A RANDOMIZED PHASE 1 TRIAL OF NEOANTIGEN DNA VACCINE ALONE VS. NEOANTIGEN VACCINE PLUS DURVALUMAB IN TRIPLE NEGATIVE BREAST CANCER PATIENTS FOLLOWING STANDARD OF CARE THERAPY

HRPO #: 201710109
Version date: 04/24/2023
IND #: 17625
clinicaltrials.gov #: NCT03199040

INVESTIGATORS

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

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

Foluso O. Ademuyiwa, M.D., MPH
Medical Oncologist
Washington University School of Medicine
660 South Euclid, Box 8056
St. Louis, MO 63110
Telephone: 314-362-7201
E-mail: bisiademuyiwa@wustl.edu

Timothy Eberlein, M.D.
Breast Surgeon
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone: 314-362-8020
E-mail: eberleint@wustl.edu

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

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

Rebecca L. Aft, M.D., Ph.D.
Breast Surgeon
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone:
E-mail: aftr@wustl.edu

Amy Cyr, M.D.
Breast Surgeon
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone: 636-916-7160
E-mail: amycyr@wustl.edu

Julie A. Margenthaler, M.D.
Breast Surgeon
Washington University School of Medicine
660 South Euclid, Box 8109
St. Louis, MO 63110
Telephone: 314-362-7534
E-mail: jmargenthaler@wustl.edu

Christopher A. Miller, Ph.D.
Genome Scientist
Washington University School of Medicine
660 South Euclid, Box 8007
St. Louis, MO 63110
Telephone: 314-286-0263
E-mail: c.a.miller@wustl.edu

Malachi Griffith, Ph.D.
Genome Scientist
Washington University School of Medicine
660 South Euclid, Box 8501
St. Louis, MO 63110
Telephone: 314-286-1274
E-mail: mgriffit@wustl.edu

Protocol Revision History

Initial Approval:	09/28/17
Amendment #1:	03/01/18
Amendment #2:	08/31/18
Amendment #3:	06/04/19
Amendment #4:	08/13/19
Amendment #5:	02/12/20
Amendment #6	05/04/20
Amendment #7	06/11/20
Amendment #8	12/09/21
Amendment #9	04/24/23

SUMMARY

Protocol: **A RANDOMIZED PHASE 1 TRIAL OF NEOANTIGEN DNA VACCINE ALONE VS. NEOANTIGEN DNA VACCINE PLUS DURVALUMAB IN TRIPLE NEGATIVE BREAST CANCER PATIENTS FOLLOWING STANDARD OF CARE THERAPY**

Study Design: This is a single institution, open-label randomized phase 1 trial of neoantigen DNA vaccine alone vs. neoantigen DNA vaccine plus durvalumab in triple negative breast cancer (TNBC) patients who have residual disease following standard of care therapy. Patients with newly diagnosed clinical stage II-III TNBC are eligible for enrollment. Patients will receive standard of care therapy including chemotherapy, surgery and radiation therapy as clinically indicated. Following standard of care therapy, patients will be randomized to receive either a neoantigen DNA vaccine alone, or a neoantigen DNA vaccine + durvalumab.

Hypotheses: Hypotheses tested in this study include: (1) neoantigen DNA vaccines can induce and/or enhance neoantigen-specific T cell responses; and (2) durvalumab will enhance the response to neoantigen DNA vaccines.

Objectives: The primary objective is to assess the safety of neoantigen DNA vaccines given alone or in combination with durvalumab. Safety assessment will include both clinical observation and laboratory evaluation. The secondary objective is to measure the immune response to neoantigen DNA vaccines given alone, or in combination with durvalumab. The immune response will be measured in the peripheral blood by luminex assay, ELISPOT, and multiparametric flow cytometry.

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

Durvalumab is a human monoclonal antibody (MAb) of the immunoglobulin G1 kappa (IgG1κ) subclass that inhibits binding of programmed cell death ligand 1 (PD-L1) to programmed cell death 1 (PD-1) and CD80 (B7-1).

Subjects: Patients with clinical stage II-III TNBC are eligible for participation. Patients must provide consent for tumor/normal exome sequencing and dbGAP-based data sharing and provide germline and tumor DNA samples of adequate quality for exome and cDNA-capture sequencing.

Study Plan: Twenty-four breast cancer patients will be enrolled. Tumor tissue and peripheral blood will be obtained prior to initiation of any treatment for sequencing and correlative studies. These analyses will inform the design of the neoantigen DNA vaccines.

The neoantigen DNA vaccines will be manufactured at Siteman Cancer Center and then administered following standard of care therapy. It is anticipated that standard of care therapy in this clinical context will include surgery, adjuvant or neoadjuvant chemotherapy (AC + T or similar regimen), and adjuvant radiation therapy, taking a total of approximately 6 months to complete.

Following standard of care adjuvant therapy, patients will be randomized to receive either a neoantigen DNA vaccine alone, or a neoantigen DNA vaccine + durvalumab. The vaccine will be administered by intramuscular injection on Day 1, Day 29 ± 7, Day 57 ± 7, Day 85 ± 7, Day 113 ± 7, and Day 141 ± 7 with at

least 21 days between injection days. Each DNA vaccination will be 4 mg vaccine administered intramuscularly using a TriGrid electroporation device.

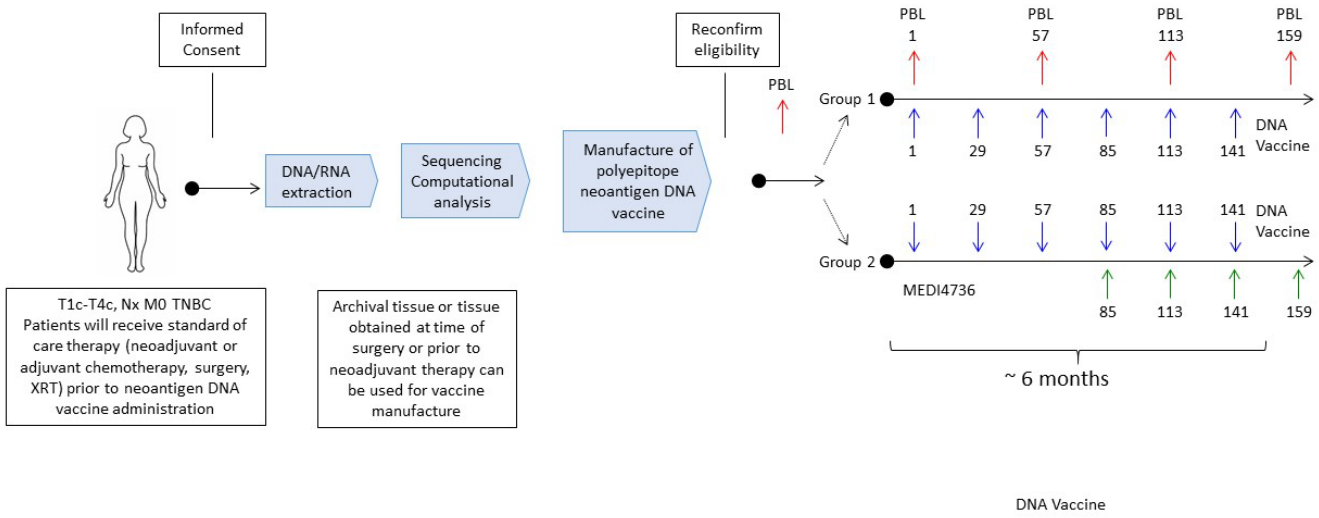
For patients who are randomized to neoantigen DNA vaccine + durvalumab, the neoantigen-specific T cell response will be assessed after two vaccinations (Day 57). If a neoantigen-specific T cell response is present, durvalumab will be administered every 28 days at a dose of 1500 mg beginning on Day 85.

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

Study Endpoints: The phenotype and function of class I and II neoantigen-specific T cell response will be assessed by ELISPOT, luminex, multiparametric flow cytometry, and CyTOF. Safety endpoints include safety of the neoantigen DNA vaccine alone, or neoantigen DNA vaccine plus durvalumab. Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.

SCHEMA

Anti-PD-L1 antibody (MEDI4736)
Adjuvant personalized breast cancer vaccine (SLP with poly IC:LC)



PROTOCOL SIGNATURE PAGE

A RANDOMIZED PHASE 1 TRIAL OF NEOANTIGEN DNA VACCINE ALONE VS. NEOANTIGEN VACCINE PLUS DURVALUMAB IN TRIPLE NEGATIVE BREAST CANCER PATIENTS FOLLOWING STANDARD OF CARE THERAPY

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

TABLE OF CONTENTS

SUMMARY 3

SCHEMA 5

PROTOCOL SIGNATURE PAGE 6

1 OBJECTIVES 10

1.1 PRIMARY OBJECTIVE 10
1.2 SECONDARY OBJECTIVE 10

2 BACKGROUND 11

2.1 CHECKPOINT BLOCKADE THERAPY 11
2.2 NEOANTIGENS 11
2.3 NEXT GENERATION SEQUENCING AND CANCER VACCINES 12
2.4 SEQUENCING STRATEGIES TO IDENTIFY NEOANTIGENS 13
2.5 PRIORITIZATION OF SEQUENCING-IDENTIFIED NEOANTIGENS 13
2.6 NEOANTIGEN DNA VACCINES 13
2.7 ELECTROPORATION 14
2.8 RATIONALE FOR VACCINATION IN THE ADJUVANT SETTING 14
2.9 RATIONALE FOR TARGETING PATIENTS WITH TRIPLE NEGATIVE BREAST CANCER 15
2.10 DURVALUMAB (MEDI4736) 16

3 PATIENT SELECTION 19

3.1 ELIGIBILITY CRITERIA 19
3.2 INCLUSION OF WOMEN AND MINORITIES 21

4 REGISTRATION PROCEDURES 22

4.1 PRIOR TO REGISTRATION 22
4.2 REGISTRATION PROCEDURES 22
4.3 CONFIRMATION OF PATIENT ELIGIBILITY 23
4.4 PATIENT REGISTRATION IN THE SITEMAN CANCER CENTER ONCORE DATABASE 23
4.5 ASSIGNMENT OF UPN 23
4.6 RANDOMIZATION 23

5 INVESTIGATIONAL AGENT: DURVALUMAB 24

5.1 DESCRIPTION 24
5.2 PHARMACOKINETICS AND DRUG METABOLISM 24
5.3 SUPPLIER(S) 24
5.4 DOSAGE FORM AND PREPARATION 24
5.5 STORAGE AND STABILITY 24
5.6 ADMINISTRATION 25

6 INVESTIGATIONAL AGENT: NEOANTIGEN DNA VACCINES 26

6.1 CHEMICAL NAME AND STRUCTURE 26
6.2 MANUFACTURING FACILITY 27
6.3 MANUFACTURING PROCESS 30
6.4 PRODUCT FORMULATION 33
6.5 PRODUCT RELEASE TESTS 34
6.6 STABILITY 35
6.7 LABELS 35
6.8 SITEMAN CANCER CENTER INVESTIGATIONAL PHARMACY 35
6.9 INVESTIGATIONAL AGENT ADMINISTRATION 36
6.10 INVESTIGATIONAL AGENT ACCOUNTABILITY 40

7 NONCLINICAL STUDIES 41
7.1 OVERVIEW 41
7.2 NONCLINICAL STUDIES 41
7.3 RATIONALE FOR NO GLP SAFETY STUDIES 42
7.4 RATIONALE FOR NO GLP BIODISTRIBUTION STUDIES 43
7.5 SEQUENCING PIPELINE 43
7.6 EPITOPE PREDICTION ALGORITHM 45
8 TREATMENT PLAN 46
8.1 NEOANTIGEN DNA VACCINE ADMINISTRATION 46
8.2 DURVALUMAB ADMINISTRATION 46
8.3 REPLACEMENT OF PATIENTS 46
8.4 GENERAL CONCOMITANT MEDICATION AND SUPPORTIVE CARE GUIDELINES 47
8.5 WOMEN OF CHILDBEARING POTENTIAL 48
8.6 BLOOD DONATION 49
8.7 DURATION OF THERAPY 49
8.8 DURATION OF FOLLOW UP 49
9 POTENTIAL TOXICITY AND DOSE MODIFICATIONS 50
9.1 POTENTIAL TOXICITY 50
9.2 TOXICITY MONITORING AND MANAGEMENT 53
9.3 DOSE MODIFICATIONS FOR DURVALUMAB 53
9.4 DOSE MODIFICATIONS FOR NEOANTIGEN DNA VACCINES 79
10 STUDY CALENDAR 80
11 CRITERIA FOR RESPONSE 82
11.1 PRIMARY OBJECTIVE: SAFETY 82
11.2 SECONDARY OBJECTIVE: NEOANTIGEN-SPECIFIC T CELL RESPONSE 82
11.3 NEOANTIGEN-SPECIFIC T CELL RESPONSE 82
12 REMOVAL OF SUBJECTS FROM PROTOCOL THERAPY 85
12.1 REMOVAL OF SUBJECTS FROM PROTOCOL THERAPY 85
12.2 VOLUNTARY SUBJECT WITHDRAWAL 85
12.3 PROCEDURE FOR DISCONTINUATION 85
13 REGULATORY AND REPORTING REQUIREMENTS 86
13.1 SPONSOR-INVESTIGATOR REPORTING REQUIREMENTS 86
14 DATA AND SAFETY MONITORING 91
14.1 PROTOCOL STOPPING CRITERIA 91
14.2 DATA SAFETY MONITORING PLAN 91
14.3 DEVELOPMENTAL THERAPEUTICS 92
14.4 NIH RECOMBINANT DNA ADVISORY COMMITTEE 92
15 STATISTICAL CONSIDERATIONS 93
15.1 STUDY OBJECTIVES AND ENDPOINTS 93
15.2 STUDY DESIGN AND SAMPLE JUSTIFICATION 93
15.3 DATA ANALYSIS 94
16 DATA MANAGEMENT 95
16.1 ADVERSE EVENT COLLECTION IN THE CASE REPORT FORMS 95
17 REGULATORY AND ETHICAL OBLIGATIONS 97

17.1 INFORMED CONSENT 97
17.2 INSTITUTIONAL REVIEW BOARD 97
17.3 SUBJECT CONFIDENTIALITY 97

18 ADMINISTRATIVE AND LEGAL OBLIGATIONS 98

18.1 STUDY DOCUMENTATION AND RETENTION OF RECORDS 98
18.2 POLICY REGARDING RESEARCH-RELATED INJURIES 98
18.3 STUDY TERMINATION 98

19 REFERENCES 99

20 APPENDICES 108

20.1 ABBREVIATIONS 108
20.2 ECOG/ZUBROD PERFORMANCE STATUS SCALE 109
20.3 NATIONAL CANCER INSTITUTE COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS 109
20.4 DEFINITIONS FOR ADVERSE EVENT REPORTING 109
20.5 REPORTING TIMELINES 111

1 OBJECTIVES

This is a single institution, open-label randomized phase 1 trial of neoantigen DNA vaccine alone vs. neoantigen DNA vaccine plus durvalumab in triple negative breast cancer (TNBC) patients following standard of care therapy. Patients with newly diagnosed clinical stage II-III TNBC are eligible for enrollment. Patients will receive standard of care therapy including chemotherapy, surgery and radiation therapy as clinically indicated. Following standard of care therapy, patients will be randomized to receive either a neoantigen DNA vaccine alone, or a neoantigen DNA vaccine + durvalumab.

Hypotheses tested in this study include: (1) neoantigen DNA vaccines can induce and/or enhance neoantigen-specific T cell responses; and (2) durvalumab will enhance the response to neoantigen DNA vaccines.

1.1 Primary objective

The primary objective is to assess the safety of neoantigen DNA vaccines given alone or in combination with durvalumab. Safety assessment will include both clinical observation and laboratory evaluation. Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03.

1.2 Secondary objective

The secondary objective is to measure the immune response to neoantigen DNA vaccines given alone, or in combination with durvalumab. The immune response will be measured in the peripheral blood by luminexssay, ELISPOT, and multiparametric flow cytometry.

2 BACKGROUND

2.1 Checkpoint blockade therapy

Activation of naïve T cells requires two signals: one signal is mediated through the T cell receptor when it interacts with a peptide-MHC complex on the antigen-presenting cell (APC) [1]. The second signal is a (positive) co-stimulatory signal through CD28 on the T cell and its ligand(s) B7.1/B7.2 on the APC [1, 2]. Physiologically-activated T cells such as those triggered by infections rapidly expand and typically eradicate infected cells. Several immune regulatory mechanisms exist that control the clonal expansion and persistence of activated T cells. CTLA4 is induced on the T cell surface upon activation; it effectively competes with CD28 for binding to B7 ligands, but transduces a negative co-stimulatory signal and abrogates T cell activation. A second regulatory pathway by which activated T cells are controlled is mediated through the Programmed Cell Death-1 (PD-1) molecule on T cells. PD-1 also has two ligands, PD-L1 and PD-L2, expressed on many cell types [1]. Since PD-1 ligation blocks signaling through the T cell receptor, PD-1 acts on the effector function of T cells rather than on stimulation. As such, CTLA4 and PD1 regulate different stages of T cell activation. These findings have been successfully exploited therapeutically using antibodies that block ligation of these checkpoint molecules [1-3]. The anti-CTLA4 antibody ipilimumab received FDA approval in 2011, and two antibodies to PD-1, pembrolizumab and nivolumab, received approval in 2014 for the treatment of malignant melanoma. Multiple checkpoint inhibition clinical trials are currently ongoing in other diseases such as lung cancer, kidney cancer, bladder, prostate cancer, and others, and are expected to lead to approval of checkpoint therapy drugs for these diseases in the coming years. It is important to realize that this form of cancer therapy is unlike others in that the target is not the tumor but rather T cell regulatory molecules, and that checkpoint blockade therapy does not activate the immune system but instead neutralizes inhibitory signals that block tumor-specific T cells. As such, there is strong rationale to combine checkpoint blockade therapy with strategies that actively induce immune effector cells, such as cancer vaccine therapy.

2.2 Neoantigens

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

Shared tumor antigens are expressed in multiple cancers, and are often self-differentiation antigens that are expressed in a limited subset of normal tissues, but overexpressed in cancers. Examples of shared tumor antigens include MAGE (melanoma) [4], prostatic acid phosphatase (prostate cancer) [5], and HER2/neu (breast cancer) [6].

Tumor-specific antigens 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 [7, 8]). As such, tumor-specific antigens represent the only antigens that are truly unique to the tumor and not expressed in normal tissues. The first human mutant tumor-specific antigen was described in 1995, resulting from a point mutation of cyclin-dependent kinase (CDK4) [9]. Since that time additional publications have described the expression of neoantigens in melanoma [10], non-small cell lung cancer [11] and other human cancers [12].

Cancer vaccine strategies targeting neoantigens have clear conceptual advantages over strategies targeting shared tumor antigens. Conceptual advantages include: (1) Targeting neoantigens is potentially safer. Neoantigens are expressed only in the tumor, decreasing the risk of autoimmunity. (2) Targeting neoantigens is potentially more effective. T cell responses to neoantigens are high in affinity, and are not limited by central mechanisms of self-tolerance. (3) Targeting neoantigens potentially limits antigen-loss, a common tumor escape mechanism. One of the hallmarks of cancer is genome instability, and one clear weakness of cancer vaccines that target a single shared tumor antigen is antigen-loss. Targeting multiple neoantigens may preclude antigen loss. In addition, many neoantigens play a functional role in neoplastic transformation (driver mutations). Immune selection resulting in loss of driver mutations may fundamentally alter the phenotype of targeted cancers. (4) Targeting neoantigens is likely to be universally applicable in solid tumors. Solid tumors appear to have a remarkable number of nonsynonymous mutations present (each nonsynonymous mutation is a candidate mutant tumor-specific

antigen), suggesting that a neoantigen DNA vaccine approach could be used in most solid tumor patients, regardless of intrinsic subtype or HLA type.

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

2.3 Next generation sequencing and cancer vaccines

Cancer genome sequencing is a major focus area for Siteman Cancer Center, and for the Genome Institute at Washington University School of Medicine (WUSM). We recently used next-generation sequencing technologies to sequence and compare four DNA samples (primary tumor, brain metastasis, xenograft of the primary tumor, and peripheral blood) from an African-American patient with basal-like breast cancer. Of note, next-generation sequencing technologies are particularly well suited to breast cancer genome sequencing. Breast cancer is a heterogenous disease, but genome sequencing at almost 40× haploid coverage allowed us to precisely calculate mutant allele frequencies, demonstrating genome remodeling, and unexpected similarities between the brain metastasis and xenograft [13].

Additional studies are currently ongoing, and we have successfully sequenced over 47 breast cancer tumor/normal genomes in postmenopausal breast cancer patients with luminal disease enrolled in the American College of Surgeons Oncology Group Z1031 clinical trial (neoadjuvant endocrine therapy) [14]. Although the main objective of this study was to define a genetic profile of susceptibility and/or resistance to aromatase inhibitor therapy, the results do provide important insights into the rational development of vaccine strategies for targeting neoantigens.

In the 47 luminal breast cancer genomes sequenced, we identified and validated 1415 nonsynonymous single-nucleotide variant (SNV) mutations. This means that approximately 31 nonsynonymous SNV mutations are present per luminal breast cancer genome, similar to the number identified in our previous studies of a basal-like breast cancer [13], and similar to the number predicted by limited exome sequencing [15], and statistical models [16]. 79 genes contained nonsynonymous SNV mutations in more than one breast cancer (recurrently mutated genes). The most common recurrently mutated genes include *PIK3CA* (mutations present in 21 breast cancers), *MT-ND5* (11 breast cancers), *TP53* (7 breast cancers), *SYNE1* (6 breast cancers), and *TTN* (5 breast cancers). Of the remaining 79 recurrently mutated genes, nonsynonymous SNV mutations were present in < 10% of the breast cancers studied. Of note, we identified 10 different *PIK3CA* mutations, and the most common mutation, p.H1047R, was present in 10 cancers. Our interpretation of these data is that a personalized vaccine approach is significantly more attractive than an off-the-shelf vaccine approach targeting recurrent mutations. Given the diversity of mutations observed, the limited number of recurrent mutations present in >10% of patients, and the fact that off-the-shelf vaccines would be restricted by HLA type, we estimate that < 10% of breast cancer patients would be eligible for an off-the-shelf vaccine targeting the most common *PIK3CA* mutation. Hence, even if multiple off-the-shelf vaccines were available for different recurrent mutations, only a limited number of patients would be eligible. However, given the number of mutations consistently observed in breast cancer, it is likely that at least a subset of these mutations could be successfully targeted by a personalized vaccine approach. These results suggest that a personalized vaccine approach is the best strategy to target neoantigens.

There are two conceptual strategies for creating personalized cancer vaccines targeting neoantigens: a candidate epitope strategy, and an unbiased strategy. The candidate epitope strategy uses computer algorithms [17, 18] and *in vitro* studies to predict immunodominant epitopes, which are then integrated into a neoantigen vaccine. In the unbiased strategy, no attempt is made to identify the immunodominant epitopes, and all candidate neoantigens are integrated into a neoantigen vaccine.

We have considered both the candidate epitope strategy and the unbiased strategy. We believe that the candidate epitope strategy is superior to the unbiased strategy for the following reasons. (1) Preliminary data from preclinical models and human correlative studies suggest that relatively few sequencing-identified neoantigens are processed, presented and effectively recognized by the immune system. (2) We have now developed and validated algorithms for the prediction and prioritization of sequencing-identified neoantigens [19, 20]. (3) Targeting a limited number of prioritized sequencing-identified neoantigens will facilitate vaccine design and manufacture, and streamline immune monitoring.

2.4 Sequencing strategies to identify neoantigens

Robust next-generation sequencing strategies for the identification of neoantigens will be required for the successful clinical translation of personalized cancer vaccine strategies. As such, a major focus of our research studies has been the development of cost-effective and accurate next-generation sequencing strategies to identify neoantigens and validate the expression of these antigens at the mRNA level. Initially a cancer genome sequencing approach was used. While cancer whole genome sequencing is informative and provides comprehensive information about both the coding and noncoding regions of the genome, this level of information may not be necessary for identifying neoantigens, or prioritizing antigens for immune intervention. We have now confirmed that tumor/normal exome sequencing is a robust and accurate strategy for the identification of neoantigens [20]. Of note, recent studies suggest that approximately 40% of mutations identified by cancer exome sequencing are not expressed at the mRNA level, so it is important to confirm expression of the mutant allele at the mRNA level. To evaluate mRNA expression, we have performed cDNA-capture sequencing analyses. We have confirmed that cDNA-capture sequencing can be used to successfully confirm expression of sequencing-identified neoantigens at the mRNA level. This analysis also provides an estimation of how highly expressed the mutated allele is expressed relative to other genes in the tumor. For the phase 1 clinical trial proposed, tumor/normal exome sequencing analysis will be used to identify mutations (single nucleotide variants, insertions and deletions) present only in the tumor, and cDNA-capture sequencing will be used to confirm mutant allele expression and expression level in the tumor mRNA.

2.5 Prioritization of sequencing-identified neoantigens

Of note, we have now developed and validated an epitope prediction algorithm for the prioritization of sequencing-identified neoantigens. Once somatic mutations have been identified and mutant mRNA expression confirmed/quantified using the sequencing strategies outlined above, neoantigens will be prioritized using an epitope prediction algorithm that has been designed to select and prioritize the most promising sequencing-identified neoantigens. Currently, the most commonly used CD8 T cell epitope prediction algorithm is NetMHC. However, collaborative work conducted by Robert Schreiber, Elaine Mardis, Max Artyomov and William Gillanders has shown that a much more accurate prediction comes from calculating a median affinity for each sequencing-predicted mutant epitope using multiple epitope prediction algorithms (NetMHC Pan; ANN; SMM; and others). We have significantly improved this epitope prediction algorithm by applying three filters to the initial prioritized output list: (a) elimination of hypothetical proteins; (b) use of an antigen processing algorithm to eliminate epitopes that are not likely to be proteolytically produced by constitutive proteasomes or immunoproteasomes; and (c) prioritization of “neo-epitopes” identified by a higher affinity binding of the mutant peptide sequence compared to the wildtype peptide sequence. The final output of these analyses is a rank-ordered list of the highest to lowest priority sequencing-identified neoantigens for each individual patient. In experiments performed using preclinical mouse sarcoma models, this refined prediction algorithm has successfully identified the major tumor rejection antigens in three out of three tumors tested to date [19, 20]. To our knowledge, this is the only algorithm that has been successfully applied to date to cancer vaccine development. Additional information about the preclinical validation of the epitope prediction algorithm is provided in Section 6.2 Nonclinical Studies. A similar process will be used to identify and prioritize HLA class II neoantigens

Once a rank-ordered list of the highest to lowest priority sequencing-identified mutant tumor-specific peptide antigens is generated, we may perform *in vitro* binding studies to confirm that the prioritized sequencing-identified mutant tumor-specific peptide antigens can bind to and stabilize the appropriate HLA class I allele. Up to 20 of the highest priority sequencing-identified neoantigens that are confirmed to bind and stabilize HLA class I or class II molecules will be targeted using the neoantigen DNA vaccine strategy detailed below.

2.6 Neoantigen DNA vaccines

The neoantigen DNA vaccine strategy is based on the DNA vaccine platform. The observation that direct administration of recombinant DNA can generate potent immune responses established the field of DNA vaccines in the early 1990s [21-26]. Since that time, DNA vaccines have remained an area of intense research interest, and vaccines targeting infectious disease agents and cancers have progressed into

clinical trials. Advantages of the DNA vaccine platform include the remarkable safety profile of DNA vaccines, and the relative ease of manufacture relative to proteins and other biologics. Perhaps most important, however, is the molecular flexibility of the DNA vaccine platform, with the ability to genetically manipulate encoded antigens, and/or incorporate other genes to amplify the immune response [27, 28]. The molecular flexibility of the DNA vaccine platform allows us to target multiple neoantigens using a single polyepitope DNA vaccine. Polyepitope DNA vaccines integrate multiple epitopes in a single construct (Figure 1). We have optimized the polyepitope DNA vaccine platform to maximize antigen presentation of neoantigens by integrating a mutant ubiquitin molecule. Because MHC class I binding peptides are initially processed in the cytosol by the ubiquitin/proteasome pathway, we have integrated a mutant form of ubiquitin (UbG76V). Fusion of UbG76V to the N-terminus of the polyepitope construct promotes epitope generation and display.

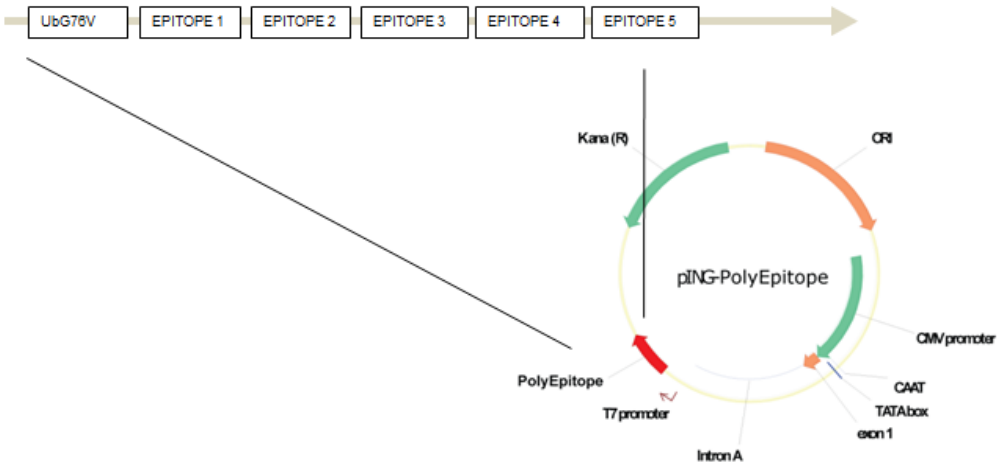


Figure 1: Neoantigen DNA vaccine design. Neoantigen DNA vaccines integrate multiple epitopes in a single construct. We have optimized the neoantigen DNA vaccine platform to maximize antigen presentation of the sequencing-identified neoantigens by integrating a mutant ubiquitin molecule.

2.7 Electroporation

Recent research provides valuable insights into why preclinical studies of DNA vaccines have been successful in rodents, but less successful in larger animals. One difficulty is scaling up DNA vaccine dose and injection volume [29]. In rodents, hydrostatic pressure from injecting DNA in a relatively large liquid volume significantly improves cellular uptake of DNA and antigen expression [30]. This effect is reduced in larger animals as the relative volume injected and hydrostatic pressure is reduced. Electroporation dramatically increases DNA uptake by muscle cells, antigen expression, and immunogenicity [31-34]. Of particular note, electroporation has now been used successfully in non-human primates, with responses at levels previously not observed with other DNA vaccine approaches and similar to or superior to responses induced by live vectors [35-42]. The importance of electroporation to the successful clinical translation of DNA vaccines was recently highlighted and emphasized in a high-profile review in *Nature Reviews* [43], and in the introduction to a special issue of *Vaccine* [44]. We have established a collaboration with Ichor Medical Systems and will use the TDS-IM electroporation device in the proposed phase 1 clinical trial.

2.8 Rationale for vaccination in the adjuvant setting

To date the majority of therapeutic cancer vaccine trials have been performed in patients with metastatic disease. Unfortunately, the results of cancer vaccines in patients with metastatic cancers have been disappointing. In a recent review of cancer vaccine trials of 440 patients at the NCI, Rosenberg et al. noted that the objective clinical response rate by standard RECIST criteria was only 2.6% [45]. It is now commonly believed that generation of an effective antitumor immune response will be difficult in patients

with metastatic cancers [46]. Metastatic breast cancer is no exception; metastatic breast cancer is associated with an increase in the prevalence of regulatory T cells [47, 48] and immature myeloid cells [49, 50] in the peripheral blood and in the tumor microenvironment, and these cells are capable of inhibiting endogenous or elicited antitumor immune responses.

These issues have resulted in a fundamental reassessment of the clinical development paradigm for therapeutic cancer vaccines, with an emphasis on early assessment of vaccine safety and efficacy in an appropriate clinical context [51-53].

The most important consideration in the design of this clinical trial is to ensure the safe translation of the neoantigen DNA vaccine strategy. The Food and Drug Administration (FDA) dictates that initial studies of biologic therapies be performed in such a way that there is a balance between the potential risks and benefits in individual patients.

2.9 Rationale for targeting patients with triple negative breast cancer

Triple-negative breast cancer (TNBC) lacks expression of estrogen receptor, progesterone receptor and HER-2. TNBC is associated with an aggressive clinical course, and there are no targeted therapies available. As such, development of innovative therapies for the treatment of TNBC is an important priority.

There is strong rationale for the use of checkpoint blockade therapy in patients with TNBC.

First, TNBC is a mutationally complex breast cancer subtype. Previous studies suggest that response to checkpoint blockade therapy is associated with mutational load. Several recent studies have provided insight into the mutational landscape in human breast cancer, including TNBC. While the average somatic mutational frequency across almost 30 different types of cancer is approximately one mutation/megabase, the frequency among all breast cancers ranges from 0.1 to 10 [54-56]. One feature of TNBC is the increased frequency of germline and somatic TP53 and BRCA1 mutations [57]. TP53 and BRCA mutations lead to defects in DNA repair mechanisms, and consequently an increased mutational frequency. Extensive analysis of the clonal and mutational spectrum of primary TNBC suggests that TNBC is characterized by a higher mutational frequency than other subtypes of breast cancer [58, 59]. The relative abundance of somatic mutations in TNBC compared to other breast cancer subtypes suggests that mutant antigens that can be targeted by the immune system are more likely to be present.

Second, tumor infiltrating lymphocytes (TILs) are more common in TNBC, and TILs are associated with improved outcome in TNBC following adjuvant or neoadjuvant chemotherapy [60-62]. The association between TILs and improved outcome in TNBC suggests that the adaptive immune system contributes to the response to chemotherapy.

Third, PD-L1 expression is higher in TNBC than other breast cancer subtypes [63, 64]. PD-L1 expression has been used as a biomarker for response to checkpoint blockade therapy [65].

We have examined the expression of PD-1 and PD-L1 in the common molecular subtypes of breast cancer using a well annotated human breast cancer tissue array [64, 66]. These studies demonstrate that expression of PD-1 on tumor-infiltrating lymphocytes and/or PD-L1 on breast cancers is more common in TNBC, and expression of these proteins is associated with a poor prognosis. Other investigators have also documented increased expression of PD-L1 in TNBC [63].

Fourth, a recent phase 1 clinical trial of atezolizumab in patients with metastatic TNBC demonstrates the safety of checkpoint blockade therapy with promising response rates. In an oral presentation at the AACR annual meeting in April 2015 (Abstract 2859), Emens et al. reported on 54 patients with metastatic TNBC enrolled in an expansion cohort. Patients received atezolizumab at 15 mg/kg, 20 mg/kg or 1200 mg flat dose IV q3w. AEs occurred in 67% of patients, most frequently fatigue (22%), pyrexia (15%), neutropenia (15%), and nausea (15%). Grade 3 AEs occurred in 11% of patients (adrenal insufficiency, neutropenia, nausea, vomiting, decreased WBC count). Twenty-one of the 54 patients were evaluable for efficacy, all of whom were PD-L1-positive (IHC 2/3). Among the 21 evaluable patients with PD-L1-expressing tumors, the unconfirmed RECIST ORR was 24% (95% CI, 8% to 47%) which included 3 PRs and 2 CRs.

Adams et al reported the results of a phase 1b clinical trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic TNBC at the 2015 SABCS Meeting (Abstract P2-11-06). Twenty-four

patients with a minimum follow-up of ≥ 3 months were evaluable for efficacy. Across all groups the ORR was 71%. Based on these tests, a randomized phase 3 trial is planned (Impassion130, NCT02425891).

2.10 Durvalumab (MEDI4736)

The non-clinical and clinical experience is fully described in the most current version of the durvalumab Investigator's Brochure (edition 10).

Durvalumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG) 1 kappa subclass that inhibits binding of PD-L1 and is being developed by AstraZeneca/MedImmune for use in the treatment of cancer. (MedImmune is a wholly owned subsidiary of AstraZeneca; AstraZeneca/MedImmune will be referred to as AstraZeneca throughout this document.) As durvalumab is an engineered mAb, it does not induce antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity. The proposed mechanism of action for durvalumab is interference of the interaction of PD-L1 with PD-1 and CD80. PD-L1 can bind to both PD-1 and CD80 on T cells. Similar to PD-L1/PD-1, the PD-L1/CD80 interaction delivers an inhibitory signal in T cells. Blockade of the PD-L1/CD80 interaction is expected to block the inhibitory signal and thereby enhance T cell responsiveness and expansion.

To date durvalumab has been given to more than 1800 subjects as part of ongoing studies either as monotherapy or in combination with other anti-cancer agents. Details on the safety profile of durvalumab monotherapy are summarized in Section 2.10.1. Refer to the current durvalumab Investigator's Brochure for a complete summary of non-clinical and clinical information including safety, efficacy and pharmacokinetics.

2.10.1 Durvalumab monotherapy dose rationale

A durvalumab dose of 20 mg/kg Q4W is supported by in-vitro data, non-clinical activity, clinical PK/pharmacodynamics, biomarkers, and activity data from Study 1108 in subjects with advanced solid tumors and from a Phase I trial performed in Japanese subjects with advanced solid tumor (D4190C00002).

2.10.2 PK/Pharmacodynamic data

Based on available PK/pharmacodynamic data from ongoing Study 1108 with doses ranging from 0.1 to 10 mg/kg Q2W or 15 mg/kg Q3W, durvalumab exhibited non-linear (dose-dependent) PK consistent with target-mediated drug disposition. The PK approached linearity at ≥3 mg/kg Q2W, suggesting near complete target saturation (membrane-bound and sPD-L1), and further shows that the durvalumab dosing frequency can be adapted to a particular regimen given the linearity seen at doses higher than 3 mg/kg. The expected half-life with doses ≥3 mg/kg Q2W is approximately 21 days. A dose-dependent suppression in peripheral sPD-L1 was observed over the dose range studied, consistent with engagement of durvalumab with PD-L1. A low level of immunogenicity has been observed. No subjects have experienced immune-complex disease following exposure to durvalumab (For further information on immunogenicity, please see the current IB).

Data from Study D4190C00006 (Phase I trial in NSCLC subjects using the combination of durvalumab and tremelimumab) also show an approximately dose-proportional increase in PK exposure for durvalumab over the dose range of 3 to 20 mg/kg durvalumab Q4W or Q2W. (For further information on PK observations in Study 006, please see the current IB).

The observed durvalumab PK data from the combination study were well in line with the predicted monotherapy PK data (5th median and 95th percentiles) for a Q4W regimen.

A population PK model was developed using the data from Study 1108 (doses=0.1 to 10 mg/kg Q2W or 15 mg/kg Q3W (Fairman et al 2014). Multiple simulations indicate that a similar overall exposure is expected following both 10 mg/kg Q2W and 20 mg/kg Q4W regimens, as represented by AUCss (4 weeks). Median Cmax,ss is expected to be higher with 20 mg/kg Q4W (~1.5 fold) and median Ctrough,ss is expected to be higher with 10 mg/kg Q2W (~1.25 fold). Clinical activity with the 20 mg/kg Q4W dosing regimen is anticipated to be consistent with 10 mg/kg Q2W with the proposed similar dose of 20 mg/kg Q4W expected to (a) achieve complete target saturation in majority of subjects; (b) account for anticipated variability in PK, pharmacodynamics, and clinical activity in diverse cancer populations;

(c) maintain sufficient PK exposure in case of ADA impact; and (d) achieve PK exposure that yielded maximal antitumor activity in animal models.

Given the similar area under the plasma drug concentration-time curve (AUC) and modest differences in median peak and trough levels at steady state, the observation that both regimens maintain complete sPD-L1 suppression at trough, and the available clinical data, the 20 mg/kg Q4W and 10 mg/kg Q2W regimens are expected to have similar efficacy and safety profiles, supporting further development with a dose of 20 mg/kg Q4W.

2.10.3 Clinical data

Refer to the current durvalumab Investigator's Brochure for a complete summary of clinical information including safety, efficacy and pharmacokinetics at the 20mg/kg Q4W regimen.

2.10.4 Rationale for fixed dosing

A population PK model was developed for durvalumab using monotherapy data from a Phase I study (study 1108; N=292; doses= 0.1 to 10 mg/kg Q2W or 15 mg/kg Q3W; solid tumors). Population PK analysis indicated only minor impact of body weight (WT) on the PK of durvalumab (coefficient of ≤ 0.5). The impact of body WT-based (10 mg/kg Q2W) and fixed dosing (750 mg Q2W) of durvalumab was evaluated by comparing predicted steady state PK concentrations (5th, median and 95th percentiles) using the population PK model. A fixed dose of 750 mg was selected to approximate 10 mg/kg (based on median body WT of ~75 kg). A total of 1000 subjects were simulated using body WT distribution of 40–120 kg. Simulation results demonstrate that body WT-based and fixed dosing regimens yield similar median steady state PK concentrations with slightly less overall between-subject variability with fixed dosing regimen.

Similar findings have been reported by others (Ng et al 2006, Wang et al 2009, Zhang et al 2012, Narwal et al 2013). Wang and colleagues investigated 12 monoclonal antibodies and found that fixed and body size-based dosing perform similarly, with fixed dosing being better for 7 of 12 antibodies (Wang et al 2009)]. In addition, they investigated 18 therapeutic proteins and peptides and showed that fixed dosing performed better for 12 of 18 in terms of reducing the between-subject variability in pharmacokinetic/pharmacodynamics parameters (Zhang et al 2012).

A fixed dosing approach is preferred by the prescribing community due to ease of use and reduced dosing errors. Given expectation of similar pharmacokinetic exposure and variability, we considered it feasible to switch to fixed dosing regimens. Based on average body WT of 75 kg, a fixed dose of 1500 mg Q4W durvalumab (equivalent to 20 mg/kg Q4W) is included in the current study.

2.10.5 Identified and potential risks

Monoclonal antibodies directed against immune checkpoint proteins, such as programmed cell death ligand 1 (PD-L1) as well as those directed against programmed cell death-1 (PD-1) or cytotoxic T-lymphocyte antigen-4 (CTLA-4), aim to boost endogenous immune responses directed against tumor cells. By stimulating the immune system however, there is the potential for adverse effects on other tissues.

Most adverse drug reactions seen with the immune checkpoint inhibitor class of agents are thought to be due to the effects of inflammatory cells on specific tissues. Potential risks are events with a potential inflammatory mechanism and which may require more frequent monitoring and/or unique interventions such as immunosuppressants and/or endocrine replacement therapy. These risks include gastrointestinal AEs such as colitis and diarrhoea, pneumonitis, nephritis and acute renal failure, hepatic AEs such as hepatitis and liver enzyme elevations, dermatitis, and endocrinopathies such as hypo- and hyper-thyroidism, hypophysitis and adrenal insufficiency.

Identified risks with durvalumab are diarrhea, increases in transaminases, pneumonitis and colitis.

Potential risks include endocrinopathies (hypo- and hyper-thyroidism, hypophysitis and adrenal insufficiency) hepatitis/hepatotoxicity, neurotoxicities, nephritis, pancreatitis, dermatitis, infusion-related reactions, anaphylaxis, hypersensitivity or allergic reactions, and immune complex disease. Further information on these risks can be found in the current version of the durvalumab IB.

In monotherapy clinical studies AEs (all grades) reported very commonly (≥ 10% of subjects) are fatigue, nausea, decreased appetite, dyspnea, cough, constipation, diarrhea, vomiting, back pain, pyrexia, abdominal pain, anemia, arthralgia, peripheral edema, headache, rash, and pruritus. Approximately 10% of subjects experienced an AE that resulted in permanent discontinuation of durvalumab and approximately 3.5% of subjects experienced an SAE that was considered to be related to durvalumab by the study investigator.

The majority of treatment-related AEs were manageable with dose delays, symptomatic treatment, and in the case of events suspected to have an immune basis, the use of established treatment guidelines for immune-mediated.

A detailed summary of durvalumab monotherapy AE data can be found in the current version of the durvalumab IB

3 PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Inclusion criteria

A patient will be eligible for inclusion in this study only if ALL of the following criteria apply:

(1) Histologically confirmed diagnosis of invasive breast cancer.

(2) ER and PR less than Allred score of 3 or less than 1% positive staining cells in the invasive component of the tumor. Patients not meeting this pathology criteria, but have been clinically treated as having TNBC, may be enrolled at treating physician's discretion.

(3) HER2 negative by FISH or IHC staining 0 or 1+.

(4) Consented for genome sequencing.

(5) Clinical stage T1c-T4c, any N, M0 primary tumor by AJCC 7th edition clinical staging prior to neoadjuvant chemotherapy, with residual invasive breast cancer after neoadjuvant therapy.

(6) At least 18 years of age.

(7) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1.

(8) Adequate organ and marrow function no more than 14 days prior to registration as defined below:

absolute neutrophil count	≥1,500/μL
platelets	≥100,000/μL
hemoglobin	≥ 9.0 g/dL
total bilirubin	≤1.5 X institutional upper limit of normal
AST/ALT	≤2.5 X institutional upper limit of normal
serum creatinine clearance	> 40 mL/min by the Cockcroft-Gault formula or by 24-hour urine collection for determination of creatinine clearance

(9) Body weight > 30 kg.

(10) Evidence of post-menopausal status or negative urine or serum pregnancy test for female pre-menopausal subjects. Women will be considered post-menopausal if they have been amenorrheic for 12 months without an alternative medical cause. The following age-specific requirements apply:

- Women < 50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and if they have luteinizing hormone and follicle-stimulating hormone levels in the post-menopausal range for the institution or underwent surgical sterilization (bilateral oophorectomy or hysterectomy).
- Women ≥ 50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments, had radiation-induced menopause with last menses > 1 year ago, had chemotherapy-induced menopause with last menses > 1 year ago, or underwent surgical sterilization (bilateral oophorectomy, bilateral salpingectomy, or hysterectomy).

(11) Able to understand and willing to sign an IRB-approved written informed consent document.

3.1.2 Exclusion criteria

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

(1) Received chemotherapy, radiotherapy (to more than 30% of the bone marrow or with a wide field of radiation), or biologic therapy within the last 30 days.

(2) Concurrent enrollment in another clinical study, unless it is an observational (non-interventional) clinical study or during the follow-up period of an interventional study.

(3) Receiving any other investigational agent(s) or has received an investigational agent within the last 30 days.

(4) Receipt of live attenuated vaccination within 6 months prior to study entry or within 30 days of receiving durvalumab.

(5) Major surgical procedure within 28 days prior to the first dose of durvalumab. Local surgery of isolated lesions for palliative intent is acceptable.

(6) Current use or prior use of immunosuppressive medication within 28 days before the first dose of durvalumab, with the exceptions of intranasal, inhaled, and intra-articular corticosteroids or systemic corticosteroids at physiological doses which are not to exceed 10 mg/day of prednisone or an equivalent corticosteroid.

(7) Known metastatic disease.

(8) Invasive cancer in the contralateral breast.

(9) Known allergy, or history of serious adverse reaction to vaccines such as anaphylaxis, hives, or respiratory difficulty.

(10) History of hypersensitivity to durvalumab or any excipient.

(11) Mean QT interval corrected for heart rate using Fridericia's formula (QTcF) ≥ 470 ms calculated from 3 electrocardiograms (ECGs) (within 15 minutes at 5 minutes apart).

(12) Any unresolved toxicity NCI CTCAE grade ≥ 2 from previous anticancer therapy with the exception of alopecia, vitiligo, and the laboratory values defined in the inclusion criteria. Subjects with grade ≥ 2 neuropathy will be evaluated on a case-by-case basis after consultation with the study physician. Subjects with irreversible toxicity not reasonably expected to be exacerbated by treatment with durvalumab may be included only after consultation with the study physician.

(13) Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia, interstitial lung disease, serious chronic gastrointestinal conditions associated with diarrhea, evidence of any acute or chronic viral illness or disease, or psychiatric illness/social situation that would limit compliance with study requirements or compromise the ability of the subject to give written informed consent.

(14) Active or prior documented autoimmune or inflammatory disorders (including inflammatory bowel disease [e.g., colitis or Crohn's disease], diverticulitis [with the exception of diverticulosis], systemic lupus erythematosus, Sarcoidosis syndrome, or Wegener syndrome [granulomatosis with polyangiitis, Graves' disease, rheumatoid arthritis, hypophysitis, uveitis, etc.]). The following are exceptions to this criterion:

- Subjects with vitiligo or alopecia
- Subjects with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement
- Any chronic skin condition that does not require systemic therapy
- Subjects without active disease in the last 5 years may be included but only after consultation with the study physician
- Subjects with celiac disease controlled by diet alone

(15) History of pneumonitis or interstitial lung disease.

(16) History of active primary immunodeficiency.

(17) Active infection including tuberculosis (clinical evaluation that includes clinical history, physical examination, and radiographic findings, and TB testing in line with local practice), hepatitis B (known positive HBV surface antigen (HBsAg) result), hepatitis C, or human immunodeficiency virus (positive HIV 1/2 antibodies). Subjects with a past or resolved HBV infection (defined as the presence of

hepatitis B core antibody (anti-HBc) and absence of HBsAg) are eligible. Subjects positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA.

(18)History of allogeneic organ transplantation.

(19)Pregnant or breastfeeding. A negative serum pregnancy test is required no more than 14 days before study entry.

(20)Subjects of reproductive potential who are not willing to employ effective birth control from screening to 1 year after last dose of vaccine.

(21)History of another primary malignancy except for:

- Malignancy treated with curative intent and with no known active disease ≥ 5 years before the first dose of study treatment and low potential for risk of recurrence
- Adequately treated non-melanoma skin cancer of lentigo maligna without evidence of disease
- Adequately treated carcinoma in situ without evidence of disease

(22)History of leptomeningeal carcinomatosis.

(23)Patient must have no active major medical or psychosocial problems that could be complicated by study participation.

(24)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.

(25)Individuals in whom a skinfold measurement of the cutaneous and subcutaneous tissue for eligible injection sites (left and right medial deltoid region) exceeds 40 mm.

(26)Individuals in whom the ability to observe possible local reactions at the eligible injection sites (deltoid region) is, in the opinion of the investigator, unacceptably obscured due to a physical condition or permanent body art.

(27)Therapeutic or traumatic metal implant in the skin or muscle of either deltoid region.

(28)Acute or chronic, clinically significant hematologic, pulmonary, cardiovascular, or hepatic or renal functional abnormality as determined by the investigator based on medical history, physical examination, EKG, and/or laboratory screening test

(29)Any chronic or active neurologic disorder, including seizures and epilepsy, excluding a single febrile seizure as a child, or chronic seizure disorder which is well controlled by medication with no seizures within the last 2 years.

(30)Syncopal episode within 12 months of screening

(31)Current use of any electronic stimulation device, such as cardiac demand pacemakers, automatic implantable cardiac defibrillator, nerve stimulators, or deep brain stimulators.

3.2 Inclusion of women and minorities

Women 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 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 deemed eligible for study, the principal investigator (or co-investigators) will discuss the Washington University Human Research Protection Office-approved informed consent with the patient. This written informed consent will be signed and dated by the patient and the principal investigator (or co-investigators). The original consent will be placed in the patient's permanent record and a copy will be given to the patient.

Washington University School of Medicine (WUSM) 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 clinical stage II or III triple negative breast cancer will be screened for eligibility for entry into the study. 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.

4.1.3 Presentation of informed consent

Consent will be obtained by either the principal investigator or by individuals approved by the principal investigator and whose names and copy of their *curriculum vitae* have been filed. The initial consent should be the IRB-approved version corresponding to the version of the protocol approved when the screening was initiated. Informed consent is to be obtained from the subject according to **Section 18.1 Informed Consent** of this protocol.

4.2 Registration procedures

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

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

(1) Confirmation of patient eligibility

(2) Registration of patient in the Siteman Cancer Center OnCore database

(3) Assignment of unique patient number (UPN)

4.3 Confirmation of patient eligibility

Confirm patient eligibility by collecting the information listed below:

(1) The 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

Twenty-four patients will be enrolled. Patients will be randomized for participation in a 1:1 ratio to receive either neoantigen DNA vaccine alone or neoantigen DNA vaccine plus durvalumab. The randomization table will be generated using the SAS program PROC PLAN. Randomization will take place after the manufacture of the vaccine and after eligibility has been reconfirmed.

5 INVESTIGATIONAL AGENT: DURVALUMAB

5.1 Description

Durvalumab is a human monoclonal antibody (mAb) of the immunoglobulin G1 kappa (IgG1κ) subclass that inhibits binding of programmed cell death ligand 1 (PD-L1) (B7-H1, CD274) to programmed cell death 1 (PD-1; CD279) and CD80 (B7-1). Durvalumab is composed of 2 identical heavy chains and 2 identical light chains, with an overall molecular weight of approximately 149 kDa. Durvalumab contains a triple mutation in the constant domain of the immunoglobulin (Ig) G1 heavy chain that reduces binding to complement protein C1q and the fragment crystallizable gamma (Fcγ) receptors involved in triggering effector function.

5.2 Pharmacokinetics and Drug Metabolism

Please refer to Section 2.10.2.

5.3 Supplier(s)

The Investigational Products Supply section of AstraZeneca/MedImmune will supply durvalumab (MEDI4736) to the investigator as a 500-mg vial solution for infusion after dilution.

5.4 Dosage Form and Preparation

Durvalumab (MEDI4736) will be supplied by AstraZeneca as a 500-mg vial solution for infusion after dilution. The solution contains 50 mg/mL durvalumab (MEDI4736), 26 mM histidine/histidine-hydrochloride, 275 mM trehalose dihydrate, and 0.02% (weight/volume) polysorbate 80; it has a pH of 6.0. The nominal fill volume is 10 mL. Investigational product vials are stored at 2°C to 8°C (36°F to 46°F) and must not be frozen. Durvalumab (MEDI4736) must be used within the individually assigned expiry date.

Preparation of infusion bags

Total time from needle puncture of the durvalumab vial to the start of administration should not exceed:

- 24 hours at 2°C to 8°C (36°F to 46°F)
- 4 hours at room temperature

If in-use storage time exceeds these limits, a new dose must be prepared from new vials. Infusion solutions must be allowed to equilibrate to room temperature prior to commencement of administration.

A dose of 1500 mg (for patients >30 kg in weight) will be administered using an IV bag containing 0.9% (w/v) saline or 5% (w/v) dextrose, with a final durvalumab (MEDI4736) concentration ranging from 1 to 20 mg/mL, and delivered through an IV administration set with a 0.2- or 0.22-µm in-line filter. Add 30.0 mL of durvalumab (MEDI4736) (ie, 1500mg of durvalumab [MEDI4736]) to the IV bag. The IV bag size should be selected such that the final concentration is within 1 to 20 mg/mL. Mix the bag by gently inverting to ensure homogeneity of the dose in the bag. No incompatibilities between durvalumab and polyvinylchloride or polyolefin IV bags have been observed. Subjects whose weight drops to ≤ 30 kg should not receive durvalumab.

In the event that either preparation time or infusion time (see Section 5.6) exceeds the time limits, a new dose must be prepared from new vials. Durvalumab does not contain preservatives, and any unused portion must be discarded.

5.5 Storage and Stability

Total in-use storage time from needle puncture of durvalumab vial to start of administration should not exceed 4 hours at room temperature or 24 hours at 2-8°C (36-46°F). If in-use storage time exceeds these limits, a new dose must be prepared from new vials. Infusion solutions must be allowed to equilibrate to room temperature prior to commencement of administration. Durvalumab does not contain preservatives and any unused portion must be discarded.

5.6 Administration

Following preparation of durvalumab, the entire contents of the IV bag should be administered as an IV infusion at room temperature (approximately 25°C) over approximately 60 minutes (±5 minutes), using a 0.2-µm or 0.22-µm in-line filter.

Standard infusion time is 1 hour. However, if there are interruptions during infusion, the total allowed time should not exceed 8 hours at room temperature. The IV line will be flushed with a volume of IV diluent equal to the priming volume of the infusion set used after the contents of the IV bag are fully administered, or complete the infusion according to institutional policy to ensure the full dose is administered and document if the line was not flushed.

Since the compatibility of durvalumab with other IV medications and solutions, other than normal saline (0.9% [weight/volume] sodium chloride for injection) or 5% (weight/volume) dextrose, is not known, the durvalumab solution should not be infused through an IV line in which other solutions or medications are being administered.

Subjects will be monitored before, during, and after the infusion with assessment of vital signs at the times specified in the Schedule of Assessments. Subjects are monitored (pulse rate, blood pressure) every 30 minutes during the infusion period (including times where infusion rate is slowed or temporarily stopped).

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event (up to 4 hours) and re-initiated at 50% of the initial rate until completion of the infusion. For subjects with a ≤Grade 2 infusion-related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion-related reaction is ≥Grade 3 or higher in severity, study drug will be discontinued. The standard infusion time is one hour, however if there are interruptions during infusion, the total allowed time from infusion start to completion of infusion should not exceed 8 hours at room temperature. For management of subjects who experience an infusion reaction, please refer to the toxicity and management guidelines.

As with any antibody, allergic reactions to dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit subjects to an intensive care unit if necessary.

6 INVESTIGATIONAL AGENT: NEOANTIGEN DNA VACCINES

6.1 Chemical name and structure

The neoantigen DNA vaccines are also known as DNA plasmid vector expressing tumor-specific antigens.

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

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

(2) Tumor/normal exome sequencing and tumor cDNA-capture sequencing will be performed to identify candidate neoantigens.

(3) Candidate neoantigens will be prioritized based on epitope prediction algorithms and *in vitro* studies.

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

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

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

(7) The neoantigen DNA vaccines will undergo product release tests at WUSM and other sites prior to investigational use.

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

Each neoantigen DNA vaccine drug product is composed of a deoxyribonucleic acid (DNA) plasmid purified from *E. Coli*. The pING parent vector was obtained from Alan Houghton, M.D. at Memorial Sloan Kettering Cancer Center (Figure 2). The pING vector has been used extensively in preclinical DNA vaccine studies [71, 72], and in human clinical trials, including a clinical trial at WUSM (ClinicalTrials.gov identifier: NCT00807781). The mammaglobin-A DNA vaccine is based on the pING vector and was manufactured at WUSM in the SCC Biologic Therapy Core Facility using manufacturing processes almost identical to the ones outlined here).

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

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

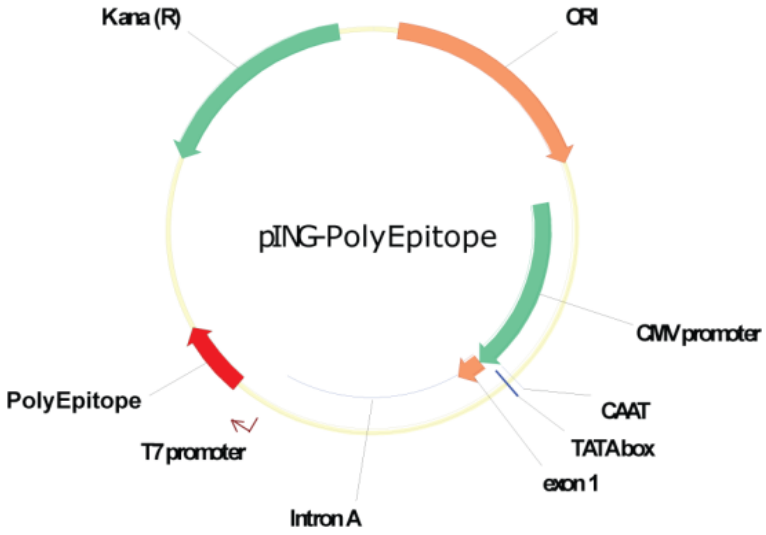


Figure 2: Neoantigen DNA vaccine design. Neoantigen DNA vaccines are based on the pING parent vector. The pING vector contains the following elements: (1) a eukaryotic promoter and enhancer from the Towne strain of CMV; (2) a polylinker region to facilitate cloning of a variety of DNA fragments; (3)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

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

6.2 Manufacturing facility

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

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

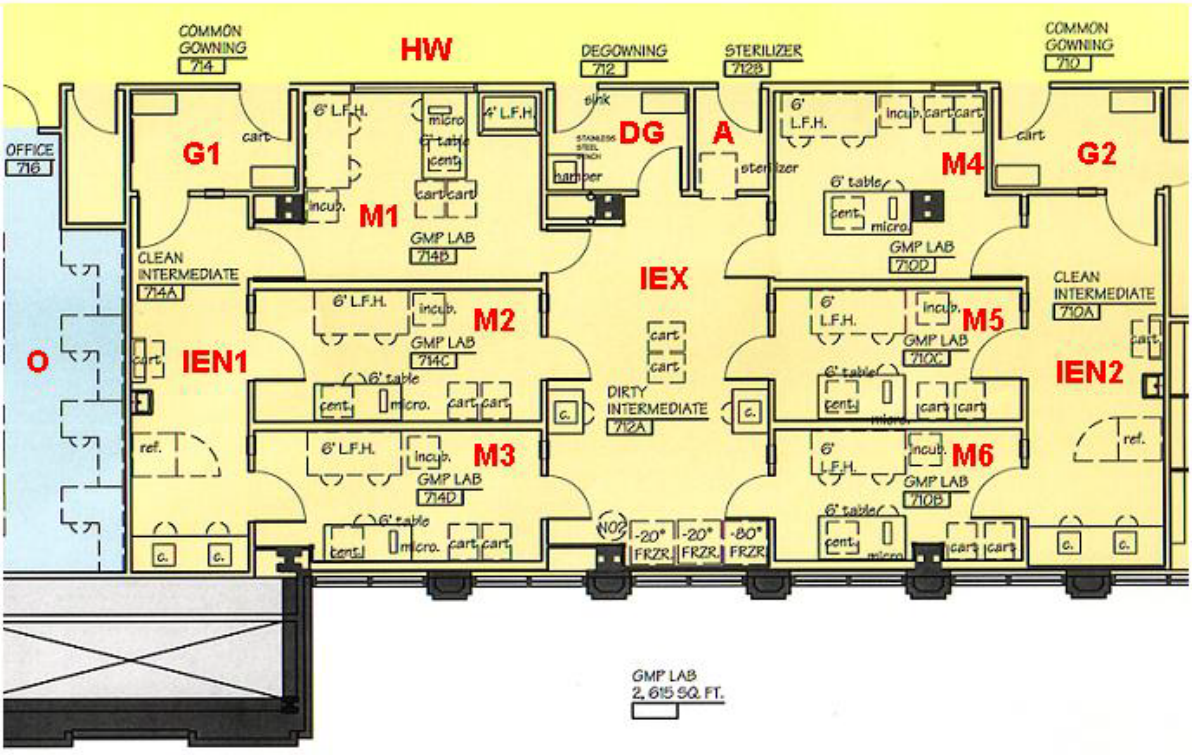


Figure 3: Siteman Cancer Center Biologic Therapy Core Facility floor plan. HW: Hallway, G1: Gowning Room 1; G2: Gowning Room 2; IEN 1: Intermediate Entry Room 1; IEN 2: Intermediate Entry Room 2; M1 to M3: Manufacturing Rooms 1 to 3; M4 to M6: Manufacturing Rooms 4 to 6; IEX: Intermediate Exit Room;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

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

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

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

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

Every manufacturing lab is equipped with a 6-foot biosafety cabinet and a dual chamber incubator. Sufficient room is available per manufacturing lab to accommodate specialized equipment (e.g. clinical grade magnetic bead cell separator) for different manufacturing processes. In the largest manufacturing

room, besides the 6ft biosafety cabinet, a second 4ft biosafety cabinet is installed for work with infectious agents. Manufacturing room 1 can also be switched to negative air pressure and BSL-3 containment (vector manufacturing), and an IBC approved protocol to accomplish this task is in place.

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

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

6.3 Manufacturing process

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

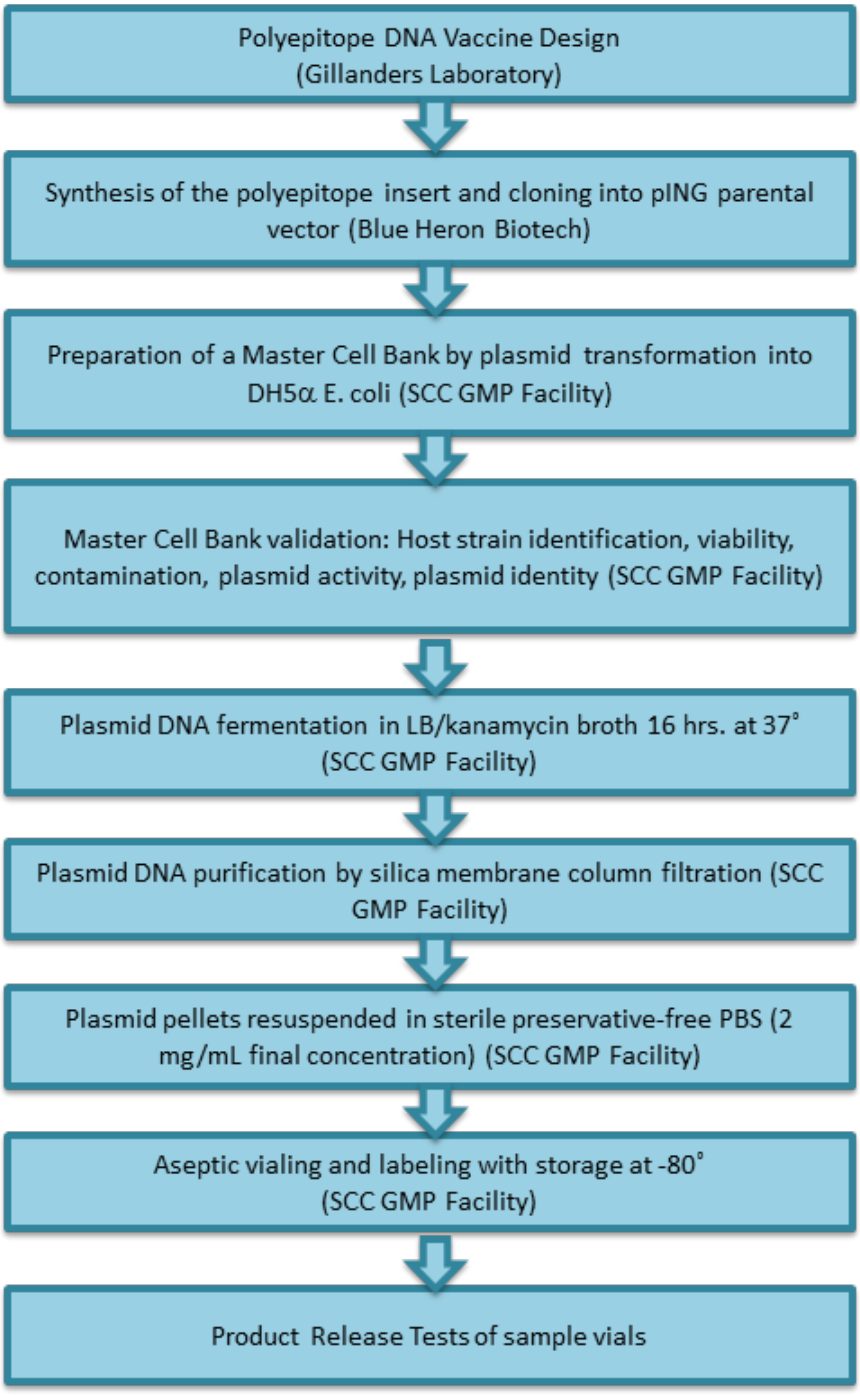


Figure 4: Overview of the neoantigen DNA vaccine manufacturing process. Schematic outline of product manufacture, in-process testing, and product release tests.

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

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

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

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

TABLE 1: Master cell bank validation for neoantigen DNA vaccines

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

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

Plasmid DNA purification. The plasmid DNA will be purified using reagents obtained from Sigma Aldrich (Saint Louis, MO). Distinct lots of the Genelute HP Select Plasmid Gigaprep Kit from Sigma-Aldrich (St. Louis, MO) will be used for the manufacture of the plasmids. The following narrative is a brief description

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

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

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

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

6.4 Product formulation

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

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

Following vialing, we anticipate that 4 vials (#1, 4, 10, 19) will be used for product release testing. Vials 1 and 19 will be used to test sterility and represent the beginning and end of the vialing procedure. Two additional vials will be used for the other product release tests listed in Table 3.

If any contamination would be detected in our plasmid samples, we would initiate an alcohol precipitation step. This procedure is basically the final stage of the gigaprep purification, where the soluble DNA is

precipitated using 2x volumes of cold isopropanol and the DNA is pelleted by centrifugation. The pellet is washed in 70% ethanol and air dried. This procedure is a standard method for eliminating any microbiologic contamination in DNA.

6.5 Product release tests

A key issue in the quality control of plasmid DNA for human use is the development of analytical methodologies capable of fully characterizing the product in its final form, ensuring the production of a consistent product. The following list of release criteria, analytical methodologies, and specifications has been adapted from [73]. Neoantigen DNA vaccines will be tested for the following specifications.

TABLE 3: Neoantigen DNA vaccine product release tests

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

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

6.6 Stability

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

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

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

6.7 Labels

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

SCC Biologic Therapy Core Facility
Saint Louis, MO
Store at -80°C
WUSM PPDV #2

Subject Study #, Subject Initials

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

CAUTION Investigational drug limited by federal law to investigational use

6.8 Siteman Cancer Center Investigational Pharmacy

The Investigational Pharmacy is located on the 7th floor of the Center for Advanced Medicine within the chemotherapy administration area of the Siteman Cancer Center. The pharmacy is locked and secured with entry only via an authorized swipe card entry system. Three pharmacists, including a pharmacist with responsibility for investigational agents, and two technicians are employed full-time. Two freezers (-70ºC and -20ºC) and one refrigerator have been designated for studies. Room temperature shelving extends over 300 feet. There are three IV admixture hoods. Two vertical flow hoods are used for preparation of chemotherapy agents and a laminar flow hood is used for non-chemotherapy agents. There were a total of 13,260 chemotherapy prescriptions filled in 2004. Pharmacy hours of operation are Monday through Friday from 7:00 am to 5:30 pm. The investigational pharmacist will obtain the vaccine from the TBC Facility and dispense the vaccine to the nurse who will be administering the agent to the patient.

6.9 Investigational agent administration

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

6.9.1 Introduction to electroporation

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

Although DNA vaccines encoding antigens from a variety of cancers and infectious pathogens have exhibited considerable promise in non-clinical studies, clinical experience to date suggests that current administration methods may have insufficient potency to achieve the desired immune responses in humans. Poor delivery efficiency and low levels of antigen expression have been implicated as factors contributing to the sub-optimal responses observed to date [74, 75].

Electroporation (EP) is a potent physical delivery technique based on the *in vivo* application of electrical fields. The propagation of electrical fields of a sufficient magnitude within a target tissue induces a transient increase in cell membrane permeability, resulting in a significant improvement of intracellular uptake of exogenous substances. Shortly after pulse delivery, the cell membrane function stabilizes and cells within the affected tissue resume normal function. EP has been shown in non-clinical studies to be a potent method for delivery of DNA vaccines [40, 76-78].

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

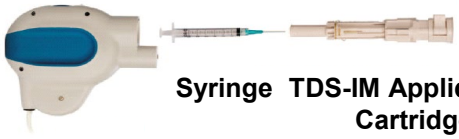
6.9.2 The TDS-IM Electrode Array System

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

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

TDS-IM Components

TDS Pulse Stimulator



Syringe **TDS-IM Application Cartridge**

TDS-IM Integrated Applicator

6.9.3 Summary of TDS-IM Clinical Experience to Date

To date, formal safety studies have been conducted to assess the TDS-IM as the means for delivery of nine DNA vaccine candidates. This includes studies of a melanoma DNA vaccine encoding a xenogeneic form of the tyrosinase antigen that uses the same vector backbone (pING) as the Mammaglobin-A DNA vaccine candidate (see Table 1). Safety studies for the various DNA vaccine candidates have included repeat dose studies in rabbits to assess safety and toxicology, and studies in rats to assess DNA vaccine biodistribution, persistence, and potential for integration into host DNA.

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

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

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

6.9.4 Summary of TDS-IM clinical experience to date

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

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

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

TDS-IM Based DNA Vaccine Administration: Ongoing Clinical Trials		
ClinicalTrials.gov Study #	**Vaccine Candidate**	**Subject Population**
NCT01138410	Epitope-based TRP-2 melanoma vaccine (SCIB-1)	Patients that are HLA A1, A2, A24, or B35 positive with AJCC stage III-IV melanoma
NCT01859325	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to administration of an vesicular stomatitis virus vector (rVSVgag)	HIV infected adult volunteers
NCT01578889	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX) prior to administration of an vesicular stomatitis virus vector (rVSVgag)	Healthy adult volunteers
NCT01266616	Multi-antigen HIV DNA vaccine (HIV-MAG) administered with or without a DNA-based human IL-12 adjuvant (GENEVAX)	HIV infected adult volunteers
NCT01634503	Multi-antigen HPV DNA vaccine administered with a DNA-based human IL-12 adjuvant (GX-188E)	Patients with Grade 3 Cervical Intraepithelial Neoplasia
NCT01493154	HPV DNA vaccine administered with a DNA-based calreticulin adjuvant (pNGVL-4a-CRT/E7 (detox))	HPV-associated squamous cell carcinoma of the head and neck
NCT01984983	VEEV DNA Vaccine Candidate	Healthy adult volunteers

To date, the 13 clinical trials that have been completed or are currently ongoing have enrolled over 350 subjects in the electroporation arms of the studies (including subjects receiving either the DNA vaccine

candidate or placebo). The device has been used for administration of DNA injections of up to 1.0 ml volume and 4.0 mg DNA dose per injection site. Subjects have received the vaccine candidate either as a single injection in one muscle site (total DNA dose up to 4.0 mg per administration time point) or as 2 injections in 2 separate muscle sites (total DNA dose up to 8.0 mg per administration time point). Subjects given the DNA dose as a single injection have received up to 5 TDS-IM injections at up to 4.0 mg DNA, and subjects administered the DNA dose in 2 injections have received up to 5 administrations (i.e., 10 total TDS-IM injections).

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

The results of the completed HIV-1 ADVAX vaccine study in healthy subjects have been published [79]. As reported in the abstract, eight volunteers each received 0.2 mg, 1 mg, or 4 mg ADVAX or saline placebo via electroporation (EP), or 4 mg ADVAX via standard intramuscular injection at weeks 0 and 8. A third vaccination was administered to eleven volunteers at week 36. EP was safe, well-tolerated and considered acceptable for a prophylactic vaccine. EP delivery of ADVAX increased the magnitude of HIV-1-specific cell mediated immunity by up to 70-fold over IM injection, as measured by gamma interferon ELISpot. The number of antigens to which the response was detected improved with EP and increasing dosage. Intracellular cytokine staining analysis of ELISpot responders revealed both CD4+ and CD8+ T cell responses, with co-secretion of multiple cytokines. Briefly, results from this study indicate that EP-based delivery with the TDS-IM device at ADVAX DNA doses ranging from 0.2–4.0 mg was safe and effective in improving the magnitude, breadth and durability of cellular immune responses to a DNA vaccine candidate. Assessment of the tolerability of the EP procedure by questionnaire after each administration indicates that the procedure is generally acceptable for use in healthy subjects.

6.9.5 Preparation of the vaccine

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

6.9.6 Vaccine administration

Detailed instructions for procedure administration using the TDS-IM device are included in the TDS-IM User's Guide. Briefly, the TDS-IM Application Cartridge is loaded into the Integrated Applicator. The Pulse Stimulator is connected to an appropriate power source, turned on, and then connected to the Integrated Applicator through the supplied cable. Prior to administration, the skin at the site of administration is prepared according to the standard procedures for a conventional intramuscular injection. Once the skin has been disinfected, the skin around the injection site is held firmly while the Application Cartridge is placed against the injection site at a 90° angle. An indicator light on the device notifies the user that sufficient pressure has been applied. The activation button is then depressed, causing the electrodes and injection needle to be inserted into the target tissue. An automated safety check is performed, and, if

passed, allows the injection of the study vaccine into the muscle. The procedure will conclude with a series of brief, localized muscle contractions at the administration site. The procedure will require approximately 7-10 seconds to complete, during which time the site is held firmly and with the device depressed against the skin. Once the indicator light on the device indicates that the procedure is complete, the device is withdrawn and site is covered with a sterile covering and pressure applied with 3 fingers for 1 minute.

6.10 Investigational agent accountability

6.10.1 Documentation

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

6.10.2 Disposition

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

7 NONCLINICAL STUDIES

7.1 Overview

The neoantigen DNA vaccine strategy is designed to target neoantigens present in the cancer, but absent in normal tissues.

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

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

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

The dosing described in this study is based on previous DNA vaccine studies as well as an ongoing phase I clinical trial (NCT02348320).

7.2 Nonclinical studies

It has long been known that there is a dynamic relationship between the immune system and cancer. This dynamic relationship has been studied in detail, ultimately resulting in the establishment of the cancer immunoediting concept [80-87].

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

In initial studies we used a combination of next-generation sequencing and epitope prediction algorithms to identify neoantigens in the d42m1 MCA sarcoma line. These algorithms identified one particular mutation (an R913L mutation of SPTBN2) as a top candidate, and subsequent analyses confirmed that this mutant tumor-specific antigen functioned as an immunodominant tumor rejection antigen. These studies were published in *Nature* [20].

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

next-generation sequencing and epitope prediction algorithms be used to prioritize antigens for immune targeting and/or neoantigen vaccine therapy?

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

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

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

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

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

7.3 Rationale for no GLP safety studies

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

<u>First, it is impossible to know *a priori* what mutations will be present and/or prioritized in patients.</u> Next-generation sequencing of epithelial cancers has demonstrated that there are very few recurrent mutations

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

Second, to our knowledge, no mammary tumor models exist that would be relevant for the proposed preclinical studies. In order to study neoantigens, sequencing analyses of paired tumor/normal tissues are required. As such, we would need to study spontaneous tumors in mice, as tumors propagated as cell lines do not have corresponding normal DNA to evaluate. There are very few models of spontaneous tumor development in wildtype mice. Spontaneous tumors do develop in genetically engineered mice, but these oncogene-driven tumors in genetically engineered murine models of cancer typically have a very limited number of mutations. We have performed extensive studies in genetically engineered mice, and have found that there are very few mutations present in tumors derived from these mice. For example, we have performed extensive studies in the p53-null transplant mammary tumor model. p53 is a tumor suppressor gene and plays an important role in maintaining genome stability. As such one might expect that there would be a significant number of mutations present in p53-null transplant mammary tumors. However, we have sequenced > 10 p53-null transplant mammary tumors and have found that there are only a limited number of mutations present in each tumor. This is not at all representative of human triple-negative breast cancers where significantly more mutations are present. Of note, a significant number of mutations must be present to reliably identify neoantigens that are immunogenic, as many candidate neoantigens are not processed and presented by the immune system, or cannot be recognized efficiently by T cells. If only a limited number of mutations is present, it is possible that no neoantigens will be identified that are immunogenic. For meaningful GLP safety and toxicology studies of a neoantigen DNA cancer vaccine, one of the key considerations is to assess any potential toxicity associated with immune responses to the vaccine. If no neoantigens are identified that are immunogenic, the GLP safety and toxicology studies will have only limited value as no immune response to the mutant tumor-specific antigen will be generated. As such, we are not aware of any mammary tumor models in mice that would be appropriate for the GLP safety and toxicology studies.

7.4 Rationale for no GLP biodistribution studies

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

7.5 Sequencing pipeline

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

A key collaborator on this project is Dr. Elaine Mardis, Co-Director of the Genome Institute at Washington University School of Medicine. Mardis is an internationally-known expert in genomics, and cancer genomics. The Genome Institute has published seminal papers describing somatic mutations present in tumors, providing significant insights into the biology of AML [88, 89] and breast cancer [13, 14]. Mardis and her team have developed and optimized the sequencing and data analysis pipelines that will be used to identify expressed mutations in subjects' breast cancers.

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

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

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

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

Exome data analysis:

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

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

Breast cancer-specific mutations that meet these filters will be prioritized. We will further prioritize indels over missense mutations. The mutations will be further analyzed for epitopes using the epitope prediction algorithms detailed below (Please see Section 6.4).

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

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

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

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

7.6 Epitope prediction algorithm

We have developed and optimized an epitope prediction algorithm for the identification and prioritization of neoantigens. This optimized epitope prediction algorithm is described below and in the Gubin 2014 manuscript [19]. We have established this algorithm is collaboration with Dr. Robert Schreiber, an internationally-known expert in tumor immunology [20, 80-87]. Schreiber was one of the first to use next generation sequencing technologies to identify neoantigens, demonstrating that these antigens are important tumor rejection antigens [20]. Schreiber and Gillanders have now optimized the epitope prediction algorithm and have demonstrated that cancer vaccines targeting neoantigens are associated with antitumor immunity [19].

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

Mutations that are expressed in the breast cancer will be identified using the sequencing pipeline outlined in the document titled “Sequencing Pipeline.” The predicted amino acid sequences corresponding to the expressed mutations will be pipelined through three class I MHC epitope-binding algorithms provided by the Immune Epitope Database and Analysis Resource (http://www.immuneepitope.org): (i) Stabilized Matrix Method (SMM) [90], (ii) Artificial Neural Network (ANN) [91], and (iii) NetMHCpan [92].

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

Filters will be applied to the list to (a) eliminate epitopes that are not processed efficiently by the immunoproteosome based on the NetChop algorithm [93] (peptides with a NetChop score $\geq$ 0.6 will be prioritized), (b) deprioritize epitopes from hypothetical proteins or that form a weaker predicted binding epitope than that expressed by the corresponding wild type sequence, (c) prioritize mutant epitopes that have the highest difference in predicted binding affinity compared to their wild type counterpart, and (d) incorporate expression profiles of wild type genes that correspond to the mutant antigen candidates in normal vs tumor cells.

To confirm the ability of prioritized neoantigens to bind to the corresponding MHC class I allele, we will synthesize peptides corresponding to the prioritized list of candidate epitopes. Synthetic peptides (> 95% purity) will be tested for binding to the respective class I MHC molecule using the T2 cell line, or one of its derivatives expressing common class I MHC alleles. The T2 cell line is deficient in transporters for antigen presentation (TAP), and as such is commonly used to assess binding of exogenously added peptides [94, 95]. Expression of class I MHC alleles is measured by staining of T2 cells with antibody specific for the class I MHC allele followed by flow cytometric analysis to measure the fluorescence intensity of the class I MHC allele on the cell surface. Data are typically expressed as Mean Fluorescence Intensity (MFI) by subtracting the baseline fluorescence intensity of the class I MHC allele to that measured after addition of exogenous peptide. Neoantigens that do not bind to the corresponding class I MHC allele will be deprioritized.

8 TREATMENT PLAN

8.1 Neoantigen DNA vaccine administration

The first neoantigen DNA vaccine injection will take place following completion of SOC therapy. The day of the first vaccine injection will be referred to as Day 1.

All subjects will be treated as outpatients in the Siteman Cancer Center.

The schedule of vaccination is Day 1, Day 29 ± 7, Day 57 ± 7, Day 85 ± 7, Day 113 ± 7, and Day 141 ± 7 with at least 21 days between injection days. All study injections will be given intramuscularly using an integrated electroporation device (TDS-IM system, Ichor Medical Systems). At each vaccination time point, patients will receive two injections of the neoantigen DNA vaccine, one injection into each deltoid or lateralis. If both injections cannot be given on the same day, the patient will be asked to return to complete the second injection. Standard aseptic technique and precautions will be utilized in site preparation, vaccine administration, and medical waste disposal to ensure maximal safety of subjects and study personnel. Participants' vital signs will be monitored for 30 minutes post vaccine for safety purposes.

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

The sites of immunization may be rotated for each of the immunizations. No injection will be given at a location in which the draining lymph nodes have been removed.

At the discretion of the treating physician, patients may be pre-medicated with lorazepam 1mg PO (or similar) 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) (or similar) 10 minutes prior to injection. Patients may also receive pain medication at treating physicians' discretion PO at least 30 minutes but no greater than 60 minutes prior to the first injection.

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

Please see **Section 10 Study Calendar** for details on study visit procedures and monitoring.

8.2 Durvalumab administration

For patients who are randomized to the neoantigen DNA vaccine plus durvalumab arm, the neoantigen-specific T cell response will be assessed prior to Day 85. If a neoantigen-specific T cell response is present, durvalumab will be started on Day 85, and will be administered Q4W (± 7 days) at a dose of 1500 mg over the course of 60 minutes. If a neoantigen-specific T cell response is not present, these patients will be replaced but may continue to receive the neoantigen DNA vaccine on study. They will not be transferred to the vaccine-only arm.

On days when subjects receive both vaccine injections and durvalumab infusions, they will receive the vaccine first, followed by observation for 30 minutes, followed by administration of durvalumab. Subjects will be monitored before, during and after the infusion with assessment of vital signs at the times specified in the Schedule of Assessment. Subjects are monitored (pulse rate, blood pressure) every 30 minutes during the infusion period (including times where infusion rate is slowed or temporarily stopped). Every effort should be made to give durvalumab and vaccine on the same day.

If weight falls to 30 kg or less, durvalumab will no longer be administered.

8.3 Replacement of patients

Patients who initially meet eligibility criteria and have a vaccine manufactured but either fail eligibility re-check based on durvalumab-specific criteria (but are still able to receive neoantigen vaccine safely) or are randomized to receive durvalumab but do not experience a neoantigen-specific T cell response (and thus

cannot receive durvalumab) may receive the neoantigen DNA vaccine but will be considered inevaluable and will be replaced. Safety and response data will be collected and analyzed separately.

8.4 General Concomitant Medication and Supportive Care Guidelines

In the event of a ≤ grade 2 infusion-related reaction, the infusion rate of durvalumb may be decreased by 50% or interrupted until resolution of the event and re-initiated at 50% of the initial rate until completion of the infusion. For subjects with a ≤ grade 2 infusion-related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion-related reaction is grade 3 or higher in severity, study drug will be discontinued. The standard infusion time is one hour, however if there are interruptions during infusion, the total allowed time from infusion start to completion of infusion should not exceed 8 hours at room temperature. For management of subjects who experience an infusion reaction, please refer to the toxicity and management guidelines.

As with any antibody, allergic reactions to dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit subjects to an intensive care unit if necessary.

8.4.1 Permitted Concomitant Medications

Supportive medication/class of drug:	**Usage:**
Concomitant medications or treatments (eg, acetaminophen or diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care, except for those medications identified as “prohibited,” as listed above	To be administered as prescribed by the Investigator
Best supportive care (including antibiotics, nutritional support, correction of metabolic disorders, optimal symptom control, and pain management [including palliative radiotherapy to non-target lesions, etc])	Should be used, when necessary, for all subjects
Inactivated viruses, such as those in the influenza vaccine	Permitted

8.4.2 Excluded Concomitant Medications (Durvalumab Arm Only)

Prohibited medication/class of drug:	**Usage:**
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
mAbs against CTLA-4, PD-1, or PD-L1 other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment

Prohibited medication/class of drug:	**Usage:**
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment. (Concurrent use of hormones for non-cancer-related conditions [eg, insulin for diabetes and hormone replacement therapy] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable [eg, by local surgery or radiotherapy])
Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding <<10 mg/day>> of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-α blockers	Should not be given concomitantly. (Use of immunosuppressive medications for the management of IP-related AEs or in subjects with contrast allergies is acceptable). In addition, use of inhaled, topical, and intranasal corticosteroids is permitted.
Drugs with laxative properties and herbal or natural remedies for constipation	Should be used with caution through to 90 days after the last dose of durvalumab during the study
Sunitinib	Should not be given concomitantly or through 90 days after the last dose of durvalumab (acute renal failure has been reported with combination therapy of tremelimumab and sunitinib)
EGFR TKIs	Should not be given concomitantly. Should be used with caution in the 90 days post last dose of durvalumab. Increased incidences of pneumonitis (with third generation EGFR TKIs) and increased incidence of transaminase increases (with 1st generation EGFR TKIs) has been reported when durvalumab has been given concomitantly.
Live attenuated vaccines	Should not be given through 30 days after the last dose of IP (including SoC)

8.5 Women of Childbearing Potential

Females of childbearing potential who are sexually active with a non-sterilized male partner must use at least 1 highly effective methods of contraception (see table below) from the time of screening, and must agree to continue using such precautions for one year after the last dose of neoantigen vaccine. Cessation of birth control after this point should be discussed with a responsible physician. Not engaging in sexual activity for the total duration of the drug treatment and the drug washout period is an acceptable practice; however, periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control. Female subjects should also refrain from breastfeeding throughout this period.

Females of childbearing potential are defined as those who are not surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal. Women will be considered post-menopausal if they have been amenorrheic for 12 months without an alternative medical cause. The following age-specific requirements apply:

- Women < 50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and if they have

luteinizing hormone and follicle-stimulating hormone levels in the post-menopausal range for the institution or underwent surgical sterilization (bilateral oophorectomy or hysterectomy).

- Women ≥ 50 years of age would be considered post-menopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatments, had radiation-induced menopause with last menses > 1 year ago, had chemotherapy-induced menopause with last menses > 1 year ago, or underwent surgical sterilization (bilateral oophorectomy, bilateral salpingectomy, or hysterectomy).

Highly effective methods of contraception, defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly are described in the table below. Note that some contraception methods are not considered highly effective (e.g., male or female condom with or without spermicide; female cap, diaphragm, or sponge with or without spermicide; non-copper containing intrauterine device; progestogen-only oral hormonal contraceptive pills where inhibition of ovulation is not the primary mode of action [excluding Cerazette/desogestrel which is considered highly effective]; and triphasic combined oral contraceptive pills).

Highly effective methods of contraception (< 1% failure rate)	
Barrier/Intrauterine Methods	**Hormonal Methods**
• Copper T intrauterine device • Levonorgestrel-releasing intrauterine system (e.g., Mirena ®)[a]	• Etonogestrel implants, e.g., Implanon or Norplant • Intravaginal device, e.g., ethinylestradiol and etonogestrel • Medroxyprogesterone injection, e.g., Depo-Provera • Normal and low dose combined oral contraceptive pill • Norelgestromin/ethinylestradiol transdermal system • Cerazette (desogestrel)

[a] This is also considered a hormonal method.

8.6 Blood donation

Patients should not donate blood while participating in this study or for at least 90 days following the last infusion of durvalumab.

8.7 Duration of therapy

In the absence of treatment delays due to adverse events, treatment may continue for 6 months (6 doses of neoantigen DNA vaccine). Patients receiving durvalumab may receive up to 4 doses. 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 12 Removal of Patients from Protocol Therapy** for additional details.

8.8 Duration of follow up

Patients will be followed for 52 weeks or until death, whichever occurs first. Additional follow-up visits or telephone contact will be scheduled annually for 5 years if the patient is alive and available for follow-up. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

9 POTENTIAL TOXICITY AND DOSE MODIFICATIONS

9.1 Potential toxicity

9.1.1 Experience with the pING parent vector

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

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

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

Note that on April 25, 2019, NIH revised the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, to streamline oversight of human gene transfer research.

Under the amended *NIH Guidelines*, the NIH Office of Science Policy (OSP) will not:

- accept new human gene transfer protocols for the protocol registration process under the *NIH Guidelines*
- accept annual reports, safety reports, amendments or other documentation for any previously registered human gene transfer protocols under the *NIH Guidelines* (Appendix M-I-C).

Additional information regarding the changes to the *NIH Guidelines* is available at this URL: https://osp.od.nih.gov/biotechnology/nih-guidelines/.

We have used this information to prepare Table 4 below. Of note, there was only one serious adverse event reported. One patient with metastatic prostate cancer developed urinary retention. It is not clear if this was related to the DNA vaccine. Review of the available annual reports confirms minimal toxicity associated with DNA vaccination in these trials. Over 90% of the toxicities related to treatment were grade 1 and included diarrhea, dizziness, edema, fatigue, injection site reaction, nausea, vomiting, and rigors.

We have also discussed these trials with Jedd Wolchock, M.D., at Memorial Sloan-Kettering Cancer Center, by e-mail correspondence January 15, 2009. Dr. Wolchock is the PI or co-investigator on all of these trials. He confirmed that there has been minimal toxicity associated with the DNA vaccines based on the pING parent vector.

Table 4 Clinical Experience with pING parent vector

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

9.1.2 Potential toxicity related to the neoantigen DNA vaccine

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

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

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

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

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

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

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

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

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

9.1.3 Potential toxicity related to the TDS-IM device

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

Local Reactions

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

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

Systemic Reactions

Systemic adverse events reported during the studies utilizing the TDS-IM and judged to be possibly related to the study product and/or delivery device have been generally mild to moderate in severity and include flu-like symptoms, headache, fever, dizziness, malaise, fatigue, arthralgia, myalgia, and aphthous

stomatitis. Transient elevation in serum creatine phosphokinase of mild to moderate severity and judged to be associated with procedure administration have been reported in a small minority of subjects. Two subjects have reported the onset of severe fatigue within 24 hours of dosing that was resolved by the following day and was not observed following subsequent doses.

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

A theoretical risk associated with the use of DNA vaccines is the possibility of uptake of the vaccine candidate into non-target tissues and/or integration of the vaccine DNA into the genomic DNA of the test subject. As described in Section 8.2 below, nonclinical studies have been performed to assess the potential for systemic biodistribution and genomic integration following TDS based delivery multiple DNA vaccine candidates, including a tyrosinase melanoma DNA vaccine candidate utilizing the same vector backbone (pING) as the Mammaglobin-A DNA vaccine candidate. To date, there has been no evidence of significant systemic uptake or genomic integration events following TDS based DNA vaccine administration.

9.2 Toxicity monitoring and management

Toxicity will be characterized according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (CTCAE).

Toxicities associated or possibly associated with durvalumab treatment should be managed according to standard medical practice or suggested management tables provided in this protocol in Section 9.3 below. Additional tests, such as autoimmune serology or biopsies, should be used to determine a possible immunogenic etiology.

Although most immune-related adverse events observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications. Discontinuation of durvalumab may not have an immediate therapeutic effect and in severe cases, immune-related toxicities may require acute management with topical corticosteroids, systemic corticosteroids, mycophenolate, or tumor necrosis factor−α inhibitors. The Investigator should consider the benefit-risk balance a given patient may be experiencing prior to further administration of durvalumab. Durvalumab should be permanently discontinued in patients with life-threatening immune-related adverse events.

Subjects who are immunized with the DNA vaccine will be evaluated at the time of each vaccination. Follow up on subject well-being will be performed by telephone on the first or second day after each vaccination. All information will be recorded on case report forms. Adverse events will be reported to the Quality Assurance and Safety Monitoring Committee of the Siteman Cancer Center, the Institutional Review Board, the Institutional Biosafety Committee, the Office of Biotechnology Activities and the Food and Drug Administration as detailed in **Section 13 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. Autoimmune involvement of the breast will be treated conservatively with analgesics; more aggressive intervention (systemic corticosteroids) will be used as necessary

9.3 Dose modifications for durvalumab

For adverse events (AEs) that are considered at least partly due to administration of durvalumab the following dose adjustment guidance may be applied:

- Treat each of the toxicities with maximum supportive care (including holding the agent suspected of causing the toxicity where required).
- If the symptoms promptly resolve with supportive care, consideration should be given to continuing the same dose of durvalumab along with appropriate continuing supportive care. If

medically appropriate, dose modifications are permitted for durvalumab (see below).

- All dose modifications should be documented with clear reasoning and documentation of the approach taken.

All toxicities will be graded according to NCI CTCAE v4.03.

9.3.1 Dosing Modification and Toxicity Management Guidelines for Immune-Mediated, Infusion-Related, and Non-Immune-Mediated Reactions

Drug administration modifications of study drug/study regimen will be made to manage potential immune-related AEs based on severity of treatment-emergent toxicities graded per NCI CTCAE v4.03.

In addition to the criteria for permanent discontinuation of study drug/study regimen based on CTCAE grade/severity (table below), permanently discontinue study drug/study regimen for the following conditions:

- Inability to reduce corticosteroid to a dose of ≤10 mg of prednisone per day (or equivalent) **within 12 weeks** of the start of the immune-mediated adverse event (imAE)
- Grade 3 recurrence of a previously experienced treatment-related imAE following resumption of dosing

See tables below for dosing modifications and toxicity management guidelines for specific immune-mediated reactions, other immune-mediated reactions, infusion-related reactions, and non-immune mediated reactions.

Dosing Modification and Toxicity Management Guidelines (TMGs) for Durvalumab Monotherapy, Durvalumab in Combination with other Products, or Tremelimumab Monotherapy – October 2022

General Considerations Regarding Immune-Mediated Reactions

These guidelines are provided as a recommendation to support investigators in the management of potential immune-mediated adverse events (imAEs).

Immune-mediated events can occur in nearly any organ or tissue, therefore, these guidelines may not include all the possible immune-mediated reactions. Investigators are advised to take into consideration the appropriate practice guidelines and other society guidelines (e.g., National Comprehensive Cancer Network (NCCN), European Society of Medical Oncology (ESMO)) in the management of these events. Refer to the section of the table titled "Other -Immune-Mediated Reactions" for general guidance on imAEs not noted in the "Specific Immune-Mediated Reactions" section.

Early identification and management of imAEs is essential to ensure safe use of the study drug. Monitor patients closely for symptoms and signs that may be clinical manifestations of underlying imAEs. Patients with suspected imAEs should be thoroughly evaluated to rule out any alternative etiologies (e.g., disease progression, concomitant medications, infections). In the absence of a clear alternative etiology, all such events should be managed as if they were immune-mediated. Institute medical management promptly, including specialty consultation as appropriate. In general, withhold study drug/study regimen for severe (Grade 3) imAEs. Permanently discontinue study drug/study regimen for life-threatening (Grade 4) imAEs, recurrent severe (Grade 3) imAEs that require systemic immunosuppressive treatment, or an inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks of initiating corticosteroids.

Based on the severity of the imAE, durvalumab and/or tremelimumab should be withheld and corticosteroids administered. Upon improvement to Grade ≤ 1, corticosteroid should be tapered over ≥ 28 days. More potent immunosuppressive agents should be considered for events not responding to systemic steroids. Alternative immunosuppressive agents not listed in this guideline may be considered at the discretion of the investigator based on clinical practice and relevant guidelines. With long-term steroid and other immunosuppressive use, consider the need for glucose monitoring.

Dose modifications of study drug/study regimen should be based on severity of treatment-emergent toxicities graded per NCI CTCAE version in the applicable study protocol.

Considerations for Prophylaxis for Long Term use of Steroids for Patients Receiving Immune Checkpoint Inhibitor Immunotherapy

- **Infection Prophylaxis: Pneumocystis jirovecii pneumonia (PJP), antifungal and Herpes Zoster reactivation**
- **Gastritis: Consider prophylaxis for patients at high risk of gastritis (e.g. NSAID use, anticoagulation)**

Relevant Society Guidelines for Management of imAEs

These society guidelines are provided as references to serve in support of best clinical practice and the TMGs. Please note, these were the current versions of these guidelines at the time of updating TMGs. Please refer to the most up to date version of these guidelines.

1. Brahmer JR, et al. Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immune checkpoint inhibitor-related adverse events. J Immunother Cancer 2021;9:e002435
2. Brahmer JR, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 2018;36(17):1714-1768.
3. Haanen JBAG, et al. Management of toxicities for immunotherapy: European Society for Medical Oncology (ESMO) clinical practice guidelines for diagnosis, treatment, and follow-up. Annals Oncol 2017;28(Suppl4):i119-i1142.
4. Sangro B, et al. Diagnosis and management of toxicities of immune checkpoint inhibitors in hepatocellular carcinoma. J Hepatol 2020;72(2):320-341.
5. Thompson JA, et al. National Comprehensive Cancer Network Guidelines: Management of immunotherapy-related toxicities version 1.2022. Published February 28, 2022.

Event	Grade	Dose Modifications	Toxicity Management
Pneumonitis / Interstitial ILD	Any Grade	General guidance	– Patients should be thoroughly evaluated to rule out any alternative etiology with similar clinical presentation (e.g. infection, progressive disease) – Monitor patients for signs (e.g. tachypnoea) and symptoms of pneumonitis or ILD (new onset or worsening shortness of breath or cough). Evaluate patients with imaging and pulmonary function tests including other diagnostic procedures as described below – Suspected pneumonitis should be confirmed with radiographic imaging and other infectious and disease-related etiologies excluded, and managed as described below. – Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up (including clinically relevant culture specimens to rule out infection). and high-resolution computed tomography (CT) scan. – Consider Pulmonary and Infectious Diseases consults.
	Grade 1	No dose modification required. However, consider holding study drug/study regimen dosing as clinically appropriate and during diagnostic work-up for other etiologies	For Grade 1 - Monitor and closely follow up in 2-4 days for clinical symptoms, pulse oximetry (resting and exertion) and laboratory work-up and then as clinically indicated
	Grade 2	Hold study drug/study regimen dose until Grade 2 resolution to ≤ Grade 1.	For Grade 2 – Monitor symptoms daily and consider hospitalization, as clinically indicated. – Consider Pulmonary and Infectious Diseases Consults. – Promptly start systemic steroids (e.g., prednisone 1 to 2mg/kg/day PO or

			IV equivalent) - Consider HRCT or chest CT with contrast. Repeat imaging study as clinically indicated. - If no improvement within 2 to 3 days, additional workup should be considered and prompt treatment with IV methylprednisolone 2 to 4mg/kg/day started - If still no improvement within 2 to 3 days despite IV methylprednisone at 2 to 4/g/kg/day, promptly start immunosuppressive therapy such as tumor necrosis factor (TNF) inhibitors (e.g. infliximab at 5mg/kg once, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. - Consider discussing with Clinical Study Lead.
	Grade 3 or 4	Permanently discontinue study drug/study regimen	For Grade 3 or 4 - Hospitalize the patient - Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent - Obtain Pulmonary and Infectious Disease consults; consider, as necessary, discussing with Clinical Study Lead - Consider starting anti-infective therapy if infection is still a consideration on the basis of other diagnostic testing despite negative culture results. - Supportive Care (e.g., oxygen) - If no improvement within 2 days, additional workup should be considered and prompt treatment with additional immunosuppressive therapy such as TNF inhibitors (e.g. infliximab at 5mg/kg once, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution:** rule out sepsis and refer to infliximab label for general guidance before using infliximab
Diarrhea / Colitis	Any Grade	General guidance	- Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections), including testing for Clostridium difficile toxin, etc. - Monitor for symptoms that may be related to diarrhea/enterocolitis (abdominal pain, cramping, or changes in bowel habits such as increased frequency over baseline or blood in stool) or related to bowel perforation (such as sepsis, peritoneal signs and ileus) - Consider further evaluation with imaging study with contrast. - Consult a gastrointestinal (GI) specialist for consideration of further workup. - **WHEN SYMPTOMS OR EVALUATION INDICATE AN INTESTINAL PERFORATION IS SUSPECTED, CONSULT A SURGEON EXPERIENCED IN ABDOMINAL SURGERY IMMEDIATELY WITHOUT ANY DELAY.** - **PERMANENTLY DISCONTINUE STUDY DRUG FOR ANY GRADE OF**

			INTESTINAL PERFORATION. - Steroids should be considered in the absence of clear alternative etiology, even for low grade events, in order to prevent potential progression to higher grade event, including perforation - Use analgesics carefully; they can mask symptoms of perforation and peritonitis
	Grade 1	No dose modifications	For Grade 1 diarrhea: - Monitor closely for worsening symptoms - Consider symptomatic treatment including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), loperamide, and other supportive care measures. - If symptoms persist, consider checking lactoferrin; if positive, treat as Grade 2 below. If negative and no infection, continue Grade 1 management.
	Grade 2	Hold study drug/study regimen until resolution to ≤ Grade 1	For Grade 2 diarrhea: - Consider symptomatic treatment including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), and loperamide and/or budesonide - Consider further evaluation with imaging study with contrast. - Consider consult of a gastrointestinal (GI) specialist for consideration of further workup. - Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent - If no improvement within 3 days despite therapy with 1 to 2 mg/kg IV prednisone equivalent, reconsult GI specialist and, if indicated, promptly start immunosuppressant agent such as infliximab at 5mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines. **Caution**: Important to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. - **If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.** - Consider, as necessary, discussing with Clinical Study Lead if no resolution to ≤ Grade 1 in 3 to 4 days.

	Grade 3 or 4	Grade 3: • For patients treated with durvalumab monotherapy, hold study drug/study regimen until resolution to Grade ≤1; study drug/study regimen can be resumed after completion of steroid taper (<10 mg prednisone per day, or equivalent). • For patients treated with durvalumab in combination with other products (not tremelimumab), decision to be made at the discretion of the study investigator, in discussion with AstraZeneca Clinical Study Lead. Grade 4: Permanently discontinue study drug/study regimen	For Grade 3 or 4: – Urgent GI consult and imaging/or colonoscopy as appropriate. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent – Monitor stool frequency and volume and maintain hydration – If still no improvement within 2 days, continue steroids and promptly add further immunosuppressants (e.g. infliximab at 5mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution**: Ensure GI consult to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. – **If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.**
Hepatitis *Infliximab should not be used for management of immune-related hepatitis.*	Any Grade	General guidance.	For Any Grade – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., viral hepatitis, disease progression, concomitant medications) – Monitor and evaluate transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP] and total bilirubin.
	ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN	• No dose modifications. • If it worsens, then consider holding therapy.	- Continue transaminase and total bilirubin monitoring per protocol
	ALT or AST > 3 ≤ 5 x ULN or total bilirubin > 1.5 ≤ 3 x ULN	• Hold study drug/study regimen dose until ALT or AST ≤ 3 X ULN or total bilirubin, ≤ 1.5 X ULN. Resume study drug/study regimen after completion of steroid taper (<10mg prednisone or equivalent). • Permanently discontinue study drug/study regimen for any case meeting Hy's law laboratory criteria (AST or ALT ≥3 xULN AND bilirubin ≥2 x	– Regular and frequent checking of transaminases and total bilirubin (e.g. every 1 to 2 days) until transaminases and total bilirubin elevations improve or resolve. – If no resolution to ALT or AST ≤ 3 x ULN or total bilirubin – ≤ 1.5 x ULN in 1-2 days, consider, discussing with Clinical Study Lead as needed. – If event is persistent (> 2 to 3 days) or worsens, promptly start prednisone 1 to 2mg/kg/day PO or IV equivalent.

PLEASE SEE shaded area immediately below this section to find guidance for management of "Hepatitis (elevated LFTS)" in hepatocellular carcinoma (HCC) patients (or secondary tumor involvement of the liver with abnormal baseline values [BLV]		ULN without initial findings of cholestasis (i.e., elevated ALP)) and in the absence of any alternative cause.	
	ALT or AST > 5- ≤ 10 x ULN	• Hold study drug/study regimen. Resume study drug/study regimen if elevations downgrade to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN after completion of steroid taper (<10 mg prednisone, or equivalent).	- Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent - Perform Hepatology Consult, abdominal workup, and imaging as appropriate. - If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant (i.e., mycophenolate mofetil 0.5 to 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not available. **Infliximab should NOT be used.**
	Concurrent ALT or AST > 3 x ULN and total bilirubin > 2 x ULN[d] ALT or AST > 10 x ULN OR total bilirubin > 3 x ULN	• Permanently discontinue study drug/study regimen	− Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant.(e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not available. Infliximab should NOT be used.

Hepatitis (elevated transaminases and total bilirubin) *Infliximab should not be used for management of immune-related hepatitis.* **THIS shaded area is guidance *only* for management of "Hepatitis (elevated LFTs)" in HCC patients** See instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either **increasing bilirubin or signs of DILI/liver decompensation**	Any Elevations of AST, ALT, or T. Bili as Described Below	General guidance	For Any Elevations Described – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., viral hepatitis, disease progression, concomitant medications, worsening of liver cirrhosis [e.g., portal vein thrombosis]). – Monitor and evaluate liver function test: AST, ALT, ALP, and T. Bili. – For hepatitis B (HBV) + patients: evaluate quantitative HBV viral load, quantitative Hepatitis B surface antigen (HBsAg), or Hepatitis B envelope antigen (HBeAg). – For Hepatitis C (HCV) + patients: evaluate quantitative HCV viral load. – Consider consulting Hepatology or Infectious Disease specialists regarding changing or starting antiviral HBV medications if HBV viral load is >2000 IU/ml. – Consider consulting Hepatology or Infectious Disease specialists regarding changing or starting antiviral HCV medications if HCV viral load has increased by ≥2-fold. – For HCV+ with Hepatitis B core antibody (HBcAB) +: Evaluate for both HBV and HCV as above.
	Isolated AST or ALT > ULN and ≤ 2.5 X BLV	• No dose modifications • If ALT/AST elevations represents significant worsening based on investigator assessment, then treat as described for elevations in the row below. For all transaminase elevations, see instructions at bottom of shaded area if transaminase rise is not isolated but (at any time) occurs in setting of either **increasing bilirubin or signs of DILI/liver decompensation**	
	ALT or AST ≥ 2.5- ≤ 5X BLV and ≤ 20xULN	• Hold study drug/study regimen dose until resolution to AST or ALT ≤2.5 X BLV. • If toxicity worsens, then treat as described for elevations in the rows below. If toxicity improves to AST or ALT ≤2.5 × BLV, resume study drug/study regimen after completion of steroid taper (<10 mg	– Regular and frequent checking of Transaminases and total bilirubin (e.g., every 1 to 3 days) until elevations of these are improving or resolved. – Recommend consult hepatologist consider abdominal ultrasound, including Doppler assessment of liver perfusion. – Consider, as necessary, discussing with Clinical Study Lead. – If event is persistent (>2 to 3 days) or worsens, and investigator suspects toxicity to be imAE, start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day of

		prednisone, or equivalent).	prednisone PO or IV equivalent, consider additional work up. If still no improvement within 2 to 3 days despite 2 mg/kg/day of IV methylprednisolone, consider additional abdominal work up (including liver biopsy) and imaging (i.e., liver ultrasound), and consider starting additional immunosuppressants (i.e., mycophenolate mofetil 0.5 to 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate mofetil is not available. **Infliximab should NOT be used.**
	ALT or AST >5-7X BLV and ≤ 20X ULN OR concurrent 2.5-5X BLV and ≤ 20XULN AND total bilirubin > 1.5 - < 2 x ULN[d]	• Hold study drug/study regimen dose until resolution to AST or ALT ≤2.5 × BLV. Resume study drug/study regimen if elevations downgrade to AST or ALT ≤2.2 × BLV and after completion of steroid taper (<10 mg prednisone, or equivalent). • Permanently discontinue study drug/study regimen if the elevations do not downgrade to AST or ALT ≤2.5 × BLV within 14 days.	– Regular and frequent checking of LFTs (e.g., every 1-2 days) until elevations of these are improving or resolved. – Consult hepatologist (unless investigator is hepatologist); obtain abdominal ultrasound, including Doppler assessment of liver perfusion; and consider liver biopsy. – Consider, discussing with Clinical Study Lead as needed. – If investigator suspects toxicity to be immune-mediated, promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. – If no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, obtain liver biopsy (if it has not been done already) and promptly start treatment with an additional immunosuppressant (e.g., mycophenolate mofetil 0.5 to 1 g every 12 hours then taper in consultation with a hepatologist or relevant practice guidelines). Discuss with Clinical Study Lead if mycophenolate is not available. **Infliximab should NOT be used.**
	ALT or AST > 7 × BLV OR >20 ULN, whichever occurs first OR bilirubin > 3ULN Permanently discontinue study drug/study regimen. – **Same as above (except recommended obtaining liver biopsy early)**		
Nephritis and/or Renal Dysfunction	Any Grade	General guidance	– Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, infections, recent IV contrast, medications, fluid status). – Consider consulting a nephrologist. – Consider imaging studies to rule out any alternative etiology – Monitor for signs and symptoms that may be related to changes in renal function (e.g. routine urinalysis, elevated serum BUN and creatinine, decreased creatinine clearance, electrolyte imbalance, decrease in urine output, or proteinuria). – Follow urine protein/creatinine ratio every 3-7 days
	Grade 1	No dose modifications.	For Grade 1: - Monitor serum creatinine weekly and any accompanying symptoms.

			• If creatinine returns to baseline, resume regular monitoring per study protocol. • If creatinine worsens, depending on the severity, treat as Grade 2, 3, or 4. - Consider hydration, electrolyte replacement, and diuretics. as clinically indicated. - Consider nephrologist consult if not resolved within 14 days, or earlier as clinically indicated
	Grade 2	Hold study drug/study regimen until resolution to Grade ≤ 1 or baseline.	For Grade 2: – Consider including hydration, electrolyte replacement, and diuretics as clinically indicated. – Follow urine protein/creatinine ratio every 3-7 days – Carefully monitor serum creatinine as clinically warranted. – Consult nephrologist and consider renal biopsy if clinically indicated. – Start prednisone 0.5 – 1 mg/kg/day if other causes are ruled out – If event is persistent beyond 5 days or worsens, increase to prednisone up to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 5 days or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup. When event returns to baseline, resume study drug/study regimen and routine serum creatinine monitoring per study protocol.
	Grade 3 or 4	Permanently discontinue study drug/study regimen	For Grade 3 or 4: – Carefully monitor serum creatinine daily. – Follow urine protein/creatinine ratio every 3-7 days – Consult nephrologist and consider renal biopsy if clinically indicated. – Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. – If event is not responsive within 3 to 5 days of steroids or worsens despite prednisone at 1 to 2 mg/kg/day or IV equivalent, consider additional workup and prompt treatment with an immunosuppressant.
Rash or Dermatitis (including Pemphigoid)	Any Grade (refer to NCI CTCAE v 4.03 for definition of severity / grade depending on type of skin rash)	General guidance	For Any Grade: – Patients should be thoroughly evaluated to rule out any alternative etiology. – Monitor for signs and symptoms of dermatitis (rash and pruritus). – **HOLD STUDY DRUG IF GRADE 3 PEMPHIGOID OR SEVERE CUTANEOUS ADVERSE REACTION (SCAR)[1] IS SUSPECTED.** – **PERMANENTLY DISCONTINUE STUDY DRUG IF SCAR OR GRADE 3 PEMPIGOID IS CONFIRMED.**
	Grade 1	No dose modifications.	For Grade 1: – Consider symptomatic treatment including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., emollient, lotion, or institutional standard).

	Grade 2	For persistent (> 1 week) Grade 2 events, hold scheduled study drug/study regimen until resolution to ≤ Grade 1 or baseline • If toxicity improves to Grade ≤ 1 or baseline, then resume drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	For Grade 2: – Consider Dermatology consult and skin biopsy, as indicated. – Consider symptomatic treatment including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy. – Consider moderate-strength topical steroid – If no improvement of rash/skin lesions occurs within 1 week or is worsening despite symptomatic treatment and/or use of moderate strength topical steroid, consider discussing with Clinical Study Lead, as needed, and promptly start systemic steroids prednisone 1 to 2 mg/kg/day PO or IV equivalent. –
	Grade 3	Grade 3: • Hold study drug/study regimen until resolution to Grade ≤ 1 or baseline • If toxicity improves to Grade ≤1 or baseline, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	For Grade 3 – Reconsult a dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible. – Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. – Consider hospitalization. – Monitor the extent of rash [Rule of Nines]. – Consider, as necessary, discussing with Clinical Study Lead.
	Grade 4	For Grade 4 Permanently discontinue study drug/study regimen	For Grade 4 − Reconsult a dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible. − Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. − Consider hospitalization. − Monitor the extent of rash [Rule of Nines]. Consider, as necessary, discussing with Clinical Study Lead.
Endocrinopathy (e.g., hyperthyroidism, thyroiditis, hypothyroidism, Type 1 diabetes mellitus,	Any Grade (Depending on the type of endocrinopathy, refer to CTCAE v 4.03 for definition)	General guidance	For Any Grade: – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression including brain metastases, or infections). – Consider consulting an endocrinologist for endocrine events. – Consider discussing with Clinical Study Lead, as needed. – Monitor patients for signs and symptoms of endocrinopathies. (Non-

hypophysitis, hypopituitarism, and adrenal insufficiency)			specific symptoms include headache, fatigue, behavior changes, mental status changes, photophobia, visual field cuts, vertigo, abdominal pain, unusual bowel habits, polydipsia, polyuria, hypotension and weakness.) – Depending on the suspected endocrinopathy, monitor and evaluate thyroid function tests: thyroid stimulating hormone (TSH), free T_3 and free T_4 and other relevant endocrine labs (e.g., blood glucose and ketone levels, hemoglobin A1C (HgA1c)). If a patient experiences an AE that is thought to be possibly of autoimmune nature (e.g., thyroiditis, pancreatitis, hypophysitis, diabetes insipidus), the investigator should send a blood sample for appropriate autoimmune antibody testing. – Investigators should ask subjects with endocrinopathies who may require prolonged or continued hormonal replacement, to consult their primary care physicians or endocrinologists about further monitoring and treatment after completion of the study.
	Grade 1	No dose modifications.	For Grade 1: – Monitor patient with appropriate endocrine function tests. – For suspected hypophysitis/hypopituitarism, consider consultating an endocrinologist to guide assessment of early-morning adrenocorticotropin hormone (ACTH), cortisol, TSH and free T4; also consider gonadotropins, sex hormones, and prolactin levels, as well as cosyntropin stimulation test (though it may not be useful in diagnosing early secondary adrenal insufficiency). – If TSH < 0.5 × LLN, or TSH > 2 × ULN or consistently out of range in 2 subsequent measurements, include free T4 at subsequent cycles as clinically indicated and consider consultation of an endocrinologist.

	Grade 2, 3, or 4	• For Grade 2-4 endocrinopathies other than hypothyroidism and Type 1 diabetes mellitus (T1DM), consider holding study drug/study regimen dose until acute symptoms resolve. • Study drug/study regimen can be resumed once event stabilizes and after completion of steroid taper (<10 mg prednisone, or equivalent). • Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen if the patien is clinically stable as per investigator or treating physician's clinical judgement.	For Grade 2, 3, or 4: – Consult endocrinologist to guide evaluation of endocrine function, and as indicated by suspected endocrinopathy and as clinically indicated, consider pituitary scan. – For all patients with abnormal endocrine work up, except those with isolated hypothyroidism or Type 1 DM, and as guided by an endocrinologist, consider short-term, corticosteroids (e.g., 1 to 2mg/kg/day methylprednisolone or IV equivalent) and prompt initiation of treatment with relevant hormone replacement. – Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids. – Isolated T1DM may be treated with appropriate diabetic therapy and without corticosteroids. **Only hold study drug/study regimen in setting of hyperglycemia when diagnostic workup is positive for diabetic ketoacidosis.** – For patients with normal endocrine work up (laboratory assessment or magnetic resonance imaging (MRI) scans), repeat laboratory assessments/MRI as clinically indicated.
Amylase/Lipase increased	Any Grade	General Guidance	For Any Grade: – Patients should be thoroughly evaluated to rule out any alternative etiology (e.g. disease progression, viral infection, concomitant medications, substance abuse). – For modest asymptomatic elevations in serum amylase and lipase, corticosteroid treatment is not indicated as long as there are no other signs or symptoms of pancreatic inflammation. – Assess for signs/symptoms of pancreatitis. – Consider appropriate diagnostic testing (e.g., abdominal CT with contrast, MRCP if clinical suspicion of pancreatitis and no radiologic evidence on CT). – If isolated elevation of enzymes without evidence of pancreatitis, continue immunotherapy. Consider other causes of elevated amylase/lipase. – If evidence of pancreatitis, manage according to pancreatitis recommendations.
	Grade 1	No dose modifications.	
	Grade 2, 3, or 4	For Grade 2, 3, or 4: In consultation with relevant gastroenterology specialist consider continuing study drug/study regimen if no clinical/radiologic evidence of pancreatitis ± improvement in amylase/lipase.	
Acute Pancreatitis	Any Grade	General Guidance	For Any Grade: – Patients should be thoroughly evaluated to rule out any alternative etiology. – Consider Gastroenterology referral.
	Grade 2	Consider holding study drug/regimen	Grade 2 – Consider IV hydration – Consider Gastroenterology referral

	Grade 3 or 4	For Grade 3: Hold study drug/study regimen until resolution of elevated enzymes and no radiologic findings If no elevation in enzymes or return to baseline values, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent). For Grade 4: Permanently discontinue study drug/study regimen.	For Grade 3 or 4: - Promptly start systemic steroids prednisone 1 to 2 mg/kg/day PO or IV equivalent. - IV hydration
Nervous System Disorders			
Aseptic Meningitis	Any Grade (, Refer to NCI CTCAE v4.03 for defining the CTC grade/severity)	General guidance.	For Any Grade: - Consider neurology consult. - Consider MRI brain with and without contrast and pituitary protocol and a lumbar puncture for diagnosis. - Exclude bacterial and viral infections. (ie HSV) - Symptoms may include headache, photophobia, and neck stiffness, nausea/vomiting which may resemble an infectious meningitis. - Patients may be febrile. - Mental status should be normal - Consider IV acyclovir until polymerase chain reactions are available
	Any Grade	Permanently discontinue study drug/study regimen	For Any Grade - Consider neurology consult - Consider MRI brain with and without contrast with pituitary protocol and a lumbar puncture for diagnosis - Exclude bacterial and viral infections. (ie HSV) - Consider IV acyclovir until polymerase chain reactions are available - Consider, as necessary, discussing with Clinical Study Lead. (Last bullet) - Consider hospitalization - Once infection has been ruled out promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent.

Encephalitis	Any Grade	General Guidance	For Any Grade - Consider neurology consult - Consider testing including MRI of the brain with and without contrast, lumbar puncture, electroencephalogram (EEG) to evaluate for subclinical seizures, ESR, CRP, antineutrophil cytoplasmic antibody (ANCA) (if vasculitic process suspected), thyroid panel including TPO and thyroglobulin and additional autoantibodies to rule out paraneoplastic disorders. - Exclude bacterial and viral infections. (ie HSV) - Consider IV acyclovir until polymerase chain reactions are available. - Symptoms may include Confusion, altered behavior, headaches, seizures, short-term memory loss, depressed level of consciousness focal weakness, and speech abnormality.
	Grade 2	For Grade 2 permanently discontinue study drug/study regimen	For Grade 2 - Consider, as necessary, discussing with the Clinical Study Lead. - Once infection has been ruled out methylprednisolone 1 2 mg/kg/day - For progressive symptoms or if oligoclonal bands are present consider methylprednisolone 1 g IV daily for 3-5 days plus IVIG or plasmapheresis
	Grade 3 or 4	For Grade 3 or 4 Permanently discontinue study drug/study regimen	For Grade 3 or 4 - Consider, as necessary, discussing with Clinical Study Lead. - Consider hospitalization. - Once infection is ruled out, start methylprednisolone 2 g IV daily for 3-5 days for progressive symptoms consider adding IVIG or plasmapheresis

Transverse Myelitis	Any Grade	General Guidance – Permanently discontinue immunotherapy – Consider MRI of the spine and brain – Once imaging is complete consider lumbar puncture – Consider testing to rule out additional aetiologies: B12, HIV, rapid plasma regain (RPR), ANA, anti-Ro/La antibodies, aquaporin-4 IgG, Myelin oligodendrocyte glycoprotein (MOG) IgG, paraneoplastic panel for anti-Hu and anti-CRMP5/CV2	For Any Grade – Consider neurology consult – Impatient Care – Consider prompt initiation of high methylprednisolone pulse dosing – Strongly consider IVIG or plasmapheresis
Peripheral neuropathy	Any Grade (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	General guidance	For Any Grade: – Patients should be evaluated to rule out any alternative etiology for neuropathy (e.g., disease progression, infections, metabolic syndromes and medications, etc.). It should be noted that the diagnosis of immune-mediated peripheral neuromotor syndromes can be particularly challenging in patients with underlying cancer, due to the multiple potential confounding effects of cancer (and its treatments) throughout the neuraxis. Given the importance of prompt and accurate diagnosis, it is essential to have a low threshold to obtain a neurological consult. – Neurophysiologic diagnostic testing (e.g., electromyogram and nerve conduction investigations) are routinely indicated upon suspicion of such conditions and may be best facilitated by means of a neurology consultation.
	Grade 1	No dose modifications.	For Grade 1: – Consider discussing with the Clinical Study Lead, as needed. – Monitor symptoms for interference with ADLS, gait difficulties, imbalance, or autonomic dysfunction
	Grade 2	Hold study drug/study regimen dose until resolution to Grade ≤1. .=	For Grade 2: – Consult a neurologist. – Consider EMG/NCS – Consider discussing with the Clinical Study Lead, as needed. – Observation for additional symptoms or consider initiating prednisone 0.5-1mg/kg orally – If progression initiate methylprednisolone 2-4 mg/kg/day and treat as GBS – Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin or duloxetine).

	Grade 3 or 4	For Grade 3 or 4: Permanently discontinue study drug/study regimen	For Grade 3 or 4: - Consider discussing with Clinical Study Lead, as needed. - Recommend hospitalization. - Monitor symptoms and consult a neurologist. - Treat per Guillain-Barre Syndrome recommendations
Guillain-Barre Syndrome (GBS)		General Guidance	− Recommend hospitalization − Obtain neurology consult − Obtain MRI of spine to rule out compression lesion − Obtain lumbar puncture − Antibody tests for GBS variants − Pulmonary function tests − Obtain electromyography (EMG) and nerve conduction studies − Frequently monitor pulmonary function tests and neurologic evaluations − Monitor for concurrent autonomic dysfunction − Initiate medication as needed for neuropathic pain

	Grade 2-4	Grade 2-4 Permanently discontinue	methylprednisolone 1 gram daily for 5 days, then taper over 4 weeks.
Myasthenia gravis		General Guidance	− Obtain neurology consult − Recommend hospitalization − Obtain pulmonary function tests − Obtain labs: ESR, CRP, creatine phosphokinase (CPK), aldolase and anti-striational antibodies − Consider cardiac exam, ECG, troponin, transthoracic echocardiogram for possible concomitant myocarditis − Obtain electromyography (EMG) and nerve conduction studies − Consider MRI of brain/spine to rule out CNS involvement by disease − Avoid medications that might exacerbate MG (e.g. beta blockers, some antibiotics, IV magnesium)

	Grade 2	Permanently discontinue	– Consider pyridostigmine 30mg three times daily and gradually increase based on symptoms (max dose 120mg four times daily) − Consider starting low dose prednisone 20mg daily and increase every 3-5 days. (Target dose 1mg/kg/day. Max dose 100mg daily)
	Grade 3-4	Permanently discontinue	− Start methylprednisolone 1-2mg/kg/day. Taper steroids based on symptom improvement − Start plasmapheresis or IVIG − Consider rituximab if refractory to plasmapheresis or IVIG − Frequent PFT assessments − Daily neurologic evaluations

Myocarditis	Any Grade (Refer to NCI CTCAE applicable version in study protocol for defining the CTCAE grade/severity)	General guidance Discontinue drug permanently if biopsy-proven immune-mediated myocarditis.	For Any Grade - Initial work-up should include clinical evaluation, B-type natriuretic peptide (BNP), cardiac enzymes, electrocardiogram (ECG), echocardiogram (ECHO), monitoring of oxygenation via pulse oximetry (resting and exertion), and additional laboratory work-up as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed. - Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections) - The prompt diagnosis of immune-mediated myocarditis is important, particularly in patients with baseline cardiopulmonary disease and reduced cardiac function. - Consider discussing with the Clinical Study Lead, as needed. - Monitor patients for signs and symptoms of myocarditis (new onset or worsening chest pain, arrhythmia, shortness of breath, peripheral edema). As some symptoms can overlap with lung toxicities, simultaneously evaluate for and rule out pulmonary toxicity as well as other causes (e.g., pulmonary embolism, congestive heart failure, malignant pericardial effusion). Consult a cardiologist early, to promptly assess whether and when to complete a cardiac biopsy, including any other diagnostic procedures. - As indicated, Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed.
	Grade 2, 3, or 4	If Grade 2-4, permanently discontinue study drug/study regimen.	For Grade 2-4: - Monitor symptoms daily, hospitalize. - Consider cardiology consultation and a prompt start of high-dose/pulse corticosteroid therapy - Supportive care (e.g., oxygen). - If no improvement consider immunosuppressive therapy such as TNF inhibitors (e.g., infliximab , IVIG or plasmapheresis or other therapies depending on the clinical condition of the patient, based on the discretion of the treating specialist consultant or relevant practice guidelines. **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. Infliximab is contraindicated for patients who have heart failure.**

Myositis/ Polymyositis	Any Grade	General guidance	For Any Grade: - Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections). - Monitor patients for signs and symptoms of poly/myositis. Typically, muscle weakness/pain occurs in proximal muscles including upper arms, thighs, shoulders, hips, neck and back, and; also difficulty breathing and/or trouble swallowing can occur and progress rapidly. Increased general feelings of tiredness and fatigue may occur, and there can be new-onset falling, difficulty getting up from a fall, and trouble climbing stairs, standing up from a seated position, and/or reaching up. - If poly/myositis is suspected, a Neurology consultation should be obtained early, with prompt guidance on diagnostic procedures. Myocarditis may co-occur with poly/myositis; refer to guidance under Myocarditis. Given breathing complications, refer to guidance under Pneumonitis/ILD. Given possibility of an existent (but previously unknown) autoimmune disorder, consider Rheumatology consultation. − Consider, as necessary, discussing with the Clinical Study Lead. − Initial work-up should include clinical evaluation, creatine kinase, aldolase, lactate dehydrogenase (LDH), blood urea nitrogen (BUN)/creatinine, erythrocyte sedimentation rate or C-reactive protein (CRP) level, urine myoglobin, and additional laboratory work-up as indicated, including a number of possible rheumatological/antibody tests (i.e., consider whether a rheumatologist consultation is indicated and could guide need for rheumatoid factor, antinuclear antibody, anti-smooth muscle, antisynthetase [such as anti-Jo-1], and/or signal-recognition particle antibodies). Confirmatory testing may include electromyography, nerve conduction studies, MRI of the muscles, and/or a muscle biopsy. Consider Barium swallow for evaluation of dysphagia or dysphonia.
	Grade 1	No dose modifications	For Grade 1: - Monitor and closely follow up in 2 to 4 days for clinical symptoms and initiate evaluation as clinically indicated. - Consider Neurology consult. - Consider, as necessary, discussing with the Clinical Study Lead.

	Grade 2	• Hold study drug/study regimen dose until resolution to Grade ≤1. • Permanently discontinue study drug/study regimen if it does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	For Grade 2 − Monitor symptoms daily and consider hospitalization. − Consider Rheumatology or Neurology consult, and initiate evaluation. − Consider, as necessary, discussing with the Clinical Study Lead. − If clinical course is rapidly progressive (particularly if difficulty breathing and/or trouble swallowing), promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant − If clinical course is *not* rapidly progressive, start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent); if no improvement within 2 to 3 days, continue additional work up and start treatment with IV methylprednisolone 2 to 4 mg/kg/day − If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 3 days, consider additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab, IVIG or plasmapheresis, or other therapies based on the discretion of the treating specialist consultant or relevant practice guideline. **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.**
	Grade 3	Grade 3 • Hold study drug/study regimen dose until resolution to Grade ≤1. • Permanently discontinue study drug/study regimen if Grade 3 imAE does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	For Grade 3 or 4 − Monitor symptoms closely; recommend hospitalization. − Consider Rheumatology and/or Neurology consult. − Consider discussing with the Clinical Study Lead, as needed. − Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. − If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.** − Consider whether patient may require IV IG, plasmapheresis.

	Grade 4	For Grade 4 Permanently discontinue study drug/study regimen	Grade 4 − Monitor symptoms closely; recommend hospitalization. − Consider Rheumatology and/or Neurology consult − Consider discussing with the Clinical Study Lead, as needed. − Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. − If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.

1 SCAR terms include Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), Erythema Multiforme, Acute Generalized Exanthematous Pustulosis, Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) and Drug-induced hypersensitivity syndrome.

Other-Immune-Mediated Reactions

Grade	**Dose Modifications**	**Toxicity Management**
Any Grade	Note: It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs, some of them are not noted specifically in these guidelines (e.g. immune thrombocytopenia, haemolytic anaemia, uveitis, vasculitis).	− Patients should be thoroughly evaluated to rule out any alternative etiology (e.g., disease progression, other medications, or infections). − The Clinical Study Lead may be contacted for immune-mediated reactions not listed in the "specific immune-medidated reactions" section. − Consultation with relevant specialist − Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Monitor as clinical indicated.
Grade 2	• Hold study drug/study regimen until resolution to ≤Grade 1 or baseline. • If toxicity worsens, then treat as Grade 3 or Grade 4. • Study drug/study regimen can be resumed once event stabilizes to Grade ≤1 after completion of steroid taper. • Consider whether study drug/study regimen should be permanently discontinued in Grade 2 events with high likelihood for morbidity and/or mortality when they do not rapidly improve to Grade <1 upon treatment with systemic steroids	For Grade 2, 3, or 4 Treat accordingly, as per institutional standard, appropriate clinical practice guidelines, and society guidelines.

	and following full taper.	
Grade 3	Hold study drug/study regimen	
Grade 4	Permanently discontinue study drug/study regimen.	

Infusion-Related Reactions		
Grade	**Dose Modifications**	**Toxicity Management**
Any Grade	General guidance.	– Manage per institutional standard at the discretion of investigator – Monitor patients for signs and symptoms of infusion-related reactions (e.g., fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnea or chest discomfort, or skin rashes) and anaphylaxis (e.g., generalized urticaria, angioedema, wheezing, hypotension, or tachycardia).
Grade 1 or 2	For Grade 1 The infusion rate of study drug/study regimen may be decreased by 50% or temporarily interrupted until resolution of the event. For Grade 2 • The infusion rate of study drug/study regimen may be decreased 50% or temporarily interrupted until resolution of the event. • Subsequent infusions may be given at 50% of the initial infusion rate.	For Grade 1 or Grade 2: – Acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator – Consider premedication per institutional standard or study protocol prior to subsequent doses – Steroids should not be used for routine medication of Grade ≤ 2 infusion reactions.
Grade 3 or 4	Permanently discontinue study drug/study regimen	For Grade 3 or 4: – Manage severe infusion-related reactions per institutional standard, appropriate clinical practice guidelines, and society guidelines.

Non-immune Mediated Reactions		
Grade	**Dose Modification**	**Toxicity Management**
Any Grade	Note: dose modifications are not required for adverse events not deemed to be related to study treatment (i.e. events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant.	Treat accordingly as per institutional standard
1	No dose modifications.	
2-3	Hold study drug/study regimen until resolution to Grade ≤ 1 or baseline	
4	Discontinue Study drug/study regimen (Note for Grade 4 labs, decision to discontinue would be based on accompanying clinical signs/symptoms and as per Investigator's clinical judgment and in consultation with the sponsor)	

9.4 Dose modifications for neoantigen DNA vaccines

No dose modifications are planned. If a subject develops an adverse event that is classified as possibly, probably, or definitely associated with protocol therapy, this may result in removal of the subject from protocol therapy as outlined in **Section 12 Removal of Subjects from Protocol Therapy**. Protocol Stopping Criteria are outlined in **Section 14 Data and Safety Monitoring**.

10 STUDY CALENDAR

	Screening[j]	**Eligibility Re-Check**[f]	**D1**[h]	**D29**	**D57**	**D85**	**D113**	**D141**	**D169**[n]	**EOT**[k]	**52 Weeks after** completion of neoantigen DNA vaccine therapy	**Long-term F/U**[e]
Randomization		X[g]										
Durvalumab[a,b]						X	X	X	X			
Vaccine[d]			X	X	X	X	X	X				
Concurrent meds	X		X ---------- X									
Physical exam, wt, PS	X	X	X	X	X	X	X	X	X	X		
Vital signs	X	X	X	X	X	X	X	X	X			
Medical History	X	X										
CBC w/diff, plts	X	X	X	X	X	X	X	X	X			
CMP[c]	X	X	X	X	X	X	X	X	X			
TSH, fT3, fT4	X	X				X[ab]	X[ab]	X[ab]	X[ab]			
Urinalysis	X	X				X[ab]	X[ab]	X[ab]	X[ab]			
ECG (in triplicate)	X	X				As clinically indicated[a]						
PT, aPTT, INR	X	X				As clinically indicated[a]						
Troponins I and T	X	X				Every 4 Weeks before treatment[a]						
AE evaluation[o]			X ---------- X									
Tumor measurements	X	As clinically indicated. Documentation (radiologic) must be provided for pts removed from study for PD.										
Radiologic evaluation	X	Radiologic measurements will be performed as clinically indicated.										
Conf. of persistent disease		X										
B-HCG	X	X	X	X	X	X	X	X				
Hepatitis serologies	X											
HIV testing	X											
Tissue collection	Research bx or surgical specimen or both											
Blood for sequencing	X											
Blood for immune monitoring[p]		X[m]	X	X	X	X	X		X		X	

a: Only if randomized to receive durvalumab

b: To begin on Cycle 4, if neoantigen-specific T cell responses are detectable only if randomized to get Durvalumab

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

d: +/- 7 days with at least 21 days between each injection
e: Annually for 5 years (± 2 weeks)
f: After completion of SOC therapy
g: After confirmation of eligibility and manufacture of vaccine
h: After completion of SOC therapy
j: May take place up to 28 days prior to registration.
k: Within 30 days of last day of study treatment
m: After the end of SOC chemotherapy
N: Durvalumab group only
O: In addition to completing an AE assessment at the vaccine dosing visit, an additional follow-up between 24-48 hours after vaccination must be performed (can be performed by phone). AE assessment and SAE reporting continues until 90 days after the last dose of Durvalumab or until time of initiation of other anticancer therapy, whichever occurs first.
p: ± 28 days

11 CRITERIA FOR RESPONSE

11.1 Primary objective: safety

The primary objective is to assess the safety of neoantigen DNA vaccines given alone or in combination with durvalumab.

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

(1) Local signs and symptoms

(2) Systemic signs and symptoms

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

(4) Adverse, and serious adverse events

Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03.

11.2 Secondary objective: neoantigen-specific T cell response

The secondary objective is to measure the neoantigen-specific T cell response to neoantigen DNA vaccines given alone, or neoantigen DNA vaccines plus durvalumab. The immune response will be measured in the peripheral blood by luminex assay, ELISPOT, and multiparametric flow cytometry.

11.3 Neoantigen-specific T cell response

The secondary objective is to assess the neoantigen-specific T cell response to the neoantigen DNA vaccine. To accomplish this goal, peripheral blood will be collected at multiple time points before and after vaccination. After peripheral blood and primary breast cancer tissue processing and storage, various assays will be used to assess the neoantigen-specific T cell response. Unless otherwise noted below, biologic specimens will be analyzed simultaneously upon completion of the vaccination protocol in order to minimize assay-to-assay variation.

11.3.1 Tumor tissue and peripheral blood processing

Processing of tumor: The tumor will be sampled prior to initiation of therapy.

Tumor from the research biopsy will be prioritized for tumor exome and tumor cDNA-capture sequencing in order to identify tumor-specific mutant antigens and produce the neoantigen DNA vaccine. The cores will be placed in complete RPMI or equivalent media solution on ice and transported to Dr. Gillanders' laboratory (CSRB, room 6664). Upon receipt by the lab, the tissue will then be prioritized for embedding in optimal cutting temperature (OCT) compound. The remaining cores will be placed into formalin for subsequent paraffin embedding (FFPE).

Cryomold will be filled slowly to the top with OCT compound. Tissue will be gently submerged into the OCT compound in the cryomold. OCT will then be hardened by placement on dry ice or in the vapor phase of liquid nitrogen. After hardening, the mold will be placed in a container and transferred to a -80^{o}C freezer for storage. The frozen tissue will be transferred to the Laboratory of Translation Pathology (LTP, BJC-Institute of Health, Room 5110) or the CHiiPs Immunomonitoring Laboratory for storage.

In addition to tumor from a research biopsy, tumor from surgical specimens may be acquired.

Processing of peripheral blood: Blood samples will be collected at the following time points: baseline, following completion of standard of care therapy, and Days 1, 29, 57, 85, 113, 169 and at 52 weeks after completion of neoantigen DNA vaccine therapy. Seventy mL of peripheral blood will be collected in 7 green top heparinized tubes by venipuncture (BD Vacutainer® sodium heparin, REF 367874, approximately 10 mL each for a total of approximately 70 mL needed total) The research patient coordinator will transport the blood to the laboratory of Dr. Gillanders located in the Clinical Sciences

Research Building, Room 6664. PBMC will be obtained by Ficoll-Hypaque gradient centrifugation and cryopreserved in 10% DMSO in fetal bovine serum according to standard procedures. Plasma will also be collected and stored for the various immunologic testing to be performed.

11.3.2 Neoantigen identification

DNA will be isolated from PBMC by the CHIIPs IML or Gillanders laboratory personnel for exome sequencing at The Genome Institute. To identify somatic mutations, DNA and RNA will be extracted from OCT-embedded tumor tissue. The OCT block will be sectioned for H&E staining by the CHIIPs IML and tumor-rich areas will be marked by a trained pathologist. Tumor cells will be macrodissected with or without laser capture, and DNA and RNA will be extracted. Tumor DNA and RNA will undergo tumor exome and tumor cDNA-capture sequencing, respectively, at the McDonnell Genome Institute.

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

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

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

We have developed and optimized an epitope prediction algorithm for the identification and prioritization of neoantigens. This optimized epitope prediction algorithm is described below and in our previous publications [19, 96]. We have established this algorithm in collaboration with Dr. Robert Schreiber, an internationally-known expert in tumor immunology [20, 80-87]. Schreiber and Mardis were one of the first to use next generation sequencing technologies to identify neoantigens, demonstrating that these antigens are important tumor rejection antigens [20]. Schreiber, Mardis, and Gillanders have now optimized the epitope prediction algorithm and have demonstrated that cancer vaccines targeting neoantigens are associated with antitumor immunity [19].

The goal of the optimized epitope prediction algorithm is to identify neoantigens and quantify the neoantigen load. The algorithm uses a combination of binding algorithms and processing algorithms.

Mutations that are expressed in the breast cancer will be identified using the sequencing pipeline outlined above. The predicted amino acid sequences corresponding to the expressed mutations will be pipelined through three class I MHC epitope-binding algorithms provided by the Immune Epitope Database and Analysis Resource (http://www.immuneepitope.org): (i) Stabilized Matrix Method (SMM) [90], (ii) Artificial Neural Network (ANN) [91], and (iii) NetMHCpan [92].

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

11.3.3 Neoantigen-specific T cell analysis

Peripheral blood will be processed and stored as PBMC and plasma. A frozen aliquot will be used for DNA isolation for both exome sequencing and HLA typing (the latter to be performed by ProImmune). The remainder of the samples will be used to analyze the immune response to neoantigen DNA vaccine alone vs. neoantigen DNA vaccine + durvalumab. We hypothesize that these two therapies will generate durable, neoantigen-specific CD4 and CD8 T cell responses, and affect the levels of immunomodulatory elements within the peripheral blood. We propose the following assays.

Multi-parametric flow cytometry will be used to measure an increase in polyclonal, activated T cells (CD3, CD4, CD8, HLA-DR, Ki67, PD-1, perforin, granzyme A/B), neoantigen-specific T cells (CD3, CD4, CD8,

MHC class I tetramers, MHC class II tetramers as described above) that persist over time (CD45RA, CD45RO, CCR7, CD27, CD28, CD95, CD58), and immunomodulatory elements (regulatory T cells: CD3, CD4, Foxp3, CD25; inflammatory monocytes, granulocytes: CD45, CD11b, CD115, CCR2, CD14, CD15) over various time points.

For functional analysis, Luminex and ELISPOT assays will be performed on PBMC using short peptides that mimic the minimal neoantigen epitope incorporated by the DNA vaccine with which the respective patient was vaccinated. The Luminex assay will be performed in conjunction with the Immune Monitoring Laboratory. ELISPOT assay will be performed as previously described [97-101] on PBMC from the subjects at all time points for IFN-γ. PBMC will be plated at various concentrations started at 300,000 cells per well, in triplicate, following the protocol previously described by Dr. Mohanakumar and colleagues for detection of breast cancer-specific T cells. PBMC will be co-cultured with the short peptides. 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) which contains immunodominant epitopes for multiple common MHC class I 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).

12 REMOVAL OF SUBJECTS FROM PROTOCOL THERAPY

12.1 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 or recurrent disease requiring systemic treatment or radiation therapy;

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

(3) Pregnancy;

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

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

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

(7) Grade ≥ 3 infusion reaction;

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

(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.

12.2 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.

12.3 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.

13 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to 20.4 for definitions and 20.5 for a grid of reporting timelines.

Adverse events will be tracked from start of treatment through 90 days after the last dose of Durvalumab or until initiation of new anticancer therapy, whichever comes first. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

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

Refer to the data submission schedule in Section 16 for instructions on the collections of AEs in the EDC.

Reporting requirements for Washington University study team are found in section 13.1.

13.1 Sponsor-Investigator Reporting Requirements

13.1.1 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.

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

The Washington University Sponsor Investigator (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that 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 to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

13.1.3 Reporting to Ichor Medical Systems

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

13.1.4 Reporting to AstraZeneca

All SAEs will be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). The reporting period for SAEs is the period immediately following the time that written informed consent is obtained through 90 days after the last dose of durvalumab or until the initiation of alternative anticancer therapy. The investigator and/or Sponsor are responsible for informing the Ethics Committee and/or the Regulatory Authority of the SAE as per local requirements.

The investigator and/or sponsor must inform the FDA, via a MedWatch form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32, and will concurrently forward all such reports to AstraZeneca. A copy of the MedWatch report must be emailed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the sponsor to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

***** A ***cover page*** should accompany the ***MedWatch*** form indicating the following:

- “Notification from an Investigator Sponsored Study”
- The investigator IND number assigned by the FDA
- The investigator’s name and address
- The trial name/title and AstraZeneca ISS reference number (ESR-16-12613)

***** Sponsor must also indicate, either in the SAE report or the cover page, the ***causality*** of events ***in relation to all study medications*** and if the SAE is ***related to disease progression***, as determined by the principal investigator.

**** Send SAE report and accompanying cover page by way of email to AstraZeneca's designated mailbox:*** AEMailboxClinicalTrialTCS@astrazeneca.com

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

Serious adverse events that do not require expedited reporting to the FDA still need to be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events. This information should be reported on a monthly basis and under no circumstance less frequently than quarterly.

13.1.4.1 Reporting of deaths to AstraZeneca

All deaths that occur during the study or within the protocol-defined 90-day post-last dose of durvalumab safety follow-up period must be reported to AstraZeneca as follows:

- Death that is clearly the result of disease progression should be documented but not reported as an SAE
- Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported to AstraZeneca as an SAE within **24 hours**. The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.
- Deaths with an unknown cause should always be reported as an SAE.

Deaths that occur following the protocol-defined 90-day post-last dose of durvalumab safety follow-up period will be documented but will not be reported as an SAE.

13.1.4.2 Definition of adverse events of special interest

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring and rapid communication by the investigator to the sponsor. An AESI may be serious or non-serious. The rapid reporting of AESIs allows ongoing surveillance of these events in order to characterize and understand them in association with the use of this investigational product.

AESIs for durvalumab include but are not limited to events with a potential inflammatory or immune-mediated mechanism and which may require more frequent monitoring and/or interventions such as steroids, immunosuppressants and/or hormone replacement therapy. These AESIs are being closely monitored in clinical studies with durvalumab monotherapy and combination therapy. An immune-mediated adverse event (imAE) is defined as an adverse event that is associated with drug exposure and is consistent with an immune-mediated mechanism of action and where there is no clear alternate etiology. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an imAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the imAE.

If the Investigator has any questions in regards to an adverse event (AE) being an imAE, the Investigator should promptly contact the Clinical Study Lead.

AESIs observed with durvalumab include:

- Diarrhea / Colitis and intestinal perforation
- Pneumonitis / ILD
- ALT/AST increases / hepatitis / hepatotoxicity
- Neuropathy / neuromuscular toxicity (i.e. Guillain-Barré and myasthenia gravis)
- Endocrinopathy (i.e. events of hypophysitis, adrenal insufficiency, diabetes insipidus and hyper- and hypothyroidism and type I diabetes mellitus)
- Rash / Dermatitis

- Nephritis / blood creatinine increases
- Pancreatitis (or labs suggestive of pancreatitis - increased serum lipase, increased serum amylase)
- Myocarditis
- Myositis / Polymyositis
- Other inflammatory response that are rare / less frequent with a potential immune-mediated aetiology include, but are not limited to, pericarditis, sarcoidosis, uveitis and other events involving the eyes, skin, haematological and rheumatological events, vasculitis, non-infectious meningitis and non-infectious encephalitis.

In addition, infusion-related reactions and hypersensitivity/anaphylactic reactions with a different underlying pharmacological aetiology are also considered AESIs.

Further information on these risks (e.g. presenting symptoms) can be found in the current version of the durvalumab Investigator Brochure. More specific guidelines for their evaluation and treatment are described in detail in the Dosing Modification and Toxicity Management Guidelines. These guidelines have been prepared to assist the Investigator in the exercise of his/her clinical judgment in treating these types of toxicities. These guidelines apply to AEs considered causally related to the study drug/study regimen by the reporting investigator.

13.1.4.3 Hy's Law

Cases where a patient shows elevations in liver biochemistry may require further evaluation and occurrences of AST or ALT ≥ 3x ULN together with total bilirubin ≥ 2x ULN may need to be reported as SAEs. Please refer to the Dosing Modification and Toxicity Management Guidelines for further instructions on cases of increases in liver biochemistry and evaluation of Hy's law.

13.1.4.4 Overdose

An overdose is defined as a subject receiving a dose of durvalumab in excess of that specified in the Investigator's Brochure, unless otherwise specified in this protocol.

Any overdose of a study subject with durvalumab, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to the sponsor and AstraZeneca/MedImmune Patient Safety or designee using the designated Safety e-mailbox. If the overdose results in an AE, the AE must also be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE. There is currently no specific treatment in the event of an overdose of durvalumab.

The investigator will use clinical judgment to treat any overdose.

13.1.4.5 Hepatic function abnormality

Hepatic function abnormality that fulfills the biochemical criteria of a potential Hy's Law case in a study subject, with or without associated clinical manifestations, is required to be reported as "hepatic function abnormal" ***within 24 hours of knowledge of the event*** to the sponsor and AstraZeneca Patient Safety using the designated Safety e-mailbox, unless a definitive underlying diagnosis for the abnormality (e.g., cholelithiasis or bile duct obstruction) that is unrelated to investigational product has been confirmed.

- If the definitive underlying diagnosis for the abnormality has been established and is unrelated to investigational product, the decision to continue dosing of the study subject will be based on the clinical judgment of the investigator.
- If no definitive underlying diagnosis for the abnormality is established, dosing of the study subject must be interrupted immediately. Follow-up investigations and inquiries must be initiated by the investigational site without delay.

Each reported event of hepatic function abnormality will be followed by the investigator and evaluated by the sponsor and AstraZeneca/MedImmune.

13.1.4.6 Pregnancy

If a patient becomes pregnant during the course of the study, the IPs should be discontinued immediately.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented even if the patient was discontinued from the study.

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel should inform the appropriate AstraZeneca representatives within 1 day, ie, immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative will work with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

13.1.5 Reporting to the FDA

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

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

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

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

13.1.6 Reporting to the Institutional Biosafety Committee

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

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

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

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

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

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

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

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

14 DATA AND SAFETY MONITORING

14.1 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 treatment with durvalumab or vaccination;

(2) **One** (or more) subject(s) experiences an SAE related to either durvalumab or vaccine;

(3) **Two** (or more) subjects experience the **same** Grade 2 or higher adverse event that is classified as probably or definitely related to durvalumab or 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.

(4) Death (other than death related to progressive disease) that occurs within 30 days after receiving DNA vaccination.

(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.

14.2 Data 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 beginning six months after accrual has opened (if at least one patient has been enrolled) or one year after accrual has opened (if no patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

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

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

The study principal investigator and Research Patient Coordinator 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 the HRPO and QASMC according to institutional guidelines.

14.3 Developmental therapeutics

Given the nature of this human gene transfer protocol, the principal investigator will monitor and analyze study data as they become available and will review this data on a monthly basis with the Developmental Therapeutics Group at the Siteman Cancer Center. This is an independent group that will provide more rigorous oversight than is routinely provided by the QASMC and the HRPO. The Director of the Developmental Therapeutics Group devotes 20% of their time to overseeing and managing early phase clinical trials. These trials consist of all phase 1 trials and selected Phase 2 trials. The Director will advise Dr. Gillanders in the proper conduct of this study and will review patient treatment and all toxicities in the weekly Phase 1 meeting. In addition, the Director will assist in the oversight of the regulatory and data management personnel involved in this clinical trial.

14.4 NIH Recombinant DNA Advisory Committee

14.4.1 Initiation of the clinical investigation

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

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

15 STATISTICAL CONSIDERATIONS

15.1 Study objectives and endpoints

This is a single institution, open-label randomized phase 1 trial of neoantigen DNA vaccine alone vs. neoantigen DNA vaccine plus durvalumab in triple negative breast cancer (TNBC) patients following standard of care therapy. Patients with newly diagnosed clinical stage II-III TNBC are eligible for enrollment. Patients will receive standard of care therapy including chemotherapy, surgery and radiation therapy as clinically indicated. Following standard of care therapy, patients will be randomized to receive either a neoantigen DNA vaccine alone, or a neoantigen DNA vaccine + durvalumab.

15.1.1 Primary objective

The primary objective is to assess the safety of neoantigen DNA vaccines given alone or in combination with durvalumab. Safety assessment will include both clinical observation and laboratory evaluation. Toxicity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03.

15.1.2 Secondary objectives.

The secondary objective is to measure the immune response to neoantigen DNA vaccines given alone, or in combination with durvalumab. The immune response will be measured in the peripheral blood by luminex assay, ELISPOT, and multiparametric flow cytometry.

15.2 Study design and sample justification

This is a single institution, open-label randomized phase 1 trial of neoantigen DNA vaccine alone vs. neoantigen DNA vaccine plus durvalumab in triple negative breast cancer (TNBC) patients following standard of care therapy. Patients with newly diagnosed clinical stage II-III TNBC are eligible for enrollment. Patients will receive standard of care therapy including chemotherapy, surgery and radiation therapy as clinically indicated. Following standard of care therapy, patients will be randomized to receive either a neoantigen DNA vaccine alone, or a neoantigen DNA vaccine + durvalumab

Twenty-four (24) eligible patients will be enrolled, and we expect that majority of them will receive the vaccine and will be eligible for evaluation.

15.2.1 Sample Size Calculations for Immunogenicity

A power analysis was performed using the paired t-test for over time differences (i.e., measures at Baseline vs. Day 1, Day 57) to test the first hypotheses that neoantigen DNA vaccines can induce and/or enhance neoantigen-specific T cell responses (e.g., the frequency of neoantigen-specific CD8 T cells in ELISPOT and multi-parameter flow cytometry). Assuming a moderate correlation between measures taken from the same individual, the designed sample size (n=24 patients) allows us 80% power at 1-sided 0.05 alpha level to detect a minimum of 0.5*SD for overtime changes. A power analysis was also performed using the 2-sample t-test for between-arm differences (i.e., measures at Day 113, Day 169) to test the second hypotheses that durvalumab will enhance the response to neoantigen DNA vaccines. We expect that the majority of the patients enrolled will remain in the study and lead to at least 10 evaluable patients/arm for such comparison. The designed sample size (n=10 patients/arm) provides us 80% power at 1-sided 0.05 alpha level to detect a minimum of 1.2*SD for between-group differences. Preliminary data from our phase 1 trial for mammaglobin-A DNA vaccine, though 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). Therefore, the designed sample size allows us to detect ~10% for over-time differences and ~15% for between-group differences respectively. As a study to estimate preliminary information, we expect that ~20% difference will provide an adequate signal for immunogenicity. In addition, 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.

15.2.2 Sample Size Calculations for Safety

Sample size calculations for safety are expressed in terms of the ability to detect serious adverse events. The ability of the study to identify serious adverse events is best expressed by the maximum true rate of events that would be unlikely to be observed and the minimum true rate of events that would very likely be observed. Based on extensive simulations regarding the sample size for translational studies, [102] Piantadosi recommended that a sample size of 10 to 20 patients would provide a reasonable precision for estimating preliminary information. For example, there is 80% chance of observing at least 1 serious adverse event among a sample size of n=10 patients if the "true" AE rate is at least 15%. Conversely, if the "true" AE rate is less than 3%, there is 3% chance to observe 2 or more serious adverse events.

15.3 Data Analysis

15.3.1 Safety analysis

Safety evaluation is the primary objective of the trial. The data will be descriptive, and standard toxicity definitions and criteria will be used as outlined in the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.

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

Laboratory data measured on a continuous scale will be characterized by summary statistics (mean and standard deviation). Boxplots of laboratory data will be generated for baseline values and for values measured during and after protocol therapy at each specific time point. Contingency tables will also be used to describe the change of safe data over time as appropriate.

15.3.2 Immune response

Neoantigen-specific T cell response to neoantigen DNA vaccines + durvalumab is the secondary objective of the trial. Neoantigen specific T cell responses will be measured by Luminex, ELISPOT, multiparametric flow cytometry, and CyTOF. The frequency of antigen-specific CD8 T cells in each arm at each time will be summarized using means, standard deviations and medians. The change over time as well as the difference between arms will also be compared using linear mixed model for repeated measurement data. The immunogenicity of the neoantigen DNA vaccine will also be analyzed qualitatively by summarizing the phenotypic and functional characteristics of epitope-specific CD8 T cells. Responses will be considered positive if the number of T cells after vaccination is greater than two standard deviations above the mean before vaccination [101]. The frequency of positive responses at each time point will be assessed and binomial response rates with 95% confidence interval estimates will be presented. In addition to presenting the binomial response rates, graphical and tabular summaries of the underlying distributions will be made.

To determine whether the observed difference is larger than might be expected by chance, a permutation test will be used to compare the observed test statistic to the distribution of test statistics that would be seen if there were no difference between the two arms. Specifically, we will randomly shuffle the data and calculate the test statistic from the shuffled data. This procedure will be repeated 10,000 times and the resultant testing statistics will provide an accurate representation of the null distribution. The observed test statistics of between-arm differences will be compared to the null distributions. For each outcome, the permuted p-value will be the fraction of permuted samples that resulted in a small statistic than the original sample [103].

16 DATA MANAGEMENT

Case report forms with appropriate source documentation will be completed according to the schedule below.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Demographics Form On-Study Form	Prior to starting treatment
Physical Exam Form	Baseline Day 1 Day 29 Day 57 Day 85 Day 113 Day 141
Durvalumab Form	Day 85 Day 113 Day 141 Day 169
Randomization Form	After end of SOC adjuvant therapy
Vaccine Administration Form	Day 1 Day 29 Day 57 Day 85 Day 113 Day 141
Immune Monitoring Form	Baseline End of SOC adjuvant therapy Day 1 Day 57 Day 113 Day 169 Week 52 after completion of neoantigen DNA vaccine therapy
Imaging Form	Baseline
Toxicity Form Concomitant Medications Form	Continuous
Follow Up Form	Week 52 after completion of neoantigen DNA vaccine therapy Year 2 Year 3 Year 4 Year 5
MedWatch Form	See Section 13.0 for reporting requirements

16.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 13) 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.

17 REGULATORY AND ETHICAL OBLIGATIONS

17.1 Informed consent

In accordance with US FDA regulations (21 CFR 50) and guidelines (Federal Register, May 9, 1997, Vol. 62, Number 90 - ICH Good Clinical Practice Consolidated Guideline) it is the investigator's responsibility to ensure that 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. Informed consent will be obtained from the subject after the details of the protocol have been reviewed. The individual responsible for obtaining consent will assure, prior to signing of the informed consent, that the subject has had all questions regarding therapy and the protocol answered.

17.2 Institutional Review Board

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

17.3 Subject confidentiality

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

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

18 ADMINISTRATIVE AND LEGAL OBLIGATIONS

18.1 Study documentation and retention of records

18.1.1 Study documentation

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

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

Pertinent documents in the study file include:

(1) The original protocol with all amendments

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

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

Pertinent documents in each individual subject file include:

(1) Informed consent forms

(2) Case report forms

(3) Supporting copies of source documentation

All original source documentation must be readily available.

18.1.2 Retention of records

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

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

18.2 Policy regarding research-related injuries

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

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

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

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

18.3 Study termination

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

19 REFERENCES

1. Sharma P, Allison JP: **The future of immune checkpoint therapy**. *Science* 2015, **348**(6230):56-61. http://www.ncbi.nlm.nih.gov/pubmed/25838373.

2. Shin DS, Ribas A: **The evolution of checkpoint blockade as a cancer therapy: what's here, what's next?** *Current opinion in immunology* 2015, **33**:23-35. http://www.ncbi.nlm.nih.gov/pubmed/25621841.

3. Sharma P, Allison JP: **Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential**. *Cell* 2015, **161**(2):205-214. http://www.ncbi.nlm.nih.gov/pubmed/25860605.

4. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T: **A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma**. *Science* 1991, **254**(5038):1643-1647. http://www.ncbi.nlm.nih.gov/pubmed/1840703.

5. Peshwa MV, Shi JD, Ruegg C, Laus R, van Schooten WC: **Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide**. *Prostate* 1998, **36**(2):129-138. http://www.ncbi.nlm.nih.gov/pubmed/9655265.

6. Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ: **Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide**. *Proceedings of the National Academy of Sciences of the United States of America* 1995, **92**(2):432-436. http://www.ncbi.nlm.nih.gov/pubmed/7831305.

7. Parmiani G, De Filippo A, Novellino L, Castelli C: **Unique human tumor antigens: immunobiology and use in clinical trials**. *J Immunol* 2007, **178**(4):1975-1979. http://www.ncbi.nlm.nih.gov/pubmed/17277099.

8. Sensi M, Anichini A: **Unique tumor antigens: evidence for immune control of genome integrity and immunogenic targets for T cell-mediated patient-specific immunotherapy**. *Clin Cancer Res* 2006, **12**(17):5023-5032. http://www.ncbi.nlm.nih.gov/pubmed/16951217.

9. Wolfel T, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer zum Buschenfelde KH, Beach D: **A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma**. *Science* 1995, **269**(5228):1281-1284. http://www.ncbi.nlm.nih.gov/pubmed/7652577.

10. Wang RF, Wang X, Atwood AC, Topalian SL, Rosenberg SA: **Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen**. *Science* 1999, **284**(5418):1351-1354. http://www.ncbi.nlm.nih.gov/pubmed/10334988.

11. Echchakir H, Mami-Chouaib F, Vergnon I, Baurain JF, Karanikas V, Chouaib S, Coulie PG: **A point mutation in the alpha-actinin-4 gene generates an antigenic peptide recognized by autologous cytolytic T lymphocytes on a human lung carcinoma**. *Cancer Res* 2001, **61**(10):4078-4083. http://www.ncbi.nlm.nih.gov/pubmed/11358829.

12. Brandle D, Brasseur F, Weynants P, Boon T, Van den Eynde B: **A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma**. *J Exp Med* 1996, **183**(6):2501-2508. http://www.ncbi.nlm.nih.gov/pubmed/8676070.

13. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, Harris CC, McLellan MD, Fulton RS, Fulton LL, Abbott RM, Hoog J, Dooling DJ, Koboldt DC, Schmidt H, Kalicki J, Zhang Q, Chen L, Lin L, Wendl MC, McMichael JF, Magrini VJ, Cook L, McGrath SD, Vickery TL, Appelbaum E, Deschryver K, Davies S, Guintoli T, Crowder R, Tao Y, Snider JE, Smith SM, Dukes AF, Sanderson GE, Pohl CS, Delehaunty KD, Fronick CC, Pape KA, Reed JS, Robinson JS, Hodges JS, Schierding W, Dees ND, Shen D, Locke DP, Wiechert ME, Eldred JM, Peck JB, Oberkfell BJ, Lolofie JT, Du F, Hawkins AE, O'Laughlin MD, Bernard KE, Cunningham M, Elliott G, Mason MD, Thompson DM, Jr., Ivanovich JL, Goodfellow PJ, Perou CM, Weinstock GM, Aft R, Watson M, Ley TJ, Wilson RK, Mardis ER: **Genome remodelling in a basal-like breast cancer metastasis**

and xenograft. *Nature* 2010, **464**(7291):999-1005. http://www.ncbi.nlm.nih.gov/pubmed/20393555.

14. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, Van Tine BA, Hoog J, Goiffon RJ, Goldstein TC, Ng S, Lin L, Crowder R, Snider J, Ballman K, Weber J, Chen K, Koboldt DC, Kandoth C, Schierding WS, McMichael JF, Miller CA, Lu C, Harris CC, McLellan MD, Wendl MC, DeSchryver K, Allred DC, Esserman L, Unzeitig G, Margenthaler J, Babiera GV, Marcom PK, Guenther JM, Leitch M, Hunt K, Olson J, Tao Y, Maher CA, Fulton LL, Fulton RS, Harrison M, Oberkfell B, Du F, Demeter R, Vickery TL, Elhammali A, Piwnica-Worms H, McDonald S, Watson M, Dooling DJ, Ota D, Chang LW, Bose R, Ley TJ, Piwnica-Worms D, Stuart JM, Wilson RK, Mardis ER: **Whole-genome analysis informs breast cancer response to aromatase inhibition**. *Nature* 2012, **486**(7403):353-360. http://www.ncbi.nlm.nih.gov/pubmed/22722193.

15. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE: **The consensus coding sequences of human breast and colorectal cancers**. *Science* 2006, **314**(5797):268-274. http://www.ncbi.nlm.nih.gov/pubmed/16959974.

16. Srivastava N, Srivastava PK: **Modeling the repertoire of true tumor-specific MHC I epitopes in a human tumor**. *PLoS One* 2009, **4**(7):e6094. http://www.ncbi.nlm.nih.gov/pubmed/19593447.

17. Parker KC, Bednarek MA, Coligan JE: **Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains**. *J Immunol* 1994, **152**(1):163-175. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8254189

18. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S: **SYFPEITHI: database for MHC ligands and peptide motifs**. *Immunogenetics* 1999, **50**(3-4):213-219. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10602881

19. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN, Schreiber RD: **Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens**. *Nature* 2014, **515**(7528):577-581. http://www.ncbi.nlm.nih.gov/pubmed/25428507.

20. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, Arthur CD, White JM, Chen YS, Shea LK, Hundal J, Wendl MC, Demeter R, Wylie T, Allison JP, Smyth MJ, Old LJ, Mardis ER, Schreiber RD: **Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting**. *Nature* 2012, **482**(7385):400-404. http://www.ncbi.nlm.nih.gov/pubmed/22318521.

21. Cox GJ, Zamb TJ, Babiuk LA: **Bovine herpesvirus 1: immune responses in mice and cattle injected with plasmid DNA**. *J Virol* 1993, **67**(9):5664-5667. http://www.ncbi.nlm.nih.gov/pubmed/8350420.

22. Davis HL, Michel ML, Whalen RG: **DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody**. *Hum Mol Genet* 1993, **2**(11):1847-1851. http://www.ncbi.nlm.nih.gov/pubmed/8281146.

23. Fynan EF, Webster RG, Fuller DH, Haynes JR, Santoro JC, Robinson HL: **DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations**. *Proc Natl Acad Sci U S A* 1993, **90**(24):11478-11482. http://www.ncbi.nlm.nih.gov/pubmed/8265577.

24. Tang DC, DeVit M, Johnston SA: **Genetic immunization is a simple method for eliciting an immune response**. *Nature* 1992, **356**(6365):152-154. http://www.ncbi.nlm.nih.gov/pubmed/1545867.

25. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, Dwarki VJ, Gromkowski SH, Deck RR, DeWitt CM, Friedman A, et al.: **Heterologous protection against influenza by injection of DNA encoding a viral protein**. *Science* 1993, **259**(5102):1745-1749. http://www.ncbi.nlm.nih.gov/pubmed/8456302.

26. Wang B, Ugen KE, Srikantan V, Agadjanyan MG, Dang K, Refaeli Y, Sato AI, Boyer J, Williams WV, Weiner DB: **Gene inoculation generates immune responses against human immunodeficiency virus type 1**. *Proc Natl Acad Sci U S A* 1993, **90**(9):4156-4160. http://www.ncbi.nlm.nih.gov/pubmed/8483929.

27. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA: **DNA vaccines**. *Annu Rev Immunol* 1997, **15**:617-648. http://www.ncbi.nlm.nih.gov/pubmed/9143702.

28. Gurunathan S, Klinman DM, Seder RA: **DNA vaccines: immunology, application, and optimization***. *Annual review of immunology* 2000, **18**:927-974. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10837079

29. Stevenson FK, Rice J, Ottensmeier CH, Thirdborough SM, Zhu D: **DNA fusion gene vaccines against cancer: from the laboratory to the clinic**. *Immunol Rev* 2004, **199**:156-180. http://www.ncbi.nlm.nih.gov/pubmed/15233733.

30. Dupuis M, Denis-Mize K, Woo C, Goldbeck C, Selby MJ, Chen M, Otten GR, Ulmer JB, Donnelly JJ, Ott G, McDonald DM: **Distribution of DNA vaccines determines their immunogenicity after intramuscular injection in mice**. *J Immunol* 2000, **165**(5):2850-2858. http://www.ncbi.nlm.nih.gov/pubmed/10946318.

31. Aihara H, Miyazaki J: **Gene transfer into muscle by electroporation in vivo**. *Nat Biotechnol* 1998, **16**(9):867-870. http://www.ncbi.nlm.nih.gov/pubmed/9743122.

32. Mathiesen I: **Electropermeabilization of skeletal muscle enhances gene transfer in vivo**. *Gene Ther* 1999, **6**(4):508-514. http://www.ncbi.nlm.nih.gov/pubmed/10476210.

33. Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D: **High-efficiency gene transfer into skeletal muscle mediated by electric pulses**. *Proc Natl Acad Sci U S A* 1999, **96**(8):4262-4267. http://www.ncbi.nlm.nih.gov/pubmed/10200250.

34. Widera G, Austin M, Rabussay D, Goldbeck C, Barnett SW, Chen M, Leung L, Otten GR, Thudium K, Selby MJ, Ulmer JB: **Increased DNA vaccine delivery and immunogenicity by electroporation in vivo**. *J Immunol* 2000, **164**(9):4635-4640. http://www.ncbi.nlm.nih.gov/pubmed/10779767.

35. Boyer JD, Robinson TM, Kutzler MA, Parkinson R, Calarota SA, Sidhu MK, Muthumani K, Lewis M, Pavlakis G, Felber B, Weiner D: **SIV DNA vaccine co-administered with IL-12 expression plasmid enhances CD8 SIV cellular immune responses in cynomolgus macaques**. *J Med Primatol* 2005, **34**(5-6):262-270. http://www.ncbi.nlm.nih.gov/pubmed/16128921.

36. Chong SY, Egan MA, Kutzler MA, Megati S, Masood A, Roopchard V, Garcia-Hand D, Montefiori DC, Quiroz J, Rosati M, Schadeck EB, Boyer JD, Pavlakis GN, Weiner DB, Sidhu M, Eldridge JH, Israel ZR: **Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques**. *Vaccine* 2007, **25**(26):4967-4982. http://www.ncbi.nlm.nih.gov/pubmed/17335943.

37. Hirao LA, Wu L, Khan AS, Hokey DA, Yan J, Dai A, Betts MR, Draghia-Akli R, Weiner DB: **Combined effects of IL-12 and electroporation enhances the potency of DNA vaccination in macaques**. *Vaccine* 2008, **26**(25):3112-3120. http://www.ncbi.nlm.nih.gov/pubmed/18430495.

38. Hirao LA, Wu L, Khan AS, Satishchandran A, Draghia-Akli R, Weiner DB: **Intradermal/subcutaneous immunization by electroporation improves plasmid vaccine delivery and potency in pigs and rhesus macaques**. *Vaccine* 2008, **26**(3):440-448. http://www.ncbi.nlm.nih.gov/pubmed/18082294.

39. Luckay A, Sidhu MK, Kjeken R, Megati S, Chong SY, Roopchand V, Garcia-Hand D, Abdullah R, Braun R, Montefiori DC, Rosati M, Felber BK, Pavlakis GN, Mathiesen I, Israel ZR, Eldridge JH, Egan MA: **Effect of plasmid DNA vaccine design and in vivo electroporation on the resulting vaccine-specific immune responses in rhesus macaques**. *J Virol* 2007, **81**(10):5257-5269. http://www.ncbi.nlm.nih.gov/pubmed/17329330.

40. Otten G, Schaefer M, Doe B, Liu H, Srivastava I, zur Megede J, O'Hagan D, Donnelly J, Widera G, Rabussay D, Lewis MG, Barnett S, Ulmer JB: **Enhancement of DNA vaccine potency in rhesus macaques by electroporation**. *Vaccine* 2004, **22**(19):2489-2493. http://www.ncbi.nlm.nih.gov/pubmed/15193413.

41. Otten GR, Schaefer M, Doe B, Liu H, Megede JZ, Donnelly J, Rabussay D, Barnett S, Ulmer JB: **Potent immunogenicity of an HIV-1 gag-pol fusion DNA vaccine delivered by in vivo electroporation**. *Vaccine* 2006, **24**(21):4503-4509. http://www.ncbi.nlm.nih.gov/pubmed/16181711.

42. Schadeck EB, Sidhu M, Egan MA, Chong SY, Piacente P, Masood A, Garcia-Hand D, Cappello S, Roopchand V, Megati S, Quiroz J, Boyer JD, Felber BK, Pavlakis GN, Weiner DB, Eldridge JH, Israel ZR: **A dose sparing effect by plasmid encoded IL-12 adjuvant on a SIVgag-plasmid DNA vaccine in rhesus macaques**. *Vaccine* 2006, **24**(21):4677-4687. http://www.ncbi.nlm.nih.gov/pubmed/16288822.

43. Rice J, Ottensmeier CH, Stevenson FK: **DNA vaccines: precision tools for activating effective immunity against cancer**. *Nat Rev Cancer* 2008, **8**(2):108-120. http://www.ncbi.nlm.nih.gov/pubmed/18219306.

44. Weiner DB: **DNA vaccines: Crossing a line in the sand Introduction to special issue**. *Vaccine* 2008. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18652867

45. Rosenberg SA, Yang JC, Restifo NP: **Cancer immunotherapy: moving beyond current vaccines**. *Nature medicine* 2004, **10**(9):909-915. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15340416

46. Finn OJ: **Cancer vaccines: between the idea and the reality**. *Nature reviews Immunology* 2003, **3**(8):630-641. http://www.ncbi.nlm.nih.gov/pubmed/12974478.

47. Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, Drebin JA, Strasberg SM, Eberlein TJ, Goedegebuure PS, Linehan DC: **Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma**. *JImmunol* 2002, **169**(5):2756. PM:12193750

48. Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B: **Increase of regulatory T cells in the peripheral blood of cancer patients**. *Clin Cancer Res* 2003, **9**(2):606-612. http://www.ncbi.nlm.nih.gov/pubmed/12576425.

49. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, Carbone DP, Gabrilovich DI: **Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer**. *J Immunol* 2001, **166**(1):678-689. http://www.ncbi.nlm.nih.gov/pubmed/11123353.

50. Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED, Carbone DP, Gabrilovich DI: **Clinical significance of defective dendritic cell differentiation in cancer**. *Clin Cancer Res* 2000, **6**(5):1755-1766. http://www.ncbi.nlm.nih.gov/pubmed/10815894.

51. Hoos A, Parmiani G, Hege K, Sznol M, Loibner H, Eggermont A, Urba W, Blumenstein B, Sacks N, Keilholz U, Nichol G, Cancer Vaccine Clinical Trial Working G: **A clinical development paradigm for cancer vaccines and related biologics**. *Journal of immunotherapy* 2007, **30**(1):1-15. http://www.ncbi.nlm.nih.gov/pubmed/17198079.

52. Schlom J, Arlen PM, Gulley JL: **Cancer vaccines: moving beyond current paradigms**. *Clin Cancer Res* 2007, **13**(13):3776-3782. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17606707

53. Simon RM, Steinberg SM, Hamilton M, Hildesheim A, Khleif S, Kwak LW, Mackall CL, Schlom J, Topalian SL, Berzofsky JA: **Clinical trial designs for the early clinical development of therapeutic cancer vaccines**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2001, **19**(6):1848-1854. http://www.ncbi.nlm.nih.gov/pubmed/11251017.

54. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, Carter SL, Stewart C, Mermel CH, Roberts SA, Kiezun A, Hammerman PS, McKenna A, Drier Y, Zou L, Ramos AH, Pugh TJ, Stransky N, Helman E, Kim J, Sougnez C, Ambrogio L, Nickerson E, Shefler E, Cortes ML, Auclair D, Saksena G, Voet D, Noble M, DiCara D, Lin P, Lichtenstein L, Heiman DI, Fennell T, Imielinski M, Hernandez B, Hodis E, Baca S, Dulak AM, Lohr J, Landau DA, Wu CJ, Melendez-Zajgla J, Hidalgo-Miranda A, Koren A, McCarroll SA, Mora J, Lee RS, Crompton B, Onofrio R, Parkin M, Winckler W, Ardlie K, Gabriel SB, Roberts CW, Biegel JA, Stegmaier K, Bass AJ, Garraway LA, Meyerson M, Golub TR, Gordenin DA, Sunyaev S, Lander ES, Getz G: **Mutational heterogeneity in cancer and the search for new cancer-associated genes**. *Nature* 2013, **499**(7457):214-218. http://www.ncbi.nlm.nih.gov/pubmed/23770567.

55. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjord JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jager N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, Lopez-Otin C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdes-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR, Australian Pancreatic Cancer Genome I, Consortium IBC, Consortium IM-S, PedBrain I, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR: **Signatures of mutational processes in human cancer**. *Nature* 2013, **500**(7463):415-421. http://www.ncbi.nlm.nih.gov/pubmed/23945592.

56. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, Leiserson MD, Miller CA, Welch JS, Walter MJ, Wendl MC, Ley TJ, Wilson RK, Raphael BJ, Ding L: **Mutational landscape and significance across 12 major cancer types**. *Nature* 2013, **502**(7471):333-339. http://www.ncbi.nlm.nih.gov/pubmed/24132290.

57. Anders CK, Winer EP, Ford JM, Dent R, Silver DP, Sledge GW, Carey LA: **Poly(ADP-Ribose) polymerase inhibition: "targeted" therapy for triple-negative breast cancer**. *Clin Cancer Res* 2010, **16**(19):4702-4710. http://www.ncbi.nlm.nih.gov/pubmed/20858840.

58. Liedtke C, Bernemann C, Kiesel L, Rody A: **Genomic profiling in triple-negative breast cancer**. *Breast care* 2013, **8**(6):408-413. http://www.ncbi.nlm.nih.gov/pubmed/24550748.

59. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G, Bashashati A, Prentice LM, Khattra J, Burleigh A, Yap D, Bernard V, McPherson A, Shumansky K, Crisan A, Giuliany R, Heravi-Moussavi A, Rosner J, Lai D, Birol I, Varhol R, Tam A, Dhalla N, Zeng T, Ma K, Chan SK, Griffith M, Moradian A, Cheng SW, Morin GB, Watson P, Gelmon K, Chia S, Chin SF, Curtis C, Rueda OM, Pharoah PD, Damaraju S, Mackey J, Hoon K, Harkins T, Tadigotla V, Sigaroudinia M, Gascard P, Tlsty T, Costello JF, Meyer IM, Eaves CJ, Wasserman WW, Jones S, Huntsman D, Hirst M, Caldas C, Marra MA, Aparicio S: **The clonal and**

mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 2012, **486**(7403):395-399. http://www.ncbi.nlm.nih.gov/pubmed/22495314.

60. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, Martino S, Wang M, Jones VE, Saphner TJ, Wolff AC, Wood WC, Davidson NE, Sledge GW, Sparano JA, Badve SS: **Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2014, **32**(27):2959-2966. http://www.ncbi.nlm.nih.gov/pubmed/25071121.

61. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, Rouas G, Francis P, Crown JP, Hitre E, de Azambuja E, Quinaux E, Di Leo A, Michiels S, Piccart MJ, Sotiriou C: **Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013, **31**(7):860-867. http://www.ncbi.nlm.nih.gov/pubmed/23341518.

62. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, Pfitzner BM, Salat C, Loi S, Schmitt WD, Schem C, Fisch K, Darb-Esfahani S, Mehta K, Sotiriou C, Wienert S, Klare P, Andre F, Klauschen F, Blohmer JU, Krappmann K, Schmidt M, Tesch H, Kummel S, Sinn P, Jackisch C, Dietel M, Reimer T, Untch M, Loibl S: **Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015, **33**(9):983-991. http://www.ncbi.nlm.nih.gov/pubmed/25534375.

63. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, Su X, Wang Y, Gonzalez-Angulo AM, Akcakanat A, Chawla A, Curran M, Hwu P, Sharma P, Litton JK, Molldrem JJ, Alatrash G: **PD-L1 expression in triple-negative breast cancer**. *Cancer immunology research* 2014, **2**(4):361-370. http://www.ncbi.nlm.nih.gov/pubmed/24764583.

64. Muenst S, Schaerli AR, Gao F, Daster S, Trella E, Droeser RA, Muraro MG, Zajac P, Zanetti R, Gillanders WE, Weber WP, Soysal SD: **Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer**. *Breast cancer research and treatment* 2014, **146**(1):15-24. http://www.ncbi.nlm.nih.gov/pubmed/24842267.

65. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS: **Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients**. *Nature* 2014, **515**(7528):563-567. http://www.ncbi.nlm.nih.gov/pubmed/25428504.

66. Muenst S, Soysal SD, Gao F, Obermann EC, Oertli D, Gillanders WE: **The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer**. *Breast cancer research and treatment* 2013, **139**(3):667-676. http://www.ncbi.nlm.nih.gov/pubmed/23756627.

67. Ng CM, Lum BL, Gimenez V, Kelsey S, Allison D: **Rationale for fixed dosing of pertuzumab in cancer patients based on population pharmacokinetic analysis**. *Pharm Res* 2006, **23**(6):1275-1284. http://www.ncbi.nlm.nih.gov/pubmed/16715358.

68. Wang DD, Zhang S, Zhao H, Men AY, Parivar K: **Fixed dosing versus body size-based dosing of monoclonal antibodies in adult clinical trials**. *J Clin Pharmacol* 2009, **49**(9):1012-1024. http://www.ncbi.nlm.nih.gov/pubmed/19620385.

69. Zhang S, Shi R, Li C, Parivar K, Wang DD: **Fixed dosing versus body size-based dosing of therapeutic peptides and proteins in adults**. *J Clin Pharmacol* 2012, **52**(1):18-28. http://www.ncbi.nlm.nih.gov/pubmed/21233304.

70. Narwal R, Roskos LK, Robbie GJ: **Population pharmacokinetics of sifalimumab, an investigational anti-interferon-alpha monoclonal antibody, in systemic lupus erythematosus**. *Clin Pharmacokinet* 2013, **52**(11):1017-1027. http://www.ncbi.nlm.nih.gov/pubmed/23754736.

71. Bowne WB, Srinivasan R, Wolchok JD, Hawkins WG, Blachere NE, Dyall R, Lewis JJ, Houghton AN: **Coupling and uncoupling of tumor immunity and autoimmunity**. *The Journal of experimental medicine* 1999, **190**(11):1717-1722. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10587362

72. Hawkins WG, Gold JS, Dyall R, Wolchok JD, Hoos A, Bowne WB, Srinivasan R, Houghton AN, Lewis JJ: **Immunization with DNA coding for gp100 results in CD4 T-cell independent antitumor immunity**. *Surgery* 2000, **128**(2):273-280. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10923004

73. Prazeres DM, Monteiro GA, Ferreira GN, Diogo MM, Ribeiro SC, Cabral JM: **Purification of plasmids for gene therapy and DNA vaccination**. *Biotechnol Annu Rev* 2001, **7**:1-30. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11686041

74. Manoj S, Babiuk LA, van Drunen Littel-van den Hurk S: **Approaches to enhance the efficacy of DNA vaccines**. *Crit Rev Clin Lab Sci* 2004, **41**(1):1-39. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15077722.

75. Donnelly J, Berry K, Ulmer JB: **Technical and regulatory hurdles for DNA vaccines**. *Int J Parasitol* 2003, **33**(5-6):457-467. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12782047.

76. Scheerlinck JP, Karlis J, Tjelle TE, Presidente PJ, Mathiesen I, Newton SE: **In vivo electroporation improves immune responses to DNA vaccination in sheep**. *Vaccine* 2004, **22**(13-14):1820-1825. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15068866.

77. Babiuk S, Baca-Estrada ME, Foldvari M, Storms M, Rabussay D, Widera G, Babiuk LA: **Electroporation improves the efficacy of DNA vaccines in large animals**. *Vaccine* 2002, **20**(27-28):3399-3408. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12213410.

78. Luxembourg A, Hannaman D, Ellefsen B, Nakamura G, Bernard R: **Enhancement of immune responses to an HBV DNA vaccine by electroporation**. *Vaccine* 2006, **24**(21):4490-4493. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16140436

79. Vasan S, Hurley A, Schlesinger SJ, Hannaman D, Gardiner DF, Dugin DP, Boente-Carrera M, Vittorino R, Caskey M, Andersen J, Huang Y, Cox JH, Tarragona-Fiol T, Gill DK, Cheeseman H, Clark L, Dally L, Smith C, Schmidt C, Park HH, Kopycinski JT, Gilmour J, Fast P, Bernard R, Ho DD: **In vivo electroporation enhances the immunogenicity of an HIV-1 DNA vaccine candidate in healthy volunteers**. *PLoS One* 2011, **6**(5):e19252. https://www.ncbi.nlm.nih.gov/pubmed/21603651.

80. Dunn GP, Bruce AT, Sheehan KC, Shankaran V, Uppaluri R, Bui JD, Diamond MS, Koebel CM, Arthur C, White JM, Schreiber RD: **A critical function for type I interferons in cancer immunoediting**. *Nature immunology* 2005, **6**(7):722-729. http://www.ncbi.nlm.nih.gov/pubmed/15951814.

81. Dunn GP, Koebel CM, Schreiber RD: **Interferons, immunity and cancer immunoediting**. *Nature reviews Immunology* 2006, **6**(11):836-848. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17063185

82. Dunn GP, Old LJ, Schreiber RD: **The three Es of cancer immunoediting**. *Annual review of immunology* 2004, **22**:329-360. http://www.ncbi.nlm.nih.gov/pubmed/15032581.

83. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD: **IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity**. *Nature* 2001, **410**(6832):1107-1111. http://www.ncbi.nlm.nih.gov/pubmed/11323675.

84. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, Lee H, Arthur CD, White JM, Kalinke U, Murphy KM, Schreiber RD: **Type I interferon is selectively required by dendritic cells for immune rejection of tumors**. *J Exp Med* 2011, **208**(10):1989-2003. http://www.ncbi.nlm.nih.gov/pubmed/21930769.

85. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD: **Cancer immunoediting: from immunosurveillance to tumor escape**. *Nature immunology* 2002, **3**(11):991-998. http://www.ncbi.nlm.nih.gov/pubmed/12407406.

86. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ: **Natural innate and adaptive immunity to cancer**. *Annual review of immunology* 2011, **29**:235-271. http://www.ncbi.nlm.nih.gov/pubmed/21219185.

87. Vesely MD, Schreiber RD: **Cancer immunoediting: antigens, mechanisms, and implications to cancer immunotherapy**. *Annals of the New York Academy of Sciences* 2013, **1284**:1-5. http://www.ncbi.nlm.nih.gov/pubmed/23651186.

88. Klco JM, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O'Laughlin M, Fronick C, Magrini V, Demeter RT, Fulton RS, Eades WC, Link DC, Graubert TA, Walter MJ, Mardis ER, Dipersio JF, Wilson RK, Ley TJ: **Functional heterogeneity of genetically defined subclones in acute myeloid leukemia**. *Cancer cell* 2014, **25**(3):379-392. http://www.ncbi.nlm.nih.gov/pubmed/24613412.

89. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, Wartman LD, Lamprecht TL, Liu F, Xia J, Kandoth C, Fulton RS, McLellan MD, Dooling DJ, Wallis JW, Chen K, Harris CC, Schmidt HK, Kalicki-Veizer JM, Lu C, Zhang Q, Lin L, O'Laughlin MD, McMichael JF, Delehaunty KD, Fulton LA, Magrini VJ, McGrath SD, Demeter RT, Vickery TL, Hundal J, Cook LL, Swift GW, Reed JP, Alldredge PA, Wylie TN, Walker JR, Watson MA, Heath SE, Shannon WD, Varghese N, Nagarajan R, Payton JE, Baty JD, Kulkarni S, Klco JM, Tomasson MH, Westervelt P, Walter MJ, Graubert TA, DiPersio JF, Ding L, Mardis ER, Wilson RK: **The origin and evolution of mutations in acute myeloid leukemia**. *Cell* 2012, **150**(2):264-278. http://www.ncbi.nlm.nih.gov/pubmed/22817890.

90. Peters B, Sette A: **Generating quantitative models describing the sequence specificity of biological processes with the stabilized matrix method**. *BMC bioinformatics* 2005, **6**:132. http://www.ncbi.nlm.nih.gov/pubmed/15927070.

91. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M: **NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11**. *Nucleic acids research* 2008, **36**(Web Server issue):W509-512. http://www.ncbi.nlm.nih.gov/pubmed/18463140.

92. Hoof I, Peters B, Sidney J, Pedersen LE, Sette A, Lund O, Buus S, Nielsen M: **NetMHCpan, a method for MHC class I binding prediction beyond humans**. *Immunogenetics* 2009, **61**(1):1-13. http://www.ncbi.nlm.nih.gov/pubmed/19002680.

93. Nielsen M, Lundegaard C, Lund O, Kesmir C: **The role of the proteasome in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage**. *Immunogenetics* 2005, **57**(1-2):33-41. http://www.ncbi.nlm.nih.gov/pubmed/15744535.

94. Thomas AM, Santarsiero LM, Lutz ER, Armstrong TD, Chen YC, Huang LQ, Laheru DA, Goggins M, Hruban RH, Jaffee EM: **Mesothelin-specific CD8(+) T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients**. *J Exp Med* 2004, **200**(3):297-306. http://www.ncbi.nlm.nih.gov/pubmed/15289501.

95. Dannull J, Diener PA, Prikler L, Furstenberger G, Cerny T, Schmid U, Ackermann DK, Groettrup M: **Prostate stem cell antigen is a promising candidate for immunotherapy of advanced prostate cancer**. *Cancer Res* 2000, **60**(19):5522-5528. http://www.ncbi.nlm.nih.gov/pubmed/11034097.

96. Hundal J, Carreno BM, Petti AA, Linette GP, Griffith OL, Mardis ER, Griffith M: **pVAC-Seq: A genome-guided in silico approach to identifying tumor neoantigens**. *Genome Med* 2016, **8**(1):11. http://www.ncbi.nlm.nih.gov/pubmed/26825632.

97. Jaramillo A, Majumder K, Manna PP, Fleming TP, Doherty G, Dipersio JF, Mohanakumar T: **Identification of HLA-A3-restricted CD8+ T cell epitopes derived from mammaglobin-A, a tumor-associated antigen of human breast cancer**. *International journal of cancer Journal international du cancer* 2002, **102**(5):499-506. http://www.ncbi.nlm.nih.gov/pubmed/12432553.

98. Jaramillo A, Narayanan K, Campbell LG, Benshoff ND, Lybarger L, Hansen TH, Fleming TP, Dietz JR, Mohanakumar T: **Recognition of HLA-A2-restricted mammaglobin-A-derived epitopes by CD8+ cytotoxic T lymphocytes from breast cancer patients**. *Breast cancer research and treatment* 2004, **88**(1):29-41. http://www.ncbi.nlm.nih.gov/pubmed/15538043.

99. Manna PP, Jaramillo A, Majumder K, Campbell LG, Fleming TP, Dietz JR, Dipersio JF, Mohanakumar T: **Generation of CD8+ cytotoxic T lymphocytes against breast cancer cells by stimulation with mammaglobin-A-pulsed dendritic cells**. *Breast cancer research and treatment* 2003, **79**(1):133-136. http://www.ncbi.nlm.nih.gov/pubmed/12779090.

100. Narayanan K, Jaramillo A, Benshoff ND, Campbell LG, Fleming TP, Dietz JR, Mohanakumar T: **Response of established human breast tumors to vaccination with mammaglobin-A cDNA**. *Journal of the National Cancer Institute* 2004, **96**(18):1388-1396. http://www.ncbi.nlm.nih.gov/pubmed/15367572.

101. Hobeika AC, Morse MA, Osada T, Ghanayem M, Niedzwiecki D, Barrier R, Lyerly HK, Clay TM: **Enumerating antigen-specific T-cell responses in peripheral blood: a comparison of peptide MHC Tetramer, ELISpot, and intracellular cytokine analysis**. *Journal of immunotherapy* 2005, **28**(1):63-72. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15614046

102. Piantadosi S: **Translational clinical trials: an entropy-based approach to sample size**. *Clinical trials* 2005, **2**(2):182-192. http://www.ncbi.nlm.nih.gov/pubmed/16279140.

103. Westfall PH, Young SS: **Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment**. New York: John Wiley & Sons, Inc.; 1993.

20 APPENDICES

20.1 Abbreviations

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

20.2 ECOG/Zubrod performance status scale

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

20.3 National Cancer Institute Common Terminology Criteria for Adverse Events

This study will collect adverse events using the NCI Common Terminology Criteria for Adverse Events v4.03 (CTCAE), if applicable. The CTCAE provides a descriptive terminology that is to be used for adverse event reporting. A grading (severity) scale is also provided in the CTCAE for each adverse event term. An electronic version of the CTCAE may be accessed through the web at http://ctep.cancer.gov. Alternatively, a full copy is available from the principal investigator.

20.4 Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

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

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

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

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

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

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

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not

include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

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

As defined in 21 CFR 312.32:

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

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

E. Protocol Exceptions

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

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

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

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

20.5 Reporting Timelines

Expedited Reporting Timelines						
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Ichor Medical Systems**	**AstraZeneca**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	Report at time of HRPO submission; IBC will retrieve information from myIRB and will follow up with study team if needed		
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			If related to device, 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.	
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					

Expedited Reporting Timelines						
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Ichor Medical Systems**	**AstraZeneca**
	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.					
SAEs, deaths, AESIs, Hy's Law events or hepatic function abnormalities, pregnancy						Reported to AstraZeneca within 24 hours on FDA MedWatch form.