. In the largest study to date, neoantigen synthetic long peptide vaccines were associated with (1) durable neoantigen-specific immune responses, (2) induction of neoantigen-specific cytotoxic T cells with the ability to traffic to the tumor, and (3) induction of epitope spreading consistent with vaccine-mediated tumor cytotoxicity [38].

Neoantigen synthetic long peptide vaccines have a remarkable safety profile in early phase clinical trials. The most common adverse events are injection-site reactions and influenza-like illness occurring in approximately 50% and 35% of patients, respectively. Injection site reactions typically include localized warmth, and erythema. Most adverse events observed have been mild (CTCAE grade 1).

2.6 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" [39].

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

2.7 Neoantigen prioritization

Next-generation sequencing now provides robust technologies to identify genetic alterations present in individual patient's cancer and candidate neoantigens. As several thousand mutations may be present in an individual cancer, algorithms are required to prioritize mutations for inclusion in neoantigen vaccines. The pVACtools suite of software tools will be used to identify and prioritize cancer neoantigens in this protocol [27, 28].

The first step to prioritize cancer neoantigens is to confirm expression of the genetic alterations in the tumor. Up to 40% of the genetic alterations identified by exome sequencing are not expressed due to

early termination of transcripts or other reasons. Expression of the genetic alteration at the mRNA level can be confirmed by sequencing tumor mRNA (cDNA-capture or bulk RNA sequencing). The second step to prioritize neoantigens is to estimate potential binding to the appropriate HLA alleles. For this, computer-based algorithms that quantitate the predicted binding affinity of candidate epitopes to class I HLA alleles (such as http://www.iedb.org/ [40]) can be used. The pVACtools suite of software tools integrates multiple binding affinity algorithms.

To date, algorithms for neoantigen identification have focused on class I neoantigens, and neoantigen vaccine trials have focused primarily on targeting class I neoantigens. Part of the issue is that current class II binding prediction tools are inaccurate, partially because the open structure of the class II binding groove leads to substantial variation in epitope length. We recently developed a hidden Markov model-based class II binding prediction tool that inherently accommodates peptide sequences of variable length and is trained on recent IEDB content [20]. We then developed algorithms for class II neoantigen prioritization based on the hidden Markov binding prediction tool, mutant:wild-type binding ratios, and transcript abundance. Using these algorithms, we demonstrated that optimized neoantigen vaccines integrating both class I and class II epitopes increase the number of activated neoantigen-specific CD8 and CD4 T cells in the tumor, and the reciprocal cross-stimulation facilitates tumor rejection [20]. We have now integrated these class II algorithms into pVACtools, and the proposed trial represents the first-in-human evaluation of optimized neoantigen vaccines

The pVACtools neoantigen prediction algorithms are described in detail in an Appendix to this protocol and in the IND application.

3 PATIENT SELECTION

3.1 Step 0 Inclusion criteria

A patient will be eligible for evaluation and sequencing of tissue for vaccine development only if ALL of the following criteria apply:

1. Histologically or cytologically confirmed, newly diagnosed, treatment-naïve patients with localized or borderline resectable adenocarcinoma of the pancreas for whom neoadjuvant chemotherapy is considered appropriate; mixed histology (including adenosquamous) will be allowed. Patients with clinical suspicion of pancreatic adenocarcinoma can be enrolled for pre-treatment biopsy, and must be histologically confirmed to have adenocarcinoma before being treated on study. Patients with squamous carcinoma or neuroendocrine tumor will be excluded.
2. Evaluable disease, in the opinion of the treating investigator or PI.
3. Tissue specimens available in sufficient quantity to allow for sequencing.
4. At least 18 years of age.
5. Life expectancy of > 12 months.
6. ECOG performance status ≤ 1
7. Adequate bone marrow and organ function as defined below:

a.	WBC	≥1.5 K/cumm
b.	absolute neutrophil count	≥1.0 K/cumm
c.	platelets	≥50 K/cumm
d.	hemoglobin	≥ 8.0 g/dL
e.	total bilirubin	≤5.0 X institutional upper limit of normal
f.	AST/ALT	≤5.0 X institutional upper limit of normal
g.	creatinine OR creatinine clearance	≤2.5 X institutional upper limit of normal ≥ 30 mL/min by Cockcroft-Gault for patients with creatinine levels above institutional normal

Note: labs can be repeated prior to chemo if needed.
Note: Patients who have had a stent placed for biliary obstruction and whose liver function is expected to improve may enroll provided serum bilirubin at time of enrollment is within the limits above.

8. International Normalized Ratio (INR) and activated partial thromboplastin time (PTT) < 2.0 x ULN provided the patient is not on anticoagulation therapy.
9. Women of childbearing potential and men must agree to use two forms of adequate contraception (see Section 6.5) prior to study entry, for the duration of study participation, and for 3 months after completion of the study. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.
10. Able to understand and willing to sign an IRB approved written informed consent document.

3.2 Step 0 Exclusion criteria

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

1. Evidence of predominantly neuroendocrine (defined by > 50% histology) tumor, pure neuroendocrine tumor, duodenal adenocarcinoma, or ampullary adenocarcinoma.
2. 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.
3. Receiving any other investigational agents, or planning to receive other investigational agents as

part of neoadjuvant therapy.

4. Known allergy, or history of serious adverse reaction to vaccines such as anaphylaxis, hives, or respiratory difficulty.
5. Acute or chronic, clinically significant hematologic, pulmonary, cardiovascular, hepatic renal, and/or other functional abnormality that might 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. If needed and appropriate. the final determination related to study eligibility prior to the administration of the first vaccine will be documented by both the PI and Sub-Investigators in consultation with a specialist.
6. 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.
7. 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. Premedication for chemotherapy does not apply to this criteria and may be administered as per SOC practice. Any patients receiving steroids should be discussed with the PI to determine if eligible.
8. Pregnant and/or breastfeeding.
9. Known HIV-positive status.
10. History of positive test for Hepatitis B virus surface antigen (HBsAg) and/or positive Hepatitis C antibody result with detectable hepatitis C virus (HCV) ribonucleic acid (RNA) indicating acute or chronic infection.

3.3 Step 1 eligibility

At Step 1 eligibility confirmation prior to vaccination, the above criteria in Sections 3.1 and 3.2 must continue to be met plus:

1. Completed at least 4 months of neoadjuvant chemotherapy such as FOLFIRINOX, modified FOLFIRINOX, or gemcitabine + nab-paclitaxel. Dose modifications and/or delays in neoadjuvant chemotherapy may be made at the discretion of the treating physician.
2. Reimaging within 4 weeks of last dose of chemotherapy demonstrates no evidence of progressive disease. Patients who progress on mFOLFIRINOX and transition to gemcitabine + nab-paclitaxel may still be eligible for vaccine administration at discretion of PI and treating MD provided they do not show progression following completion of chemotherapy and the patient continues to be eligible for surgical resection. Patients who, in the opinion of the treating physician, require SBRT prior to surgery will receive vaccine after surgery regardless of randomization.
    a. Patients who progress or recur following neoadjuvant chemotherapy or who are otherwise ineligible or unable to complete a surgical resection, but who still meet other Step 1 criteria, may still be eligible for vaccine administration with documented treating physician and PI approval.
3. There is a 1 week washout prior to Day 1 of vaccine for patients on daily systemic steroids at doses exceeding 10 mg prednisone.

4. Must not be receiving or have received any other investigational agents within the last 30 days. Note that patients who are receiving or will be receiving adjuvant chemotherapy are permitted to continue on study and are considered eligible.
5. Patients may not have received a live vaccine within 30 days prior to the first day of treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette-Guerin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g. FluMist) are live attenuated vaccines and are not allowed.
6. Sufficient wound healing per the evaluation of the treating physician.

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 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 determined to be a potential candidate for study, the principal investigator (or co-investigators) will discuss the Washington University IRB-approved informed consent with the patient. This written informed consent will be signed and dated by the patient and a member of the study team delegated to consent. 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 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 shortly after their initial consult with the surgeon or 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 should not knowingly be registered. Adherence of the Siteman 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.

4.2 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 4 weeks of initiation of neoadjuvant chemotherapy; delays beyond 4 weeks are allowed at the discretion of the PI. 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 (Step 0 enrollment will be "On Study" in the OnCore database. The date of the first vaccine treatment will be "On Treatment" in the OnCore database.)

(3) Assignment of unique patient number (UPN)

4.3 Confirmation of patient eligibility

Confirm patient eligibility for both Step 0 and Step 1 by collecting the information listed below:

(1) Registering MD's name

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

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

(4) Copy of signed consent form

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

(6) Copy of appropriate source documentation confirming patient eligibility

4.4 Patient registration in the Siteman Cancer Center OnCore database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.5 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

4.6 Randomization

Subjects will be randomized at time of registration to Step 0 on a 1:1 basis to either Arm 1 (n=18) or Arm 2 (n=18). Subjects randomized to Arm 1 will not receive any treatment during the chemotherapy holiday prior to surgical resection. Subjects randomized to Arm 2 will receive the neoantigen peptide vaccine during the chemotherapy holiday. Patients who are randomized (to either arm) who progress but still continue on to treatment will receive the vaccine as described in Section 6.2.1.

5 INVESTIGATIONAL AGENTS

5.1 Neoantigen synthetic long peptide vaccine

5.1.1 Physical, chemical and biological characteristics

The synthetic long peptide neoantigen 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 and manufactured by a CMO 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 clinical trial.

(2) DNA and RNA will be extracted from representative tissue samples.

(3) Tumor/normal exome sequencing and tumor cDNA-capture sequencing, if feasible, will be performed to identify candidate neoantigens. Candidate neoantigens will be prioritized based on epitope prediction algorithms using the pVACtools suite of software tools.

(4) 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 the CMO.

(5) The neoantigen peptide vaccines will be vialed at Washington University School of Medicine. Please see the CMC section of the IND for complete details.

Neoantigen vaccines will be provided on a patient-specific basis. Each patient specific peptide pool vial label and secondary package label will contain the protocol number, subject ID number, and initials.

Neoantigen vaccine supplies MAY NOT be transferred from one study subject to another.

Up to four peptide pools will be available for each subject. Patient-specific vials for each peptide pool (Pools A, B, C, and D) will be in separate boxes for each subject.

Peptides will be supplied in 1.8 mL cryovials sufficient for at least 7 doses, with one cryovial per pool planned for use at each injection time point. Each cryovial will contain 1.5 mL of pooled peptide dissolved in a 26.7% DMSO/sterile water solution (final peptide concentration is 400 µg/mL of each peptide in 26.7% DMSO).

The personalized synthetic long peptide will be synthesized by a CMO as detailed below.

5.1.2 Storage conditions

The vaccine study drug will be supplied by a CMO. The vaccine study drug will be received by designated personnel in the Biologic Therapy Core Facility or Siteman Cancer Center Investigational Pharmacy at Washington University School of Medicine, handled and stored safely and properly, and kept in a secure, limited-access location. The vaccine study drug may be dispensed only by the Investigator or designated personnel specifically authorized by the Investigator.

The synthetic long peptide vials will be stored frozen at -75ºC +/- 10°C . Do not remove vials from the peptide pool box until dose preparation.

The investigator will be notified of any expiry or retest date extension of clinical study material during the study conduct. Changes to retest dates will be communicated via memo from WUSM, Oncovir, or a designee. The retest dates of the study drug 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.

5.1.3 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. Prior to mixing, verify that the patient ID number on the vial and the patient ID number on the patient label match.

- 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 syringes 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 mcg/mL of each peptide in the pool in a solution of 26.7% DMSO/5 mM Sodium Succinate Hexahydrate in Dextrose 5% in water (C S Bio).
- Remove one vial of the poly-ICLC (adjuvant) from the refrigerated storage, remove from box so that air can circulate around 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. The suspension will have an opalescent 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 21 gauge 1 ½ inch needle into the poly-ICLC 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 0.25 mL 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 for a minimum of 5 full passes (1 pass = back and forth).
    - Draw the entire 1 mL into one of the syringes, remove the syringe from the device and attach a 25 gauge x 1 inch stainless steel needle for IM 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.

5.2 Sequencing pipeline

Robust next-generation sequencing strategies for the identification of somatic genetic alterations present in cancers will be required for the successful clinical translation of cancer neoantigen 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 genetic alterations and validate the expression of genetic alterations at the mRNA level.

Key collaborators on this project are Dr. David Spencer and Dr. Malachi Griffith. Dr. Spencer is the Medical Director of the CAP-accredited, CLIA-licensed Clinical Laboratory Environment (CLE) at the McDonnell Genome Institute at WUSM and has designed and validated the sequencing assay that will be used. Dr. Griffith is an expert in bioinformatics and cancer genomics and a key contributor to the development of these genomic analysis pipelines. The McDonnell Genome Institute has published seminal papers describing somatic mutations present in tumors, providing significant insights into the biology of AML [41, 42], breast cancer [43, 44], and dozens of other tumor types. Spencer and Griffith and others in the Bioinformatics and Genome Analysis Group at the McDonnell Genome Institute have developed and optimized the sequencing and data analysis pipelines that will be used to identify expressed mutations in subjects' cancers.

Detailed SOPs are included in the IND application.

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

Exome sequences from tumor and normal DNA are compared separately to the human reference sequence and then to one another to identify somatic variation using a suite of computational tools, including Mutect, Varscan, Strelka, and Pindel, followed by a custom filtering approach to remove false positive variant calls.

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

The third step in the sequencing pipeline is data analysis to identify the expressed somatic mutations. RNA or cDNA-capture sequencing data will be used to quantify not only the expression of each gene, but the expression level of each specific mutation. This will be performed using algorithms such as Kallisto and HISAT [45, 46].

5.3 Epitope prediction algorithm

We have developed and optimized an epitope prediction algorithm for the identification and prioritization of mutant tumor-specific antigens called pVAC-seq. This optimized epitope prediction algorithm is described below and in the Hundal 2016 manuscript [27]. pVAC-seq is part of a broader computational toolkit, pVACtools, that includes different tools needed for neoantigen characterization and vaccine design. We have established this algorithm is collaboration with Dr. Robert Schreiber, an internationally-known expert in tumor immunology [13, 47-54]. Schreiber was one of the first to use next generation sequencing technologies to identify mutant tumor-specific antigens, demonstrating that these antigens are important tumor rejection antigens [13]. Our team has now optimized the epitope prediction algorithm and have demonstrated that cancer vaccines targeting mutant tumor-specific antigens are associated with antitumor immunity [14]. In follow-up studies, we developed novel algorithms for identification of class II neoantigens, demonstrating the critical importance of class II neoantigens to the success of neoantigen vaccines (*Nature*, 2019; cited > 250 times) [20]. We have now integrated these novel class II prediction algorithms into the pVACtools software suite [27-31].

The goal of the epitope prediction algorithm is to identify and prioritize mutant tumor-specific antigens. The algorithm uses a combination of binding and antigen processing algorithms.

Detailed SOPs are included in the IND application.

The predicted amino acid sequences corresponding to the expressed mutations will then 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) and other publically available databases including: (i) Stabilized Matrix Method (SMM) [55], (ii) NetMHC/Artificial Neural Network (ANN) [56], (iii) NetMHCpan [57], (iv) MHCflurry [58], and (v) MHCnuggets [59].

Each peptide's "combined binding score" will be defined as the median binding affinity score from all algorithms to each peptide for each allele. In some cases, especially on rare HLA alleles with little training data, there can be substantial disagreement between prediction algorithms. By using the median score, we essentially require that several of the predictors agree, and increase our confidence in the results.

For the 38 most common HLA-A and HLA-B alleles, we will use recommended binding affinity (ic50) cutoffs, as published by the IEDB consortium [15]. For HLA-C alleles, we will use an ic50 value of less than 500nM and for MHC Class II we will use a value of < 1000nM as recommended by the same source.

Initial prioritization of candidate neoantigens will be performed by creating a condensed list, where each mutation is assigned the best (lowest) binding affinity score over all peptide lengths and alleles (MHC class I and class II). This table will also integrate the following information: readcount and Variant Allele Frequency (VAF) of each mutation in both the DNA (copy-number corrected) and RNA, Expression level of the gene (TPM value via Kallisto), the fold-change in binding between the wild-type and mutant peptides, and the predicted mutation position.

Each of the following 4 criteria will be assigned a rank-ordered value (1 = worst)

B = binding affinity

F = Fold Change between MT and WT alleles

M = mutant allele expression, calculated as (geneCPM * MT_allele_RNA_VAF)

D = DNA VAF

Then a final ranking will be based on a score obtained from combining these values: Priority Score = B+F+(M*2)+(D/2). This formula codifies our understanding of the relative importance of the factors and has good concordance with manually ranked variants from our clinical and genomics working group. Additionally, minimum criteria for inclusion will be set as follows:

- A binding affinity score below the recommended cutoff for that allele

- Presence in the founding clone or a significant percentage of cells of the tumor

- Observed expression of the gene and mutant allele in the RNA

If the ranked, filtered list contains more peptides than can be accommodated in a vaccine, we will select the highest ranked peptides. In cases where RNA quality is poor and expression of most genes and mutant alleles cannot be confirmed, we may include peptides without evidence of expression, if the other criteria are met. In cases where fewer peptides are identified than desired for the vaccine, we may relax binding thresholds slightly to include peptides that were up to twice the suggested binding score, provided other criteria are met. We will also consider mutation position in the selection process.

A panel that includes the clinical and genomics investigators meets regularly to review the sequencing and neoantigen prediction data and will make the final decision and document which candidate neoantigen peptides are selected for inclusion in the neoantigen vaccines.

5.4 Poly-ICLC (Hiltonol)

5.4.1 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.

5.4.2 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

5.4.3 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.

5.4.4 Supplier

Poly-ICLC will be supplied by Oncovir, Inc.

5.4.5 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.

5.4.6 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

5.4.7 Administration

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

6 TREATMENT PLAN

6.1 Standard of Care Neoadjuvant Chemotherapy Administration

Patients will receive standard of care neoadjuvant chemotherapy according to institutional practice either at Siteman Cancer Center or an outside cancer center. This protocol does not dictate the neoadjuvant chemotherapy administration.

Neoadjuvant chemotherapy may include mFOLFIRINOX according to institutional practice, including administration of pre-medications. Standard dosing at our site is as follows:

- Oxaliplatin at a dose of 85 mg/m^2 intravenously over 2 hours
- Leucovorin at a dose of 400 mg/m^2 intravenously over 2 hours
- Irinotecan at a dose of 150 mg/m^2 intravenously over 90 minutes (starting 30 minutes after the start of the leucovorin infusion)
- 5-FU at a dose of 2400 mg/m^2 continuous intravenous infusion over 46 hours

This is repeated on Day 1 of each 14-day cycle for 8 cycles (16 weeks in the absence of dosing delays). Delays, dose modifications, omission of certain agent such as irinotecan or oxaliplatin due to intolerability or adverse reactions, and deviations can occur at the discretion of the treating physician.

Should a patient progress on mFOLFIRINOX or experience unacceptable toxicity after adjustments by the treating physician, the patient may transition to another standard of care neoadjuvant chemotherapy regimen, such as treatment with gemcitabine + nab-paclitaxel per institutional practice. Standard dosing at our site is as follows:

- Gemcitabine at a dose of 1000 mg/m^2 intravenously over 30 minutes
- Nab-paclitaxel at a dose of 125 mg/m^2 intravenously over 30-40 minutes

This is repeated on Days 1, 8, and 15 of each 28-day cycle (up to 16 weeks total for mFOLFIRINOX followed by gemcitabine + nab-paclitaxel in the absence of dosing delays). Delays, dose modifications and deviations can occur at the discretion of the treating physician.

Up to 4 additional weeks of either standard of care mFOLFIRINOX or gemcitabine + nab-paclitaxel may be given for patients randomized to Arm 2 whose vaccine is not yet available after completion of neoadjuvant chemotherapy. If it is not possible for Arm 2 patients to receive additional chemotherapy, they may initiate vaccination on the Arm 1 schedule (with Day 1 occurring post-operatively rather than during the chemotherapy holiday) at the discretion of the treating physician if the physician is convinced that additional chemotherapy will not compromise care and is within institutional treatment norms.

6.2 Peptide Vaccine Administration

Vaccine administration will occur on an outpatient basis in the Siteman Cancer Center.

For patients randomized to Arm 1, the schedule for vaccination will be Days 1, 4, 8, 15, 22, 50, and 78, with Day 1 being approximately 1 month after surgery. Patients are typically seen post-op about 4 weeks after discharge. Optimal timing for Day 1 is 1 month after surgery, but it may occur up to 12 weeks after surgery depending on length of adequate recovery needed following surgery or other unforeseen vaccine manufacturing or scheduling delays. Delay of more than 12 weeks after surgery may be allowed on a case by case basis after discussion with the treating physician and PI.

For patients randomized to Arm 2, the schedule for vaccination will be Days 1, 4, 8, 15, and 22 during the chemotherapy holiday, and Days 50 and 78 post-operatively. Optimal timing for Day 1 for Arm 2 patients is 1 week after the end of chemotherapy, but Day 1 may be given up to 3 weeks after the end of chemotherapy. If there is a significant delay in vaccine manufacture, it is permissible for all vaccine doses to be given post-operatively on the Arm 1 schedule. Any changes to planned treatment on protocol will be carefully coordinated with the treating physician.

Refer to Section 6.2.1 for vaccine administration instructions for participants whose disease progresses or recurs during neoadjuvant chemotherapy or who are otherwise unable to complete a surgical resection.

All study injections will be given intramuscularly and co-administered with poly-ICLC by a trained healthcare provider. Injections should be given in deltoid and dorsogluteal muscles, but other sites are possible (vastus lateralis, ventrogluteal, recto femoris), depending on treating physician and patient preference. A log will be kept of each pool, injection site and time point. At each vaccination time point, patients will receive up to four injections of the neoantigen peptide vaccine + adjuvant. Each peptide pool should be given to the same injection site at each injection time point. 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 5.1.3 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 in accordance with the study calendar.

Post-vaccination follow-up visits are at 4 weeks following last injection (+/- 7 days) and 1 and 2 years following last injection (+/- 30 days). Additional follow-up visits or telephone contact may be scheduled annually thereafter if the patient is alive and available for follow-up as per SOC.

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

6.2.1 Vaccination Administration for Step 1 Ineligible Patients

Patients who are not eligible for vaccine administration per Step 1 but for whom a personalized neoantigen vaccine has been successfully manufactured, or for which significant progress has been made as determined by treating physician, are permitted to receive vaccine injections on study. Injections may be given concurrently with adjuvant chemotherapy. Patients can proceed with vaccine administration when their disease has been stabilized on standard treatment (one radiographic assessment showing stable disease or partial response). They will then have a temporary treatment break from chemotherapy during which vaccine Days 1 through 22 will take place, with Day 1 occurring at least 7 days after last chemotherapy dose. After the Day 22 vaccination, standard of care chemotherapy may resume at the discretion of their treating physician. Resumption of chemotherapy should occur no less than 7 days after Day 22 vaccination. These patients will receive Day 50 and 78 vaccinations with a +/-7 day window to allow them to occur during an off week of subsequent chemotherapy. Blood for immune monitoring will be collected on these patients as well (as described in Section 9.2).

6.3 Standard of Care Adjuvant Chemotherapy

Adjuvant chemotherapy is permitted at the discretion of the treating medical oncologist. If a patient is receiving adjuvant chemotherapy, it may be given concurrently with vaccination, but it is preferred that adjuvant chemotherapy be initiated after the Day 22 injections of vaccine and that the Day 50 and 78 injections occur during an off week for chemotherapy.

6.4 Concomitant Medication Guidelines

Patients may receive other vaccinations while on study, including COVID, influenza, and pneumococcal; however, live vaccines are not allowed.

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.

Immunosuppressive medications, or corticosteroids in doses exceeding 10 mg prednisone daily, are not allowed during study treatment with the vaccine.

6.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum or urine pregnancy test within 14 days prior to the first dose of the vaccine.

Female patients are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 3 months following the last dose of the vaccine.

If a patient is suspected to be pregnant, the vaccine should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient becomes pregnant during therapy or within 3 months after the last dose of the study agent, the investigator must be notified in order to facilitate outcome follow-up.

6.6 Definitions of Evaluability

Endpoint	Definition of Evaluability
Primary	
Safety of neoantigen SLP vaccine	Must have received at least 1 dose of vaccine. Patients who are not eligible per Step 1 but receive vaccine injections as described in Section 6.2.1 are evaluable for the primary objective of safety.
Secondary	
Number and phenotype of neoantigen-specific T cells	Must have received at least one dose of vaccine and had immune monitoring sample collection at baseline and at least one time point during/post-treatment. Patients who receive vaccine injections as described in Section 6.2.1 are excluded from this analysis.
Exploratory	
Phenotype and function of neoantigen-specific T cells	Must have received at least one dose of vaccine and had immune monitoring sample collection at baseline and at least one time point during/post-treatment. Patients who receive vaccine injections as described in Section 6.2.1 are excluded from this analysis.

6.7 Duration of therapy

In the absence of treatment delays due to adverse events, treatment may continue for approximately 78 days (7 doses of neoantigen peptide pancreatic cancer vaccine). 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.

6.8 Duration of follow up

Patients receiving at least one injection of vaccine will be examined at 4 weeks and 1 and 2 years following the final vaccination. 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 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 peptide vaccines.

Risks associated with intramuscular injections include acute bleeding and/or bruising. Although highly unlikely, intramuscular injection can result in peripheral nerve damage and/or 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. All information will be recorded on case report forms. Adverse events will be reported to the Quality Assurance and Safety Monitoring Committee of the Siteman Cancer Center, the Institutional Review Board, 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 for Neoantigen Peptide Vaccine

No dose modifications are planned for the neoantigen peptide vaccine. If a subject develops an adverse event that is classified as possibly, probably, or definitely associated with the neoantigen peptide vaccine, this may result in either a delay in the dose as described below, or 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**.

In order to be dosed, patients must meet the following criteria prior to each vaccine administration:

- ECOG is 0 or 1
- AEs related to the vaccine have resolved to ≤ grade 1
- Any intercurrent illness or medical condition that could jeopardize the health or safety of the participant is resolved or adequately managed, as determined by the treating physician

Note: Patients held for an ECOG of 2 for more than 1 week may resume dosing prior to resolution of ECOG to 0 or 1 at the discretion of the treating physician and after discussion with the PI.

Patients may have their consecutive vaccine dose delayed by up to 6 weeks. If a delay of more than 6 weeks is needed, the patient will discontinue protocol therapy, proceed with EOT visit and enter follow up per protocol. Exceptions to this may be made at the discretion of the PI and the treating physicians after review of the patient's condition and if the conditions leading to the delay of vaccine dose are deemed not related to the vaccine.

8 STUDY CALENDAR

The window for Step 0 screening procedures is up to 28 days prior to enrollment. The window for Step 1 screening procedures is 14 days prior to first vaccine dose.

FOR SUBJECTS IN ARM 1:

Day 1 (first vaccination) will occur approximately 1 month after surgery (may occur up to 12 weeks after surgery).

	Step 0 Screening	Surgery[n]	Step 1 Screening	Day 1	Day 4[d]	Day 8[d]	Day 15[d]	Day 22[d]	Day 50[i]	Day 78[i]	EOT[j]	F/U[e]
Neoantigen peptide vaccine[f]				X	X	X	X	X	X	X		
Tissue Collection	X[m]	X										
Randomization	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[a]	X		X	X				X	X	X		
INR, PTT	X		X									
Radiologic evaluation	X[h]		X[h]	Radiologic measurements will be performed as clinically indicated.								
Adverse event evaluation	X---------- X[k]											
B-HCG[c]	X		X	X				X	X	X		
Immune monitoring[b]	X	X		X			X	X	X	X		X[g]
Eligibility Confirmation	X		X	X[l]								

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Immune monitoring labs to consist of seven 10mL lavender top EDTA tubes for isolation of PBMCs. This will occur prior to the start of neoadjuvant chemotherapy, at the end of neoadjuvant chemotherapy, and as indicated in the calendar. This will yield approximately 50-200 $x10^6$ PBMCs per collection.
c: Serum or urine pregnancy test (women of childbearing potential).
d: +/- 1 days with at least 24 hours between vaccinations
e: follow-up or telephone contact at 4 weeks (+/- 7 days), 1 year (+/- 30 days), and 2 years (+/- 30 days) after last injection. F/U visits may be done at the participant's local MD office.
f: See Section 7.3 for dosing guidance.
g: mandatory on Day 28 and optional for 1 and 2 years based on patient availability
h: SOC scans to be completed within 6 weeks prior to Step 0, but scans outside this window can be used with PI permission. Scans prior to surgery showing no evidence of progression can be utilized for Step 1 registration.
i: +/- 7 days
j: EOT is defined as 4 weeks after the last dose of vaccine (+/- 7 days).
k: See Section 11 for further details.
l: Patients must continue to meet eligibility criteria in order to receive Day 1 of vaccine.
m: If the tissue collected in Step 0 screening is through a fresh biopsy, the biopsy must be performed prior to the start of neoadjuvant chemotherapy.
n: surgery to be performed as per standard site practice.

FOR SUBJECTS IN ARM 2:

Day 1 (first vaccination) will occur 1 week after the end of chemotherapy, with up to 3 weeks after the end of chemotherapy permitted; PI approval required for delays.

	Step 0 Screening	Step 1 Screening	Day 1	Day 4[d]	Day 8[d]	Day 15[d]	Day 22[d]	Surgery[n]	Day 50[i]	Day 78[i]	EOT[j]	F/U[e]
Neoantigen peptide vaccine[f]			X	X	X	X	X		X	X		
Tissue Collection	X[m]							X				
Randomization	X											
Informed consent	X											
Demographics	X											
Medical history	X											
Physical exam[i]	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[a]	X	X	X				X		X	X		
INR, PTT	X	X										
Radiologic evaluation	X[h]	X[h]	Radiologic measurements will be performed as clinically indicated.									
Adverse event evaluation	X----------X[k]											
B-HCG[c]	X	X	X				X		X	X		
Immune monitoring[b]	X		X			X	X	X	X	X		X[g]
Eligibility Confirmation	X	X	X[l]									

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Immune monitoring labs to consist of seven 10mL lavender top EDTA tubes for isolation of PBMCs. This will occur prior to the start of neoadjuvant chemotherapy, at the end of neoadjuvant chemotherapy, and as indicated in the calendar. This will yield approximately 50-200 $x10^6$ PBMCs per collection.
c: Serum or urine pregnancy test (women of childbearing potential).
d: +/- 1 days with at least 24 hours between vaccinations and at least 72 hours between vaccination and day of surgery
e: follow-up or telephone contact at 4 weeks (+/- 7 days), 1 year (+/- 30 days), and 2 years (+/- 30 days) after last injection. F/U visits may be done at the participant's local MD office.
f: See Section 7.3 for dosing guidance.
g: mandatory on Day 28 and optional for 1 and 2 years based on patient availability
h: SOC scans to be completed within 6 weeks prior to Step 0, but scans outside this window can be used with PI permission. Scans prior to surgery showing no evidence of progression can be utilized for Step 1 registration.
i: +/- 7 days
j: EOT is defined as 4 weeks after the last dose of vaccine (+/- 7 days).
k: See Section 11 for further details.
l: Patients must continue to meet eligibility criteria in order to receive Day 1 of vaccine.
m: If the tissue collected in Step 0 screening is through a fresh biopsy, the biopsy must be performed prior to the start of neoadjuvant chemotherapy.
n: surgery to be performed as per standard site practice.

FOR SUBJECTS WHO ENROLL UNDER STEP 1 ELIGIBILITY CRITERION 2a:

Day 1 (first vaccination) will occur after their disease has been stabilized on standard treatment (one radiographic assessment showing stable disease or partial response). They will then have a temporary treatment break from chemotherapy during which vaccine Days 1 through 22 will take place. Day 1 must occur at least 7 days after last chemotherapy dose. After the Day 22 vaccination, SOC chemotherapy may resume at the discretion of the treating physician (no less than 7 days after Day 22).

	Day 1	Day 4[d]	Day 8[d]	Day 15[d]	Day 22[d]	Day 50[i]	Day 78[i]	EOT[j]	F/U[e]
Neoantigen peptide vaccine[f]	X	X	X	X	X	X	X		
Informed consent	X[k]								
Physical exam	X	X	X	X	X	X	X	X	X
Concurrent Meds	X ---------- X								
Vital signs	X	X	X	X	X	X	X		X
Weight	X	X	X	X	X	X	X		X
Performance status	X	X	X	X	X	X	X		
CBC w/diff, plts	X				X	X	X		
CMP[a]	X				X	X	X		
Radiologic evaluation	Radiologic measurements will be performed as clinically indicated.								
Adverse event evaluation	X----------X[l]								
B-HCG[c]	X				X	X	X		
Immune monitoring[b]	X			X	X	X	X		X[g]

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Immune monitoring labs to consist of seven 10mL lavender top EDTA tubes for isolation of PBMCs. This will yield approximately 50-200 $x10^6$ PBMCs per collection.
c: Serum or urine pregnancy test (women of childbearing potential).
d: +/- 1 days with at least 24 hours between vaccinations and at least 72 hours between vaccination and day of surgery (if applicable)
e: follow-up or telephone contact at 4 weeks (+/- 7 days), 1 year (+/- 30 days), and 2 years (+/- 30 days) after last injection. F/U visits may be done at the participant's local MD office.
f: See Section 7.3 for dosing guidance.
g: mandatory on Day 28 and optional for 1 and 2 years based on patient availability
h: First scan must show stability on new regimen.
i: +/- 7 days; must occur during an off week of adjuvant chemotherapy
j: EOT is defined as 4 weeks after the last dose of vaccine (+/- 7 days).
k: must be consented to a separate consent form once determined to be enrolling under Step 1, criterion 2a
l: See Section 11 for further details.

9 CRITERIA FOR RESPONSE

9.1 Primary objective: safety

Assessment of safety will include both clinical observation and laboratory evaluation. Safety will be closely monitored after injection:

(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. The quantity and quality of antigen-specific T cells is determined utilizing the ELISPOT analysis, which is based on measuring the frequencies of IFN-γ producing T cells in response to polyepitope antigen. In the proposed study, blood samples will be collected at multiple time points 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 tissue will be collected for research and used for generating nucleic acid (DNA and RNA) for neoantigen prediction. FFPE samples cannot be used for these analyses. Tumor tissue may come from either a research biopsy or previously banked fresh frozen tissue. Blood and tumor specimens will be processed by the SCC Tissue Procurement Core, the Immune Monitoring Laboratory, and the McDonnell Genome Institute. All tissue used for sequencing will be processed into a single-cell suspension by mechanical and enzymatic digestion, and nucleic acids (DNA and RNA) co-isolated. Tumor DNA + RNA will undergo tumor exome and tumor cDNA-capture sequencing, respectively at the McDonnell Genome Institute. Normal genomic DNA will be isolated from PBMCs by the SCC Tissue Procurement Core for normal exome sequencing at The McDonnell 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. Immune monitoring labs will be drawn at the end of chemotherapy and on Days 1, 15, 22, 50, and 78. Immune monitoring labs will also be drawn at the time of surgery and optionally at the time of follow-up. Seven collection tubes (BD Vacutainer® EDTA (lavender top), 10 mL each for a total of approximately 70 mL) are filled by venipuncture at each time point. Blood samples will be transported to the SCC Tissue Procurement Core, preferably within 90 minutes of collection. PBMC will be obtained by Ficoll-Hypaque gradient centrifugation and cryopreserved in 10% DMSO according to standard procedures. Exome sequencing of PBMC will be performed to obtain germline sequences.

9.2.3 ELISPOT Analysis

ELISPOT analysis, will be performed as previously described [60-64]. 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. PBMC will be co-cultured with the mutant 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).

9.3 Exploratory objectives

As funding allows, we will complete the following exploratory studies.

In addition to the ELISPOT analysis described above, we will evaluate immunogenicity by multiparametric flow cytometry. The multiparametric flow cytometry assesses phenotypic as well as functional characteristics of epitope-specific T cells. We use coupled scRNA-seq/TCR-seq to understand the biology of neoantigen-specific T cells in the PDAC tumor microenvironment. We have used *ex vivo* culture systems and TCR-seq to identify the clonotypes of neoantigen-specific T cells responding to vaccination [65]. Using these TCR sequences as a barcode, we will investigate the transcriptional programming of neoantigen-specific T cells using coupled scRNA-seq and TCR-seq [66, 67]. By coupling scRNA-seq and TCR-seq, we will be able to determine if neoantigen-specific T cells have an effector or exhausted phenotype by assessing relative expression of the following genes: IFNG, GZMB, GZMH, XCL1, TNFRSF9 (CD137), KLRG1, TCF7, PDCD1 (PD-1), LAG3, CTLA4, and TIGIT as described by Steele et al.[68] in PDAC.

We will 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 [69, 70]. 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.

We will use both CyTOF and scRNA-seq to comprehensively catalog changes in the TME with vaccination as previously described [71]. The studies will be performed in the IML. Single-cell suspensions will be prepared and stained with a panel of >40 heavy metal-coupled antibodies specific for immune subsets and markers known to have important roles in restraining antitumor immune responses. scRNA-seq captures the mRNA expression profile of individual cells within a sample and can detect minute changes that can otherwise be lost in heterogeneous populations. Single-cell lysis of cell suspensions will be performed, and mRNA from individual cells will be captured as cDNA and tagged with a unique barcode. Pooled cDNA libraries will be sequenced via next-generation sequencing, and data will be analyzed with a variety of bioinformatics methods by the Artyomov laboratory as recently described (*Cell*, 2018) [71].

9.3.1 Tetramer assays

Patient-derived PBMC will be used for functional assays to characterize immunity to neoantigen peptide 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 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 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.

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 progressive disease prior to the initial vaccination will result in stopping vaccine development at its current stage, unless sufficient progress on the vaccine has been made and the treating physician determines the subject may still benefit.

(2) Development of progressive 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 administration. 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 3 or 4 systemic or injection site adverse event classified as possibly, probably, or definitely associated with protocol therapy;

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

(8) Unable to complete vaccine production;

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

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

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

(12) Administrative reasons, e.g., study termination by the principal investigator, Siteman 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 ADVERSE EVENT REPORTING

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Section 18.4 for definitions and Section 18.5 for a grid of reporting timelines. All investigators treating patients on this study are responsible for ensuring that serious adverse events (as defined in Section 18.4) are reported to the Sponsor-Investigator within an adequate timeframe for the event to be assessed by the Sponsor-Investigator for reporting to HRPO, QASMC, and/or FDA. **Note that for investigator-initiated studies, the term "Sponsor-Investigator" refers to the investigator holding the IND.**

All adverse events will be tracked from first dose of vaccine through 4 weeks after last vaccination. From the time of signing the initial consent to the time of the first dose of vaccine, only AEs related to study procedures need be reported. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF
- AEs related to SOC neoadjuvant chemotherapy
- AEs related to SOC adjuvant chemotherapy if also thought to be unrelated to the neoantigen peptide vaccine

Refer to the data submission schedule in Section 14 for instructions on the collection of AEs in the EDC. Reporting requirements may be found in Section 11.1 and 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 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 has 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 qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

11.1.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** The Siteman Cancer Center Protocol Development team (PD) facilitates reporting to FDA. Please see directions for preparing documentation and contacting PD below, and refer to the internal IND Safety Report SOP for additional guidance.

It is the responsibility of the Sponsor-Investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Section 18.4 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 18.4) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Section 18.4) 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.

Each report should be developed 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 (PD team, protocoldevelopment@wustl.edu) of each potentially reportable event within 1 business day after initial receipt of the information, and must send the completed FDA Form 3500A to PD 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 study. 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”).

11.2 Sub-Investigator Reporting Requirements

All investigators treating patients on this study are responsible for ensuring that serious adverse events (as defined in Section 18.4) are reported to the Sponsor-Investigator within 24 hours of learning of the event in order for the event to be assessed by the Sponsor-Investigator for reporting to HRPO, QASMC, and/or FDA.

11.3 Exceptions to Expedited Reporting

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

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

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

12 DATA AND SAFETY MONITORING

12.1 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.

12.2 Data and safety monitoring plan

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually. The first report is required either 30 days after the enrollment of the 5th participant (if sooner than 6 months after study activation) or 6 months after study activation (provided at least one patient has been enrolled; if zero patients have been enrolled at the 6-month mark, the first report will be required one year after accrual opens provided at least one patient has been enrolled).

For phase I studies, the Principal Investigator will review all patient data at least monthly, and provide a semi-annual report to the Quality Assurance and Safety Monitoring Committee (QASMC). This report will include:

- Study demographic information (local protocol number, protocol title, list of primary study team members, study sites, primary and secondary sponsors, IND/IDE status, date of most recent QA audit, and study status and history (including activation and suspension dates)
- Accrual information, including study-wide target accrual and actual accrual, anticipated and/or actual accrual end date, and accrual by year by site (if applicable)
- Subject status information presented in both cumulative format (total number of subjects who consented, enrolled, screen failed, started intervention, discontinued intervention, went off study, expired) and current format (number of subjects in screening, on intervention, in follow-up, or off study at time of report)
- Protocol objectives and the number of participants who are evaluable for each objective

- History of study (including summaries of substantive amendments, accrual suspensions and reasons, protocol exceptions, errors, and breaches of confidentiality)
- Summary of exceptions, noncompliance reports, and unanticipated problems reported to the IRB
- Early stopping rules and data describing whether the stopping rules have been met (if applicable)
- Interim analysis plans and the results of the interim analysis (if applicable)
- Separate SAE and worst grade toxicity tables, each separated by site (if applicable) and arm/cohort/dose level (if applicable)
- Participant-level response and survival data by arm/cohort/dose level (if applicable)
- Summary of specimen collection (percentage of participants who have had specimens collected at each required time point)
- Abstract submissions/publications

Summary of any recent literature that may affect the safety of participants or the ethics of the study The study team will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to HRPO and QASMC according to institutional guidelines.

13 STATISTICAL CONSIDERATIONS

13.1 Study objectives and endpoints

The primary objective of the phase 1 clinical trial is to assess the safety of neoantigen vaccine therapy in patients with pancreatic cancer following neoadjuvant chemotherapy. Safety assessment will include both clinical observation and laboratory evaluation. The secondary objective of the trial is to assess the number and phenotype of neoantigen-specific T cells in the peripheral blood of patients pre- and post-vaccination, as measured by ELISPOT. Exploratory objectives are focused on the phenotype and function of neoantigen-specific T cells in the peripheral blood as measured by CyTOF analysis and tetramer analysis and the phenotype and function of neoantigen-specific T cells in the tumor as measured by coupled scRNA-seq/TCR-seq and CODEX.

13.2 Overall experimental design

A total of 36 subjects will be enrolled in this study. We anticipate that there will be 18 evaluable subjects. The trial will include two arms: (1) neoantigen vaccine following neoadjuvant chemotherapy and surgery, (2) neoantigen vaccine following neoadjuvant chemotherapy in the window prior to surgery. The randomization will be performed upfront at the initiation of neoadjuvant chemotherapy, and subjects will be randomized to either Arm1 (N=18 randomized, anticipate 9 evaluable subjects) or Arm 2 (N=18 randomized, anticipate 9 evaluable subjects) group.

Patients who are treated after progression (as detailed in Section 3.3, criterion 2a) will be evaluated separately by descriptive statistics.

13.3 Sample size justification

The designed sample size (at least 9 evaluable patients/arm) will provide a reasonable precision to estimate the safety of neoantigen vaccine therapy in patients with pancreatic cancer following neoadjuvant chemotherapy. For example, there is 87% chance of observing at least 1 serious adverse event (SAE) out of 9 evaluable patients if the "true" SAE rate is 20% or higher. Conversely, if the "true" SAE rate is <5%, there is only 7% chance to observe 2 or more SAE. We expect <20% toxicity rate.

A power analysis is also performed using paired t-test for differences across time to test the hypothesis that neoantigen peptide vaccines can induce and/or enhance neoantigen-specific T-cell responses. Assuming a moderate correlation between measures taken from the same individual, the designed sample size (N=9/arm) gives 80% power at a two-sided 0.05 significance level to detect a minimum of 1.06 x SD (standard deviation of neoantigen-specific T-cells) for temporal changes. Preliminary data from our phase 1 trial for mammaglobin-A DNA vaccine on patients with stable metastatic breast cancer and targeting a tissue-specific antigen showed that the frequency of antigen-specific CD8 T cells can be measured with ~20% coefficient of variation (CV=SD/mean). We anticipate that more power could be achieved because the actual data analysis will be performed using mixed model, which uses data more efficiently and allows us to borrow information across multiple time points.

13.4 Statistical analysis

The number and percentage of subjects experiencing each type of SAE will be tabulated by severity and relationship to treatment. If appropriate, confidence intervals will be used to characterize the precision of the estimate. The frequency of neoantigen-specific T cells in each arm will be summarized using means, SDs, and medians. Responses will be considered positive if the number of T cells after vaccination is greater than two standard deviations above the mean before vaccination. The frequency of positive responses at each time point will be assessed and binomial response rates with 95% confidence interval estimates will be presented. Temporal changes and differences between arms will be compared using linear mixed model for repeated measurement data.

13.5 Interim analysis for futility

A Bayesian stopping rule will be implemented for futility in each vaccination arm when the secondary endpoint is available in 6 patients. The futility rule is defined as $Pr(\theta_E < 0.8 \mid data) > 0.9$, where θ_E denotes the incidence of immune response (defined as the number of T cells after vaccination is greater than two

standard deviations above the mean before vaccination [51]). That is, enrollment will be stopped early whenever, given the accumulated observed data, there is a high probability (>90%) that the “true” response rate is less than 80%. The stopping boundaries are obtained using the online study design tools from M.D. Anderson Cancer Center (https://www.trialdesign.org/one-page-shell.html#BTOX). Specifically, we assume that θ_E follows a beta distribution, beta ($\alpha + x$, $\beta + n - x$), where x and n-x represent the observed numbers of responders and non-responders (with response defined in Section 13.4 above), while α and β are parameters for prior distribution. We set the prior as beta (0.5, 0.5), i.e., with a non-informative prior.

- An arm will be recommended to stop for futility if we observe 3 or less responses out of 6 patients during the interim analysis

There is 82% chance of terminating earlier for futility if the "true" response rate is 40% or less. Conversely, if the "true" response rate is 80%, there is only 10% chance to stop early for futility.

14 DATA MANAGEMENT

Data collected will be collected using electronic data capture forms. Forms must be completed within 28 days of time point.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Demographics Form On Study Form Medical History Form	At time of Step 0 screening
Neoadjuvant Chemotherapy Summary Form	At time of Step 1 screening
Surgery Form	Time of surgery
Vaccine Form	Day 1 Day 4 Day 8 Day 15 Day 22 Day 50 Day 78
Immune Monitoring Form	Step 0 screening Time of surgery Day 1 Day 15 Day 22 Day 50 Day 78 Follow-up (optional)
Toxicity Form Concomitant Medications Form	Continuous
Adjuvant Chemotherapy Summary Form (if applicable; only for patients who receive adjuvant chemotherapy concurrently with vaccine)	At time of completion of adjuvant chemotherapy
Follow Up Form	4 Weeks 1 Year 2 Years
MedWatch Form	See Section 11.0 for reporting requirements
Progression Form	Time of progression
Death Form	Time of death

14.1 Adverse Event Collection in the Case Report Forms

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

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

15 REGULATORY AND ETHICAL OBLIGATIONS

15.1 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.

15.2 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.

16 ADMINISTRATIVE AND LEGAL OBLIGATIONS

16.1 Study documentation and retention of records

16.1.1 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 monitoring or 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.

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored by the study sponsor.

Monitors under contract will review aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points in the electronic data capture system, and prompt reporting of all SAEs to the appropriate entities; 3) to compare data captured to individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol and applicable regulations.

16.1.2 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.

16.2 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 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.

16.3 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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18 APPENDICES

18.1 Abbreviations

AE	Adverse Event
CBER	Center for Biologics Evaluation and Research
CFR	Code of Federal Regulations
CPA	Cooperative Project Assurance
CR	Complete response
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
PRMC	Protocol Review and Monitoring Committee
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

18.2 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

18.3 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.

18.4 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 Unexpected Suspected Adverse Reaction (SUSAR)

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.

18.5 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.		

18.6 Neoantigen Prediction SOP

Please see attached detailed SOP for neoantigen identification and prioritization.

18.7 Study Specific DSM Tables

Protocol Objectives and Subject Evaluability	
Objective	**# of patients evaluable for this endpoint to date**
Primary	
To assess the safety of optimized neoantigen SLP vaccines in patients with pancreatic cancer following neoadjuvant chemotherapy.	
Secondary	
To assess the number and phenotype of neoantigen-specific T cells in the peripheral blood of patients pre- and post-vaccination, as measured by ELISPOT.	
Exploratory	
To explore the phenotype and function of neoantigen-specific T cells in the peripheral blood as measured by CyTOF analysis and tetramer analysis, and the phenotype and function of neoantigen-specific T cells in the tumor as measured by coupled scRNA-seq/TCR-seq and CODEX	

Interim Analysis and Early Stopping Rules
Does the study design include an interim toxicity analysis? No
Does the study design include an interim futility analysis? Yes
If yes, please insert text describing analysis from the protocol A Bayesian stopping rule will be implemented for futility in each vaccination arm when the secondary endpoint is available in 6 patients. The futility rule is defined as $Pr(\theta_E < 0.8 \mid data) > 0.9$, where θ_E denotes the incidence of immune response (defined as the number of T cells after vaccination is greater than two standard deviations above the mean before vaccination [51]). That is, enrollment will be stopped early whenever, given the accumulated observed data, there is a high probability (>90%) that the "true" response rate is less than 80%. The stopping boundaries are obtained using the online study design tools from M.D. Anderson Cancer Center (https://www.trialdesign.org/one-page-shell.html#BTOX). Specifically, we assume that θ_E follows a beta distribution, beta ($\alpha + x$, $\beta + n - x$), where x and n-x represent the observed numbers of responders and non-responders, while α and β are parameters for prior distribution. We set the prior as beta (0.5, 0.5), i.e., with a non-informative prior. • An arm will be recommended to stop for futility if we observe 3 or less responses out of 6 patients during the interim analysis There is 82% chance of terminating earlier for futility if the "true" response rate is 40% or less. Conversely, if the "true" response rate is 80%, there is only 10% chance to stop early for futility.
If yes, please insert results of the interim analysis:
Are there early stopping rules that outline circumstances under which the study must be suspended or closed? Yes
If yes, please insert text describing early stopping rules from the protocol 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.

If yes, please provide data describing whether the stopping rules have been met; this should include a table for all toxicities falling under the stopping rule guidance:

Have any subjects experienced a G3 or G4 AE that is probably or definitely related to vaccination?	If yes, provide UPN(s).	If yes, specify AE.
Have any subjects experienced an SAE that is possibly, probably, or definitely related to vaccination?	If yes, provide UPN(s).	If yes, specify SAE.
Have any subjects died within 30 days of vaccination?	If yes, provide UPN(s).	If yes, specify cause of death.
Have any subjects experienced the same G2 AE that is probably or definitely related to vaccination, with the exceptions of subjective local or systemic symptoms of pain/tenderness, malaise, fatigue, headache, chills, nausea, myalgia, or arthralgia?	If yes, provide UPN(s).	If yes, specify AE.
Has the PI recommended halting enrollment based on any other observations?	If yes, provide details.	

Treatment/Study Discontinuation					
UPN	**On Study Date (Step 0)**	**On Tx Date (Step 1)**	**Arm**	**Off Tx Date**	**Reason for not enrolling to Step 1, or reason off tx**

Survival		
UPN	**Vital Status**	**If dead, cause**

Summary of Specimen Collections			
Type of specimen	**Time point**	**# of patients eligible for collection at this time point**	**% of patients who have reached this time point and had the specimen collected**
Tissue	**Step 0 Screening**		
Tissue	**Surgery**		
Blood	**Step 0 Screening**		
Blood	**Surgery**		
Blood	**Day 1**		
Blood	**Day 15**		
Blood	**Day 22**		
Blood	**Day 50**		
Blood	**Day 78**		
Blood	**28-day follow-up**		
Blood	**1-year follow-up (optional)**		
Blood	**2-year follow-up (optional)**		