A Pilot Study to Assess the Safety, Feasibility, and Immunogenicity of a Neoantigen-based Personalized DNA Vaccine in Patients with Newly Diagnosed, Unmethylated Glioblastoma

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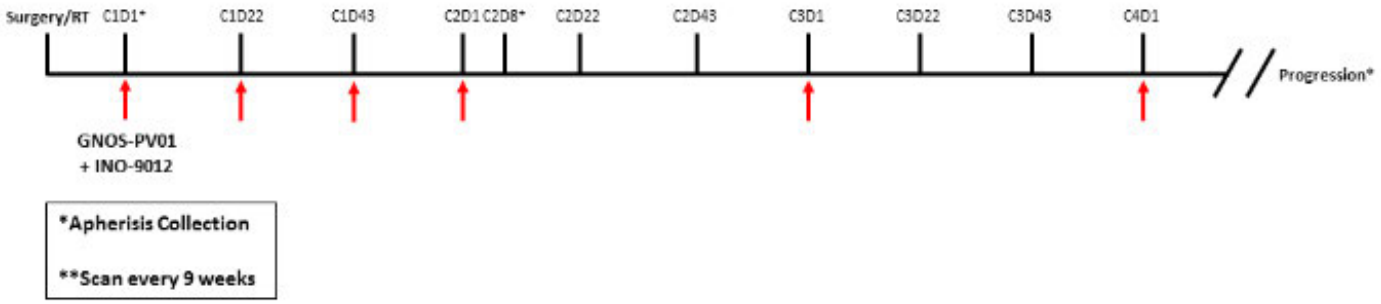
SCHEMA



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1.0 BACKGROUND AND RATIONALE

1.1 Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor accounting for approximately 13,000 new cases per year[1]. Unfortunately, it also carries the worst prognosis of primary gliomas with a dismal median survival of 12-15 months despite aggressive combinatorial treatment[1, 2]. Current standard of care therapy involves post-resection concurrent radiotherapy plus temozolomide followed by six months of maintenance temozolomide[2, 3]. Unfortunately, the vast majority of patients will eventually relapse, and since there is a relative paucity of proven effective second-line options available, patients rapidly succumb to their disease with the current 5-year survival rate <10%[2]. Thus, new therapeutic modalities are desperately needed.

1.2 Immunotherapy in Glioblastoma: Focus on Neoantigens

A recent and active area of interest in cancer research is immunotherapy, which involves the use of various strategies to evoke an anti-tumor immune response. One such immunotherapeutic approach, termed immunogenomics, which employs DNA whole exome sequencing and RNA-seq data to identify a patient's tumor-specific missense coding mutations that result in the expression of novel peptides, or neoantigens, which are predicted to bind with high affinity to the patient's specific HLA molecules. This represents a method by which patient tumor-specific immunogenic epitopes can be identified to be used in a personalized vaccine strategy. While the identification of neoantigens has largely been demonstrated in proof-of-principle *in silico* prediction studies, several recent *in vivo* studies have identified neoantigens in a number of human cancer types: melanoma[4-7], AML[8], CLL[9], cholangiocarcinoma[10], and NSCLC[11]. Thus, these finding support the applicability of this approach, but further studies will be necessary to determine if there is clinical benefit.

Interestingly, the targeting of neoantigens has shown some efficacy in the treatment of GBM. This is best illustrated by the peptide-based vaccine rindopepimut[12]. Rindopepimut targets the tumor-specific neoantigen, EGFRvIII, which is a naturally-occurring variant of EGFR resulting from an 801 base pair in-frame deletion of the extracellular domain creating a novel antigenic junction by the fusion of two distant portions of the molecule[13, 14]. Importantly, EGFRvIII is not expressed on normal cells, therefore it represents a tumor-specific immunologic target. Rindopepimut is a peptide-based vaccine that combines a 14-mer peptide encompassing the novel extracellular fusion portion of EGFRvIII coupled to the hapten carrier molecule, keyhole limpet hemocyanin (KLH)[15]. To date, there have been three phase II studies assessing the role of rindopepimut combined with standard concurrent radiation and temozolomide followed by adjuvant temozolomide in patients with newly diagnosed EGFRvIII-expressing GBM following gross total resection[16-18]. Similar results were obtained in all three studies showing significant improvement in both progression free survival (12.3, 14.2, 15.2 months vs 6.3 months) and overall survival (23.6, 24.6, 26.0 months vs 15.0 months) in patients treated with rindopepimut compared to matched historical controls, respectively.

Although the recently completed phase III trial was negative[19], an ongoing approach was the identification of genomic alterations in GBM that can represent vaccine targets. Importantly, EGFRvIII is only expressed in 20-30% of GBMs[20, 21], and thus the majority of patients with GBM are not candidates for treatment with rindopepimut leading to a need to identify new tumor-specific antigenic targets. In a recent study assessing the neoantigen landscape of tumors from The Cancer Genome Atlas, a total of 147 pre-treatment GBM samples were sequenced with an average of 10 (range 0-51) neoantigens identified supporting the feasibility of this approach to identify novel neoantigens to target in the majority of patients with glioblastoma[22]. Furthermore, the identification of "hypermutated" tumors in the recurrent GBM setting suggests that a patient subset may be particularly amenable to this approach[23]. Thus, the immunogenomic-based approach to identify neoantigens represents a feasible strategy to identify targets for personalized vaccines in patients with GBM.

Recently, Zhang et al., set out to determine a neoantigen quality fitness model to stratify GBM patients with more favorable clinical outcomes[24]. The authors found that this neoantigen model together with $CD8^+$ T lymphocytes tumor infiltration, identified a GBM subgroup with the longest survival, which is characterized by distinct genomic and transcriptomic features. Interestingly, neither tumor neoantigen burden from a quantitative model nor the isolated enrichment of $CD8^+$ T lymphocytes alone were able to predict survival of GBM patients highlighting the need for selection of neoantigens able to drive a robust $CD8^+$ T cell response for maximum response to immunotherapy in GBM.

Keskin et al., demonstrated that a personalized neoantigen vaccine using multi-epitopes, which has previously been tested in patients with high-risk melanoma, was feasible in GBM even though GBM is characterized by a low mutation load and an immunologically 'cold' tumor microenvironment[25]. The authors developed a personalized neoantigen vaccine which was tested in a phase 1/1b study in newly diagnosed GBM patients following surgical resection and radiotherapy. Interestingly, in the absence of steroids, patients generated circulating polyfunctional neoantigen-specific CD4+ and CD8+ T cell responses with a memory phenotype. The authors found an increase of infiltrating T cells which were further characterized using single-cell T cell receptor analysis and determined that neoantigen-specific T cells from the peripheral blood can migrate intracranially.

1.3 Definition of a Neoantigen

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)[34], prostatic acid phosphatase (prostate cancer)[35], and HER2/neu (breast cancer)[36]. On the other hand, tumor-specific antigens, or neoantigens, are uniquely expressed in individual cancers and are typically the result of point mutations or other genetic changes that are present only in the tumor (reviewed in [37, 38]). 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)[39]. Since that time additional publications have described the expression of mutant tumor-specific antigens in melanoma[40], non-small cell lung cancer[41] and other human cancers[42].

Cancer vaccine strategies targeting mutant tumor-specific antigens have clear conceptual advantages over strategies targeting shared tumor antigens. Conceptual advantages include: (1) Targeting mutant tumor-specific antigens is potentially safer. Mutant tumor-specific antigens are expressed only in the tumor, decreasing the risk of autoimmunity. (2) Targeting mutant tumor-specific antigens is potentially more effective. T cell responses to mutant tumor-specific antigens are high in affinity and are not limited by central mechanisms of self-tolerance. (3) Targeting mutant tumor-specific antigens 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 mutant tumor-specific antigens may preclude antigen loss. In addition, many mutant tumor-specific antigens 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 mutant tumor-specific antigens 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 personalized vaccine approach could be used in most solid tumor patients, regardless of intrinsic subtype or HLA type.

1.4 Immunogenomics: A Sequencing-based Strategy to Identify Neoantigens

Cancer genome sequencing is a major focus area for Siteman Cancer Center and for the Genome Institute at Washington University School of Medicine (WUSM). Cancer immunogenomics is a strategy that uses the information obtained from these DNA and RNA sequencing efforts to identify expressed patient tumor-specific mutations, or neoantigens. [redacted] Therefore, a major focus of our research studies has been the development of cost-effective and accurate next-generation sequencing strategies to identify mutant tumor-specific antigens 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 mutant tumor-specific antigens, 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 mutant tumor-specific antigens[43]. 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 mutant tumor-specific antigens 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 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.

1.5 Prioritization of Sequencing-Identified Neoantigens

Of note, epitope prediction algorithms for the prioritization of sequencing-identified mutant tumor-specific antigens have been developed and validated. Once somatic mutations have been identified and mutant mRNA expression confirmed/quantified using the sequencing strategies outlined above, mutant tumor-specific antigens will be prioritized using an epitope prediction algorithm that has been designed to select and prioritize the most promising sequencing-identified mutant tumor-specific antigens. Currently, the most commonly used CD8 and CD4 T cell epitope prediction algorithms are NetMHC and IEDB (Immune Epitope DataBase)[44, 45]. However, collaborative work conducted by Drs. Robert Schreiber, Elaine Mardis, Max Artyomov and William Gillanders has shown that a much more accurate prediction comes from calculating a median affinity for each sequencing-predicted mutant epitope using multiple available epitope prediction algorithms[46]. 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 neoantigens 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 mutant tumor-specific antigens 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[47]. To our knowledge, this is the only algorithm that has been successfully applied to date to cancer vaccine development.

1.6 Neoantigen Discovery in Glioblastoma: Preclinical and Clinical

We next applied the cancer immunogenomics approach discussed above to identify tumor-specific neoantigens in the murine-derived GL261 and SMA-560 glioblastoma tumor models[48]. Following DNA whole exome and RNA sequencing, high-affinity candidate neoepitopes were predicted and screened for immunogenicity by ELISPOT and tetramer analyses. GL261 and SMA-560 harbored 4,932 and 2,171 non-synonymous exome mutations, respectively, of which less than half were expressed. To validate the immunogenicities of candidate neoantigens predicted for H-2K^b and H-2D^b, the MHC class I molecules expressed by both tumor cell lines evaluated, we assessed the ability of the highest priority epitopes to activate tumor-infiltrating T cells harvested from established GL261 and SMA-560 tumors. Using IFN-γ ELISPOT as a functional readout of reactivity, we confirmed H-2D^b-restricted Imp3$_{D81N}$ (GL261) and Odc1$_{Q129L}$ (SMA-560) along with H-2K^b-restricted E2f8$_{K272R}$ (SMA-560) as endogenous tumor-specific neoantigens that are functionally immunogenic. Furthermore, neoantigen-specific T cells to Imp3$_{D81N}$ and Odc1$_{Q129L}$ were detected within intracranial tumors as well as cervical draining lymph nodes by tetramer analysis. Together, these preclinical studies support the cancer immunogenomics approach as a viable strategy to identify candidate neoantigens in glioblastoma.

In recent years, several reports have been published describing the application of this immunogenomics approach to the identification of neoantigen candidates to be used in a personalized peptide vaccine for patients with newly diagnosed glioblastoma[25, 49, 50]. Together, neoantigen-specific CD8 and CD4 T cell responses were observed to several, though not all, of the immunized neoantigens. Interestingly, it was noted that in the presence of concurrent dexamethasone use, no responses were detected to immunized neoantigen peptides[25]. Furthermore, no serious adverse events were observed, with the most common side effects being grade 1 injection site reactions[25, 49]. Overall, these studies support the feasibility and safety of the proposed study.

1.7 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[51-56]. 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, including notably a DNA plasmid encoded tumor multi-antigen vaccine INO-5401 in combination with plasmid encoded IL-12 (INO-9012) and anti-PD1 therapy (REGN2810; Cemiplimab) in subjects with newly diagnosed glioblastoma [redacted]. The INO-5401 Phase 1/2 study is based on the identical plasmid DNA backbone as is proposed in this study and also uses the identical plasmid encoded IL-12 (INO-9012) as an adjuvant. 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[57-59]. 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. We have optimized the polyepitope DNA vaccine to maximize antigen presentation of neoantigens[60] and the induction of antigen specific T cells using plasmid encoded IL-12 as a molecular adjuvant [redacted].

1.8 Use of IL-12 Plasmid with DNA Vaccines

Preclinical studies showed that the immunogenicity of DNA vaccines can be substantially increased by the use of cytokine adjuvants[61-69]. Co-administration of cytokine plasmids with DNA vaccines has been studied in rodents[65-67] and provided the basis for evaluation of INO-5401 + INO-9012 in humans. IL-12 is a heterodimeric cytokine encoded by two separate genes, IL-12A (p35) and IL-12B (p40). Although p35 IL-12 gene transcripts are ubiquitous, p40 transcripts are unique to cells producing biologically active IL-12, which include monocytes, macrophages, dendritic cells (DCs), polymorphonuclear leukocytes (PMN), and B cells[70]. These studies demonstrated a dramatic increase in specific cytotoxic T-lymphocyte (CTL) activity when a DNA plasmid was co-administered with an IL-12 plasmid, as compared with results in animals receiving therapeutic plasmid alone. The molecular adjuvant activity of several Th1 cytokines (GM-CSF, IL-2, IL-12, IL-15, and IL-18) was evaluated in mice in a subsequent study[67]. This study revealed that the IL-12 plasmid was the best driver of major histocompatibility complex (MHC)-restricted $CD8^+$ CTL activity. Co-delivery of IL-12 DNA with DNA vaccines was also evaluated in macaques and chimpanzees with enhanced responses to DNA immunogens with IL-12 plasmid injections[71-73]. A substantial enhancement of cellular immune responses with IL-12 DNA and DNA therapeutic plasmid administration compared with a DNA therapeutic plasmid alone in macaques has also been demonstrated[63, 74]. Of note, no significant additional toxicity has been observed when cytokine adjuvants such as plasmid encoded IL-12 were co-administered with DNA vaccines in preclinical studies.

In a clinical trial with a vaccine of HIV-1 DNA immunogen, co-administration of IL-12 DNA was associated with an enhanced $CD8^+$ antigen-specific immune response[75, 76]. The use of IL-12 DNA has also been associated with expansion of antigen-specific interferon-gamma (IFN-γ) positive effector cells, as well as granzyme B production. The induced immunity included both a $CD8^+$ as well a $CD4^+$ component[72, 77]. The safety and tolerability of IL-12 in humans is discussed in Section 6.

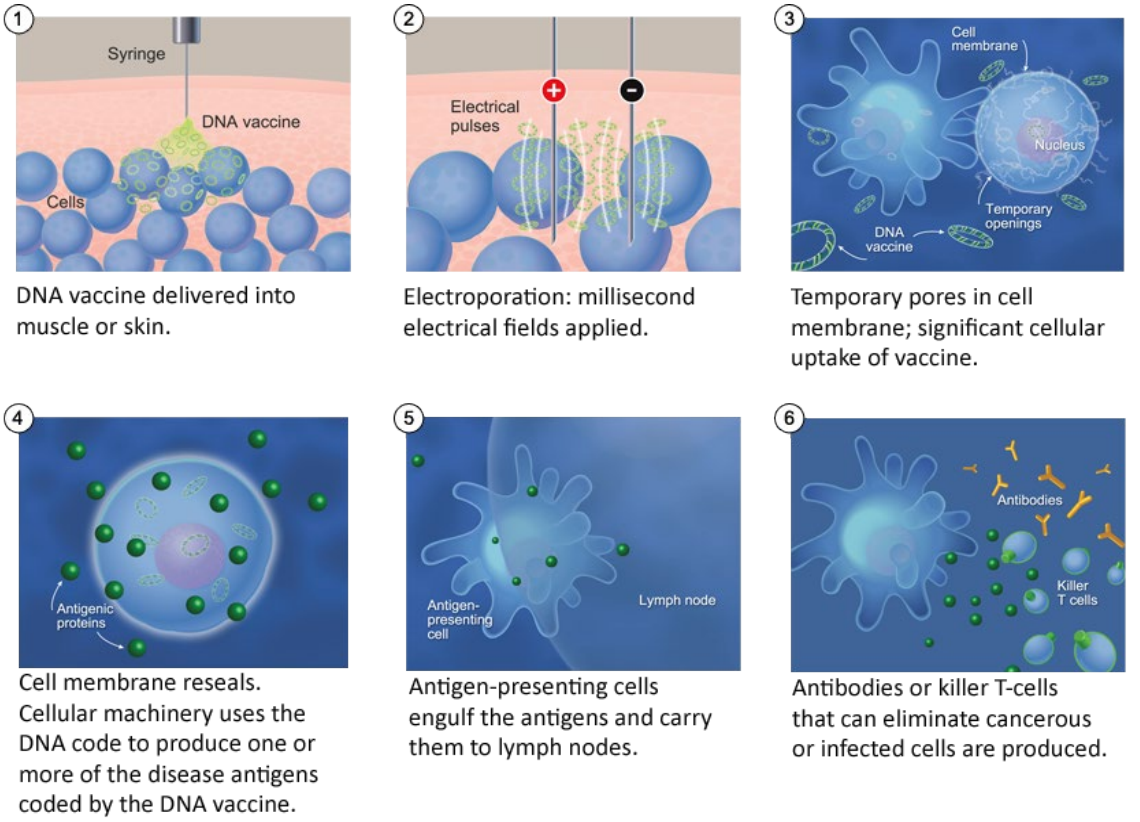
1.9 Use of Electroporation with DNA Vaccines

Several groups are developing methods to improve the immune responses of DNA vaccines, using genetic optimization, cytokine adjuvants, and alternative cellular delivery with devices such as electroporation (EP)[78]. Geneos employs all these methods with an emphasis on improving the transfection of DNA using *in vivo* EP. This physical process exposes the target tissue to a brief electric field pulse that induces temporary and reversible pores in the cell membrane to enhance the cellular uptake of large molecules such as DNA.

By temporarily increasing the permeability of cell membranes, EP has been shown to be an efficient way to introduce DNA into cells[79, 80] and to increase the expression level of antigens encoded by DNA[81, 82]. This technology has been used for more than three decades by molecular biologists for *in vitro* cell transfection including use of gene editing approaches for development of personalized therapies such CAR-T therapy. The proposed device to be used in this study, Cellectra®2000 has been licensed from Inovio Pharmaceuticals for the purpose of enabling the uptake of the plasmid DNA encoding the personalized neoantigen vaccines.

The hypothesis is that EP will increase the uptake and expression of plasmid DNA (pDNA), generating significantly increased immunogenicity. Electroporation enhances both cellular and humoral immune responses, using less DNA than intramuscular immunizations alone[83]. Studies have demonstrated the ability of EP to augment specific cellular immune responses in mice[84] and in macaques[85]. It has been found that simian immunodeficiency virus (SIV) DNA vaccines potency can be increased 50 to 200-fold when delivered intramuscularly (IM) followed by EP. There are various means of delivering EP (i.e. constant current vs. constant voltage) and testing these strategies in mice and pigs has shown a constant current device may be the most effective at generating immune responses[86]. Studies in macaques found that EP of SIV DNA + IL-12 plasmids yielded 10-fold higher responses than DNA without EP[81], and that this immune response was boosted with additional doses. These responses are also polyfunctional, as defined by the ability of immune cells from treated animals to generate IFN-γ, tumor necrosis factor alpha (TNF-α) and IL-2. Furthermore, the functional consequence of EP delivery of DNA encoding an antigen in combination with IL-12 is an improved ability of specific $CD8^+$ T cells to proliferate in culture in response to antigenic stimulation compared to delivery without EP[81]. Additionally, it has been shown that delivering DNA vaccines with electroporation has a significant dose-sparing effect, along with superior immunogenicity when compared to DNA vaccines delivered without the use of electroporation[75, 76]. Figure 2 describes how EP works in the body.

Figure 2: How electroporation works in the body



Following successful proof-of-concept studies in animals, we have optimized both pulse pattern and voltage to increase transfection efficiency. More recently, clinical applications of DNA or drug delivery via EP have been tested in the treatment of cancer and in gene therapy[87-90]. To date, however, EP remains experimental in humans; it has not been licensed by the FDA for clinical use.

1.9.1 Nonclinical Studies to Inform Neoantigen Identification and Selection

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

Regarding nonclinical studies to be performed, for the reasons outlined below, 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 pGX0001 parental vector has proven to be safe in phase 1 clinical trials [redacted]

Similarly, 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 CELLECTRA®2000 device. This includes GLP information about biodistribution and integration of DNA vaccines using the pGX0001 parent vector. Additional information about the biodistribution and integration of DNA vaccines following electroporation with the CELLECTRA®2000 device is summarized in the below and described in greater detail in the investigator brochure and IND application.

The dosing described in this study is based on previous DNA vaccine studies as well as an ongoing phase I clinical trial (NCT03491683).

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[91-98].

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 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*[43].

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 □4 (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*, prophylactic vaccination of mice with long peptides (~30mer) corresponding to either the mLama4 epitope alone, or both the mLama4 and mAlg8 epitopes induced protection against subsequent challenge with d42m1-T3 tumor cells. The combined peptide vaccine was more protective than the vaccine containing the mLama4 long peptide alone.

Furthermore, in a separate preclinical model, neoantigens selected based on predictions for high MHC I binding following immunization generated CD8+ or CD8+/CD4+ neoantigen-specific immune responses 75% of the time[60]. Upon further analysis, it was found that many of the neoantigen epitopes yielded specific CD8 + T cells that were polyfunctional, with production of multiple cytokines simultaneously (IFNγ, TNFα, and IL-2), and had expression of the degranulation marker CD107a, indicating cytolytic potential. These vaccines induced neoantigen specific T cells demonstrated *in vivo* and *in vitro* cytolytic activity and showed efficacy in pre-clinical tumor challenge models. Importantly, formulations containing as many as 60 neoepitope sequences were successfully developed and demonstrated their ability to induce immune responses and confer tumor challenge protection even in the presence of potential neoantigenic competition. Additional details regarding other pre-clinical studies that were performed can be found in the investigator brochure.

[redacted]

[redacted]

[redacted]

[redacted]

1.11.4.1 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 [redacted]

The first step in the sequencing pipeline is whole exome sequencing of

cancer and normal DNA. Current protocol involves exome fragment capture 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 cancer and normal DNA and processed independently. Exome sequencing is performed using the Illumina platform. Due to the mutational spatial heterogeneity in glioblastoma, we will perform whole exome sequencing on 2-4 spatially distinct regions of resected tumors to attempt to identify the full breadth of clonal and subclonal somatic mutations.

Exome sequences from 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 cancer DNA.

The second step in the sequencing pipeline is RNA sequencing. To validate the results of the exome sequencing and confirm expression of the somatic mutations in the cancer, RNA sequencing of the cancer RNA will be performed for each of the tumor regions. cDNA was generated using the TruSeq Stranded Total RNA kit (Illumina Inc) and captured similar to DNA exome sequences described above. RNA sequencing is performed using the Illumina platform. RNA-sequencing data is processed using Tophhat v2.0.8 and Cufflins v2.1.1 to generate gene expression values.

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 Count < 10 Reads

Tumor cDNA Capture Data
(5) Tumor Coverage $\geq$ 10x
(6) FPKM > 1

GBM-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.

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.

1.11.4.2 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[47]. We have established this algorithm in collaboration with Dr. Robert Schreiber, an internationally-known expert in tumor immunology[91-98]. Schreiber was one of the first to use next generation sequencing technologies to identify neoantigens, demonstrating that these antigens are important tumor rejection antigens[43]. Griffith and colleagues have now optimized the epitope prediction algorithm and have demonstrated that cancer vaccines targeting neoantigens are associated with antitumor immunity[47].

The goal of the optimized epitope prediction algorithm is to identify and prioritize up to 40 neoantigens. In a recent study assessing the neoantigen landscape of tumors from The Cancer Genome Atlas, a total of 147 pre-treatment GBM samples were sequenced with an average of 10 (range 0-51) neoantigens identified supporting the feasibility of this approach to identify novel neoantigens to target in the majority of patients with glioblastoma[22]. Additionally, we recently received approval to proceed

from the FDA under Compassionate use [redacted] – to treat the patient with up to 35 identified tumor neoantigens in the patient tumor using our neoantigen prediction pipeline. Using the proposed modestly expanded neoantigen target set of up to 40 neoantigens per tumor, we would be able to treat the patient with their full set of predicted neoantigens. As noted below, the stringent selection criteria built into our neoantigen prediction pipeline – including deprioritizing neoantigens that form a weaker binding epitope than the corresponding wild type epitope mitigate the incremental risk associated with the development of an autoimmune response by targeting a larger range of neo-epitopes. In our pre-clinical studies we have demonstrated that inclusion of up to 60 tumor specific and tumor irrelevant neoantigens did not impair the ability of the specific neoantigens to drive in vivo protective immune responses and positively impact tumor challenge in the TC1 mouse tumor challenge model[60].

The algorithm uses a combination of binding algorithms, processing algorithms and *in vitro* binding assays.

Mutations that are expressed in the cancer will be identified using the sequencing pipeline outlined in the document titled "Sequencing Pipeline." The predicted amino acid sequences corresponding to the expressed mutations will be pipelined through multiple publicly-available *in silico* class I and class II MHC epitope-binding algorithms using the pVAC-Seq program (www.pvactools.org)[46].

A prioritized list of candidate neoantigens for vaccine incorporation will be generated taking into multiple variables including: median binding affinity value (i.e. IC_{50}), RNA expression values, representation across all patient-specific HLA alleles, clonal versus subclonal mutations. Selection of final neoantigens will be performed by Selection Committee Review, a group of physician-scientists and computational biologists, who are experts in the field of neoantigens, that will vet each candidate.

1.11.5 Clinical Safety and Potential Toxicity

1.11.5.1 Experience with the pGX0001 parent vector and the plasmid encoded IL-12 adjuvant (INO-9012)

[redacted] is a single patient anaplastic astrocytoma compassionate use study that opened on 20Feb2019. The patient has received 6 doses of GEN-PV-001 + INO-9012 monotherapy, followed by CELLECTRA®2000 EP. No serious adverse events have been reported to date and the patient continues to receive treatment every 8 weeks. Conventional DNA cancer vaccines based on the pGX0001 parent vector have been used extensively in phase 1, 2, and 3 human clinical trials. INO-9012 has been used as an adjuvant in multiple studies across multiple therapeutic areas. A description

of the safety findings related to INO-5108, INO-1401, INO-1404, and INO-9012, as well as with the DNA plasmid backbone and EP, are presented in this Section.

In studies with INO-based DNA vaccine products administered IM with EP, the most common finding is mild to moderate administration site pain that usually spontaneously resolves within 5 to 10 minutes. Administration of INO-5108, INO-1401, INO-1404, and INO-9012 has otherwise been clinically unremarkable, and adverse events (AE) and serious adverse events (SAE) have been consistent with the population under investigation. No EP-related safety issues have been identified other than transient injection site pain mentioned above. A brief description of these studies and the summary of the safety data for these individual studies is described below.

1.11.5.2 INO-1400 + INO-9012: TRT-001 Phase 1 Study of INO-1400 with or without INO-9012 for Treatment of Solid Tumors at High Risk of Relapse Post Definitive Surgery and Standard Therapy

TRT-001 is a phase 1 study of INO-1400 or INO-1401 in combination with or without INO-9012 in patients with solid tumors. Patients with advanced solid tumors receive doses of 2 mg or 8 mg of INO-1400 or INO-1401, with or without INO-9012 at either 0.5 mg or 2 mg. Up to four doses of vaccine are administered, each four weeks apart.

As of the data cut-off date of December 22, 2018, a total of 93 subjects had enrolled in the study, assigned to one (1) of ten (10) treatment arms, regardless of tumor type. INO-1400 with or without INO-9012 has been well-tolerated. As of that date, a treatment-related SAE, cellulitis of the breast, CTCAE Grade 3, in a patient with a history of breast cancer, mastectomy, and breast reconstruction, has been reported. A second treatment-related SAE of abdominal pain associated with elevated lipase reported on June 30, 2017 is further described in Section 6.5.9. A summary of AEs by SOCs, PT and severity grade for treatment arms combined, as of December 22, 2018, is provided in the IB. The most frequent AE was injection site pain (76; 81.7%), all of which were ≤Grade 2. Additionally, in the IB a summary of SAEs for all treatment arms combined, as of December 22, 2018 is reported.

1.11.5.3 INO-5150 + INO-9012: PCa-001 Phase I study of INO-5150 with or without INO-9012 for Treatment of Biochemically Relapsed Prostate Cancer

PCa-001 is a phase 1 study of INO-5150 (INO-5108 + INO-5101) in combination with or without INO-9012 in patients with prostate cancer. Up

to four doses of vaccine are administered, at Day 0, and at Weeks 3, 12 and 24 of the study.

As of April 11, 2018, 62 subjects have been enrolled in this trial and enrollment is now closed. Safety data is available for all subjects. INO-5150 with or without INO-9012 has been well tolerated with no treatment-related CTCAE Grade 3 or higher AEs reported. A summary of AEs reported in this trial as of April 11, 2018 by treatment arm is provided in the IB and additionally, the IB provides a summary of AEs reported by severity for all treatment arms combined. In the IB, the treatment-emergent serious adverse events for this study, as of April 11, 2018 are shown as well.

1.11.5.4 IL-12 Plasmid DNA and Protein Safety

IL-12 DNA is being or has been previously studied as a cytokine adjuvant in combination with other antigen-specific DNA products in multiple clinical trials (INO-9012 in EBOV-001, HPV-004, HPV-005, HPV-006, HVTN-098, PCa-001, TRT-001, and INO-9112 in HBV-001).

The combined safety data from all concluded and ongoing trials with IL-12 DNA plasmids are summarized in Appendix 1. Only one SAE thought related to the combination of INO-1400 and INO-9012 has been reported (cellulitis, Grade 3, in Trial TRT-001 as reported above). In addition, up to 5.8 mg of IL-12 DNA has been administered intratumorally followed by EP, and no safety signals were observed[113].

In a phase I study of intravenous recombinant human interleukin 12 protein in patients with advanced malignancies, high doses of recombinant IL-12 protein have exhibited some dose-related toxicities[114]. In contrast, to date, IL-12 plasmid DNA has shown an acceptable safety profile in clinical studies when delivered intramuscularly either alone or when followed by electroporation[75, 76].

1.11.5.5 Electroporation Experience and Safety

As of December 31, 2018, in collated reporting of 37 different studies of ongoing and completed trials in human subjects, 1305 subjects received IM and 638 subjects received ID treatment followed by electroporation using the Inovio CELLECTRA®2000 device. More than 6300 doses have been administered, including 5299 injections (IM or ID) of DNA followed by EP, 752 injections (IM or ID) of placebo followed by EP, and 336 injections (IM or ID) followed by EP in blinded studies, see Table 2 below.

Table 2. Total Subjects and Doses Administered via CELLECTRA® Device IM, ID and Overall (Number of Studies Included = 37)

Device	Injection Method	Dose Type	Subjects	Doses
CELLECTRA®2000	IM	Active	1070	3291
		Placebo/EP Only	81	194
		Blinded	16	33
		All	1158[a]	3518
	ID	Active	532	1984
		Placebo/EP Only	106	540
		All	638	2524
	All	Active	1580[b]	5275
		Placebo/EP Only	187	734
		Blinded	16	33
		All	1774	6042
CELLECTRA 5PSP	IM	Active	12	24
		Placebo/EP Only	18	18
		Blinded	117	303
		All	147	345
	All	Active	12	24
		Placebo/EP Only	18	18
		Blinded	117	303
		All	147	345

Abbreviations: ID = Intradermal; IM = Intramuscular

a: 9 HPV-101 subjects received Placebo/EP Only IM in a Safety Lead-in Phase followed by Active IM in a Treatment Phase.

b: 22 FLU-001/FLU-002 rollover subjects received both Active IM and Active ID. These subjects are counted in both Active IM and Active ID but only once for Active All.

Source: squpdose2 Data Cutoff: 12/31/2018 Table Generation: 01/09/2019 9:06

1.11.5.6 Overview of DNA backbone and Adverse Events

Appendix 1 summarizes the number of subjects who received at least one dose of vaccination in 37 ongoing and completed trials as of the data cutoff date of 31 December 2018. Two ongoing blinded clinical trials are excluded from Appendix 1. The treatment groups included subjects with or without IL-12, subjects who received DNA treatment either through ID or IM administration, and subjects who received EP alone. There are no significant differences in subjects with the treatment related or unrelated Grade 3 or higher AEs across all treatment groups. There is slightly higher number of Grade 3 or higher treatment unrelated events in EP alone treatment group. Number of subjects with at least one treatment emergent AE are 1143 (80.5%). There were no major differences in numbers of subjects who discontinued from study. The primary reason for discontinuation was lost to follow-up for all but one treatment group. Overall, there were no major differences in the safety profile of each treatment group.

1.11.5.7 Summaries of AEs observed in 5 or >5% of subjects with CELLECTRA® 5P

The most frequently reported AEs across studies in which various tumor associated antigens and plasmids with a DNA backbone identical to that of GNOS-PV01 and delivered via IM+EP alone or in combination with IL-12 DNA plasmid (e.g. INO-9012) are listed in Table 3. As discussed earlier, injection site pain is the most frequently reported study treatment related AE across all studies, in 514 (56%) of subjects.

Table 3. All AEs Reported in Studies of Inovio DNA Delivered IM with CELLECTRA®2000 or 5PSP Occurring at Frequency of 5% or Greater (N=1082)

System Organ Class Preferred Term	Total N (%)
Gastrointestinal disorders	252 (23.3)
Nausea	126 (11.6)
General disorders and administration site conditions	737 (68.1)
Injection site pain	592 (54.7)
Fatigue	247 (22.8)
Injection site erythema	203 (18.8)
Injection site swelling	139 (12.8)
Injection site pruritus	113 (10.4)
Injection site bruising	85 (7.9)
Malaise	80 (7.4)
Infections and infestations	332 (30.7)
Upper respiratory tract infection	131 (12.1)
Musculoskeletal and connective tissue disorders	293 (27.1)
Myalgia	161 (14.9)
Arthralgia	99 (9.1)
Nervous system disorders	274 (25.3)
Headache	194 (17.9)

*CELLECTRA®2000 and 5PSP are different models of the electroporation device
Subject events are only counted once at both the preferred term and system organ class levels.
Data Cutoff: 12/31/2018

Summary of Most Common AEs
Data from the clinical studies evaluating plasmids that share the identical DNA backbone of the INO-5401 have not identified any clinically significant or treatment related safety concerns other than injection site reactions (primarily mild to moderate pain).

1.11.5.8 Injection Site Reactions

Injection site reactions were the most common AE across all studies. These were transient in nature and reported as mild to moderate in severity. Almost all were assessed as Grade 1 or Grade 2 and resolved without sequelae.

In summary, data from the clinical studies evaluating various tumor associated antigens and plasmids that share the identical DNA backbone of the GNOS-PV01 have not identified any safety concerns other than injection site reactions (primarily mild to moderate pain). Antibodies to IL-12 were not detected in the HVTN 080 study in subjects receiving PENNVAX®-B with IL-12 plasmid.

Taken together, the above data indicate that synthetic consensus antigenic sequences inserted into this highly optimized plasmid backbone generate significant cellular and humoral immune responses when delivered using the CELLECTRA®2000 EP device without compromising the acceptable safety profile of naked DNA delivery. The generally acceptable risk/benefit profile of DNA vaccines delivered using electroporation supports further study in selected diseases such as prostate cancer.

1.11.5.9 Death and Serious Adverse Events

Summaries of SAEs observed in previous clinical studies using INO-5101, INO-1400, INO-1401, and other DNA vaccines with an identical DNA backbone to that of the components of GNOS-PV01 are summarized above. SAEs observed in clinical studies including IL-12 plasmid DNA are summarized above. No related deaths have been reported in any studies.

One possibly related Suspected Unexpected Serious Adverse Reaction (SUSAR) of Grade 3 skin infection-right breast cellulitis was reported for one subject in TRT-001, exposed to INO-1400 + INO-9012. The subject had a history of Stage IIIC breast cancer of the right breast, status-post right-sided axillary lymph node dissection and radiotherapy, and bilateral mastectomy and bilateral breast reconstruction with silicone implantation. The subject had a history of non-serious right breast cellulitis post-exposure to INO-1400 + INO-9012 and developed the reported SAE of skin infection-right breast cellulitis 22 days after receiving a third dose of INO-1400 + INO-9012. Despite the history of axillary lymph node dissection of the right arm, right-sided chest radiation, right breast implant, and concurrent breast implant exchange with capsulotomy, the Investigator considered the SAE to be possibly related to study treatment.

A second possibly related SUSAR of Grade 3 pancreatitis was reported for one subject with a history of pancreatic cancer, in TRT-001, exposed to INO-1401. The subject had a history of pancreatitis at the time of diagnosis of pancreatic cancer. The subject had a history of exocrine pancreatic insufficiency and developed the reported SAE of pancreatitis 13 days after receiving a fourth dose of INO-1401. Despite the history of pancreatitis, the Investigator considered the SAE to be possibly related to study treatment.

1.11.5.10 Effects on Cardiac Depolarization/Repolarization

No abnormalities have been noted by ECGs performed before and after treatment with DNA vaccines followed by EP.

1.12 Investigational Device - CELLECTRA®2000 IM Device

CELLECTRA®2000 Device is a system indicated for use to enhance the uptake and expression of plasmid-based biologics in order to enhance vaccine efficacy. The electroporation is accomplished through a sterile, disposable needle array attached to an applicator. The system includes the CELLECTRA®2000 Pulse Generator, applicator, and disposable sterile array. The plasmid is delivered separately through injection, in the area delineated by the electrodes immediately prior to the electroporation treatment. Information regarding the CELLECTRA®2000 Device is available in the CELLECTRA®2000 device manual and investigator brochure.

1.13 Study Design

This is a single institution, open-label, single arm, phase I study assessing the safety, feasibility, and immunogenicity of a personalized neoantigen-based DNA vaccine in subjects with newly diagnosed, MGMT unmethylated glioblastoma. Subjects will be consented for the study, and resected tumors will undergo multi-sector sequencing of approximately 3 to 4 tumor regions. Following standard radiation therapy (without temozolomide), subjects will receive GNOS-PV01 + INO-9012 via IM injection followed by EP once every 3 weeks for 3 doses during the first cycle, then every 9 weeks beginning with the second cycle. Each cycle is 9 weeks. Cycle 1 is the “Priming” phase; Cycle 2 and beyond is the “Boosting” phase.

Immunogenicity will be assessed following completion of enrollment. Since there are presently no available standardized criteria to objectively evaluate immunogenicity, we will assess immunogenicity based on the following parameters:

1) the number of subjects who develop at least one demonstrable neoantigen-specific CD8 T cell response by 6 months after administration of the first dose of vaccine, and
2) the percentage of neoantigens that elicit a neoantigen-specific CD8 T cell response out of the total number of neoantigens vaccinated against (i.e. total # neoantigens with detectable T cell response/total # neoantigens vaccinated against).

Overall, total accrual is expected to 12 subjects. If a subject withdraws early from participation voluntarily without a DLT or due to disease progression before the end of the DLT observation period, the subject will be substituted to allow adequate assessment of the safety and immunogenicity.

1.14 Study Population

Subjects newly diagnosed with glioblastoma will be eligible for screening. Since adjuvant temozolomide will be withheld during radiation therapy and in the adjuvant setting to reduce the potential impact of temozolomide-induced lymphopenia on vaccine efficacy, eligible subjects will be limited to those whose tumors have unmethylated MGMT promoter loci as determined by standard assays. Temozolomide can be safely withheld in patients with unmethylated glioblastoma without any clinically meaningful impact as the addition of temozolomide to standard radiation therapy does not result in a statistically significant improvement in either progression-free survival (PFS) or overall survival (OS)[120]. Furthermore, it is reasonable to eliminate temozolomide from first line therapy given the increased side effect profile, immunosuppressive properties, and lack of efficacy in this patient population. Additionally, the removal of temozolomide has already been adopted in ongoing clinical trials for MGMT unmethylated glioblastoma subjects using nivolumab (Checkmate-498) and personalized vaccines (NCT02287428).

Enrollment will be based on the ability to identify candidate neoantigens suitable for the personalized vaccine development and completion of standard radiation therapy with preserved functional status (KPS >60) and end organ function.

1.15 Study Rationale

The goal of this protocol is to test the safety, feasibility, and immunogenicity of GNOS-PV01 in patients with glioblastoma. GNOS-PV01 is a novel DNA-based neoantigen vaccine platform that differs from other DNA-based vaccines used clinically or investigationally to date. First, GNOS-PV01 is combined with INO-9012, a plasmid encoding IL-12, which has been shown to generate more robust T cell priming when given as a vaccine adjuvant. IL-12 adjuvant has not been evaluated with neoantigen vaccines previously. Second, GNOS-PV01 will aim to incorporate up to 40 neoantigens per plasmid per patient. No neoantigen vaccine has evaluated more than 20-25 neoantigens previously. Third, neoantigen vaccines have not been tested in patients with brain tumors. As we expect the GNOS-PV01 + INO-9012 platform to generate a more robust neoantigen-specific T cell response and we are targeting a significantly larger number of neoantigens, it is not clear if the anticipated increased intratumoral immune cell infiltration in the CNS will lead to increased intracranial inflammation and edema. Therefore, we proposed a pilot study that will allow us to evaluate the safety of this novel DNA vaccine platform in patients with glioblastoma and to assess the feasibility of generating the vaccine in our GMP facility in a time frame that will be clinically relevant for this patient population.

2.0 OBJECTIVES

2.1 Primary Objectives

1. To determine the safety and tolerability of a personalized neoantigen DNA vaccine in patients with newly diagnosed, MGMT unmethylated GBM.

2. To determine the feasibility of generating a personalized neoantigen DNA vaccine for patients with newly diagnosed, MGMT unmethylated GBM. The feasibility assessment will be based on:
    i. The ability to identify candidate tumor-specific neoantigens.
    ii. The ability to manufacture a neoantigen-based DNA vaccine.
    iii. The ability to administer the vaccine to a patient at 4 weeks post-completion of radiotherapy. This time point is the current expected start date for adjuvant chemotherapy following radiation therapy in patients with newly diagnosed glioma based on current standard of care recommendations.

2.2 Secondary Objectives

1. To assess the immunogenicity of a personalized neoantigen DNA vaccine in patients with newly diagnosed, MGMT unmethylated GBM. The immunogenicity evaluation will consist of:
    i. The ability to generate a measurable neoantigen-specific CD8 T cell response in vaccinated patients by Week 10 post-vaccination.
    ii. The number of individual neoantigens per number of neoantigens vaccinated against, with which a measurable CD8 T cell-specific response is identified by Week 10 post-vaccination.
    iii. T-cell phenotype (e.g. activation and cytolytic markers), myeloid derived suppressor cell frequency (MDSC) in PBMC by flow cytometry
    iv. T cell receptor (TCR) sequencing from PBMC to assess diversity of clonality and putative antigen specificity
    v. Pro- and anti-inflammatory chemokine and cytokine analysis in plasma by multiplex ELISA
2. To determine the number of high-quality candidates neoantigens present in patients with newly diagnosed GBM. High quality neoantigens will be defined as those that meet criteria for inclusion into the personalized vaccine.
3. To evaluate preliminary clinical activity by determining the 6-month progression-free survival rate (PFS-6) and 12-month overall survival rate (OS-12).

2.3 Exploratory Objectives

1. To characterize tumor-infiltrating lymphocytes (TIL) derived from tissue specimens from patients who have received a personalized neoantigen DNA vaccine before and after vaccination.
2. To identify pre- and post-vaccination biomarkers associated with response to personalized neoantigen vaccine.
3. To perform gene expression analysis of TIL and PBMC to determine activation states before and after vaccination compared to unvaccinated controls.
4. To evaluate antigen-specific cellular and humoral immune responses in the peripheral blood against non-immunized tumor-associated antigens (i.e., epitope spreading).
5. To characterize recurrent tumor samples and cell-free circulating tumor DNA to assess for antigenic maintenance or loss before, during, and after treatment.

3.0 PATIENT SELECTION

The consent process can take place any time prior to the acquisition of tissue to be sent for sequencing and the development of the neoantigen DNA vaccine. This will allow freshly collected or previously collected tissue to be analyzed as needed under this protocol, as part of routine care, or under another research project. Participants will be rescreened within 14 days to confirm eligibility prior to administration of the first dose of the vaccine. Please refer to the study calendar in Section 10.0.

3.1 Inclusion Criteria

1. Newly diagnosed histologically confirmed MGMT unmethylated glioblastoma multiforme (WHO grade IV). Patients with secondary glioblastoma, in particular those who are IDH1 or IDH2 mutant, will not be excluded. High grade gliomas with molecular features of glioblastoma will be included. MGMT methylation status must be determined by a standard PCR-based assay.

2. Patients who had prior craniotomy with biopsy, subtotal resection, total gross resection, or re-resection will be permitted.

3. Consent to genome sequencing and dbGaP-based data sharing and has provided or will provide germline (PBMC) and tumor DNA/RNA samples of adequate quality for sequencing. (Acquisition of specimens for sequencing and the sequencing itself may be done as part of routine care or another research project.)

4. At least 18 years of age.

5. Karnofsky performance status ≥ 60% (see Appendix A)

6. Normal bone marrow and organ function as defined below:
   a. Absolute neutrophil count ≥ 1,500/mcL
   b. Platelets ≥ 100,000/mcL
   c. Total bilirubin ≤ 1.5 x IULN
   d. AST(SGOT)/ALT(SGPT) ≤ 3.0 x IULN
   e. Creatinine ≤ IULN OR creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

7. Systemic corticosteroid therapy is permitted provided dosing is no greater than 2 mg per day (dexamethasone or equivalent) on the day of vaccine administration. See Section 6.3 for further details.

8. Bevacizumab will be allowed if given for symptomatic control of vasogenic edema and to avoid high dose of corticosteroids.

9. Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for

the duration of study participation, including at least 5 months (for women of childbearing potential) and at least 7 months (for men) after last dose of study drug. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

10. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. As this study aims to assess the immunogenicity of a personalized neoantigen DNA vaccine plus plasmid encoded IL-12 as an adjuvant, no prior immunotherapy will be permitted.

2. Inadequate tissue acquisition to allow for neoantigen screening.

3. No candidate neoantigen identified during screening.

4. A history of other malignancy ≤ 3 years previous with the exception of non-melanoma skin cancer, any *in situ* cancer that has been successfully resected and cured, treated superficial bladder cancer, or any early-stage solid tumor that was successfully resected without need for adjuvant radiation or chemotherapy.

5. Receiving any other investigational agents within 4 weeks of beginning study treatment.

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

7. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to any agents used in the study.

8. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

9. History of immunodeficiency disorder or autoimmune condition requiring active immunosuppressive therapy. This includes inflammatory bowel disease, ulcerative colitis, Crohn's disease, systemic vasculitis, scleroderma, psoriasis, multiple sclerosis, hemolytic anemia, immune-mediated thrombocytopenia, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, sarcoidosis, or other rheumatologic disease or any other medical condition or use of medication which might make it difficult for the patient to complete the full course of treatments or to generate an immune response to vaccines.

10. Presence of clinically significant increased intracranial pressure (e.g. impending herniation) or hemorrhage, uncontrolled seizures, or requirement for immediate palliative treatment.

11. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 7 days of first dose of vaccine.

12. Presence of acute or chronic bleeding or clotting disorder that would contraindicate IM injections.

13. Fewer than 2 acceptable sites available for IM injection and CELLECTRA®2000 EP considering the deltoid and anterolateral quadriceps muscles:

    a. Tattoos, keloids, or hypertrophic scars located within 2 cm of intended administration site
    b. Implantable-cardioverter-defibrillator (ICD) or pacemaker (to prevent a life-threatening arrhythmia) that is located ipsilateral to the deltoid injection site (unless deemed acceptable by a cardiologist)
    c. Any metal implants or implantable medical device within the intended treatment site (i.e. electroporation area).

3.3 Inclusion of Women and Minorities

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

4.0 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 database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team

6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

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

4.3 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.

4.4 Treatment Staggering

For the first three patients treated on study, treatment of each subsequent patient will not be initiated until the prior patient has completed the DLT observation period (i.e. 30 days after Cycle 1 Day 1). If no unexpected adverse events attributed to study treatment are observed after the third patient completes the DLT observation period, treatment initiation may occur without restrictions for the remaining patients. If an unexpected adverse event attributed to study treatment is observed in one of the first three patients, the PI will review the event and make a determination regarding the need for additional treatment staggering or other monitoring.

Because the consent process can take place any time prior to the acquisition of tissue for the development of the neoantigen DNA vaccine, registration will not be staggered in order to allow the analysis of tissue and the manufacture of the vaccine to move forward.

5.0 TREATMENT PLAN

5.1 Agent Administration

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

Standard radiation therapy will be administered per standard of care and is outside the scope of this study. Concurrent and adjuvant temozolomide will not be given due to: 1) lack of efficacy in MGMT unmethylated glioblastoma and 2) potential risk of temozolomide-induced lymphopenia impacting vaccine efficacy. Vaccination will begin no sooner than 3 weeks after completion of radiotherapy; note that vaccination may take place any time after 3 weeks post-completion of radiotherapy, and there is no time point beyond which the vaccine may not be initiated as long as the patient continues to be eligible prior to first administration of vaccine. A baseline MRI will be obtained prior to initiating vaccine administration.

The vaccine (GNOS-PV01 + INO-9012) will be administered on a 9-week cycle. GNOS-PV01 + INO-9012 will be given on Days 1, 22 (+/- 3 days), and 43 (+/- 3 days) during Cycle 1 (i.e. C1D1, C1D22, C1D43) as a priming phase followed by booster injections on

Day 1 (+/- 7 days) of each subsequent cycle (i.e. C2D1, C3D1, etc.). GNOS-PV01 + INO-9012 administration will continue until either progression, intolerance, physician's discretion or end of supply.

All study injections will be given intramuscularly using an integrated electroporation device (CELLECTRA®2000 Device, Geneos Therapeutics). At each vaccination time point, patients will receive one injection of the neoantigen DNA vaccine into the deltoid or lateralis muscle. Injection sites will be alternated (if first injection is given in the left deltoid or vastus lateralis, the second injection will be given in the right deltoid or vastus lateralis). 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 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 training on the use of the CELLECTRA®2000 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.

Regarding the EP procedure, subjects may be offered topical anesthetic (e.g. EMLA or equivalent medicine), to limit significant discomfort from the EP procedure. If EMLA (lidocaine 2.5% and prilocaine 2.5%) is used, an approximately 1.5 cm diameter amount will be applied with occlusion to the site of injection ~30 minutes prior to EP. Subjects may be offered sedative (e.g, lorazepam) ~30 minutes prior to EP. Subjects may be offered an analgesic (e.g. acetaminophen, ibuprofen) after EP.

Subjects may also be offered a mild sedative (e.g. 0.5-1.0 mg lorazepam), or equivalent, for anxiety related to the EP procedure. Mild sedatives may be administered approximately 1 hour prior to EP. Subjects who receive a mild sedative should not operate a motor vehicle for 3-4 hours after receiving medication and should have arranged transportation to depart from the trial site.

Subjects who are allergic to or have contraindications to EMLA, acetaminophen, ibuprofen, or a mild sedative will be offered a suitable alternative upon discussion with the Sponsor. Do not apply ice to the injection area before or after the injection of GNOS-PV02 + INO-9012.

5.2 Definition of Dose Limiting Toxicities (DLTs)

A DLT will be defined as any grade 3 toxicity or greater according to CTCAE v5 considered at least possibly related to study treatment. The DLT observation period begins with Cycle 1 Day 1 (date of first vaccine administration) and continues for 30 days. Acceptable safety will be defined as a < 33% treatment-related DLT rate (1/6 subjects) for this study.

5.3 Definition of Evaluability

All patients who have tissue analyzed for candidate neoantigens are evaluable for the feasibility endpoint.

All patients who receive any study treatment are evaluable for the safety endpoint. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death.

All patients are evaluable for disease response unless they discontinue treatment due to treatment related adverse events(s) prior to completion of Dose 1 or have not had any disease assessment.

A patient is evaluable for DLT assessment only during the DLT observation period.

Patients who do not receive any vaccine injections or are deemed unevaluable will be replaced for the safety endpoint but may be evaluable for the feasibility endpoint.

5.4 General Concomitant Medication and Supportive Care Guidelines

There are no absolute contraindications to concomitant medications with any of the study medications. However, the following medications should be used cautiously as they have been shown to impair or increase the adverse events associated with the study medications:

1. Lithium
2. Leflunomide, Teriflunomide
3. Clozapine
4. Deferiprone
5. Etanercept, Adalimumab, Infliximab
6. Fingolimod
7. Lomitapide
8. Mipomersen
9. Thalidomide
10. Tofacitinib
11. Azithromycin
12. Bupropion
13. Black Cohosh
14. Fluoroquinolones
15. Ganciclovir
16. Naltrexone

Steroid or other immunosuppressive agents and anti-seizure medications will be tracked specifically.

5.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative pregnancy test within 7 days prior to the first dose of the vaccine.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study.

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

If a female patient or female partner of a male patient becomes pregnant during therapy or within 6 months after the last dose of vaccine, the investigator must be notified in order to facilitate outcome follow-up.

5.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue with vaccination until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which precludes continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study / physician discretion
- The Siteman Cancer Center decides to close the study
- Exhaustion of vaccine reagents

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.7 Duration of Follow-up

Participants will be followed for up to 24 months or until death after their last dose of vaccine. Additional follow-up visits or telephone contact will be scheduled annually thereafter if the patient is alive and available for follow-up. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

6.1 Vaccine

Vaccine dose will be 1 mg of GNOS-PV01 and 1 mg of INO-9012. No dose modifications are planned. If a subject develops an adverse event that is classified as possibly, probably, or definitely associated with vaccine administration, this may result in discontinuation of vaccine treatment.

Patients who develop radiographic evidence of progressive disease following radiation therapy AND have not yet received a dose of GNOS-PV01 are able to undergo re-resection or local therapy (includes stereotactic radiosurgery and laser interstitial thermal ablation) while remaining on study. However, initiation of study treatment will be delayed 2-4 weeks until recovery from procedure. Recovery time frame will be determined by treating neurosurgeon or radiation oncologist. Alternatively, a substitution for any patient that progresses prior to receiving study treatment will be permitted. The final decision will be made by the study PI.

6.2 Corticosteroids and Bevacizumab

One of the expected side effects of immunotherapy in general is increased inflammation at the site of disease due to immune cell infiltration. As such, subjects may experience adverse effects related to increased intracranial vasogenic edema due to inflammation. The standard treatment is a short course of corticosteroids such as dexamethasone. While subjects must not require more than 2 mg daily of dexamethasone or equivalent at time of initiation of study treatment (i.e. C1D1), a short course of higher dose dexamethasone is permitted in the setting of a flare following GNOS-PV01. Given the immunosuppressive effects of corticosteroids, all vaccinations will be held until patients are able to be tapered to at least 4 mg daily of dexamethasone or equivalent before reinitiating therapy.

If a subject is unable to be weaned from high doses of corticosteroids either prior to enrollment or following initiation of treatment, a short course of bevacizumab (dosing per treating medical oncologist) is acceptable. This is based off of preliminary results from the phase II study of rindopepimut with bevacizumab in patients with recurrent GBM which reported no significant increase in adverse events with preservation of vaccine efficacy[121].

There will be no maximum time limit treatment can be held before subjects must be withdrawn for study protocol as long as there is no evidence of disease progression that requires alternative treatment options. Of note, bevacizumab given for disease progression is not permitted and subsequent vaccinations will be discontinued.

7.0 REGULATORY AND REPORTING REQUIREMENTS

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

Adverse events will be tracked from start of treatment through 100 days following the last day of treatment. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

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

Refer to the data submission schedule in Section 11 for instructions on the collection of AEs in the EDC.

7.1 Sponsor-Investigator Reporting Requirements

7.1.1 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.

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

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

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to [redacted] Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

7.1.3 Reporting to the FDA

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

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to 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 B) 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").

7.1.4 Reporting to Geneos Therapeutics/Inovio Pharmaceuticals

All serious adverse events that occur following Day 1 of study treatment through 100 days after discontinuation of dosing must be reported to Geneos Therapeutics. SAEs, whether related or not to study drug, and pregnancies must be reported to Geneos Therapeutics [redacted] within 24 hours of discovery. If only limited information is available, follow up reports are required.

7.1.5 Reporting to the Institutional Biosafety Committee

In accordance with institutional policies and NIH guidelines, any unanticipated problems must be reported to the Institutional Biological and Chemical Safety Committee (IBC) at Washington University School of Medicine.
The Washington University Sponsor-Investigator (or designee) must report the following events to the Biosafety Officer at the time of submission to HRPO:

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

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

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

The Biosafety Officer may be contacted at [redacted]

When submitting reports to the IBC, the FDA MedWatch form will be used.

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.

7.2 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 7.1 include:

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

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

8.0 PHARMACEUTICAL INFORMATION

8.1 Neoantigen DNA Vaccine, GNOS-PV01

8.1.1 Chemical name and structure

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

Tumor tissue sequencing will be performed under CLIP/CLIA conditions. Neoantigen identification and prioritization will be performed by WUSM. Neoantigen DNA vaccines will be designed by Geneos Therapeutics and manufactured based on the following general steps:

1) Cancer tissue and normal lymphocytes will be obtained from patients who are eligible for the phase 1 clinical trial.
2) Tumor/normal exome sequencing and tumor RNA sequencing will be performed to identify candidate neoantigens.
3) Candidate neoantigens will be prioritized based on epitope prediction algorithms.
4) Personalized polyepitope inserts integrating the prioritized (up to 40) neoantigens will be designed and then synthesized and cloned into the pGX0001 parent vector.
5) The parent vector will be transformed into *E. coli* and the neoantigen DNA vaccines will be manufactured and vialed.
6) The neoantigen DNA vaccines, GNOS-PV01, will undergo product release tests prior to investigational use.
7) GNOS-PV01 will be administered in combination with INO-9012 (plasmid IL-12) using a pharmacy protocol for on-site mixing of the two drug products. The two plasmid DNA products will be combined and administered using the CELLECTRA®-2000 electroporation system.

The neoantigen DNA vaccines will be manufactured in the [redacted] 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."

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

8.1.2 Manufacturing facility

The neoantigen DNA vaccines (GNOS-PV01) will be prepared in the [redacted]

The facility adheres to cGMP practices with regard to documentation, facility maintenance, and QC/QA review. Segregated manufacturing rooms are available for clinical grade manufacturing of cellular therapy products, recombinant DNA or gene therapy products.

8.1.3 Manufacturing process

[redacted]

[redacted]

8.1.8 Investigational agent accountability

The investigational agent will be prepared by the study pharmacist at the [redacted] 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.

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.

9.0 CORRELATIVE STUDIES

The basis for the proposed correlative studies is to gain further mechanistic insight into the immunogenicity and efficacy of personalized neoantigen-based DNA vaccines. Immune monitoring (see Section 9.2.1) will characterize the ability of a personalized neoantigen vaccine to generate a robust tumor-specific T cell response. This will be accomplished by characterizing the expansion and functional characteristics of neoantigen-specific T cells primed by the personalized vaccine. Additionally, this study aims to identify potential biomarkers predictive of patients who will likely benefit from vaccination by characterizing the tumor microenvironment (see Section 9.2.2). Overall, these data will provide insight into the immunologic mediators of protection against glioblastoma that can be used in future trials to generate more potent anti-tumor vaccines.

9.1 Sample Collection Plan

At time of initial study enrollment (+/- 3 days), a research blood collection will be performed consisting of one 10 mL SST tube and six 10 mL EDTA tubes obtained by venipuncture. Samples will be labeled “pre-RT”.

After the completion of radiation therapy, and up to 7 days after the first vaccine dose, patients will undergo the second research blood collection (labeled “C1D1”). One 10 mL serum separator tube (SST; tiger or marble top) will be collected from all enrolled patients for serum isolation. Additionally, patients will have 5 L apheresis product collected for isolation of PBMC. Leukapheresis will be performed by the Apheresis Center on the 4th Floor of the Center for Advanced Medicine or equivalent substitute. Collection will be performed by either peripheral venipuncture (preferable) or through placement of a temporary pheresis catheter. In patients who are not candidates for leukapheresis via peripheral venipuncture, a formal vein assessment will be conducted by the Apheresis Center and a temporary pheresis catheter will be placed by Interventional Radiology per standard protocol prior to apheresis session. The pheresis catheter will be removed by

Interventional Radiology or equivalent substitute following leukapheresis per standard protocol. If apheresis is not possible for any reason, a standard venipuncture to collect six 10 mL EDTA tubes will be performed instead.

A third research blood collection (labeled "C2D8") will be performed at Day 8 of Cycle 2 (+/- 7 days) with serum and PBMC obtained by SST tube and leukapheresis, respectively, similar to the second research blood collection described previously.

At time of progression or discontinuation of study participation, an optional research blood collection (labeled "progression") will be obtained and will include one 10 mL SST tube plus 2-5 L apheresis product or 60 mL (six 10 mL EDTA tubes) peripheral blood.

Additional research blood collections (for serum [10 mL SST tube] or PBMC [up to 60 mL EDTA tubes]) will be performed on Day 1 (+/- 3 days) of Cycle 2 through 8 (labeled "C2D1," "C3D1," etc.). Furthermore, similar optional blood draws at other time points of interest throughout the study can be obtained if deemed pertinent to assessment of exploratory objectives per the discretion of the study Principal Investigator.

All blood samples will be transported to the [redacted] within one hour of collection. Serum will be isolated following centrifugation and aliquoted into individual 1.5 mL cryovials for storage in -80ºC freezer or liquid nitrogen. Apheresis product (PBMC) will be centrifuged, resuspended in freezing media containing 20% heat-inactivated serum plus 10% DMSO, and aliquoted into individual 1.5 mL cryovials for storage in -80ºC freezer or liquid nitrogen. PBMC from EDTA tubes will be obtained by Ficoll-Hypaque gradient centrifugation. Cryopreserved samples can be stored in the TPC, the IML, the [redacted] until analysis.

Serum will be used to assess humoral immune responses post-vaccination, which includes cytokine and chemokine analyses. PBMC will be used to assess the cellular immune response pre- and post-vaccination. Additionally, exome sequencing of PBMC will be performed to obtain germline DNA.

In addition to blood samples, tumor tissue samples will be collected during surgical resection or biopsy and will be prioritized for tumor DNA whole exome and tumor RNA sequencing (for vaccine manufacture). These samples are obtained under the study tissue consent or Neuro-Oncology tumor bank consent (HRPO# 201111001), which authorizes permission for genomic characterization and return of information. The tumor bank consent also permits collection of additional tissue samples obtained from re-resection or biopsies performed clinically to confirm diagnosis of progression or at time of recurrence. All tumor tissue will be transported to the Johanns laboratory, the [redacted] for processing:

Fresh tissue: two sections of approximately 2-4 mm^3 each from each region or resection will be flash frozen in a 1.5 mL cryovial (labeled "non-viable"). One additional section of

approximately 2-5 mm^3 will be added to a 1.5 mL cryovial containing RPMI 1640 supplemented with 20% heat-inactivated serum and 10% DMSO (labeled "viable"). Samples will be stored in -80ºC freezer or liquid nitrogen

OCT embedding: approximately 10-50 mg of tissue, sectioned into pieces no larger than 0.5 cm x 0.5 cm, will be prepared. 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 be hardened by cooling. This will be achieved by placing the cryomold in the vapor phase of liquid nitrogen or on dry ice. After the OCT has hardened, the mold will be placed in a container and transferred to a -80°C freezer for storage. The frozen tissue will be transferred to the Laboratory of Translational Pathology (LTP, BJC-Institute of Health, Room 5110) for sectioning, H&E staining, and pathology evaluation.

Single cell suspension: the remaining tumor sample will undergo mechanical and enzymatic digestion and be cryopreserved for further downstream studies such as multi-parametric flow cytometry, ELISPOT, TIL culture, and tetramer analysis.

Nucleic acid isolation: DNA will be isolated from PBMC by the [redacted] personnel for exome sequencing at [redacted]. To identify somatic mutations, DNA and RNA will be extracted from OCT-embedded tissue. The OCT block will be delivered to the LTP where the block will be sectioned and stained in order to confirm the presence of tumor, determine tumor/normal ratio, and guide isolation of tumor cells by, for instance, laser capture microdissection (LCM). All tissue selected for sequencing will be processed into a single-cell suspension by mechanical and enzymatic digestion and used to extract nucleic acids. Tumor DNA + RNA will then undergo whole exome and RNA sequencing, respectively, at [redacted].

Tumor-derived tissue will be used for genomic and molecular studies. Additionally, tumor-infiltrating lymphocytes will be used to assess the cellular immune response prior to and following vaccination.

9.2 Immune Monitoring

9.2.1 Neoantigen-specific T cell response

Patient-derived PBMC will be used to quantify and characterize the neoantigen-specific T cell response at baseline (pre-vaccination) and at various time points post-vaccination. Single cell suspensions will be isolated as described above. All correlative studies will be performed in the [redacted]

The T cell repertoire pre- and post- vaccination will be characterized tetramer and analyzing the TCR-beta region of circulating PBMCs. This will be done by multi-

parametric flow cytometry, spectratyping, and sequencing. Phenotypic and polyfunctional characterization of neoantigen-specific T cells will be performed by ELISPOT, multi-parametric flow cytometry and mass cytometry (CyTOF). Ex vivo cytotoxicity assays will be performed to determine the cytolytic activity of neoantigen-specific T cells pre- and post-vaccination. T cells may also be assessed for gene expression programs via RNA-seq analysis at [redacted].

Additionally, correlative studies may be performed on patient samples as deemed pertinent to the assessment of the outlined objectives per the discretion of the study Principal Investigator.

9.2.2 Microenvironment characterization

Tumor samples obtained from resection or initial biopsy at diagnosis will be analyzed for biomarkers that are associated with response to personalized vaccination to identify individuals who may benefit most from this therapeutic approach.

IHC staining of OCT embedded tissue will characterize the location of various infiltrating lymphocytes relative to tumor. Furthermore, single cell suspensions can be made from fresh frozen tissue to characterize the phenotype of various populations of infiltrating leukocytes (T cells, B cells, NK cells, monocytes/macrophages, dendritic cells) by multi-parametric flow cytometry and mass cytometry as well as gene expression analysis. Expression of various activation and inhibitory markers within the tumor microenvironment will also be assessed. These data will be correlated with treatment response to be used to generate a prediction algorithm to identify patients most likely to receive benefit from personalized vaccination. Additionally, this data will provide information to help guide future clinical trials incorporating various immunomodulatory molecules like checkpoint inhibitors.

TIL cultures will be generated by microdissection or magnetic bead-based isolation of single lymphocytes from tumor tissue. A baseline (pre-standard chemoradiation) assessment of the phenotype and functionality of these cells will be performed in a similar fashion as described above for PBMCs. Consideration will be given for T cell receptor sequencing of these TIL as well.

Additionally, correlative studies may be performed on patient samples as deemed pertinent to the assessment of the outlined objectives per the discretion of the study Principal Investigator.

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 2 weeks prior to registration and acquisition of tissue for sequencing and vaccine manufacture. Eligibility must be reconfirmed no more than 2 weeks prior to the first vaccine injection. Cycles are 9 weeks.

	Screening	**Eligibility Re-Check[2]**	**C1 D1[3]**	**C1 D22[3]**	**C1 D43[3]**	**C2 D1[4]**	**C2 D8**	**C3 D1[4]**	**C4 D1[4]**	**C5 D1[4]**	**C6 D1[4]**	**C7 D1[4]**	**C8 D1[4]**	**Progression**	**End of Tx**	**F/U[6]**
Informed consent	X															
H&P, KPS		X	X	X	X	X		X	X	X	X	X	X		X	
VS		X	X[5]	X[5]	X[5]	X[5]		X[5]	X[5]	X[5]	X[5]	X[5]	X[5]			
CBC		X	X	X	X	X		X	X	X	X	X	X			
CMP		X	X	X	X	X		X	X	X	X	X	X			
β-hCG[2]		X														
Brain MRI		X				X		X	X	X	X	X	X			
GNOS-PV01[9]			X	X	X	X		X	X	X	X	X	X			
MGMT methylation status	X															
Tissue for sequencing for vaccine manufacture	X[1]															
Blood for correlatives[3]	X	X[7]				X	X	X	X	X	X	X	X	X		
Apheresis for correlatives[4]		X					X							X		
Concomitant medications[8]					X ---------- X											
AE assessment					X ---------- X											

1. Once determined to be eligible.
2. No more than 2 weeks prior to the first vaccine injection (with the exception of MRI which can be up to 4 weeks prior).
3. +/- 3 days, only needed through Cycle 8
4. +/- 7 days
5. 30 minutes post-vaccine
6. 24M after EOT by review of medical record
7. After eligibility re-check and up to 7 days after first vaccine administration
8. Steroid or other immunosuppressive agents and anti-seizure medications will be tracked specifically
9. Treatment may continue until either progression, intolerance, physician's discretion, or end of supply

11.0 DATA SUBMISSION SCHEDULE

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

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form Treatment Assignment Form Medical History Form	Prior to starting treatment
Vaccine Form Vital Signs Form	With every vaccination
DLT Form	End of Cycle 1
Correlatives Form	Per protocol
Toxicity Form	Continuous
Concomitant Medications Form	Continuous
Treatment Summary Form	Completion of treatment
Long-Term Follow Up Form	Per protocol
Tumor Measurements Form	Baseline, end of every even numbered cycle, and end of treatment
Progression Form	Time of disease progression
Death Form	At time of death
MedWatch Form	See Section 7.0 for reporting requirements

11.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 7.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

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

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients will be re-evaluated for recurrence or progression every 9 (+/- 1) weeks. In addition to a baseline scan following surgical resection, a confirmatory scan will be obtained 4 (+/- 1) weeks following completion of radiation therapy to document ongoing objective response prior to study treatment. These will be performed per standard of care.

Criteria for response: Response will be evaluated in this study using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) working group guideline[122].

Criteria for Response Assessment Incorporating MRI and Clinical Factors (Adapted from [122])

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and no measurable disease sustained for at least 4 weeks. • No new lesions; stable or improved nonenhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: • ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. • No progression of nonmeasurable disease. • Stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan. • Stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: • Does not qualify for complete response, partial response, or progression. • Stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: • ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*. The absolute increase in any dimension must be at least 5mm when calculating the products.

Response	Criteria
	• Significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). • Any new measurable lesion. • Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose. • Failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.

- NOTE. All measurable and nonmeasurable lesions must be assessed using the same techniques as at baseline.
- Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
- Stable doses of corticosteroids include patients not on corticosteroids.

Criteria for progression: Pseudoprogression is a phenomenon where radiographic features are consistent with tumor recurrence/progression but is instead related to treatment effect. This is a common occurrence in glioblastoma following radiation that is generally seen in the first 3-6 months after completing therapy. While pseudoprogression may be associated with clinical features consistent with tumor progression, it is generally asymptomatic, self-limiting, or easily manageable with a short course of corticosteroids. Given that patients in this study will be treated with immune therapy within 3-6 months post-radiation, there is a high suspicion for pseudoprogression during this time frame. As such, consistent with iRECIST and irRC guidelines used to assess progression in patients with non-CNS disease treated with immune-based therapy, a confirmatory MRI performed no sooner than 4 weeks after initial radiographic evidence of progression will be required to document progression as long as the following criteria are met:

0. No new or significantly worsening neurologic deficits are clinically apparent that cannot be attributed to a co-morbid event or concurrent medication, and
1. It has been $\leq$ 6 months from initiation of immunotherapy.

If follow-up imaging confirms progression, the date of actual progression should be back dated to the date of initial radiographic evidence of progression.

Alternatively, progressive disease can be defined as radiographic evidence of progression PLUS significant clinical decline that is felt to be unrelated to a co-morbid event or concurrent medication, OR if there is radiographic evidence of progression > 6 months after initiation of immunotherapy.

12.2 Disease Parameters

Measurable disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable or evaluable disease: Uni-dimensionally measurable lesions or lesions with margins not clearly defined such as areas of T2/FLAIR signal abnormality or poorly defined enhancing abnormality.

Note: For cystic lesions, the only measurable part is any enhancement area around the cyst that is clearly defined and bi-dimensionally measurable. The cyst itself should not be considered measurable or non-measurable disease.

Target lesions: All measurable lesions should be identified as target lesions and recorded and measured. Target lesions should be selected on the basis of their size (lesions with the longest diameter), but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion, which can be measured reproducibly should be selected. When there are too many measurable lesions, choose the largest 3 lesions as target lesions to follow. The other measurable lesions should be considered evaluable for the purpose of objective status determination.

Non-target lesions: All non-measurable lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 2 weeks before the beginning of the treatment.

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are ≥ 5 mm diameter as assessed using a ruler.

Histology: This technique can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases when biopsy or surgical resection of a measurable lesion is clinically indicated.

Perfusion/CBV: This advanced brain MRI technique can be used as an adjunct test to determine treatment response or disease status. However, it should not be used as the primary or sole method to determine response or disease status.

Brain FDG-PET coupled with head CT or brain MRI: This advanced metabolic imaging technique can be used as an adjunct test to determine response or disease status. However, it should be used as the primary or sole method of determining response or disease status.

12.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions.

Partial Response (PR): ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all target lesions sustained for at least 4 weeks.

Progressive Disease (PD): At least a 25% increase in the sum of products of perpendicular diameters of at least 1 target lesion, taking as reference the smallest sum of products of perpendicular diameters on study (this includes the baseline sum if that is the smallest on study). The absolute increase in any dimension must be at least 5mm when calculating the products of perpendicular diameters.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of products of perpendicular diameters while on study.

12.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

12.3.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the

treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Summary of the RANO Response Criteria (Adapted from [122])

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.
* Progression occurs when this criterion is present.
† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

12.3.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.3.5 Neurological Exam and Performance Status

Patients will be graded using the Karnowski Performance Status scale and their neurological function evaluated as improved, stable or deteriorated in addition to objective measurement of tumor size. These parameters will be used to determine the overall response assessment.

12.3.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

13.0 DATA AND SAFETY MONITORING

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 five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least monthly (or before each treatment escalation if occurring sooner than monthly) 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 with the number of dose-limiting toxicities indicated
- 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.

14.0 STATISTICAL CONSIDERATIONS

14.1 Study Design

This is a single arm pilot study to investigate the safety and immunogenicity of DNA vaccine administration in patients with newly diagnosed, MGMT unmethylated GBM following radiation therapy. The DNA GBM cancer vaccine strategy, GNOS-PV01, is designed to target prioritized mutant tumor-specific antigens present in individual patient's malignancy but not in corresponding normal tissues. The hypothesis of this study is that GNOS-PV01 can be generated in a reasonable time frame that is feasible for use as maintenance therapy following radiation. The overall goal of this study is to identify the optimal DNA vaccine platform that can be tested in a subsequent phase II study to determine the efficacy of a personalized neoantigen vaccine approach in patients with GBM.

14.2 Study Accrual

There are approximately 90-100 patients seen at Siteman Cancer Center at Washington University in St. Louis with newly diagnosed glioblastoma, and 55-65% of glioblastoma have an unmethylated MGMT promoter. Thus, there are roughly 8 potentially eligible patients per month that can be screened for enrollment. Currently, only about 30-40% of patients are actively participating in a clinical trial and there are no competing clinical trials for this patient population. Therefore, we anticipate enrolling 2 patients per month. Roughly 60% of newly diagnosed glioblastomas are MGMT unmethylated, therefore, we anticipate screening 30 subjects in order to enroll 12 patients. Enrollment will not be staggered, but treatment of the first 3 patients will be staggered as described in Section 4.4.

14.3 Study Duration

The total estimated duration of the study is 24 months based on a 6-month accrual period and a minimum of 18-month follow-up to complete evaluation of all study objectives.

14.4 Primary Endpoint

The primary endpoints are safety and feasibility. All participants meeting the eligibility criteria who have signed a consent form and have received at least one dose of the study treatment will be evaluable for safety and feasibility. Safety will be defined as a < 33% treatment-related DLT rate related to vaccination by the end of the DLT observation period. Feasibility will be defined as the ability to identify, generate, and administer a neoantigen-specific DNA vaccine from time of resection to 4 weeks post-radiation therapy (approximately 10-14 weeks).

14.5 Secondary Endpoints

Secondary endpoints include immunogenicity of various vaccine-adjuvant strategies, 6-month progression-free survival (6-PFS), and 12-month overall survival (12-OS) for all the

patients receiving the proposed treatment regimen. Correlative studies will be assessed to identify biomarkers of response.

14.6 Power Analysis

As a pilot safety/feasibility study, power analysis is not applicable. The sample size determination for this study is mainly based on clinical feasibility rather than statistical power[124]. However, the proposed sample size (n = 12) will provide a reasonably reliable estimate of the safety and immunogenicity of the vaccine approach. This information will provide an estimation of the rate of neoantigen expression in GBM to allow for calculations of number of patients needed to screen for accrual in future trials.

14.7 Data Analysis

This trial is designed to allow preliminary assessments of feasibility, safety, and immunogenicity of DNA vaccine. No pre-specified hypothesis testing will be performed. Data from this study will be used to design subsequent phase II studies to further assess efficacy.

14.7.1 Safety

All Grade 3 and 4 adverse events as defined by CTCAE v5 and laboratory-based toxicities will be summarized. All causes of death or reasons for discontinuation of treatment will be reported.

14.7.2 Feasibility

Feasibility will be defined as the ability to identify, generate, and administer a neoantigen-specific DNA vaccine from time of resection to 4 weeks post-radiation therapy (approximately 14 weeks).

14.7.3 Immunogenicity

There are no presently available validated objective measures to assess immunogenicity. Therefore, in this study, immunogenicity will be evaluated as a relative value. The parameters that will be compared between groups include: 1) the number of subjects who develop at least one demonstrable neoantigen-specific CD8 T cell response by 6 months after administration of the first dose of vaccine, and 2) the percentage of neoantigens that elicit a neoantigen-specific CD8 T cell response out of the total number of neoantigens vaccinated against (i.e. total # neoantigens with T cell response/total # neoantigens vaccinated against).

14.7.4 Efficacy

Endpoints include: 6-month PFS and 12-month OS. Tumor response and progression will be defined by RANO and iRANO criteria.

Demographic and clinical characteristics will be summarized using descriptive statistics.

Outcomes will be correlated to immunologic parameters to identify biomarkers that predict response.

14.7.5 Correlative Studies

Independent t-test or Wilcoxon Rank Sum test will be used to compare the gene expression of TIL/PBMC between patients treated with vaccine and control (patients treated with conventionally method; data obtained from tissue bank). Paired t-test or paired-sample Wilcoxon Signed Rank test will be used to compare the gene expression of TIL and immune response in blood as measured before and after treatment.

14.8 Toxicity and Plans for Data and Safety Monitoring

Toxicity will be reviewed on a continuous basis using the go/no-go decision as outlined in the Section 1.11 (Study Design). Early stopping of this trial will be based on the excessive vaccine-related toxicity defined as anaphylaxis or clinically significant edema around head/neck region, irreversible end organ impairment, neurologic impairment not secondary to underlying malignancy, severe skin reactions around injection site, or autoimmune disease requiring treatment. If a grade 5 toxicity (death) is observed within 30 days of vaccine administration due to any cause other than progressive disease, accrual will be suspended, and the event will be reviewed by the study chair.

14.9 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) expires within 30 days of vaccine administration due to **any** cause other than progressive disease
2. **One** (or more) subject(s) experiences a Grade 3 or 4 adverse event that is classified as probably or definitely related to vaccination;
3. **One** (or more) subject(s) experiences a vaccine-related SAE;
4. **Two** (or more) subjects experience the **same** Grade 2 or higher adverse event that is classified as probably or definitely related to vaccination: this criterion applies to fever, vomiting, laboratory abnormalities or other clinical adverse experiences, but does not apply to the subjective local or systemic symptoms of pain/tenderness, malaise, fatigue, headache, chills, nausea, myalgia, or arthralgia.
5. Any other observation occurs that in the opinion of the PI results in a recommendation to halt enrollment.

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

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

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

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APPENDIX A: Karnofsky Performance Scale

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

APPENDIX B: 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. Unanticipated (Serious) Adverse Device Effect

Definition: Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Per the definition above, a UADE is a type of SAE that requires expedited reporting.

F. Protocol Exceptions

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

G. 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.

APPENDIX C: Reporting Timelines

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Geneos**
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			
Unanticipated adverse device effect			Report no later than 10 business days.		Report to [redacted] no later than 10 business days for UADE.
Serious adverse event					Report to [redacted] within 24 hours of discovery.
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	Report within 10 working days.				

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Geneos**
continuing noncompliance					
Pregnancy					Report to [redacted] within 24 hours of discovery.
Protocol exception	Approval must be obtained prior to implementing the change				
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting		
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.				
Breach of confidentiality	Within 10 working days.				
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue				

Expedited Reporting Timelines					
Event	**HRPO**	**QASMC**	**FDA**	**IBC**	**Geneos**
	and the patient will be withdrawn, report at continuing review.				

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