

COMBINATION THERAPY TO TREAT BRAIN CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Application No. 63/070,987, filed August 27, 2020; U.S. Application No. 63/018,060, filed April 30, 2020; U.S. Application No. 62/988,102 filed March 11, 2020; and U.S. Application No. 62/930,417, filed November 4, 2019. Each of these applications is incorporated herein by reference in their entireties.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 4, 2020, is named 104409_000581_SL.txt and is 89,861 bytes in size.

TECHNICAL FIELD

[0003] The present invention relates to combination therapies and methods for treating brain cancer.

BACKGROUND

[0004] Despite advances in therapy, glioblastoma (GBM) remains one of the most deadly cancers. The current standard treatment for GBM is surgery, followed by concurrent radiation therapy (RT) and temozolomide (TMZ) chemotherapy administered daily during RT and then for 6-12 maintenance (adjuvant) cycles following the completion of RT for select patients [Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-996].

[0005] Checkpoint inhibitors, such as programmed cell death-1 (PD-1) inhibitors, have increased response rates in many cancers, but have not yet shown clinical benefit in GBM.

[0006] Accordingly, a need exists for the identification and development of methods for the treatment of GBM to facilitate clinical management and progression of disease. Furthermore, more effective treatments are required to delay disease progression and/or decrease mortality in subjects suffering from cancer.

SUMMARY

[0007] Provided herein are vaccines and methods of their use to prevent or treat cancer. The cancer can be brain cancer, for example, glioblastoma. The vaccine preferably includes at least three cancer antigens, hTERT, WT-1, and PSMA. In certain embodiments, the vaccine also includes an adjuvant, such as IL-12, and an anti-PD-1 antibody. The methods involve administering cancer antigens hTERT, WT-1, and PSMA, an adjuvant, and a programmed death receptor-1 (PD-1) checkpoint inhibitor, such as an anti-PD-1 antibody to a subject diagnosed with the cancer. In some embodiments, the methods prevent tumor growth. In some embodiments, the methods can reduce tumor growth and/or mass. In some embodiments, the methods can prevent metastasis of tumor cells. In some embodiments, the methods can increase a cellular immune response in the subject. In some embodiments, the methods increase tumor-free survival, progression-free survival, overall survival, or any combination thereof, of the subject.

[0008] In certain embodiments, IL-12 is encoded by a DNA plasmid, for example, INO-9012 or a biosimilar or bioequivalent thereof. In certain embodiments, hTERT, WT-1, and PSMA are encoded by one or more DNA plasmids, for example, INO-5401 or a biosimilar or bioequivalent thereof. In certain embodiments, the anti-PD-1 antibody is cemiplimab or a biosimilar or bioequivalent thereof. In certain embodiments, the methods further comprise administering radiation therapy and/or a chemotherapeutic agent, for example, temozolomide or a bioequivalent thereof.

[0009] In certain embodiments, the methods are clinically proven safe, clinically proven effective, or both.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosed methods, there are shown in the drawings exemplary embodiments of the thereof; however, the methods are not limited to the specific embodiments disclosed. In the drawings:

[0011] **Figure 1** illustrates the study design for the example.

[0012] **Figure 2** shows the study population demographics for the example.

[0013] **Figure 3** shows representative MRI Images from two patients demonstrating increase in MRI signal at timepoints following first dose of INO-5401 + INO-9012 and cemiplimab- rwlc, suggestive of edema or tumor. Biopsy on several patients shows treatment-related changes with necrosis and mixed inflammation; absence of mitotic activity; and no evidence of viable tumor. The subject represented by the MRI images in the lower panel showed evidence of disease progression at Week 9 but resolution at Week 21. Subjects with similar findings on MRI who were resected showed only immune infiltrate with an absence of viable tumor.

[0014] **Figure 4** demonstrates ELISpot results supporting the combination of INO-5401 and cemiplimab-rwlc as immunogenic- with IFN-g magnitudes above baseline to all 3 antigens in 5/11 subjects and to at least one antigen in 9 subjects obtained at the 12-month data cut-off.

[0015] **Figures 5A, 5B, and 5C** show the lytic granule loading assay results demonstrating frequencies of live, antigen-specific, activated (CD38+) CD3+CD8+ T cells with lytic potential (expressing Granzyme A, Perforin) obtained at the 12-month data cut-off. Figure 5A shows the frequencies of live, antigen specific, activated (CD38+) CD3+CD8+ T cells with lytic potential (expressing Granzyme A, Perforin) from before treatment (pre) and the highest magnitude (peak) after treatment with INO-5401 and cemiplimab-rwlc. Each subject is represented by an open circle, bars represent the mean. The difference from pre to peak, delta, is shown for each antigen graph as well as together for 8 subjects assayed (Figure 5B) and for the 5 subjects with sample available to week 12 (Figure 5C). INO-5401 is the sum of WT1, PSMA and hTERT. Box plots extend from the 25th to 75th percentile, with a horizontal line at the median, and “+” at the mean.

[0016] **Figure 6** shows the visual representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort A, patients with the O6- methylguanine methyltransferase gene promoter unmethylated in their tumor cells. The curve shows the probability of an event at a certain time interval. The probability of the event is represented numerically on the y-axis, and the time interval on the x-axis. The event shown is progression-free survival. Progression-free survival is the absence of progression of disease at a given time point for a given subject.

[0017] **Figure 7** shows the visual representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort B, patients with the O6- methylguanine methyltransferase gene promoter methylated in their tumor cells. The curve shows the probability of an event at a certain time interval. The probability of the event is represented numerically on the y-axis, and the time interval on the x-axis. The event shown is progression-free survival. Progression-free survival is the absence of progression of disease at a given time point for a given subject.

[0018] **Figure 8** shows the visual representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort A and Cohort B, patients with the O6- methylguanine methyltransferase gene promoter unmethylated or methylated in their tumor cells. The curve shows the probability of an event at a certain time interval. The probability of the event is represented numerically on the y-axis, and the time interval on the x-axis. The event shown is progression-free survival. Progression-free survival is the absence of progression of disease at a given time point for a given subject.

[0019] **Figure 9** shows the tabular representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort A, Cohort B, and both cohorts combined.

The total number of subjects per cohort, number of events, estimation of the event (PFS6), and the 95% confidence interval (CI) in which the numerical estimate of the event (PFS6) exists are all provided.

[0020] **Figure 10A** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over twelve months for Cohort A, for patients with the O6-methylguanine methyltransferase gene promoter unmethylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. **Figure 10B** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over eighteen months for Cohort A, for patients with the O6-methylguanine methyltransferase gene promoter unmethylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. Median follow-up in Cohort A is 17.8 months. mITT includes any subject who received ≥ 1 dose of study therapy. Shading represents confidence band on point estimate for survival at that timepoint.

[0021] **Figure 11A** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over twelve months for Cohort B, for patients with the O6-methylguanine methyltransferase gene promoter methylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. **Figure 11B** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over eighteen months for Cohort B, for patients with the O6-methylguanine methyltransferase gene promoter methylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. Median follow-up in Cohort B is 15.6 months. Censored; two subjects in Cohort B withdrew consent for follow-up at Week 3. mITT includes any subject who received ≥ 1 dose of study therapy. Shading represents confidence bands on point estimate for survival at that timepoint.

[0022] **Figure 12** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over twelve months for Cohorts A + B combined. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis.

[0023] **Figure 13** shows the efficacy data of the overall survival at 12 months and 18 months for Cohort A, for Cohort B, and combined. The figure shows the total number of subjects who were reported alive at 12 months and at 18 months. The total number of subjects, estimation of the event (OS12 or OS18), and the 95% confidence interval (CI) in which the numerical estimate of the event (OS12 or OS18) exists are all provided. The 95% CI were calculated using the exact Clopper-Pearson method.

[0024] **Figure 14** illustrates all Adverse Events as defined by the clinical study protocol $\geq$ NCI CTCAE Grade 3 from the example.

Figure 15 illustrates Immune Related Adverse Events as defined by the clinical study protocol from the example.

[0025] **Figures 16A and 16B** provide ELISpot results by Cohort at the 18-month data cut-off. In Cohort A, 19/22 (86%) subjects tested to date had an IFN-g magnitude above baseline to one or more of the antigens INO-5401 (Fig. 16A). In Cohort B, 16/17 (94%) subjects tested to date had an IFN-g magnitude above baseline to one or more of the antigens in INO-5401 (Fig. 16B). Baseline values from the peak timepoint following treatment are plotted. Samples collected Q3 weeks x 4 and then Q12 weeks.

[0026] **Figures 17A and 17B** provide results of assessment of post-INO-5401 peripheral immune responses by Cohort by flow cytometry (the expansion of antigen specific CD8+ T cells with lytic potential) at the 18-month data cut-off. In Cohort A, 13/19 (68%) subjects tested to date had a frequency of CD38+GrzA+Prf+ CD8+T cells above baseline to one or more of the antigens in INO-5401 (Figure 17A). In Cohort B, 8/10 (80%) subjects tested to date had a frequency of CD38+GrzA+Prf+ CD8+T cells above baseline to one or more of the antigens in INO-5401 (Figure 17B). Baseline values from the peak timepoint following treatment are plotted. Samples were collected Q3 weeks x 4 and then Q12 weeks.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0027] The disclosed nucleic acid molecules, proteins, vaccines, and methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed nucleic acid molecules, proteins, vaccines, and methods are not limited to the specific nucleic acid molecules, proteins, vaccines, and methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed nucleic acid molecules, proteins, vaccines, and methods.

[0028] Unless specifically stated otherwise, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the disclosed nucleic acid molecules, proteins, vaccines, and methods are not to be constrained by the correctness or incorrectness of any such suggested mechanism or mode of action or reason for improvement.

[0029] Throughout this text, the descriptions refer to compositions and methods of using said compositions. Where the disclosure describes or claims a feature or embodiment associated with a composition, such a feature or embodiment is equally applicable to the methods of using said composition. Likewise, where the disclosure describes or claims a feature or embodiment associated

with a method of using a composition, such a feature or embodiment is equally applicable to the composition.

[0030] It is to be appreciated that certain features of the disclosed nucleic acid molecules, proteins, vaccines, and methods which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment.

[0031] Conversely, various features of the disclosed nucleic acid molecules, proteins, vaccines, and methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

[0032] Provided herein are vaccines and methods of their use to prevent or treat cancer. The cancer can be brain cancer, for example, glioblastoma. The vaccine preferably includes at least three cancer antigens, hTERT, WT-1, and PSMA. In certain embodiments, the vaccine also includes an adjuvant, such as IL-12, and an anti-PD-1 antibody. The methods involve administering cancer antigens hTERT, WT-1, and PSMA, an adjuvant, and a programmed death receptor-1 (PD-1) checkpoint inhibitor, such as an anti-PD-1 antibody to a subject in need thereof. In some embodiments, the methods prevent tumor growth. In some embodiments, the methods can reduce tumor growth and/or mass. In some embodiments, the methods can prevent metastasis of tumor cells. In some embodiments, the methods can increase a cellular immune response in the subject. In some embodiments, the methods increase tumor-free survival, progression-free survival, overall survival, or any combination thereof, of the subject.

[0033] In certain embodiments, IL-12 is encoded by a DNA plasmid, for example, INO-9012 or a biosimilar or bioequivalent thereof. In certain embodiments, hTERT, WT-1, and PSMA are encoded by one or more DNA plasmids, for example, INO-5401 or a biosimilar or bioequivalent thereof. In certain embodiments, the anti-PD-1 antibody is cemiplimab or a biosimilar or bioequivalent thereof. In certain embodiments, the methods further comprise administering radiation therapy and/or a chemotherapeutic agent, for example, temozolomide or a bioequivalent thereof.

[0034] In certain embodiments, the methods are clinically proven safe, clinically proven effective, or both.

[0035] The recombinant cancer antigens can induce antigen-specific T cell and/or high titer antibody responses, thereby inducing or eliciting an immune response that is directed to or reactive against the cancer or tumor expressing the antigen. In some embodiments, the induced or elicited immune response can be a cellular, humoral, or both cellular and humoral immune responses. In some embodiments, the induced or elicited cellular immune response can include induction or secretion of interferon-gamma (IFN- γ) and/or tumor necrosis factor alpha (TNF- α). In other embodiments, the induced or elicited immune response can reduce or inhibit one or more immune suppression factors that promote growth of the tumor or cancer expressing the antigen, for example, but not limited to,

factors that down regulate MHC presentation, factors that up regulate antigen-specific regulatory T cells (Tregs), PD-L1, FasL, cytokines such as IL-10 and TFG- β , tumor associated macrophages, tumor associated fibroblasts, soluble factors produced by immune suppressor cells, CTLA-4, PD-1, MDSCs, MCP-1, and an immune checkpoint molecule.

[0036] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0037] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of,” and “consisting essentially of” the embodiments or elements presented herein, whether explicitly set forth or not.

[0038] For recitation of numeric ranges herein, each intervening number therebetween with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0039] Some of the quantitative expressions given herein are not qualified with the term “about”. It is understood that, whether the term “about” is used explicitly or not, every quantity given is intended to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including approximations due to the experimental and/or measurement conditions for such value.

[0040] “Adjuvant” as used herein means any molecule added to the immunogenic compositions described herein to enhance the immunogenicity of the antigens encoded by the nucleic acid molecules and the encoding nucleic acid sequences described hereinafter.

[0041] “Biosimilar” (of an approved reference product/biological drug, i.e., reference listed drug) refers to a biological product that is highly similar to the reference product notwithstanding minor differences in clinically inactive components with no clinically meaningful differences between the biosimilar and the reference product in terms of safety, purity and potency, based upon data derived from

(a) analytical studies that demonstrate that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components; (b) animal studies (including the assessment of toxicity); and/or (c) a clinical study or studies (including the assessment of immunogenicity and pharmacokinetics or pharmacodynamics) that are sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which the reference product is licensed and intended to be used and for which licensure is sought for the biosimilar. The biosimilar may be an interchangeable product that may be substituted for the reference product at the pharmacy without the intervention of the prescribing healthcare professional. To meet the additional standard of "interchangeability," the biosimilar is to be expected to produce the same clinical result as the reference product in any given patient and, if the biosimilar is administered more than once to an individual, the risk in terms of safety or diminished efficacy of alternating or switching between the use of the biosimilar and the reference product is not greater than the risk of using the reference product without such alternation or switch. The biosimilar utilizes the same mechanisms of action for the proposed conditions of use to the extent the mechanisms are known for the reference product. The condition or conditions of use prescribed, recommended, or suggested in the labeling proposed for the biosimilar have been previously approved for the reference product. The route of administration, the dosage form, and/or the strength of the biosimilar are the same as those of the reference product and the biosimilar is manufactured, processed, packed or held in a facility that meets standards designed to assure that the biosimilar continues to be safe, pure and potent. The biosimilar may include minor modifications in the amino acid sequence when compared to the reference product, such as N- or C-terminal truncations that are not expected to change the biosimilar performance.

[0042] The term "antibody," as used herein, includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (e.g., IgM). In a typical antibody, each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0043] The term “antibody,” as used herein, also includes antigen-binding fragments of full antibody molecules. The terms “antigen-binding portion” of an antibody, “antigen-binding fragment” of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, e.g., from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, e.g., commercial sources, DNA libraries (including, e.g., phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0044] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')₂ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3- CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g. monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression “antigen-binding fragment,” as used herein.

[0045] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a VH domain associated with a VL domain, the VH and VL domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain VH-VH, VH-VL or VL-VL dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric VH or VL domain.

[0046] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) VH-CH1; (ii) VH-CH2; (iii) VH-CH3; (iv) VH-CH1-CH2; (v) VH-CH1-CH2-CH3; VH-CH2-CH3; (vii) VH-CL; (viii) VL-CH1; (ix) VL-CH2;

(x) VL-CH3; (xi) VL-CH1-CH2; (xii) VL-CH2-CH2-CH3; (xiii) VL-CH2-CH3; and (xiv) VL-CL. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (e.g., 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric VH or VL domain (e.g., by disulfide bond(s)).

[0047] “Coding sequence” or “encoding nucleic acid” as used herein means the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to which the nucleic acid is administered.

[0048] “Complement” or “complementary” as used herein means a nucleic acid can mean Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

[0049] “Consensus” or “consensus sequence” as used herein means a polypeptide sequence based on analysis of an alignment of multiple sequences for the same gene from different organisms. Nucleic acid sequences that encode a consensus polypeptide sequence can be prepared. Immunogenic compositions comprising proteins that comprise consensus sequences and/or nucleic acid molecules that encode such proteins can be used to induce broad immunity against an antigen.

[0050] “Electroporation,” “electro-permeabilization,” or “electro-kinetic enhancement” (“EP”) as used interchangeably herein means the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

[0051] “Fragment” as used herein with respect to nucleic acid sequences means a nucleic acid sequence or a portion thereof, that encodes a polypeptide capable of eliciting an immune response in a mammal that cross reacts with an antigen disclosed herein. The fragments can be DNA fragments selected from at least one of the various nucleotide sequences that encode protein fragments set forth below. Fragments can comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of one or more of the nucleic acid sequences set forth below. In some embodiments, fragments can comprise at least 20 nucleotides or

more, at least 30 nucleotides or more, at least 40 nucleotides or more, at least 50 nucleotides or more, at least 60 nucleotides or more, at least 70 nucleotides or more, at least 80 nucleotides or more, at least 90 nucleotides or more, at least 100 nucleotides or more, at least 150 nucleotides or more, at least 200 nucleotides or more, at least 250 nucleotides or more, at least 300 nucleotides or more, at least 350 nucleotides or more, at least 400 nucleotides or more, at least 450 nucleotides or more, at least 500 nucleotides or more, at least 550 nucleotides or more, at least 600 nucleotides or more, at least 650 nucleotides or more, at least 700 nucleotides or more, at least 750 nucleotides or more, at least 800 nucleotides or more, at least 850 nucleotides or more, at least 900 nucleotides or more, at least 950 nucleotides or more, or at least 1000 nucleotides or more of at least one of the nucleic acid sequences set forth below.

[0052] "Fragment" or "immunogenic fragment" with respect to polypeptide sequences means a polypeptide capable of eliciting an immune response in a mammal that cross reacts with an antigen disclosed herein. The fragments can be polypeptide fragments selected from at least one of the various amino acid sequences below. Fragments of consensus proteins can comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% of a consensus protein. In some embodiments, fragments of consensus proteins can comprise at least 20 amino acids or more, at least 30 amino acids or more, at least 40 amino acids or more, at least 50 amino acids or more, at least 60 amino acids or more, at least 70 amino acids or more, at least 80 amino acids or more, at least 90 amino acids or more, at least 100 amino acids or more, at least 110 amino acids or more, at least 120 amino acids or more, at least 130 amino acids or more, at least 140 amino acids or more, at least 150 amino acids or more, at least 160 amino acids or more, at least 170 amino acids or more, at least 180 amino acids or more of a protein sequence disclosed herein.

[0053] As used herein, the term "genetic construct" refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operably linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

[0054] The term "homology," as used herein, refers to a degree of complementarity. There can be partial homology or complete homology (i.e., identity). A partially complementary sequence that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid is referred to using the functional term "substantially homologous." When used in

reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous," as used herein, refers to a probe that can hybridize to a strand of the double-stranded nucleic acid sequence under conditions of low stringency. When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous," as used herein, refers to a probe that can hybridize to (i.e., is the complement of) the single-stranded nucleic acid template sequence under conditions of low stringency.

[0055] "Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences means that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent. Identity can be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[0056] "Substantially complementary" as used herein means that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 180, 270, 360, 450, 540, or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

[0057] "Substantially identical" as used herein means that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 180, 270, 360, 450, 540 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

[0058] The term "therapeutically effective amount" refers to a therapeutically effective amount of a biologic, compound, or composition that can produce a therapeutic effect in a human subject. A therapeutically effective amount is an amount that can treat, ameliorate, or prevent an identified disease or condition, or to exhibit a detectable therapeutic effect. A therapeutically effective amount is an amount that results in one or more of: (a) a reduction in the severity or duration of a symptom or an indication of

a cancer, e.g., glioblastoma; (b) inhibition of tumor growth, or an increase in tumor necrosis, tumor shrinkage and/or tumor disappearance; (c) delay in tumor growth and development; (d) inhibition of tumor metastasis; (e) prevention of recurrence of tumor growth; (f) increase in survival of a subject with a cancer; and/or (g) a reduction in the use or need for conventional anti-cancer therapy (e.g., reduced or eliminated use of chemotherapeutic or cytotoxic agents) as compared to an untreated subject or a subject administered the anti-cancer therapy as monotherapy. The precise effective amount for a subject will depend upon the subject's body weight, size, and health; the nature and extent of the condition; and the therapeutic selected for administration. Therapeutically effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician.

[0059] As used herein, “therapeutic effect” is a consequence of a medical treatment of any kind, the results of which are judged to be desirable and beneficial. This is true whether the result was expected, unexpected, or even an unintended consequence of the treatment. A therapeutic effect may also be an objectively identifiable improvement as noted by the clinician or other qualified observer.

[0060] “Variant” used herein with respect to a nucleic acid means (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto. A variant may be a nucleic acid sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The nucleic acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof.

[0061] “Variant” with respect to a polypeptide is one that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retains at least one biological activity of the reference polypeptide. Variant can also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A variant may be an amino acid sequence that is substantially identical over the full length of the amino acid sequence or fragment thereof. The amino acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino acid sequence or a fragment thereof.

[0062] “Vector” as used herein means a nucleic acid sequence containing an origin of replication. A vector can be a viral vector, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector can be a DNA or RNA vector. A vector can be a self-replicating extrachromosomal vector, and in one embodiment, is an expression plasmid. The vector can contain or include one or more heterologous nucleic acid sequences.

[0063] “Immune response” as used herein means the activation of a host’s immune system, e.g., that of a mammal, in response to the introduction of antigen. The immune response can be in the form of a cellular or humoral response, or both.

[0064] “Nucleic acid” or “oligonucleotide” or “polynucleotide” as used herein means at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid can be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that can hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

[0065] Nucleic acids can be single stranded or double-stranded or can contain portions of both double-stranded and single-stranded sequence. The nucleic acid can be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods.

[0066] “Operably linked” as used herein means that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter can be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene can be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance can be accommodated without loss of promoter function.

[0067] A “peptide,” “protein,” or “polypeptide” as used herein can mean a linked sequence of amino acids and can be natural, synthetic, or a modification or combination of natural and synthetic.

[0068] “Promoter” as used herein means a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter can comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter can also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents.

Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

[0069] “Signal peptide” and “leader sequence” are used interchangeably herein and refer to an amino acid sequence that can be linked at the amino terminus of a protein set forth herein. Signal peptides/leader sequences typically direct localization of a protein. Signal peptides/leader sequences used herein can facilitate secretion of the protein from the cell in which it is produced. Signal peptides/leader sequences are often cleaved from the remainder of the protein, often referred to as the mature protein, upon secretion from the cell. Signal peptides/leader sequences are linked at the amino terminus (i.e., N terminus) of the protein.

[0070] As used herein, the expression "a subject in need thereof" means a human or non-human mammal that exhibits one or more symptoms or indications of brain cancer, and/or who has been diagnosed with brain cancer, including for example glioblastoma, and who needs treatment for the same. In many embodiments, the term "subject" may be interchangeably used with the term "patient". For example, a human subject may be diagnosed with a primary or a metastatic tumor and/or with one or more symptoms or indications including, but not limited to, unexplained weight loss, general weakness, persistent fatigue, loss of appetite, fever, night sweats, bone pain, shortness of breath, swollen abdomen, chest pain/pressure, enlargement of spleen, and elevation in the level of a cancer-related biomarker (e.g., CA125). The expression includes subjects with primary or established tumors. The term includes subjects with primary or metastatic tumors (advanced malignancies). For example, the expression includes subjects who have been newly diagnosed. In some embodiment, the expression includes subjects for whom treatment in accordance with the disclosed methods is an initial treatment (e.g., “first line” treatment, wherein the patient has not received prior systemic treatment for the cancer). In certain embodiments, the expression includes subjects for whom treatment in accordance with the disclosed methods is “second-line” treatment, wherein the patient has been previously treated with “standard-of-care” therapy including, but not limited to chemotherapy, surgery and radiation.

[0071] As used herein, the term "treat", "treating", or the like, means to alleviate symptoms, eliminate the causation of symptoms either on a temporary or permanent basis, to delay or inhibit tumor growth, to reduce tumor cell load or tumor burden, to promote tumor regression, to cause tumor shrinkage, necrosis and/or disappearance, to prevent tumor recurrence, to prevent or inhibit metastasis, to inhibit metastatic tumor growth, and/or to increase duration of survival of the subject.

[0072] As used herein, the phrase “in combination with” means that the cancer antigens hTERT, PSMA, and WT-1 are administered to the subject at the same time as, just before, or just after

administration of the adjuvant, the programmed death receptor-1 (PD-1) checkpoint inhibitor, radiation therapy, and/or chemotherapeutic agent. In certain embodiments, the cancer antigens are administered as a co-formulation with the adjuvant.

[0073] As used herein, unless otherwise noted, the term "clinically proven" (used independently or to modify the terms "safe" and/or "effective") shall mean that it has been proven by a clinical trial wherein the clinical trial has met the approval standards of U.S. Food and Drug Administration, EMA or a corresponding national regulatory agency. For example, proof may be provided by the clinical trial described in the example provided herein.

[0074] The term "clinically proven safe", as it relates to a dose, dosage regimen, treatment or method with cancer antigens hTERT, PSMA, WT1 (for example, administered as INO-5401 or a biosimilar or bioequivalent thereof) in combination with the adjuvant, such as IL-12 (for example, administered as INO-9012 or a biosimilar or bioequivalent thereof) and a programmed death receptor-1 (PD-1) checkpoint inhibitor, such as an anti-PD-1 antibody (e.g., the anti-PD-1 antibody REGN2810 or a biosimilar or bioequivalent thereof), refers to a favorable risk:benefit ratio with an acceptable frequency and/or acceptable severity of treatment-emergent adverse events (referred to as AEs or TEAEs) compared to the standard of care or to another comparator. An adverse event is an untoward medical occurrence in a patient administered a medicinal product. One index of safety is the National Cancer Institute (NCI) incidence of adverse events (AE) graded per Common Toxicity Criteria for Adverse Events CTCAE v4.03.

[0075] The terms "clinically proven efficacy" and "clinically proven effective" as used herein in the context of a dose, dosage regimen, treatment or method refer to the effectiveness of a particular dose, dosage or treatment regimen. Efficacy can be measured based on change in the course of the disease in response to an agent of the present invention. For example, a combination of cancer antigens hTERT, PSMA, WT1, and adjuvant, (for example, INO-5401 or a biosimilar or bioequivalent thereof in combination with INO-9012 or a biosimilar or bioequivalent thereof) with a PD-1 checkpoint inhibitor, such as an anti-PD-1 antibody (e.g., the anti-PD-1 antibody cemiplimab or a biosimilar or bioequivalent thereof), is administered to a patient in an amount and for a time sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder that is being treated. Various indicators that reflect the extent of the subject's illness, disease or condition may be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for example, clinically recognized indicators of disease severity, symptoms, or manifestations of the disorder in question. The degree of improvement generally is determined by a physician, who may make this determination based on signs, symptoms, biopsies, or other test results, and who may also employ questionnaires that are administered to the subject, such as quality-of-life questionnaires developed for a given disease. For

example, the combination of cancer antigens hTERT, PSMA, WT1, and adjuvant, (for example, INO-5401 or a biosimilar or bioequivalent thereof in combination with INO-9012 or a biosimilar or bioequivalent thereof) with an anti-PD-1 antibody (e.g., the anti-PD-1 antibody cemiplimab or a biosimilar or bioequivalent thereof), may be administered to achieve an improvement in a patient's condition related to brain cancer, such as glioblastoma (GBM). Improvement may be indicated by an improvement in an index of disease activity, by amelioration of clinical symptoms or by any other measure of disease activity.

[0076] As used herein, "INO-5401" refers to an immunologic composition of three DNA plasmids: a DNA plasmid comprising an insert encoding hTERT operably controlled by a promoter, a DNA plasmid comprising an insert encoding WT1 operably controlled by a promoter, and a DNA plasmid comprising an insert encoding PSMA operably controlled by a promoter.

[0077] As used herein, the term "radiation therapy", also referred to as "XRT," means using ionizing radiation to kill cancer cells, generally as part of anti-cancer therapy. X-rays, gamma rays or charged particles (e.g., protons or electrons) are used to generate ionizing radiation.

[0078] Radiation therapy may be delivered by a machine placed outside the patient's body (external- beam radiation therapy), or by a source placed inside a patient's body (internal radiation therapy or brachytherapy), or through systemic radioisotopes delivered intravenously or orally (systemic radioisotope therapy). Radiation therapy may be planned and administered in conjunction with imaging-based techniques such as a computed tomography (CT), magnetic resonance imaging (MRI) to accurately determine the dose and location of radiation to be administered. In various embodiments, radiation therapy is selected from the group consisting of total all-body radiation therapy, conventional external beam radiation therapy, stereotactic radiosurgery, stereotactic body radiation therapy, 3-D conformal radiation therapy, intensity- modulated radiation therapy, image-guided radiation therapy, tomotherapy, brachytherapy, and systemic radiation therapy. Depending upon the intent, in certain embodiments, radiation therapy is curative, adjuvanting or palliative. In specific embodiments, the term "radiation therapy" refers to hypofractionated radiation therapy. Hypofractionated radiation therapy refers to a radiation treatment schedule in which the total dose of radiation is divided into large doses and treatments are given once a day or less often. Hypofractionated radiotherapy may provide more radiation per dose in fewer doses than standard radiotherapy. In various embodiments, each fraction comprises 2-20 Gy. For example, a radiation dose of 50 Gy may be split up into 10 fractions, each comprising 5 Gy. In certain embodiments, the 2 or more fractions are administered on consecutive or sequential days. In certain other embodiments, the 2 or more fractions are administered once in 2 days, once in 3 days, once in 4 days, once in 5 days, once in 6 days, once in 7 days, or in a combination thereof.

[0079] According to certain embodiments, provided herein are methods for treating cancer, such as brain cancer (for example, glioblastoma) in a subject. The disclosed methods comprise administering to the subject an immunogenic composition of cancer antigens human telomerase reverse transcriptase (hTERT), Wilms Tumor-1 (WT-1), and prostate specific membrane antigen (PSMA); an adjuvant; and an anti-programmed cell death receptor 1 (PD-1) antibody or antibody-binding fragment thereof.

[0080] Disclosed herein are optimized consensus sequences of cancer antigens hTERT, WT-1, and PSMA. In one embodiment, the antigen encoded by the optimized consensus sequence is capable of eliciting an immune response in a mammal. In one embodiment, the antigen encoded by the optimized consensus sequence can comprise an epitope(s) that makes it particularly effective as an immunogen against which an immune response can be induced.

[0081] In one embodiment is provided an optimized consensus PSMA designed to break tolerance to native human PSMA. In one embodiment, a human optimized consensus PSMA encoding sequence is as set forth in SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO: 21, or SEQ ID NO: 28. In one embodiment, a human optimized consensus PSMA encoded antigen has an amino acid sequence as set forth in SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO: 28.

[0082] In one embodiment, an optimized consensus WT-1 is designed to break tolerance to native human WT-1. In one embodiment, a human optimized consensus WT-1 encoding sequence is as set forth in SEQ ID NO:15 or SEQ ID NO: 27. In one embodiment, a human optimized consensus WT-1 encoded antigen has an amino acid sequence as set forth in SEQ ID NO:16 or SEQ ID NO: 26.

[0083] In one embodiment, an optimized consensus TERT is designed to break tolerance to native human TERT. In one embodiment, a human optimized consensus TERT encoding sequence is as set forth in SEQ ID NO:17 or SEQ ID NO:19. In one embodiment, a human optimized consensus TERT encoded antigen has an amino acid sequence as set forth in SEQ ID NO:18 and SEQ ID NO:20.

[0084] The disclosed vaccines may further comprise an adjuvant. In certain embodiments, the disclosed methods of treatment further comprise administering to the subject an adjuvant. In certain embodiments, the adjuvant is IL12. IL12 may be included in a vaccine in the form of its p35 and p40 subunits. The adjuvant IL 12 may be administered to the subject as its p35 and p40 subunits. The IL12 p35 and p40 subunits may be encoded by the same expression vector or by separate expression vectors. In one embodiment, the IL12 p35 encoding sequence is as set forth in SEQ ID NO:22. In one embodiment, the IL12 p35 subunit has an amino acid sequence as set forth in SEQ ID NO:23. In one embodiment, the IL12 p40 encoding sequence is as set forth in SEQ ID NO:24. In one embodiment, the IL12 p40 subunit has an amino acid sequence as set forth in SEQ ID NO:25.

[0085] The cancer antigens TERT, WT-1, PSMA, and/or adjuvant can be present in the vaccine or administered to the subject as the polypeptide, fragment thereof, variant thereof, nucleic

acid sequence encoding the polypeptide, fragment or variant thereof, or any combination thereof. The cancer antigen can be any form that induces an immune response in a subject. The nucleic acid sequence can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The nucleic acid sequence can also include additional sequences that encode linker or tag sequences that are linked to the antigen by a peptide bond. The amino acid sequence can be a protein, a peptide, a variant thereof, a fragment thereof, or a combination thereof.

[0086] The cancer antigens TERT, WT-1, PSMA, and/or adjuvant can be included in a vaccine or administered to the subject as the polypeptide, fragment thereof, variant thereof, nucleic acid sequence encoding the polypeptide, fragment or variant thereof, or any combination thereof. The cancer antigen can be any form that induces an immune response in a subject. The nucleic acid sequence can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The nucleic acid sequence can also include additional sequences that encode linker or tag sequences that are linked to the antigen by a peptide bond. The amino acid sequence can be a protein, a peptide, a variant thereof, a fragment thereof, or a combination thereof.

[0087] The cancer antigens TERT, WT-1, PSMA and/or IL-12 can be included in a vaccine or administered as one or more nucleic acid molecules, for example but not limited to, an expression vector(s). An expression vector can be a circular plasmid or a linear nucleic acid. An expression vector is capable of directing expression of a particular nucleotide sequence in an appropriate subject cell. An expression vector can have a promoter operably linked to the antigen-encoding nucleotide sequence, which may be operably linked to termination signals. An expression vector can also contain sequences required for proper translation of the nucleotide sequence. The expression vector comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development.

[0088] In one embodiment, the nucleic acid is an RNA molecule. Accordingly, in one embodiment, the invention provides an RNA molecule encoding one or more polypeptides of interest. The RNA may be plus-stranded. Accordingly, in some embodiments, the RNA molecule can be translated by cells without needing any intervening replication steps such as reverse transcription. A RNA molecule useful with the invention may have a 5' cap (e.g. a 7- methylguanosine). This cap can enhance in vivo translation of the RNA. The 5' nucleotide of a RNA molecule useful with the invention may have a 5' triphosphate group. In a capped RNA this may be linked to a 7-methylguanosine via a 5'-to-5' bridge. A RNA molecule may have a 3' poly-A tail. It may also include

a poly-A polymerase recognition sequence (e.g., AAUAAA) near its 3' end. A RNA molecule useful with the invention may be single- stranded. In some embodiments, the RNA molecule is a naked RNA molecule. In one embodiment, the RNA molecule is comprised within a vector.

[0089] In one embodiment, the RNA has 5' and 3' UTRs. In one embodiment, the 5' UTR is between zero and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

[0090] The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the gene of interest. Alternatively, UTR sequences that are not endogenous to the gene of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the gene of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of RNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

[0091] In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous gene. Alternatively, when a 5' UTR that is not endogenous to the gene of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many RNAs is known in the art. In other embodiments, the 5' UTR can be derived from an RNA virus whose RNA genome is stable in cells. In other embodiments, various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the RNA.

[0092] In one embodiment, the RNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability of RNA in the cell.

[0093] In one embodiment, the RNA is a nucleoside-modified RNA. Nucleoside-modified RNA have particular advantages over non-modified RNA, including for example, increased stability, low or absent innate immunogenicity, and enhanced translation.

[0094] The expression vector may be a circular plasmid, which may transform a target cell by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication). The vector can be pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing DNA encoding the antigen and enabling a cell to translate the sequence to an antigen that is recognized by the immune system.

[0095] Also provided herein is a linear nucleic acid immunogenic composition, or linear expression cassette ("LEC"), that is capable of being efficiently delivered to a subject via electroporation and expressing one or more desired antigens. The LEC may be any linear DNA devoid of any phosphate backbone. The DNA may encode one or more antigens. The LEC may contain a promoter, an intron, a stop codon, and/or a polyadenylation signal. The expression of the antigen may be controlled by the promoter. The LEC may not contain any antibiotic resistance genes and/or a phosphate backbone. The LEC may not contain other nucleotide sequences unrelated to the desired antigen gene expression. The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the antigen. The plasmid can be pNP (Puerto Rico/34) or pM2 (New Caledonia/99). The plasmid may be WLV009, pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing DNA encoding the antigen and enabling a cell to translate the sequence to an antigen that is recognized by the immune system. The LEC can be pcrM2. The LEC can be pcrNP. pcrNP and pcrMR can be derived from pNP (Puerto Rico/34) and pM2 (New Caledonia/99), respectively.

[0096] The vector can comprise heterologous nucleic acid encoding the above described antigens and can further comprise an initiation codon, which can be upstream of the one or more cancer antigen coding sequence(s), and a stop codon, which can be downstream of the coding sequence(s) of the above described antigens.

[0097] The vector may have a promoter. A promoter may be any promoter that is capable of driving gene expression and regulating expression of the isolated nucleic acid. Such a promoter is a cis-acting sequence element required for transcription via a DNA dependent RNA polymerase, which transcribes the antigen sequence described herein. Selection of the promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter may be positioned about the same distance from the transcription start in the vector as it is from the transcription start site in its natural setting. However, variation in this distance may be accommodated without loss of promoter function.

[0098] The initiation and termination codon can be in frame with the coding sequence(s) of the above described antigens. The vector can also comprise a promoter that is operably linked to the coding sequence(s) of the above described antigens. The promoter operably linked to the coding sequence(s) of the above described antigens can be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter can also be a promoter from a human gene such as human actin, human

myosin, human hemoglobin, human muscle creatine, or human metallothionein. The promoter can also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

[0099] The vector can also comprise a polyadenylation signal, which can be downstream of the coding sequence(s) of the above described antigens and/or antibodies. The polyadenylation signal can be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β -globin polyadenylation signal. The SV40 polyadenylation signal can be a polyadenylation signal from a pCEP4 vector (Invitrogen, San Diego, CA).

[0100] The vector can also comprise an enhancer upstream of the above described antigens.

[0101] The enhancer can be necessary for expression. The enhancer can be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, HA, RSV or EBV.

[0102] The vector may include an enhancer and an intron with functional splice donor and acceptor sites. The vector may contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

[0103] The disclosed methods may comprise administration of a plurality of copies of a single nucleic acid molecule such as a single plasmid, or a plurality of copies of two or more different nucleic acid molecules such as two or more different plasmids. For example, the methods may comprise administration of two, three, four, five, six, seven, eight, nine or ten or more different nucleic acid molecules.

[0104] The nucleic acid molecules used in accordance with the disclosed methods, such as plasmids, may collectively contain coding sequence for a single antigen or for multiple antigens. As an example, in one embodiment, the antigens are multiple antigens selected from TERT and one or more additional cancer antigens. In one exemplary embodiment, the antigens are TERT and WT-1. In one exemplary embodiment, the antigens are TERT and PSMA. In one exemplary embodiment, the antigens are PSMA and one or more additional cancer antigens. In one exemplary embodiment, the antigens are PSMA and WT-1. In another exemplary embodiment, the antigens are TERT, WT-1 and PSMA.

[0105] The vector can further comprise elements or reagents that inhibit it from integrating into the chromosome. The vector can comprise a mammalian origin of replication in order to maintain the vector extrachromosomally and produce multiple copies of the vector in a cell. The vector can be pVAX1, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which can comprise the Epstein Barr

virus origin of replication and nuclear antigen EBNA-1 coding region, which can produce high copy episomal replication without integration. The vector can be pVAX1 or a pVax1 variant with changes such as the variant plasmid described herein. The variant pVax1 plasmid is a 2998 base pair variant of the backbone vector plasmid pVAX1 (Invitrogen, Carlsbad CA). The CMV promoter is located at bases 137-724. The T7 promoter/priming site is at bases 664-683. Multiple cloning sites are at bases 696-811.

[0106] Bovine GH polyadenylation signal is at bases 829-1053. The Kanamycin resistance gene is at bases 1226-2020. The pUC origin is at bases 2320-2993.

[0107] Based upon the sequence of pVAX1 available from Invitrogen, the following mutations were found in the sequence of pVAX1 that was used as the backbone for plasmids 1-6 set forth herein:

C>G241 in CMV promoter

C>T 1942 backbone, downstream of the bovine growth hormone polyadenylation signal (bGHpolyA)

A> - 2876 backbone, downstream of the Kanamycin gene

C>T 3277 in pUC origin of replication (Ori) high copy number mutation (see Nucleic Acid Research 1985)

G>C 3753 in very end of pUC Ori upstream of RNaseH site

Base pairs 2, 3 and 4 are changed from ACT to CTG in backbone, upstream of CMV promoter. The backbone of the vector can be pAV0242. The vector can be a replication defective adenovirus type 5 (Ad5) vector.

[0108] The vector can also comprise a regulatory sequence, which can be well suited for gene expression in a mammalian or human cell into which the vector is administered. The one or more cancer antigen sequences disclosed herein can comprise a codon, which can allow more efficient transcription of the coding sequence in the host cell.

[0109] The vector can be pSE420 (Invitrogen, San Diego, Calif.), which can be used for protein production in Escherichia coli (E. coli). The vector can also be pYES2 (Invitrogen, San Diego, Calif.), which can be used for protein production in Saccharomyces cerevisiae strains of yeast. The vector can also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which can be used for protein production in insect cells. The vector can also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells. The vector can be expression vectors or systems to produce protein by routine techniques and readily available starting materials including Sambrook et al., Molecular Cloning and Laboratory Manual, Second Ed., Cold Spring Harbor (1989), incorporated fully herein by reference.

[0110] Exemplary DNA plasmids encoding the cancer antigens hTERT, WT-1, and/or PSMA are disclosed in U.S. Application No. 62/899,543, filed September 12, 2019, the entire contents of which are disclosed herein by reference.

[0111] In accordance with the disclosed methods, the subject may be administered about 5 nanograms to about 20 mg of a nucleic acid molecule(s) encoding an antigen or antigens. In some embodiments, the subject may be administered about 5 mg to about 15 mg of a nucleic acid molecule(s) encoding an antigen or antigens. In some embodiments, the subject may be administered about 9 mg to about 11 mg of a nucleic acid molecule(s) encoding an antigen or antigens. In some embodiments, the subject may be administered about 10 mg of a nucleic acid molecule(s) encoding an antigen or antigens.

[0112] The DNA plasmid(s) can be delivered via a variety of routes. Typical delivery routes include parenteral administration, e.g., intradermal, intramuscular or subcutaneous delivery. Other routes include oral administration, intranasal, and intravaginal routes. For the DNA of the vaccine in particular, the vaccine can be delivered to the interstitial spaces of tissues of an individual (Felgner et al., U.S. Pat. Nos. 5,580,859 and 5,703,055, the contents of all of which are incorporated herein by reference in their entirety). The DNA plasmid(s) can also be administered to muscle, or can be administered via intradermal or subcutaneous injections, or transdermally, such as by iontophoresis. Epidermal administration of the DNA plasmid(s) can also be employed. Epidermal administration can involve mechanically or chemically irritating the outermost layer of epidermis to stimulate an immune response to the irritant (Carson et al., U.S. Pat. No. 5,679,647, the contents of which are incorporated herein by reference in its entirety).

[0113] The DNA plasmid(s) can be a liquid preparation such as a suspension, syrup or elixir. The vaccine can also be a preparation for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as a sterile suspension or emulsion.

[0114] The DNA plasmid(s) can be incorporated into liposomes, microspheres or other polymer matrices (Felgner et al., U.S. Pat. No. 5,703,055; Gregoriadis, Liposome Technology, Vols. I to III (2nd ed. 1993), the contents of which are incorporated herein by reference in their entirety). Liposomes can consist of phospholipids or other lipids, and can be nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

[0115] The DNA plasmid(s) can be administered via electroporation, such as by a method described in U.S. Pat. No. 7,664,545, the contents of which are incorporated herein by reference. The electroporation can be by a method and/or apparatus described in U.S. Pat. Nos. 6,302,874; 5,676,646; 6,241,701; 6,233,482; 6,216,034; 6,208,893; 6,192,270; 6,181,964; 6,150,148;

6,120,493; 6,096,020; 6,068,650; and 5,702,359, the contents of which are incorporated herein by reference in their entirety. The electroporation may be carried out via a minimally invasive device.

[0116] The minimally invasive electroporation device (“MID”) may be an apparatus for injecting the vaccine described above and associated fluid into body tissue. The device may comprise a hollow needle, DNA cassette, and fluid delivery means, wherein the device is adapted to actuate the fluid delivery means in use so as to concurrently (for example, automatically) inject DNA into body tissue during insertion of the needle into the said body tissue. This has the advantage that the ability to inject the DNA and associated fluid gradually while the needle is being inserted leads to a more even distribution of the fluid through the body tissue. The pain experienced during injection may be reduced due to the distribution of the DNA being injected over a larger area.

[0117] The MID may inject the DNA plasmid(s) into tissue without the use of a needle. The MID may inject the vaccine as a small stream or jet with such force that the vaccine pierces the surface of the tissue and enters the underlying tissue and/or muscle. The force behind the small stream or jet may be provided by expansion of a compressed gas, such as carbon dioxide through a micro-orifice within a fraction of a second. Examples of minimally invasive electroporation devices, and methods of using them, are described in published U.S. Patent Application No. 20080234655; U.S. Pat. Nos. 6,520,950; 7,171,264; 6,208,893; 6,009,347; 6,120,493; 7,245,963; 7,328,064; and 6,763,264, the contents of each of which are herein incorporated by reference.

[0118] The MID may comprise an injector that creates a high-speed jet of liquid that painlessly pierces the tissue. Such needle-free injectors are commercially available. Examples of needle-free injectors that can be utilized herein include those described in U.S. Pat. Nos. 3,805,783; 4,447,223; 5,505,697; and 4,342,310, the contents of each of which are herein incorporated by reference.

[0119] A desired vaccine in a form suitable for direct or indirect electrotransport may be introduced (e.g., injected) using a needle-free injector into the tissue to be treated, usually by contacting the tissue surface with the injector so as to actuate delivery of a jet of the agent, with sufficient force to cause penetration of the vaccine into the tissue. For example, if the tissue to be treated is mucosa, skin or muscle, the agent is projected towards the mucosal or skin surface with sufficient force to cause the agent to penetrate through the stratum corneum and into dermal layers, or into underlying tissue and muscle, respectively.

[0120] Needle-free injectors are well suited to deliver DNA plasmid(s) to all types of tissues, particularly to skin and mucosa. In some embodiments, a needle-free injector may be used to propel a liquid that contains the DNA plasmid(s) to the surface and into the subject's skin or mucosa. Representative examples of the various types of tissues that can be treated using the invention methods include pancreas, larynx, nasopharynx, hypopharynx, oropharynx, lip, throat, lung, heart,

kidney, muscle, breast, colon, prostate, thymus, testis, skin, mucosal tissue, ovary, blood vessels, or any combination thereof.

[0121] The MID may have needle electrodes that electroporate the tissue. By pulsing between multiple pairs of electrodes in a multiple electrode array, for example set up in rectangular or square patterns, provides improved results over that of pulsing between a pair of electrodes. Disclosed, for example, in U.S. Pat. No. 5,702,359 entitled “Needle Electrodes for Mediated Delivery of Drugs and Genes” is an array of needles wherein a plurality of pairs of needles may be pulsed during the therapeutic treatment. In that application, which is incorporated herein by reference as though fully set forth, needles were disposed in a circular array, but have connectors and switching apparatus enabling a pulsing between opposing pairs of needle electrodes. A pair of needle electrodes for delivering recombinant expression vectors to cells may be used. Such a device and system is described in U.S. Pat. No. 6,763,264, the contents of which are herein incorporated by reference. Alternatively, a single needle device may be used that allows injection of the DNA and electroporation with a single needle resembling a normal injection needle and applies pulses of lower voltage than those delivered by presently used devices, thus reducing the electrical sensation experienced by the patient.

[0122] The MID may comprise one or more electrode arrays. The arrays may comprise two or more needles of the same diameter or different diameters. The needles may be evenly or unevenly spaced apart. The needles may be between 0.005 inches and 0.03 inches, between 0.01 inches and 0.025 inches; or between 0.015 inches and 0.020 inches. The needle may be 0.0175 inches in diameter. The needles may be 0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm, 2.5 mm, 3.0 mm, 3.5 mm, 4.0 mm, or more spaced apart.

[0123] The MID may consist of a pulse generator and a two or more-needle injectors that deliver the DNA plasmid(s) and electroporation pulses in a single step. The pulse generator may allow for flexible programming of pulse and injection parameters via a flash card operated personal computer, as well as comprehensive recording and storage of electroporation and patient data. The pulse generator may deliver a variety of volt pulses during short periods of time. For example, the pulse generator may deliver three 15-volt pulses of 100 ms in duration. An example of such a MID is the Elgen 1000 system by Inovio Biomedical Corporation, which is described in U.S. Pat. No. 7,328,064, the contents of which are herein incorporated by reference.

[0124] The MID may be a CELLECTRA® (Inovio Pharmaceuticals, Blue Bell Pa.) device and system, which is a modular electrode system, that facilitates the introduction of a macromolecule, such as a DNA, into cells of a selected tissue in a body or plant. The modular electrode system may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to

the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The macromolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the macromolecule into the cell between the plurality of electrodes. Cell death due to overheating of cells is minimized by limiting the power dissipation in the tissue by virtue of constant-current pulses. The CELLECTRA® device and system is described in U.S. Pat. No. 7,245,963, the contents of which are herein incorporated by reference.

[0125] The MID may be an Elgen 1000 system (Inovio Pharmaceuticals). The Elgen 1000 system may comprise device that provides a hollow needle; and fluid delivery means, wherein the apparatus is adapted to actuate the fluid delivery means in use so as to concurrently (for example automatically) inject fluid, the described DNA plasmid(s) herein, into body tissue during insertion of the needle into the said body tissue. The advantage is the ability to inject the fluid gradually while the needle is being inserted leads to a more even distribution of the fluid through the body tissue. It is also believed that the pain experienced during injection is reduced due to the distribution of the volume of fluid being injected over a larger area.

[0126] In addition, the automatic injection of fluid facilitates automatic monitoring and registration of an actual dose of fluid injected. This data can be stored by a control unit for documentation purposes if desired.

[0127] It will be appreciated that the rate of injection could be either linear or non-linear and that the injection may be carried out after the needles have been inserted through the skin of the subject to be treated and while they are inserted further into the body tissue.

[0128] Suitable tissues into which fluid may be injected by the apparatus of the present invention include tumor tissue, skin or liver tissue but may be muscle tissue.

[0129] The apparatus further comprises needle insertion means for guiding insertion of the needle into the body tissue. The rate of fluid injection is controlled by the rate of needle insertion. This has the advantage that both the needle insertion and injection of fluid can be controlled such that the rate of insertion can be matched to the rate of injection as desired. It also makes the apparatus easier for a user to operate. If desired means for automatically inserting the needle into body tissue could be provided.

[0130] A user could choose when to commence injection of fluid. Ideally however, injection is commenced when the tip of the needle has reached muscle tissue and the apparatus may include means for sensing when the needle has been inserted to a sufficient depth for injection of the fluid to commence. This means that injection of fluid can be prompted to commence automatically

when the needle has reached a desired depth (which will normally be the depth at which muscle tissue begins). The depth at which muscle tissue begins could for example be taken to be a preset needle insertion depth such as a value of 4 mm which would be deemed sufficient for the needle to get through the skin layer.

[0131] The sensing means may comprise an ultrasound probe. The sensing means may comprise a means for sensing a change in impedance or resistance. In this case, the means may not as such record the depth of the needle in the body tissue but will rather be adapted to sense a change in impedance or resistance as the needle moves from a different type of body tissue into muscle. Either of these alternatives provides a relatively accurate and simple to operate means of sensing that injection may commence. The depth of insertion of the needle can further be recorded if desired and could be used to control injection of fluid such that the volume of fluid to be injected is determined as the depth of needle insertion is being recorded.

[0132] The apparatus may further comprise: a base for supporting the needle; and a housing for receiving the base therein, wherein the base is moveable relative to the housing such that the needle is retracted within the housing when the base is in a first rearward position relative to the housing and the needle extends out of the housing when the base is in a second forward position within the housing. This is advantageous for a user as the housing can be lined up on the skin of a patient, and the needles can then be inserted into the patient's skin by moving the housing relative to the base.

[0133] As stated above, it is desirable to achieve a controlled rate of fluid injection such that the fluid is evenly distributed over the length of the needle as it is inserted into the skin. The fluid delivery means may comprise piston driving means adapted to inject fluid at a controlled rate. The piston driving means could for example be activated by a servo motor. However, the piston driving means may be actuated by the base being moved in the axial direction relative to the housing. It will be appreciated that alternative means for fluid delivery could be provided. Thus, for example, a closed container which can be squeezed for fluid delivery at a controlled or non-controlled rate could be provided in the place of a syringe and piston system.

[0134] The apparatus described above could be used for any type of injection. It is however envisaged to be particularly useful in the field of electroporation and so it may further comprises means for applying a voltage to the needle. This allows the needle to be used not only for injection but also as an electrode during, electroporation. This is particularly advantageous as it means that the electric field is applied to the same area as the injected fluid. There has traditionally been a problem with electroporation in that it is very difficult to accurately align an electrode with previously injected fluid and so users have tended to inject a larger volume of fluid than is required over a larger area and to apply an electric field over a higher area to attempt to guarantee an overlap

between the injected substance and the electric field. Using the present invention, both the volume of fluid injected and the size of electric field applied may be reduced while achieving a good fit between the electric field and the fluid.

[0135] Upon administration of nucleic acid molecule(s) encoding cancer antigens hTERT, PSMA, and WT-1 to the subject, the transfected cells will express and secrete one or more of the cancer antigens. These secreted proteins, or synthetic antigens, will be recognized as foreign by the immune system, which will mount an immune response that can include: antibodies made against the one or more cancer antigens, and T-cell response specifically against the one or more cancer antigens. In some examples, a mammal administered the immunogenic composition discussed herein will have a primed immune system and when challenged with the one or more cancer antigens as disclosed herein, the primed immune system will allow for rapid clearing of subsequent cancer antigens as disclosed herein, whether through the humoral, cellular, or both cellular and humoral immune responses.

[0136] The recombinant cancer antigen can induce antigen-specific T cell and/or high titer antibody responses, thereby inducing or eliciting an immune response that is directed to or reactive against the cancer or tumor expressing the antigen. In some embodiments, the induced or elicited immune response can be a cellular, humoral, or both cellular and humoral immune responses. In some embodiments, the induced or elicited cellular immune response can include induction or secretion of interferon-gamma (IFN- γ) and/or tumor necrosis factor alpha (TNF- α). In other embodiments, the induced or elicited immune response can reduce or inhibit one or more immune suppression factors that promote growth of the tumor or cancer expressing the antigen, for example, but not limited to, factors that down regulate MHC presentation, factors that up regulate antigen-specific regulatory T cells (Tregs), PD-L1, FasL, cytokines such as IL-10 and TGF- β , tumor associated macrophages, tumor associated fibroblasts, soluble factors produced by immune suppressor cells, CTLA-4, PD-1, MDSCs, MCP-1, and an immune checkpoint molecule.

[0137] The disclosed vaccines may further comprise an anti-PD-1 antibody. The disclosed methods of treatment may further comprise administering to the subject an anti-PD-1 antibody. According to certain embodiments of the present invention, the anti-PD-1 antibody comprises a heavy chain variable region (HCVR), light chain variable region (LCVR), and/or complementarity determining regions (CDRs) comprising the amino acid sequences of any of the anti-PD-1 antibodies as set forth in US Patent Publication No. 20150203579, hereby incorporated in its entirety. In certain exemplary embodiments, the anti-PD-1 antibody that can be used in the context of the disclosed methods comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 1 and the light chain complementarity determining regions (LCDRs) of a light chain variable region (LCVR) comprising

the amino acid sequence of SEQ ID NO: 2. According to certain embodiments, the anti-PD-1 antibody comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 4; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 5; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 6; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 7; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 8. In yet other embodiments, the anti-PD-1 antibody comprises an HCVR comprising SEQ ID NO: 1 and an LCVR comprising SEQ ID NO: 2. In certain embodiments, the methods of the present invention comprise the use of an anti-PD-1 antibody, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the anti-PD-1 antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 10. An exemplary antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 9 and a light chain comprising the amino acid sequence of SEQ ID NO: 10 is the fully human anti-PD-1 antibody known as REGN2810 and also known as cemiplimab or cemiplimab-rwlc.

[0138] According to certain exemplary embodiments, the methods of the present invention comprise the use of REGN2810, or a biosimilar or bioequivalent thereof. The term “bioequivalent”, as used herein, refers to anti-PD-1 antibodies or PD-1-binding proteins or fragments thereof that are pharmaceutical equivalents or pharmaceutical alternatives whose rate and/or extent of absorption do not show a significant difference with that of REGN2810 when administered at the same molar dose under similar experimental conditions, either single dose or multiple dose. In the context of the invention, the term refers to antigen-binding proteins that bind to PD-1 which do not have clinically meaningful differences with REGN2810 in their safety, purity and/or potency.

[0139] According to certain embodiments of the present invention, the anti-human PD-1 antibody comprises a HCVR having 90%, 95%, 98% or 99% sequence identity to SEQ ID NO: 1.

[0140] According to certain embodiments of the present invention, the anti-human PD-1 antibody comprises a LCVR having 90%, 95%, 98% or 99% sequence identity to SEQ ID NO: 2.

[0141] According to certain embodiments of the present invention, the anti-human PD-1 antibody comprises a HCVR comprising an amino acid sequence of SEQ ID NO: 1 having no more than 5 amino acid substitutions. According to certain embodiments of the present invention, the anti-human PD-1 antibody comprises a LCVR comprising an amino acid sequence of SEQ ID NO: 2 having no more than 2 amino acid substitutions.

[0142] Sequence identity may be measured by any method known in the art (e.g., GAP, BESTFIT, and BLAST).

[0143] The present invention also includes use of anti-PD-1 antibodies in methods to treat cancer, wherein the anti-PD-1 antibodies comprise variants of any of the HCVR, LCVR and/or CDR amino acid sequences disclosed herein having one or more conservative amino acid substitutions. For example, the present invention includes use of anti-PD-1 antibodies having HCVR, LCVR and/or CDR amino acid sequences with, e.g., 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR and/or CDR amino acid sequences disclosed herein.

[0144] The amount of anti-PD-1 antibody administered to a subject according to the disclosed methods can be a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" of anti-PD-1 antibody is an amount that results in one or more of: (a) a reduction in the severity or duration of a symptom or an indication of a cancer, e.g., glioblastoma; (b) inhibition of tumor growth, or an increase in tumor necrosis, tumor shrinkage and/or tumor disappearance; (c) delay in tumor growth and development; (d) inhibition of tumor metastasis; (e) prevention of recurrence of tumor growth; (f) increase in survival of a subject with a cancer; and/or (g) a reduction in the use or need for conventional anti-cancer therapy (e.g., reduced or eliminated use of chemotherapeutic or cytotoxic agents) as compared to an untreated subject or a subject administered the antibody as monotherapy.

[0145] In the case of an anti-PD-1 antibody or antigen-binding fragment thereof, a therapeutically effective amount can be from about 0.05 mg to about 600 mg, from about 1 mg to about 500 mg, from about 10 mg to about 450 mg, from about 50 mg to about 400 mg, from about 75 mg to about 350 mg, or from about 100 mg to about 300 mg of the antibody. For example, in various embodiments, the amount of the anti-PD-1 antibody is about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, or about 600 mg, of the anti-PD-1 antibody. In one embodiment, 250 mg of an anti-PD-1 antibody is administered according to the methods of the present invention. In one embodiment, 200 mg of an anti-PD-1 antibody is administered according to the methods of the present invention. In one

embodiment, 350 mg of an anti-PD- 1 antibody is administered according to the methods of the present invention.

[0146] The anti-PD-1 antibody may be administered to the subject in multiple doses, e.g., as part of a specific therapeutic dosing regimen. For example, the therapeutic dosing regimen may comprise administering one or more doses of an anti-PD-1 antibody to the subject at a frequency of about once a day, once every two days, once every three days, once every four days, once every five days, once every six days, once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, or less frequently.

[0147] In some embodiments, the anti-PD-1 antibody is contained within a pharmaceutical composition. The pharmaceutical compositions of the invention may be formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0148] Various delivery systems are known and can be used to administer the anti-PD-1 antibody, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262: 4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0149] The anti-PD-1 antibody can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering the anti- PD-1 antibody. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition of the anti-PD-1 antibody. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical

composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0150] In certain situations, the anti-PD-1 antibody can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used; see, *Medical Applications of Controlled Release*, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Fla. In yet another embodiment, a controlled release system can be placed in proximity of the target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, 1984, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, *Science* 249:1527-1533.

[0151] Injectable preparations of the anti-PD-1 antibody may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0152] In certain embodiments, the anti-PD-1 antibody is formulated in a pharmaceutical composition for use in intravenous administration.

[0153] In certain embodiments, the methods further comprise administering radiation therapy to the subject. In certain embodiments, the one or more doses of radiation therapy are administered to the subject at a frequency of about once a day, once every two days, once every three days, once every four days, once every five days, once every six days, once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, or less frequently.

[0154] In certain embodiments, the radiation therapy is hypofractionated radiation therapy. In some embodiments, the subject is administered 20-60 Gy in 2-20 fractions. In certain embodiments, the hypofractionated radiation therapy comprises 15 fractions. In certain embodiments, the 15

fractions are administered on 15-25 consecutive days. In certain embodiments, the 15 fractions are administered on 21 consecutive days.

[0155] In certain embodiments, the methods further comprise administering a chemotherapeutic agent to the subject, for example, temozolomide (TMZ). The chemotherapeutic agent can be administered with the radiation therapy. For example, TMZ is administered at a daily dose of 75 mg/m² concomitant with hypofractionated radiation therapy. In some embodiments, subjects having a tumor with a methylated MGMT promoter will be administered maintenance therapy of the chemotherapeutic agent. For example, following radiation therapy, subjects having a tumor with a methylated MGMT promoter may receive TMZ at a starting dose of 150 mg/m²/day for 6 cycles on the first 5 days of a 28-day cycle (5 days “on,” 23 days “off”) with increased each maintenance cycle by 50 mg/m²/dose to a maximum of 200 mg/m²/dose, in the absence of hematologic toxicity. In some embodiments, the maintenance therapy will start approximately three to five weeks, preferably about 4 weeks, after the last dose of radiation therapy.

[0156] In particular embodiments, the disclosed methods can mediate clearance or prevent growth of tumor cells by inducing (1) humoral immunity via B cell responses to generate antibodies that block monocyte chemoattractant protein-1 (MCP-1) production, thereby retarding myeloid derived suppressor cells (MDSCs) and suppressing tumor growth; (2) increase cytotoxic T lymphocyte such as CD8+ (CTL) to attack and kill tumor cells; (3) increase T helper cell responses; (4) and increase inflammatory responses via IFN- γ and TFN- α ; or (5) any combination of the aforementioned. The methods can increase progression-free survival by 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, and 45%. The methods can reduce tumor mass by 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, and 60% after immunization. The methods can prevent and block increases in monocyte chemoattractant protein 1 (MCP- 1), a cytokine secreted by myeloid derived suppressor cells. The methods can increase tumor survival by 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, and 60%.

[0157] The disclosed methods can increase a cellular immune response in a subject by about 50-fold to about 6000-fold, about 50-fold to about 5500-fold, about 50-fold to about 5000- fold, about 50-fold to about 4500-fold, about 100-fold to about 6000-fold, about 150-fold to about 6000-fold, about 200-fold to about 6000-fold, about 250-fold to about 6000-fold, or about 300-fold to about 6000-fold as compared to a cellular immune response in a subject not administered the method or administered a standard-of-care treatment method. In some embodiments the methods can increase the cellular immune response in the subject by about 50-fold, 100-fold, 150-fold, 200-fold, 250-fold, 300-fold, 350-fold, 400-fold, 450-fold, 500- fold, 550-fold, 600-fold, 650-fold, 700-fold, 750-fold,

800-fold, 850-fold, 900-fold, 950-fold, 1000-fold, 1100-fold, 1200-fold, 1300-fold, 1400-fold, 1500-fold, 1600-fold, 1700-fold, 1800-fold, 1900-fold, 2000-fold, 2100-fold, 2200-fold, 2300-fold, 2400-fold, 2500-fold, 2600-fold, 2700-fold, 2800-fold, 2900-fold, 3000-fold, 3100-fold, 3200-fold, 3300-fold, 3400-fold, 3500-fold, 3600-fold, 3700-fold, 3800-fold, 3900-fold, 4000-fold, 4100-fold, 4200-fold, 4300-fold, 4400-fold, 4500-fold, 4600-fold, 4700-fold, 4800-fold, 4900-fold, 5000-fold, 5100-fold, 5200-fold, 5300-fold, 5400-fold, 5500-fold, 5600-fold, 5700-fold, 5800-fold, 5900-fold, or 6000-fold as compared to the cellular immune response in the subject not administered the method or administered a standard-of-care treatment method.

[0158] In some embodiments, the methods can increase tumor-free survival, reduce tumor mass, increase progression-free survival, increase overall survival, or a combination thereof in the subject. The methods can increase tumor-free survival by 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, and 60% in the subject. The methods can reduce tumor mass by 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, and 70% in the subject. The methods can increase progression-free survival by 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, and 60% in the subject. The methods can increase overall survival by 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, and 60% in the subject.

[0159] In certain embodiments, the methods are clinically proven safe, clinically proven effective, or both.

EXAMPLE

Objectives

[0160] **Primary Objective:** To evaluate the safety and tolerability of INO-5401 and INO-9012 delivered by intramuscular (IM) injection followed by EP with CELLECTRA® 2000 in combination with cemiplimab-rwlc in adult subjects with newly-diagnosed GBM.

[0161] **Primary Endpoint(s) and Assessments:**

- Incidence of adverse events (AE) graded per Common Toxicity Criteria for Adverse Events (CTCAE) v4.03, classified by system organ class, preferred term, severity, and relationship to

trial treatment.

- Clinically significant changes in safety laboratory parameters from baseline.

[0162] Secondary Objectives:

▪To evaluate preliminary clinical efficacy and immunogenicity of INO-5401 and INO- 9012 delivered by IM injection followed by EP with CELLECTRA® 2000 in combination with cemiplimab-rwlc in adult subjects with newly-diagnosed GBM.

▪To evaluate preliminary immunogenicity of INO-5401 and INO-9012 delivered by IM injection followed by EP with CELLECTRA® 2000 in combination with REGN2810 in adult subjects with newly-diagnosed GBM.

[0163] Secondary Endpoint(s) and Assessments:

- Overall survival at 18 months (OS18);
- Antigen-specific cellular immune responses assessed by:
 - Interferon- γ secreting T lymphocytes in peripheral blood mononuclear cells (PBMC) by ELISpot;
 - T-cell phenotype (e.g. activation and cytolytic cell, myeloid derived suppressor cell frequency (MDSC)) in PBMC by Flow Cytometry;
 - T cell receptor (TCR) sequencing from PBMCs to assess diversity and putative antigen specificity;
- Antigen-specific humoral responses (e.g. B cell activation/antibody secretion).

[0164] Exploratory Objective(s):

▪To explore correlative association between clinical efficacy and tumor genetics and/or biomarkers.

▪To further evaluate efficacy of INO-5401 and INO-9012 delivered by IM injection followed by EP with CELLECTRA® 2000 in combination with REGN2810 and hypofractionated radiation therapy in adult subjects with newly-diagnosed GBM. Exploratory Endpoint(s):

- Tumor infiltrating lymphocytes (TILs) and immunosuppressive elements, where feasible;
- Expression of tumor oncoproteins, including but not limited to tumor expression of hTERT, WT1, and PSMA by IHC, immunofluorescence (IF) or genome sequencing;
- MicroRNA signatures in blood plasma and/or sera;
- Circulating tumor cells, circulating endothelial cells, and/or circulating cancer associated macrophage-like cells from peripheral blood where feasible;

- Assessment of tumor-associated antigen (TAA)-specific peripheral T cells by RNAseq;
- Assessment of cytokine profiles from plasma and/or sera;
- Progression-Free Survival, as assessed by RANO (Response Assessment in Neurooncology) criteria and Immunotherapy Response Assessment in Neuro- oncology (iRANO) criteria;
- Overall Survival (OS).

[0165] *Study Design*

[0166] The study described in this example corresponds to ClinicalTrials.gov identifier NCT03491683. The data presented herein as associated with this study reflects the state of this study as of the time of this filing. In this study, antigen-specific T cell-generating therapy, INO-5401, combined with INO-9012, followed by electroporation with the CELLECTRA® 2000 device, together with a PD-1 checkpoint inhibitor, cemiplimab-rwlc, was given to patients with newly-diagnosed GBM, together with radiation and temozolomide, in order to evaluate tolerability, immunogenicity and anti-tumor activity of the combination. Ethics Approval by NYU Ethics Board; approval number 17-00764.

[0167] This is a Phase 1/2, open-label, multi-center trial to evaluate the safety, immunogenicity, and preliminary efficacy of INO-5401 and INO-9012 in combination with cemiplimab (also known as REGN2810) in subjects with newly-diagnosed GBM. All patients provided written informed consent.

[0168] Subjects started immunotherapy with REGN2810 upon definitive histopathological diagnosis of GBM and adequate recovery from surgical intervention. Subjects were assigned to a cohort based on the results of the MGMT gene methylation assay performed in a CLIA-certified laboratory, which was available prior to the completion of RT. The start of immunotherapy is designated as Day 0. REGN2810 was administered intravenously (IV) every three weeks until disease progression defined by iRANO (Immune Response Assessment in Neuro-Oncology), unacceptable toxicity, withdrawal of consent, or death.

[0169] On Day 0, subjects received INO-5401 and INO-9012 intramuscularly (IM) followed by electroporation (EP). INO-5401 and INO-9012 were administered, followed by EP, every three weeks for four doses, and then every 9 weeks until disease progression defined by iRANO, unacceptable toxicity, withdrawal of consent, or death.

[0170] Temozolomide was administered to all subjects both with and without MGMT promoter methylation, unless clinically contraindicated, during radiation therapy. Radiation therapy (RT) began no later than 42 days after surgical intervention. Radiation therapy started approximately

1 to 2 weeks after Day 0, and was continue(d) for approximately three weeks. Temozolomide (TMZ) was given daily during radiation therapy (TMZ/RT). Subjects with MGMT promoter methylation received maintenance (adjuvant) TMZ for 6 cycles, following recovery from TMZ/RT. Maintenance (adjuvant) TMZ was administered for the first 5 days of a 28-day cycle. This study had two cohorts: Cohort A, consisting of subjects with an unmethylated MGMT promoter, and Cohort B consisting of subjects with a methylated MGMT promoter.

[0171] *Study Population*

[0172] Each potential subject satisfied all of the following criteria to be enrolled in the study:

Overview of Patient Eligibility

[0173] Adults with newly-diagnosed GBM who are post-definitive surgery, and are able to receive standard therapy. Estimated Number of Subjects: 52. Cohort A: MGMT promoter unmethylated (N=32 for 30 evaluable subjects). Cohort B: MGMT promoter methylated (N=20 for 19 evaluable subjects).

[0174] *Inclusion Criteria:*

- Subjects must provide written IRB approved informed consent in accordance with institutional guidelines;
- Be 18 years of age or older on the day of signing the informed consent, and able and willing to comply with all trial procedures;
- Newly-diagnosed brain cancer with histopathological diagnosis of glioblastoma (GBM);
- Karnofsky Performance Status (KPS) rating of ≥ 70 at baseline;
- Receipt of dexamethasone equivalent dose ≤ 2 mg per day, stable or decreased for $\geq$ three days prior to Day 0;
- Recovery from the effects of prior GBM surgery as defined by the Investigator;
- ECG with no clinically significant findings as assessed by the Investigator performed within 28 days of signing the informed consent form (ICF);
- Adequate organ function as demonstrated by hematological, renal, hepatic parameters as defined in the Table below, obtained within 28 days prior to the first trial treatment;

Absolute neutrophil count (ANC)	$\geq 1500/\text{mm}^3$
Platelets	$\geq 100,000/\text{mm}^3$
Hemoglobin	$\geq 9 \text{ g/dL}$
Creatinine OR Measured or calculated	$\leq 1.5 \times \text{upper limit of normal (ULN)}$, OR $\geq 50 \text{ mL/min}$ for subject with creatinine level
creatinine clearance or GFR*	$> 1.5 \times \text{institutional ULN}$
Total bilirubin	$\leq 1.5 \times \text{ULN}$
AST (SGOT) and ALT (SGPT)	$\leq 2.5 \times \text{ULN}$

*Creatinine clearance should be calculated per Cockcroft-Gault equation

- Agree that, during the trial, men will not father a child, and women cannot be or become pregnant if they are of child-bearing potential. Subjects must be of non-child bearing potential (≥ 12 months of non-therapy-induced amenorrhea, confirmed by follicle stimulating hormone [FSH], if not on hormone replacement); or surgically sterile (vasectomy in males or absence of ovaries and/or uterus in females); or agree to use one highly effective or combined contraceptive methods that result in a failure rate of $< 1\%$ per year during the treatment period and at least through week 12 after last dose. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception. Examples of contraceptive methods with an expected failure rate of $< 1\%$ per year include male sterilization and hormonal implants. Alternatively, proper use of combined oral or injected hormonal contraceptives and certain intrauterine devices (IUDs) or two methods (e.g., two barrier methods such as a condom and a cervical cap) may be combined to achieve a failure rate of $< 1\%$ per year (barrier methods must always be supplemented with the use of aspermicide);

- Ability to tolerate magnetic resonance imaging (MRI).

[0175] *Exclusion Criteria:*

- Presence of greater than 1 cm x 1 cm residual tumor enhancement on post-operative MRI;
- Multifocal disease or leptomeningeal disease (LM) disease on post-operative MRI;
- Are not able to start radiation within 42 days of surgical resection of their tumor;
- Receive dexamethasone equivalent dose $> 2 \text{ mg}$ per day;
- Prior treatment with an agent that blocks the PD-1/PD-L1 pathway at any point in the past;
- Receipt of previous approved or investigative immune modulatory agent (for example, anti-TNF, therapeutic anti-cancer vaccines, cytokine treatments (other than G-CSF or

erythropoietin), or agents that target cytotoxic T- lymphocyte antigen 4 (CTLA-4), 4-1BB (CD137), PI3K-delta, or OX-40) within 28 days of receiving the first dose of treatment;

- Have received prior treatment with idelalisib at any point in the past;
- Past, current or planned treatment with tumor treatment fields (Optune; NovoTTF); oncolytic viral treatment; or prior exposure to an investigational agent or device, including Gliadel wafer (Carmustine) implant for chemotherapy; within 28 days of receiving the first dose of treatment;
- Allergy or hypersensitivity to REGN2810 or to any of its excipients;
- History of documented allergic reactions or acute hypersensitivity reaction attributed to antibody treatments;
- Ongoing or recent (within 5 years) evidence of autoimmune disease that required treatment with systemic immunosuppressive treatments, which may suggest risk for immune-related adverse events (irAEs), with the exception of: vitiligo, childhood asthma that has resolved, type 1 diabetes, residual hypothyroidism that required only hormone replacement, or psoriasis that does not require systemic treatment;
- Diagnosis of immunodeficiency or treatment with systemic immunosuppressive therapy within 28 days prior to the first dose of trial treatment, other than dexamethasone for the underlying disease under investigation, as noted in the inclusion criteria;
- Positive serological test for human immunodeficiency virus (HIV), or a history of HIV infection; or positive tests for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV RNA) indicating active or chronic infection, as these infections may interfere with the ability to mount an appropriate immune response to vaccination;
- Current malignancy at another site, with the exception of adequately treated basal or squamous cell skin cancers, or carcinoma of the cervix in situ, with no evidence of disease within 3 years. Cancer survivors who have undergone curative therapy for a prior malignancy, have no evidence of disease for 3 years and are deemed at low risk for recurrence are eligible for the trial;
- Receipt of any vaccine within 4 weeks prior to first dose of trial treatment with the exception of the inactivated influenza vaccine, which may be given up to 2 weeks prior;
- History of clinically significant, medically unstable disease which, in the judgment of the investigator, would jeopardize the safety of the subject, interfere with trial assessments or endpoint evaluation, or otherwise impact the validity of the trial results (e.g. chronic renal failure, angina, myocardial ischemia or infarction, New York Heart Association (NYHA) class III/ IV cardiac disease); or any cardiac pre-excitation syndromes (such as Wolff- Parkinson-White; cardiomyopathy, or clinically significant arrhythmias);
- History of pneumonitis within the last 5 years;

- Acute or chronic bleeding or clotting disorder that would contraindicate IM injections or use of blood thinners (e.g. anticoagulants or antiplatelet drugs, excluding over-the-counter aspirin or non-steroidal anti-inflammatory drugs, such as ibuprofen) within 2 weeks of Day 0;
- Fewer than two acceptable sites available for IM injection considering the deltoid and anterolateral quadriceps muscles. The following are unacceptable sites:
 - Tattoos, keloids or hypertrophic scars located within 2 cm of intended treatment site;
 - Cardioverter-defibrillator or pacemaker (to prevent a life-threatening arrhythmia) that is located ipsilateral to the deltoid injection site (unless deemed acceptable by a cardiologist);
 - Metal implants or implantable medical device within the intended treatment site (i.e. EP area);
- Active drug or alcohol use or dependence that, in the opinion of the Investigator, would interfere with adherence to trial requirements;
- Imprisonment, or compulsory detention (involuntary incarceration) for treatment of either a psychiatric or physical (i.e. infectious disease) illness;
- Pregnant or current breastfeeding;
- As determined by the Investigator, any medical or psychological or non- medical condition that might interfere with the subject's ability to participate or affect the safety of the subject.

[0176] *Dosage and Administration*

Investigational Drug Products

Product	Formulation	Unit
INO-5401	10.0 ± 0.5 mg/mL total plasmid (pGX1108, pGX1404, pGX1434; 1:1:1 w/w) in 165 mM sodium chloride and 16.5 mM sodium citrate	1.4 mL/ 2-mL vial
INO-9012	10.0 ± 0.5 mg/mL pGX6001 in water for injection	0.2 mL/ 2-mL vial
REGN2810	REGN2810 is supplied as a sterile liquid solution of 5.5 mL in a 10 mL glass vial (50 mg/mL) for IV administration. REGN2810 may also be supplied as a sterile liquid solution of 7.44 mL in a 10 mL glass vial (50 mg/mL) for IV administration.	5.5 mL/ 10-mL vial Or 7.44 mL/ 10-mL vial

[0177] The active pharmaceutical ingredients (APIs) in INO-5401 are DNA plasmid sequences that were designed and constructed using proprietary synthetic consensus (SynCon®) technology. This process involves synthetically deriving consensus genes across multiple strains and

optimizing DNA inserts at the genetic level to allow high expression in human cells. The INO-5401 plasmids are as follows:

- pGX1108, a plasmid for expression of prostate-specific membrane antigen (PSMA; SEQ ID NO: 28). 3 mg of pGX1108 will be present in each 10 mg dose of trial treatment (INO-5401 + INO-9012).

- pGX1404, a plasmid for expression of Wilms' tumor gene-1 (WT1) antigen (SEQ ID NO: 26). 3 mg of pGX1404 will be present in each 10 mg dose of trial treatment.

- pGX1434, a plasmid for expression of human telomerase reverse transcriptase (hTERT) (SEQ ID NO: 20). 3 mg of pGX1434 will be present in each 10 mg dose of trial treatment.

[0178] The API in drug product INO-9012 is pGX6001, a DNA plasmid for expression of human IL-12 p35 and p40 subunit proteins. 1 mg of pGX6001 will be present in each 10 mg dose of trial treatment. Both DNA plasmid products INO-5401 and INO-9012, are administered using a syringe and the investigational CELLECTRA® 2000 electroporation (EP) device.

[0179] Cemiplimab-rwlc (REGN2810) is a covalent heterotetramer consisting of two disulfide-linked human heavy chains, each of which is covalently bonded through disulfide linkages to a human kappa light chain. The antibody possesses an approximate molecular weight of 143.6 kDa based on the primary sequence. There is a single N-linked glycosylation site on each heavy chain, located within the constant region in Fc portion of the molecule.

[0180] The REGN2810 heavy chain possesses an IgG4 isotype-constant region. The variable domains of the heavy and light chains combine to form PD-1 binding site within the antibody. Antibody generation by VelocImmune® mice is carried out using standard techniques after immunization with PD-1. The genes encoding the heavy and light chains of REGN2810 were introduced into CHO cells, and a stable expression cell line with a higher titer (Cell Line 2) was developed for this antibody. For both cell lines, the recombinant CHO cells were grown in suspension culture and chemically induced to initiate antibody expression and secretion into the cell culture medium. Antibody is harvested via filtration and purified through a series of preparative column chromatographic and filtration steps to generate drug substance. Drug substance is then formulated and sterile-filtered to produce the final drug product.

[0181] REGN2810 (50 mg/mL) is formulated in an aqueous buffered solution at pH 6.0 containing 10 mM histidine, 5% (w/v) sucrose, 1.5% (w/v) L-proline, and 0.2% (w/v) polysorbate 80. REGN2810 is supplied as a sterile liquid solution of 5.5 mL in a 10 or 20 mL glass vial for IV administration. A maximum volume of 5.0 mL can be withdrawn from each vial containing 250 mg of REGN2810. Seven mLs are needed to provide a 350 mg dose of REGN2810, thus 2 vials must be used when supplied with the 5 mL vials. REGN2810 (50 mg/mL) may also be supplied as a sterile

liquid solution of 7.44 mL in a 10 or 20 mL glass vial for IV administration. A maximum volume of 7.0 mL can be withdrawn from each vial containing 350 mg of REGN2810.

[0182] *Treatment*

[0183] Subjects who meet all of the inclusion criteria and none of the exclusion criteria started immunotherapy with REGN2810 and INO-5401 + INO-9012 on Day 0. REGN2810 was administered IV every three weeks at a dose of 350 mg per dose, in the absence of dose holding, until disease progression as defined by iRANO, unacceptable toxicity, withdrawal of consent, or death. INO-5401 and INO-9012 IM followed by EP was administered every three weeks for four doses, and then every 9 weeks, at a dose of 10 mg/DNA per dose, in the absence of dose holding, until disease progression as defined by iRANO, unacceptable toxicity, withdrawal of consent, or death. RT began no later than 42 days after surgical intervention and approximately 1 to 2 weeks after Day 0. RT continued for approximately three weeks. The total dose of RT was 40 Gy given over 3 weeks.

[0184] Daily TMZ with radiation therapy (TMZ/RT) began no later than 42 days after surgical intervention and approximately 2 weeks after Day 0. TMZ/RT continued for approximately three weeks. TMZ was given at a dose of 75 mg/m²/dose, in the absence of dose reduction. Subjects should then received maintenance (adjuvant) TMZ for an additional 6 cycles. Cohort B received TMZ following radiotherapy for up to six cycles. Maintenance (adjuvant) TMZ was administered to subjects in Cohort B for the first 5 days of a 28-day cycle at 150-200 mg/m²/dose, following peripheral blood count recovery from TMZ/RT per standard guidelines TMZ treatment.

[0185] Day 0 (first dose of INO 5401, INO 9012 and REGN2810) was at least 14 days after completion of resection of primary tumor and the subject has recovered from surgery, but no later than post-operative day 28.

[0186] For subjects who discontinued one therapy (either INO-5401 + INO-9012 or REGN2810) for reasons other than progression, the other therapy was allowed to continue after consultation with the Medical Monitor.

[0187] **Figure 1** illustrates the trial design for Cohorts A and B.

- INO-5401 (3 mg each of hTERT, WT-1 and PSMA plasmids) combined with 1 mg INO-9012 (IL-12), for a total of 10 mg of DNA, administered via intramuscular (IM) injection followed by electroporation (EP) with CELLECTRA® 2000 device, and delivered every three weeks for four doses, then every 9 weeks.

- Chemoradiation: Radiation (RT) given in a hypofractionated schedule (40 Gy over three weeks)

- Temozolomide (TMZ) concurrent with radiation (Cohorts A and B), followed by six

maintenance (adjuvant) cycles (Cohort B only)

[0188] Cemiplimab-rwlc (REGN2810) was administered IV at a dose of 350 mg every three weeks (Q3W) over approximately 30 minutes, starting at Day 0, and continued until disease progression as defined by iRANO, unacceptable toxicity, withdrawal of consent, or death.

[0189] INO-5401 is a mixture of three separate synthetic plasmids that target WT1, PSMA and hTERT proteins. Each plasmid was dosed at 3 mg DNA, for a total of 9 mg DNA per dose of INO-5401. INO-5401 was administered IM at Day 0, Week 3, Week 6, and Week 9, and then every 9 weeks thereafter, and continued until disease progression as defined by iRANO, unacceptable toxicity, withdrawal of consent, or death. INO-9012 is a synthetic plasmid that expresses human IL-12, and is dosed at 1 mg DNA, and was administered IM together with INO-5401. The total dose of DNA in each dose of INO-5401 + INO-9012 when mixed and administered together was 10 mg. INO-5401 (3 mg each of hTERT, WT-1 and PSMA plasmids) combined with 1 mg INO-9012 (IL-12), for a total of 10 mg of DNA, was administered via intramuscular (IM) injection followed by electroporation (EP) with CELLECTRA® 2000 device, and delivered every three weeks for four doses, then every 9 weeks.

[0190] All subjects received a total of 40 Gy in 15 fractions (three weeks).

[0191] Hypofractionated radiation therapy (hfRT) began no later than 42 days after surgery. Radiotherapy was given for three weeks.

[0192] All patients received TMZ, regardless of MGMT methylation status, unless clinically contraindicated, during radiotherapy. TMZ was administered at 75 mg/m² daily by mouth for 21 days concomitant (7 days a week for three weeks) with hfRT therapy.

[0193] Following radiation therapy, subjects with MGMT promoter methylation (Cohort B) continued TMZ maintenance therapy at a starting dose of 150 mg/m²/day for 6 cycles on the first 5 days of a 28-day cycle (5 days “on,” 23 days “off”), and increased each maintenance cycle by 50 mg/m²/dose to a maximum of 200 mg/m²/dose, in the absence of hematologic toxicity. Maintenance (adjuvant) TMZ started approximately four weeks after the last dose of RT ($\pm$ 3 days) and following peripheral blood count recovery, per TMZ treatment guidelines. The dose was determined using actual body surface area (BSA) as calculated in square meters at the beginning of each treatment cycle.

[0194] The daily dose was rounded to the nearest 5 mg.

[0195] *Efficacy Evaluations/Endpoints ELISpot*

[0196] ELISpot was employed to give a qualitative measure of whether antigen specific T cells are present in a peripheral blood mononuclear cell (PBMC) sample. PBMCs were collected from subjects at study weeks 0, 3, 6, 9, 12, and 24 and assayed by IFN- γ ELISpot. At the 12-month

data cut-off, antigen specific IFN γ spot forming units (SFU) per million PBMCs are shown from before (pre) and the highest magnitude (peak) after treatment with INO-5401 and cemiplimab-rwlc from 8 subjects with sample to week 24. Each subject is represented by an open circle, bars represent the mean. The difference from pre to peak, delta, is shown for each antigen graph as well as together for 11 subjects assayed and for the 8 with sample available to week 24. At the 18-month data cut-off, antigen specific IFN γ spot forming units (SFU) per million PBMCs are shown from before (pre) and the highest magnitude (peak) after treatment with INO-5401 and cemiplimab-rwlc from 39 subjects. Each subject is represented by an open circle, bars represent the mean. The difference from pre to peak, delta, is shown for each antigen graph as well as together for the 39 subjects. INO- 5401 is the sum of WT1, PSMA and hTERT. Box plots extend from the 25th to 75th percentile, with a horizontal line at the median, and “+” at the mean.

[0197] *Lytic Granule Loading*

[0198] A lytic granule loading assay was performed to explore the activation status and lytic potential of antigen-specific T cells present in PBMC samples collected from subjects at study weeks 0, 3, 6, 9, 12, and 24. PBMCs were stimulated with overlapping peptide libraries for INO-5401 antigens (hTERT, PSMA and WT1) or relevant controls in the absence of any exogenous cytokines. After 5 days, cells were stained with antibodies and assessed by flow cytometry. Frequencies of live, antigen-specific, activated (CD38+) CD3+CD8+ T cells with lytic potential (expressing Granzyme A, Perforin) from before treatment (pre) and the highest magnitude (peak) after treatment with INO-5401 and cemiplimab-rwlc from 8 subjects were determined at the 12-month data cut-off, and from 29 subjects at the 18-month data cut-off.

[0199] *Safety Assessment*

[0200] Adverse events (AE) graded per Common Toxicity Criteria for Adverse Events (CTCAE) v4.03, classified by system organ class, preferred term, severity, and relationship to trial treatment; and clinically significant changes in safety laboratory parameters from baseline.

[0201] A safety run-in was performed using a modified Rolling 6 design, which enrolled up to six subjects to each Cohort (A and B up to 12 subjects total). Enrollment was staggered, with a waiting period of one week between enrollment of the first and second subject and again between the second and third subject in each cohort. Each subject was assessed up to Week 9. By Week 9 a subject had received three doses of REGN2810, three doses of INO-5401 + INO-9012, and had completed RT.

[0202] Once the first three subjects of these six in each cohort completed Week 9 assessments, in the absence of dose-limiting toxicity (DLT), following a review of all available safety

data from these patients by the Medical Monitors from the Sponsor and from Regeneron Pharmaceuticals, and the coordinating Principal Investigator, enrollment to that cohort began in full. However, if prior to the first three subjects in each cohort completing Week 9, a single subject from the first six in that cohort experienced a DLT then enrollment was to be limited to six subjects in that cohort until all six subjects reached Week 9 and were assessed for DLT.

[0203] If a second subject within the first 6 experienced a DLT in the same cohort within the first 9 weeks, enrollment to that cohort would have been stopped, and the Sponsor's Medical Monitor, in addition to the Principal Investigator (PI) and Investigator(s) at the subjects' site(s) would have discussed the case, and decide whether to modify trial drug or concomitant drug (RT/TMZ) dose within that cohort, or to cease further enrollment to that cohort. If a change to the protocol was required, enrollment was only to be re-initiated after amendment of the protocol and approval of the amended protocol by the IRB.

[0204] A DLT is defined as:

Non-Hematologic Toxicity

- Grade ≥ 2 uveitis.
- Grade ≥ 3 non-hematologic toxicity; with the exception of:
 - Grade 3 nausea, vomiting or diarrhea unless persistent (>7 days duration) despite maximal supportive care measures as prescribed by the treating physician.
 - Grade ≥ 3 laboratory abnormalities that are considered clinically insignificant and do not meet criteria for an AE.
 - Grade 3 infusion-related reactions that respond to medical management.
 - Grade 3 Immune-Related Adverse Events (IRAE), other than uveitis, that improve within 14 days to Grade ≤ 2 with medical management (including treatment with steroids).

Hematologic Toxicity

- Grade 4 neutropenia >7 days.
- Grade 4 thrombocytopenia, or Grade 3 thrombocytopenia with bleeding.
- Grade ≥ 3 febrile neutropenia (fever $\geq 38.5^{\circ}\text{C}$ with absolute neutrophil count [ANC] $< 1000/\text{mm}^3$), or Grade ≥ 3 neutropenia with documented infection.

[0205] Events considered by the treating Investigator to be related to underlying tumor, concurrent medication or co-morbid event, and events considered unlikely related to trial therapy (INO-5401, INO-9012 or REGN 2810), but at least probably related to either temozolomide or RT were not to be considered a DLT. Patients who experienced DLT were to be discontinued from further trial

therapy and to enter the post-trial follow-up phase of the trial. Adverse events that meet DLT criteria but occur outside the DLT window were to be classified as unacceptable AEs and trial treatment was to be discontinued.

[0206] Following the safety run-in period, if at any point during the course of this clinical study, $\geq 30\%$ of subjects in either cohort experienced a dose-limiting toxicity deemed related to investigational trial drug(s), enrollment to one or both cohorts was to be stopped, and the Sponsor's Medical Monitor, in addition to the Principal Investigator (PI) and Investigator(s) at the subjects' site(s) were to discuss the safety profile of the trial drug(s), and a decision was to be made whether to modify trial drug or concomitant drug (RT/TMZ) dose within one or both cohorts, or to cease further enrollment to one or both cohorts. If a change to the protocol had been required, enrollment was only to be re-initiated after amendment of the protocol and approval of the amended protocol by the IRB.

[0207] *Medical and Clinical Assessments*

[0208] Concomitant medications were collected from the time of signing the ICF and at all subsequent trial visits through trial discharge.

[0209] Assessment of all AEs were collected from the time of signing of ICF until 30 days post last dose of INO-5401 + INO-9012 or REGN2810, whichever was later, with the exception of AESIs, and SAE's as defined in this protocol, which were to be collected until 6 months post last dose of INO-5401 + INO-9012 or REGN2810, whichever is later. AEs were assessed using the NCI CTCAE v4.03.

[0210] *Physical Assessments*

[0211] A full examination including complete neurologic exam (cranial nerve assessment, deep tendon reflexes, muscle strength and sensation), and Karnofsky Performance Scale (KPS) were conducted every 3 weeks.

[0212] *Medical Post-Treatment Reaction Assessment*

[0213] On Day 0, vital signs were collected prior to both REGN2810 and INO-5401 + INO-9012 treatment, at the end of the REGN2810 infusion, and every 30 minutes for the first 4 hours post-REGN2810 infusion, as well as 30 minutes after the INO-5401 + INO-9012 injection with EP. At all other visits, the Investigator assessed local and systemic reactions for 30 minutes post-each treatment (REGN2810 and/or INO-5401 + INO-9012) and at specified visits as per the Schedule of Events.

[0214] *Vital Signs*

[0215] Vital signs including body temperature, respiration rate, blood pressure and heart rate were measured at all the trial treatment visits.

[0216] *Weight, Height, and Body Mass Index*

[0217] Weight (kg) and height (cm) was collected at the screening visit. Weight was collected at each additional treatment visit from Day 0 through end of treatment.

[0218] Body Mass Index was assessed at Day 0, weeks 3, 6, 9, 18, and every 9 weeks after week 18 while the subject was receiving the EP procedure, secondary to the need to assess needle gauge.

[0219] *12-Lead ECG*

[0220] An ECG was performed at screening within 28 days prior to Day 0 for all subjects to determine subject eligibility. Abnormal ECGs were to be interpreted as clinically significant or not clinically significant. Abnormal ECGs at screening were to be discussed with the trial medical monitor to determine subject eligibility.

[0221] *Pregnancy Test*

[0222] For women of reproductive potential, a negative result for serum pregnancy test (test must have a sensitivity of at least 25 mIU/mL) was obtained at the screening visit and prior to each administration of INO-5401 + INO-9012 and REGN2810. If at any point, the β -HCG (pregnancy) test was positive, indicating that the subject is pregnant, no additional trial treatment was to be administered, but the subject was to be followed for the duration of the trial and beyond to determine the outcome of the pregnancy (with the subject's consent).

[0223] *Laboratory Evaluations*

[0224] Blood samples were collected as specified in the Trial Schedule of Events.

[0225] Screening labs may be used for Visit #1 (Day 0) if they were within 7 days of Day 0. Otherwise, all labs associated with any treatment visit (CBC and chemistry) were collected no more than 72 hours prior to treatment and reviewed/evaluated by the Investigator prior to treatment.

Complete blood count (CBC) should include:

- White blood cell (WBC) count with differential
- Red blood cell (RBC) count
- Hemoglobin, Hematocrit
- Platelet count

Serum chemistries should include:

- Glucose
- Albumin
- Total protein
- SGPT (serum glutamic-pyruvic transaminase; ALT)
- SGOT (serum glutamic-oxaloacetic transaminase; AST)
- Alkaline phosphatase
- Bilirubin (total)
- Direct bilirubin
- BUN (blood urea nitrogen)
- Calcium
- Creatinine
- Electrolytes (Sodium, Potassium, Chloride, Carbon Dioxide or Bicarbonate)
- Lipase
- Amylase
- CPK

Assessments to be performed at screening only:

- HIV screening test (antibody immunoassay test); or documentation of these results in the medical record;
- Hepatitis B Serology: HBsAg (hepatitis B surface antigen); or documentation of these results in the medical record;
- Hepatitis C antibody immunoassay; or documentation of these results in the medical record;
- Urinalysis including specific gravity, glucose, blood and ketones;
- Activated partial thromboplastin time (aPTT), INR.

[0226] Peripheral Blood Immunogenicity Assessments

[0227] Whole blood and serum samples were obtained. Immunology samples were drawn at Screening, Day 0, Weeks 3, 6, 9, 12, and then every 12 weeks (Weeks 24, 36, 48, etc.).

[0228] T cell responses were assessed using antigen-specific IFN- γ ELISpot assay using overlapping peptide libraries covering the INO-5401 antigens (hTERT, WT-1, and PSMA). Additionally, PBMC responses against a pool of known antigenic epitopes combined from Cytomegalovirus, Epstein Barr Virus and Influenza (CEF) were evaluated in order to track general cellular immune competence during the trial.

[0229] T cell responses were assessed via flow cytometry overlapping peptide libraries covering the INO-5401 antigens. Flow cytometric assays may include an examination of the influence of immunotherapy on the ability of subject T cells to exhibit phenotypic markers

associated with cytolytic potential, activation or exhaustion after stimulation by peptides corresponding to INO-5401 antigens. Markers used for this purpose include CD3, CD4, CD8, CD137, CD69, CD38, PD-1, Granulysin, Granzyme A, Granzyme B and Perforin. These markers may change relative to new data becoming available that is informative for this assessment.

[0230] Assessment of the presence of cells that are known to play a role in immune suppression may occur via the application of flow cytometry. Flow cytometric assays may include an examination of the influence of these cells on the induction or expansion of an immune response after immunotherapy. Markers used for this purpose include CD3, CD16, CD19, CD20, CD56, CD11b, CD14, CD15, CD33 and HLA-DR. These markers may change relative to new data becoming available that is informative for this assessment.

[0231] TCR sequencing from PBMCs to assess diversity and putative antigen specificity were performed on whole PBMCs with or without prior *in vitro* stimulation.

[0232] Humoral responses were assessed via application of Enzyme Linked Immunosorbent Assay (ELISA) or other methods for the detection of antigen specific antibody secretion and/or employment of flow cytometry for B cell phenotyping. Analysis of TAA-specific T cells may occur via isolation of these cells based on expression of markers such as CD137 or others. Upon isolation, RNAseq may be performed in order to understand the unique transcriptome of these cells. Analysis of cytokine profiles from peripheral blood may be undertaken using an assessment platform such as Luminex.

[0233] Tissue Immunology

[0234] Immune infiltration and the presence of immunomodulatory factors in neoplastic tissue prior to and following INO-5401 dosing was examined via a number of assessments which may include:

- Expression of PD-L1 and tumor oncoproteins in tumor and infiltrating immune cells by IHC, immunofluorescence (IF) or genome sequencing
- Characterization of TIL infiltrate (CD3, CD8, CD4) by RNAScope
- Assessment of tumor Treg, myeloid immune suppressive populations and T cell immune checkpoint expression by RNAScope
- T cell receptor sequencing in resected tumor tissue to correlate any pre-existing clonal T cell populations with treatment related changes in T cell diversity in peripheral blood

[0235] Biomarkers

[0236] Biomarker assessments was performed from peripheral and tissue samples referenced above and collected per lab manual instructions. Immunohistochemical assessment of the

expression of the hTERT, WT1 and/or PSMA proteins within tissue samples from enrolled subjects occurred contingent on the presence of sufficient sample quantity and continued relevance as supported by available data. IDH-1 status was to be performed on tumor tissue if available. MicroRNA signatures in blood plasma and/or sera was to be assessed in order to determine disease and/or therapy specific signatures that predict disease course and/or response to treatment with INO-5401 as well as INO-5401 driven changes. RNAseq may be used for this method.

[0237] Assessment of circulating tumor cells, circulating endothelial cells and/or circulating cancer associated macrophage-like cells from peripheral blood may occur. Use of size exclusion based filters may be used for this purpose and markers may include GFAP, CD45, vimentin, PD-L1, CD146, TIE-2 and possibly others.

[0238] Clinical Assessments

[0239] Clinical evaluations for disease response was conducted at all of the trial visits (as assessed by clinical signs and symptoms of disease progression). MRI for disease progression was obtained for all subjects 9 weeks after Day 0 ($\pm$ 3 days) and every 3 months thereafter, unless considered pseudo-progression by iRANO assessment. In that case, the repeat confirmatory MRI scan was to be performed at 3 months after the suspected pseudo-progression.

[0240] OVERALL SURVIVAL

[0241] All subjects were followed for survival. After completion of the End of Treatment visit, documentation of survival status was required every 6 months and at month 18 post day 0. The following methods were acceptable to document survival: phone call; personal contact; certified letter; or documentation of a visit confirmed in the medical record.

[0242] PROGRESSION

[0243] Progression was assessed by both RANO and iRANO. Patients that withdrew prior to progression were followed for progression and survival.

[0244] RANO AND IRANO: (IMMUNOTHERAPY) RESPONSE ASSESSMENT IN NEURO-ONCOLOGY

[0245] Both RANO and iRANO criteria were utilized in this trial. The Radiologic Assessment for Neuro-Oncology (RANO) criteria were proposed in 2010 to improve assessment of the evolving complexities of imaging for subjects with malignant glioma [Wen, et al., J Clin Oncol 2010;28:1963-1972.]. The RANO criteria provides guidance for the occurrence of pseudo-progression, which occurs in about 10-20 % of newly diagnosed subjects with GBM after radiotherapy and TMZ therapy. Clinical benefit, including long- term survival and tumor regression,

can still occur after initial disease progression or after the appearance of a new lesion. The iRANO criteria were developed by a multinational and multidisciplinary panel of neurooncology immunotherapy experts (RANO Working Group) who established a guidance for the determination of tumor progression in immunotherapy trials in neuro-oncology [Okada, et al. *Lancet Oncol* 2015,16:e534-542; Reardon, et al. *Neuro Oncology* 2014, 16 (Suppl 2)]. The iRANO working committee recommends that for subjects with early progressive findings (treatment with immunotherapy less than 6 months), including subjects who develop new lesions but who do not have substantial neurologic decline, confirmation of radiographic progression by follow-up imaging should be sought 3 months after initial radiographic evidence of progressive disease to decrease the likelihood of prematurely declaring progressive disease in subjects with pseudo-progression or delayed response. In such subjects, those with confirmation of further radiographic progression based comparison with the scan that first showed evidence of disease progression, or who develop substantial clinical decline at any time, were to be classified as having progressive disease with the date of disease progression back dated to the first date that the subjects met criteria for radiographic progression.

[0246] In this study, Investigators utilized iRANO criteria when making a decision as to whether to stop study drug in the face of suspected progression, however all subjects were to be assessed for progression by both RANO and iRANO criteria for progression.

[0247] NANO SCALE: NEUROLOGIC ASSESSMENT IN NEURO-ONCOLOGY

[0248] The NANO scale is used only in conjunction with the iRANO and RANO criteria.

[0249] Although both the RANO and the iRANO scales specify that clinical status must be incorporated for overall assessment, neither scale provides specific parameters to do so. An international group of neuro-oncologists convened to draft the NANO criteria as an objective and quantifiable metric of neurologic function evaluable during a routine examination. The NANO scale involves evaluation of eight relevant neurologic domains based on direct observation/testing conducted during routine office visits. The score defines criteria for domain-specific and overall scores of response, progression, stable disease and “not assessed.” These criteria provide a detailed and objective measure of neurologic function that can be assessed across clinical trials and therapeutic interventions [Nayak, et al., *Neuro Oncol* 2017,19:625-635]. This trial included clinical assessment utilizing the NANO scale when RANO and iRANO is assessed.

[0250] An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease

temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

[0251] In this study, AEs were monitored, classified, and summarized. Medical condition/diseases present before starting the investigational products were considered AEs only if they worsened after starting study treatment. An unexpected AE is one not identified in reference safety documents of the study drugs, or corresponding sections of the IBs. Throughout the course of the study, all AEs were monitored and reported on an AE CRF, including the event's seriousness, severity, action taken, and relationship to IP(s). AEs were followed until resolution or stable and the outcome documented on the appropriate CRF. All AEs were recorded in standard medical terminology rather than the subject's own words.

[0252] AEs include the following:

- Pre- or post-treatment complications that occur as a result of protocol mandated procedure.
- Any pre-existing condition that increases in severity, or changes in nature during or as a consequence of the trial drug phase of a human clinical trial.
- Complications of pregnancy. AEs do not include the following:
 - Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion) performed; the condition that leads to the procedure may be considered an AE.
 - Diseases, conditions, or laboratory abnormalities present or detected before the Screening visit that do not worsen.
 - Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
- Overdose of study drug without clinical sequelae
- Any medical condition or clinically significant laboratory abnormality with an onset date before the ICF is signed is not an AE, unless it worsens. It is documented on the medical history CRF.
- Uncomplicated pregnancy.
- An induced elective abortion to terminate a pregnancy without medical reason (documented on a pregnancy CRF).

[0253] All AEs that occurred from the time of signing of consent onwards and throughout the duration of the trial, and 30 days (6 months for immune-related AEs; irAEs) post-last dose were recorded.

[0254] A serious adverse event (SAE) is any AE that meets one of the following conditions:

- Death during the period of surveillance defined by the protocol (excluding death due to disease progression);
- Is immediately life-threatening (e.g., subject was, in the view of the Investigator, at immediate risk of death from the event as it occurred);
- Requires a subject's hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance (including any overnight stay in the hospital, regardless of the length of stay, even if the hospitalization is only a precautionary measure to allow continued observation). However, hospitalization (including hospitalization for an elective procedure) for a pre-existing condition that has not worsened does not constitute an SAE;
- Results in persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions);
- Results in congenital anomaly or birth defect;
- Is otherwise an important medical event that may not result in death, be life threatening, or require hospitalization, 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. Examples of such medical events include:
 - Allergic bronchospasm requiring intensive treatment in an emergency room or at home;
 - Blood dyscrasias or convulsions that do not result in hospitalization;
 - The development of drug dependency or drug abuse. Note the following clarifications of SAEs:
 - Death is an outcome of an AE, and not an AE in itself.
 - The subject may not have been on IP when the event occurred.
 - Dosing may have been given as treatment cycles or interrupted temporarily before the onset of the SAE, but may have contributed to the event.
 - "Life-threatening" means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity.
 - Complications that occur during hospitalizations are AEs. If a complication prolongs the hospitalization, it is an SAE.
 - Hospitalization means that the subject has been formally admitted to a hospital for medical reasons, for any length of time.

[0255] The Investigator was to attempt to establish a diagnosis of the event on the basis of signs, symptoms, and/or other clinical information. In such cases, the diagnosis was to be documented as the AE and/or SAE and not the individual signs/symptoms.

[0256] STATISTICAL AND ANALYTICAL PLAN

[0257] This is a single-arm (INO-5401 + INO 9012 in combination with REGN2810), open-label, multi-center trial in subjects with newly-diagnosed GBM with a tumor with an unmethylated MGMT promoter (Cohort A) and with a tumor with a MGMT methylated promoter (Cohort B). The trial's primary analyses regard the safety and tolerability of INO-5401 and INO-9012 in combination with REGN2810. The trial's secondary analyses assessed the efficacy of INO-5401 and INO-9012 in combination with REGN2810 using OS18 and using immunogenicity biomarkers (ELISpot/flow cytometry/TCR sequencing/antigen-specific humoral responses).

[0258] Exploratory analyses concern the correlative association between clinical response and tumor genetics and biomarkers. Progression-Free Survival as assessed by RANO (Response Assessment in Neuro-oncology) criteria and Overall Survival were also assessed as exploratory endpoints.

[0259] STATISTICAL HYPOTHESES

[0260] Each cohort for this trial had a separate independent hypothesis to evaluate the secondary endpoint overall survival at 18 months (OS18). The true treatment effect for OS18 is defined as p , where p denotes the true population probability of OS18 in each cohort. Then, for the MGMT promoter unmethylated subjects (Cohort A), the hypothesis of superiority to the historical control is: $H_0: p \leq 0.45$ vs. $H_1: p > 0.45$, and for the MGMT promoter methylated subjects (Cohort B), the hypothesis of superiority to the historical control is: $H_0: p \leq 0.60$ vs. $H_1: p > 0.60$.

[0261] ANALYSIS POPULATIONS/DATASETS

[0262] Analysis populations were:

- The modified intention to treat (mITT) population includes all subjects who receive at least one dose of the trial treatment. The mITT population will be used for analysis of the secondary endpoint of OS18 as well all of the exploratory efficacy endpoints, including OS and PFS.
- The per-protocol (PP) population is comprised of subjects who receive at least three of the first four doses of the trial treatment, and who have no protocol violations. Analyses on the PP population will be considered supportive of the corresponding mITT population for the analysis of efficacy. Subjects excluded from the PP population will be identified and documented prior to locking the trial database.

- The safety analysis set includes all subjects who receive at least one dose of either INO-5401 or INO-9012 or REGN2810 trial treatment. Subjects will be analyzed as to the treatment they received.

[0263] DESCRIPTION OF STATISTICAL METHODS ANALYSIS OF PRIMARY SAFETY ENDPOINT

[0264] The primary analyses for this trial are safety analyses of Treatment Emergent Adverse Events (TEAE) and clinically significant changes in safety laboratory parameters from baseline.

[0265] TEAEs are defined for this trial as any AEs that occur on or after Day 0 up to 30 days after the last dose of trial treatment, with the exception of irAEs, AESIs and SAEs, which may occur up to 6 months after the last dose of trial treatment. All TEAEs will be summarized for the subjects in the safety analysis set by frequency, percentage and 95% Clopper-Pearson confidence intervals within each cohort and across both cohorts combined.

[0266] These frequencies will be presented overall and separately by dose number, and will depict overall, by system organ class and by preferred term, the percentage of subjects affected. Additional frequencies will be presented with respect to maximum severity and to strongest relationship to trial treatment. Multiple occurrences of the same AE in a single subject will be counted only once following a worst-case approach with respect to severity and relationship to trial treatment. All serious TEAEs will also be summarized as above.

[0267] Any AEs with missing or partial onset/stop dates will be included in the overall AE summaries but excluded from the calculation of AE duration. AE duration will be calculated as AE Stop date - AE start date + 1 day. AEs, irAEs, AESIs and SAEs that are not TEAEs or serious TEAEs will be presented in listings.

[0268] Laboratory response variables will be descriptively summarized per time point and as changes from baseline including 95% confidence intervals. Shifts from baseline according to the CTCAE will also be presented. Laboratory values considered clinically significant will be presented in listings.

[0269] All of the safety analyses will be conducted on the subjects in the safety analysis set.

[0270] ANALYSIS OF SECONDARY EFFICACY ENDPOINT

[0271] The secondary endpoint of OS18 is summarized using frequency, percentage, 95% Clopper-Pearson confidence interval and p-value for each cohort. A subject is considered a survivor if they are determined to be alive after 18 months (548 days).

[0272] Superiority is concluded if the one-sided p-value is < 0.025 . OS18 was analyzed on subjects in both the mITT and per protocol population and all mITT/PP subjects were included in the OS18 denominator.

[0273] Cellular and humoral immune responses will be presented using descriptive statistics at each time point and for changes from baseline.

[0274] ANALYSIS OF OTHER SAFETY DATA

[0275] The percentage of subjects with abnormal medical history findings will be summarized by body system and preferred term for each cohort for subjects in the safety population.

[0276] Prior medications are those that were used before the start of the trial (within 28 days prior to Day 0). Concomitant medications are those used during the course of trial (on or after day 0). Partial start dates of prior and concomitant medications will be assumed to be the earliest possible date consistent with the partial date. Partial stop dates of prior and concomitant medications will be assumed to be the latest possible date consistent with the partial date. Data for all prior and concomitant medications will be summarized with percentages for each cohort for subjects in the safety population.

[0277] Measurements for vital signs as well as changes from baseline will be descriptively summarized by time point for each cohort for subjects in the safety analysis set. The percentage of subjects with abnormal physical examination findings at each time point will be descriptively summarized overall and within each cohort by body system for subjects in the safety analysis set.

[0278] ECG and viral serology at screening, and serum pregnancy at each time point will be descriptively summarized overall and within each cohort.

[0279] DISPOSITION

[0280] Subject disposition will be summarized by cohort and overall for all enrolled subjects and will include the number and percentage enrolled, the number and percentage who received each planned dose and the number who completed the trial. The number and percentage of subjects who discontinued treatment will be summarized overall and by reason. The number in each analysis population will also be presented.

[0281] DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

[0282] Demographic and baseline characteristic data were descriptively summarized by cohort for subjects in the safety analysis set.

[0283] EXPLORATORY ANALYSES

[0284] Progression-free survival as assessed by RANO (defined as the time from Day 0 until the date of death from any cause or progression whichever occurs first) and OS (defined as the time from Day 0 until the date of death from any cause) were summarized with Kaplan-Meier statistical methods within each cohort and overall. Subjects were censored for PFS at withdrawal of consent or the last progression assessment date where the subject was considered to have not progressed. Subjects who are not recorded as having died were censored for OS at withdrawal of consent or the last date the subject was known to be alive. Progression free survival and OS were analyzed in the mITT and Per Protocol populations.

[0285] Exploratory tumor genetics and/or biomarker responses were presented using descriptive statistics at each time point and as changes from baseline for the mITT population and per protocol population.

[0286] OS18, PFS, and OS were modeled using logistic regression models and Cox PH models against the exploratory responses to examine associations. Baseline variables such as patient demographics or patient disease characteristics were included in the models as potential confounders. Separately, cellular and humoral immune responses were used as explanatory variables.

[0287] RESULTS

[0288] Demographics of enrolled patients are shown in **Figure 2** (Demographics Table). Assessment of gene transcripts from tumors at diagnosis confirmed expression of antigens encoded by INO-5401 and a diverse immune gene profile. 5 of 47 subjects (811%) with available tissue for assessment exhibited transcript expression of one or more Tumor Associated Antigens encoded by INO-5401 (WT1, PSMA and hTERT). 43 of 47 subjects (89%) with available tissue for assessment exhibited transcript expression of two (2) or more Tumor Associated Antigens encoded by INO-5401 (WT1, PSMA and hTERT). 19 of 47 subjects (40%) exhibited transcript expression of all three Tumor Associated Antigens. No subject exhibited PD-1 expression without concomitant PD-L1 expression. 27 of 47 subjects (57%) exhibited PD-L1 expression with no concomitant PD-1 expression. 20 of 47 subjects (43%) showed co-expression of PD-1 and PD-L1. Normalized transcript read counts >1 were considered "positive."

[0289] *MRI Imaging*

[0290] Several patients have experienced pseudo-progression, with radiographic evidence of progression on MRI without evidence of tumor on repeat biopsy. Images from example patients demonstrate increase in MRI signal at timepoints following first dose of INO-5401 + INO-9012 and cemiplimab-rwlc, suggestive of edema or tumor. Biopsy on several patients shows treatment-related

changes with necrosis and mixed inflammation; absence of mitotic activity; and no evidence of viable tumor. Representative images from two patients are presented in **Figure 3**.

[0291] *ELISpot*

[0292] ELISpot is/was employed to give a qualitative measure of whether antigen specific T cells are present in a peripheral blood mononuclear cell (PBMC) sample. PBMCs are collected from subjects at study weeks 0, 3, 6, 9, 12, and 24 and assayed by IFN-g ELISpot. At the 12-month data cut-off, antigen specific IFNg spot forming units (SFU) per million PBMCs are shown from before (pre) and the highest magnitude (peak) after treatment with INO-5401 and cemiplimab-rwlc from 8 subjects with sample to week 24 (**Figure 4**). Each subject is represented by an open circle, bars represent the mean. The difference from pre to peak, delta, is shown for each antigen graph as well as together for 11 subjects assayed and for the 8 with sample available to week 24. INO- 5401 is the sum of WT1, PSMA and hTERT. Box plots extend from the 25th to 75th percentile, with a horizontal line at the median, and “+” at the mean. ELISpot results support the combination of INO-5401 and cemiplimab-rwlc are immunogenic- with IFN-g magnitudes above baseline to all 3 antigens in 5/11 subjects and to at least one antigen in 9 subjects as shown in **Figure 4**.

[0293] Assessment of post-INO-5401 peripheral immune responses by Cohort at the 18-month data cut-off revealed antigen-specific T cell responses by Interferon gamma ELISpot (cytokine production in response to each component of INO-5401). Results of assessment of post-INO-5401 peripheral immune responses by ELISpot at by Cohort are provided in **Figures 16A and 16B**. Baseline values from the peak timepoint following treatment are plotted. In Cohort A, 19/22 (86%) subjects tested to date had an IFN-g magnitude above baseline to one or more of the INO-5401 antigens (Figure 16A). In Cohort B, 16/17 (94%) subjects tested to date had an IFN-g magnitude above baseline to one or more of the INO-5401 antigens (Figure 16B).

[0294] *Lytic Granule Loading*

[0295] A lytic granule loading assay was performed to explore the activation status and lytic potential of antigen specific T cells present in PBMC samples collected from subjects at study weeks 0, 3, 6, 9, 12, and 24. PBMCs were stimulated with overlapping peptide libraries for INO-5401 antigens (hTERT, PSMA and WT1) or relevant controls in the absence of any exogenous cytokines. After 5 days, cells were stained with antibodies and assessed by flow cytometry. Frequencies of live, antigen-specific, activated (CD38+) CD3+CD8+ T cells with lytic potential (expressing Granzyme A, Perforin) are shown from before treatment at baseline (pre) and the highest magnitude (peak) after treatment with INO-5401 and cemiplimab-rwlc from 8 subjects for each antigen (**Figure 5A**). Each subject is represented by an open circle, bars represent the mean. The

difference from pre to peak, delta, is shown for each antigen as well as INO-5401 at the 12-month data cut-off for 8 subjects assayed (**Figure 5B**) and for the 5/8 subjects with sample available to week 12 (**Figure 5C**). INO-5401 is the sum of WT1, PSMA and hTERT. Box plots extend from the 25th to 75th percentile, with a horizontal line at the median, and “+” at the mean. Five subjects had a frequency of activated CD8+T cells with lytic potential (CD38+Prf+GrzA+) above baseline (pre) to more than one antigen; three subjects had a frequency of activated CD8+T cells with lytic potential (CD38+Prf+GrzA+) above baseline to all three antigens. Three subjects did not have a response above baseline to any antigen at any time.

[0296] Assessment of post-INO-5401 peripheral immune responses by Cohort at the 18-month data cut-off revealed antigen-specific T cell responses by flow cytometry (the expansion of antigen specific CD8+ T cells with lytic potential). In Cohort A, 13/19 (68%) subjects tested to date had a frequency of CD38+GrzA+Prf+ CD8+T cells above baseline to one or more of the INO-5401 antigens (**Figure 17A**). In Cohort B, 8/10 (80%) subjects tested to date had a frequency of CD38+GrzA+Prf+ CD8+T cells above baseline to one or more of the INO-5401 antigens (**Figure 17B**). Samples were collected Q3 weeks x 4 and then Q12 weeks. Baseline values from the peak timepoint following treatment are plotted.

[0297] *Progression-Free Survival*

[0298] **Figure 6** shows the visual representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort A, patients with the O6-methylguanine methyltransferase gene promoter unmethylated in their tumor cells. The curve shows the probability of an event at a certain time interval. The probability of the event is represented numerically on the y-axis, and the time interval on the x-axis. The event shown is progression-free survival. Progression-free survival is the absence of progression of disease at a given time point for a given subject.

[0299] **Figure 7** shows the visual representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort B, patients with the O6-methylguanine methyltransferase gene promoter methylated in their tumor cells. The curve shows the probability of an event at a certain time interval. The probability of the event is represented numerically on the y-axis, and the time interval on the x-axis. The event shown is progression-free survival. Progression-free survival is the absence of progression of disease at a given time point for a given subject.

[0300] **Figure 8** shows the visual representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort A and Cohort B, patients with the O6-methylguanine methyltransferase gene promoter unmethylated or methylated in their tumor cells. The curve shows the probability of an event at a certain time interval. The probability of the event is

represented numerically on the y-axis, and the time interval on the x-axis. The event shown is progression-free survival. Progression-free survival is the absence of progression of disease at a given time point for a given subject.

[0301] **Figure 9** shows the tabular representation of the Kaplan-Meier estimator of the progression-free survival at six months (PFS6) for Cohort A, Cohort B, and both cohorts combined. The total number of subjects per cohort, number of events, estimation of the event (PFS6), and the 95% confidence interval (CI) in which the numerical estimate of the event (PFS6) exists are all provided.

[0302] Confirmed progressive disease (PD) is determined by confirmation by consecutive PD scan ≥ 4 weeks from original PD event, or progressed according to biopsy surgery. Subjects who terminated for any reason prior to 6 months other than PD included as confirmed progressive events, including two (2) subjects in Cohort B who came off-study at week three (3), and declined long-term follow-up.

[0303] *Overall Survival*

[0304] All efficacy analyses (OS12, OS18, & Kaplan-Meier) were analyzed on subjects in the modified intent-to-treat (mITT) population which is defined as all subjects who received at least one dose of planned treatment. Overall Survival at twelve months (OS12) was tabulated as the proportion of subjects alive at 12 months out of all subjects at risk of death at the start of the study. Subjects who dropped out were considered failures (that is deaths). All subjects in Cohort A who dropped out prior to 12 months also died before 12 months of follow-up. The 95% confidence intervals (CIs) are calculated using the Clopper-Pearson exact method. Overall Survival at eighteen months (OS18) was tabulated as the proportion of subjects alive at 18 months out of all subjects at risk of death at the start of the study. The 95% confidence intervals (CIs) are calculated using the Clopper-Pearson exact method.

[0305] **Figure 10A** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over twelve months for Cohort A, for patients with the O6-methylguanine methyltransferase gene promoter unmethylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. **Figure 10B** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over eighteen months for Cohort A, for patients with the O6-methylguanine methyltransferase gene promoter unmethylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. Median follow-up in Cohort A is 17.8 months. mITT includes any subject who received ≥ 1 dose of study therapy. Shading represents confidence band on point estimate for survival at that timepoint.

[0306] **Figure 11A** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over twelve months for Cohort B, for patients with the O6-methylguanine methyltransferase gene promoter methylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. **Figure 11B** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over eighteen months for Cohort B, for patients with the O6-methylguanine methyltransferase gene promoter methylated in their tumor cells. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis. Median follow-up in Cohort B is 15.6 months. Censored; two subjects in Cohort B withdrew consent for follow-up at Week 3. mITT includes any subject who received ≥ 1 dose of study therapy. Shading represents confidence bands on point estimate for survival at that timepoint.

[0307] **Figure 12** shows the visual representation of the Kaplan-Meier estimator of the overall survival probability over twelve months for Cohorts A + B combined. The stepwise curve shows the probability of surviving up to and beyond a specific time point. The survival probability is represented numerically on the y-axis, and survival time in days on the x-axis.

[0308] **Figure 13** shows the efficacy data of the overall survival at 12 months and 18 months for Cohort A, for Cohort B, and combined. The figure shows the total number of subjects who were reported alive at 12 months and at 18 months. The total number of subjects, estimation of the event (OS12 or OS18), and the 95% confidence interval (CI) in which the numerical estimate of the event (OS12 or OS18) exists are all provided. The 95% CI were calculated using the exact Clopper-Pearson method.

[0309] *Safety Data*

[0310] Safety data was tabulated from subjects who were members of the safety analysis population, defined as having at least one dose of investigational product (IP).

[0311] All Adverse Events as defined by the Clinical Study Protocol $\geq$ NCI CTCAE Grade 3 are shown in **Figure 14**. Immune Related Adverse Events as defined by the Clinical Study Protocol are identified in **Figure 15**.

[0312] The most common Grade ≥ 3 adverse events reported in subjects were: platelet count decreased (11.5%), lymphocyte count decreased (11.5%), tumour inflammation (7.7%), seizure (7.7%), ALT increased (7.7%). One Grade 5 unrelated event of urosepsis was reported. There was only one SAE related to INO-5401 + INO-9012 reported, pyrexia. 48% of subjects reported irAEs, most frequently ALT increased (9.6%), AST increased (7.7%), diarrhea (7.7%), pyrexia (7.7%) and tumor inflammation (7.7%). 71% of the reported SAEs and irAEs occurred within the first 12 weeks of treatment.

[0313] Conclusions

[0314] In patients with newly diagnosed GBM, INO-5401 + INO-9012 in combination with cemiplimab-rwlc, given with radiation and temozolomide, has an acceptable safety profile, is immunogenic and is potentially efficacious in patients with newly diagnosed GBM. Common AEs included injection site administration events; $\geq$ Grade 3 AEs were primarily due to TMZ or radiation, and immune-related AEs were consistent with the profile of cemiplimab-rwlc. SAEs were consistent with those seen in patients with GBM (seizure).

[0315] Antigen-specific T cell responses were seen to one or more of the antigens included in INO- 5401 in almost all patients tested to date. PFS6 exceeds that of historical controls in this study, in patients with and without MGMT promoter methylation, and OS12 exceeds that of historical controls in patients without MGMT promoter methylation [Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005, 352:987-996].

[0316] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

[0317] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

SEQUENCE LISTING

SEQ ID NO: 1 R2810 HCVR

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Glu Val Gln Leu Leu Glu Ser Gly Gly Val Leu Val Gln Pro Gly Gly
 1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Phe
          20           25           30
Gly Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35           40           45
Ser Gly Ile Ser Gly Gly Gly Arg Asp Thr Tyr Phe Ala Asp Ser Val
          50           55           60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
          65           70           75           80
Leu Gln Met Asn Ser Leu Lys Gly Glu Asp Thr Ala Val Tyr Tyr Cys
          85           90           95
Val Lys Trp Gly Asn Ile Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu
          100          105          110
Val Thr Val Ser Ser
          115

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SEQ ID NO: 2 R2810 LCVR

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10           15
Asp Ser Ile Thr Ile Thr Cys Arg Ala Ser Leu Ser Ile Asn Thr Phe
          20           25           30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
          35           40           45
Tyr Ala Ala Ser Ser Leu His Gly Gly Val Pro Ser Arg Phe Ser Gly
          50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg Thr Leu Gln Pro
          65           70           75           80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Ser Asn Thr Pro Phe
          85           90           95
Thr Phe Gly Pro Gly Thr Val Val Asp Phe Arg
          100          105

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SEQ ID NO: 3 R2810 HCDR1

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Gly Phe Thr Phe Ser Asn Phe Gly
 1           5

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SEQ ID NO: 4 R2810 HCDR2

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Ile Ser Gly Gly Gly Arg Asp Thr
 1           5

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SEQ ID NO: 5 R2810 HCDR3

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Val Lys Trp Gly Asn Ile Tyr Phe Asp Tyr
 1           5           10

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SEQ ID NO: 6 R2810 LCDR1

Leu Ser Ile Asn Thr Phe
1 5

SEQ ID NO: 7 R2810 LCDR2

Ala Ala Ser
1

SEQ ID NO: 8 R2810 LCDR3

Gln Gln Ser Ser Asn Thr Pro Phe Thr
1 5

SEQ ID NO: 9 R2810 HC

Glu Val Gln Leu Leu Glu Ser Gly Gly Val Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Phe
20 25 30
Gly Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Gly Ile Ser Gly Gly Gly Arg Asp Thr Tyr Phe Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Lys Gly Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Val Lys Trp Gly Asn Ile Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125
Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
130 135 140
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190
Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn
195 200 205
Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro
210 215 220
Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe
225 230 235 240
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255
Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe
260 265 270
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285
Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
290 295 300

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Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305                      310                      315                      320
Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
                      325                      330                      335
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln
                      340                      345                      350
Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                      355                      360                      365
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
                      370                      375                      380
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
385                      390                      395                      400
Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu
                      405                      410                      415
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
                      420                      425                      430
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
                      435                      440

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SEQ ID NO: 10 R2810 LC

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1                      5                      10                      15
Asp Ser Ile Thr Ile Thr Cys Arg Ala Ser Leu Ser Ile Asn Thr Phe
20                      25                      30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
35                      40                      45
Tyr Ala Ala Ser Ser Leu His Gly Gly Val Pro Ser Arg Phe Ser Gly
50                      55                      60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg Thr Leu Gln Pro
65                      70                      75                      80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Ser Asn Thr Pro Phe
85                      90                      95
Thr Phe Gly Pro Gly Thr Val Val Asp Phe Arg Arg Thr Val Ala Ala
100                      105                      110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115                      120                      125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130                      135                      140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                      150                      155                      160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165                      170                      175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180                      185                      190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195                      200                      205
Phe Asn Arg Gly Glu Cys
210

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SEQ ID NO: 11 PSMA with no IgE leader sequence and with HA tag

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atgtggaacg cactgcatga gactgattct gctgtcgcac tgggacggag acccggtgg 60
ctgtgctgtg gagcactggt gctggccggc gggggattcc tgctgggatt cctgtttggc 120
tggttttatca aaagctccag cgaggtctacc aatattaccc ctaagcaca taagaaagca 180
ttcctggatg aactgaaagc cgagaacatc aagaaattcc tgtacaactt cacaagaatt 240
ccacatctgg ctggcactga gcagaacttc cagctggcaa aacagatcca ggtcagtg 300
aaggaatttg ggtggactc agtggagctg acccactacg atgtcctgct gtcctatcca 360
aataagactc atcccaacta catctctatc attaacgaag acggaatga gattttcaac 420
acctctctgt ttgaaccccc tccacccggc tatgagaatg tcagtgcagt ggtccctcca 480
ttctcagcct tcagccccc ggggatgcct gagggagatc tgggtgacgt caattatgct 540
agaacagaag acttcttta gctggagagg gatatgaaaa tcaactgttc cggcaagatc 600
gtgattgcc ggtacgggaa ggtgttcaga ggaaataagg tcaaaaacgc tcagctggcc 660
ggagctaccg gcgtgatcct gtacagcgac ccgctgatt attttgacc tggcgtgaag 720
tcctatccag acggatggaa tctgcccggc gggggagtgc agaggggaaa catcctgaac 780
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gctgtggtcc tgcggaagta cgcagacaag atctataaca tctccatgaa gcacccccag 1860
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gccggcgagt ctttcccagg gatctatgac gctctgtttg atattgaatc aaaggtggac 2160
cccagcaaaag catggggcga ggtcaagaga cagatcagca ttgcagcctt tacagtgcag 2220
gccgcccgcg aaacctgtgc cgaagtcgct taccatcac atgtccccga ttacgcagta 2280
taa

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SEQ ID NO: 12 PSMA with IgE leader sequence (underlined) and with HA tag

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atggactgga catggattct gttcctgggc gccgcggcaa ctgcgctgca ttcctggaac 60
gcactgcatg agactgattc tgctgtcgca ctgggacgga gaccccggtg gctgtgctgt 120
ggagcactgg tgctggccgg cgggggattc ctgctgggat tctgtttgg ctggtttatc 180
aaaagctcca gcgaggctac caatattacc cctaagcaca ataagaaagc attcctggt 240
gaactgaaag ccgagaacat caagaaattc ctgtacaact tcacaagaat tccacatctg 300
gctggcactg agcagaactt ccagctggca aaacagatcc agagtcatg gaaggaattt 360
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gcagaggccg tgggcctgcc tagcatocca gtccatccca ttggctatta cgatgccccc 960
aagctgctgg agaaaatggg cgggagcgct cccctgact ctagttggaa gggctccctg 1020

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aaagtgcctt acaatgtcgg gccaggattc actgggaact tttctacca gaaggtgaaa 1080
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gggggaatcg atccacagag cggagcagct gtggtccatg aaattgtgcg cagctttggg 1260
accctgaaga aagagggatg gcgacccagg cgcacaatcc tgttcgcatac ctgggacgcc 1320
gaggaatttg ggctgctggg cagcacagaa tgggccgagg aaaattctcg cctgctgcag 1380
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tctttcccag ggatctatga cgctctgttt gatattgaat caaaggtgga cccagcaaaa 2220
gcatggggcg aggtcaagag acagatcagc attgcagcct ttacagtgca ggccgccgcc 2280
gaaacctgtg ccgaagtcgc ttaccatac gatgtccccg attacgcatg ataa

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SEQ ID NO: 13 PSMA with no IgE leader sequence

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Met Trp Asn Ala Leu His Glu Thr Asp Ser Ala Val Ala Leu Gly Arg
Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val Leu Ala Gly Gly Gly
Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile Lys Ser Ser Ser Gly
Ala Thr Asn Ile Thr Pro Lys His Asn Lys Lys Ala Phe Leu Asp Glu
Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Arg Ile
Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln Leu Ala Lys Gln Ile
Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser Val Glu Leu Thr His
Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr Ile
Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu Phe
Glu Pro Pro Pro Gly Tyr Glu Asn Val Ser Asp Val Val Pro Pro
Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val Tyr
Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp Met
Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys Val
Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala Thr Gly
Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val Lys
Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly Val Gln Arg Gly
Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly Tyr
Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val Gly
Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln Lys
Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp Lys
Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly Asn
Phe Ser Thr Gln Lys Val Lys Met His Ile His Ser Thr Ser Glu Val
Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu Pro
Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe Gly
Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val Arg
Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr Ile
Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser Thr
Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val Ala
Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val
Asp Cys Thr Pro Leu Met Tyr Ser Leu Val Tyr Asn Leu Thr Lys Glu
Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu Ser
Trp Thr Glu Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg Ile
Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg Leu
Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr Asn

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Lys Phe Ser Ser Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr Glu
 Leu Val Glu Lys Phe Tyr Asp Pro Thr Phe Lys Tyr His Leu Thr Val
 Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile Val
 Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala
 Asp Lys Ile Tyr Asn Ile Ser Met Lys His Pro Gln Glu Met Lys Ala
 Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr
 Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Leu Asp Lys Ser
 Asn Pro Ile Leu Leu Arg Ile Met Asn Asp Gln Leu Met Phe Leu Glu
 Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg
 His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser
 Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp
 Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Ser Ile Ala Ala
 Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala

SEQ ID NO: 14 PSMA with IgE leader sequence (underlined)

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
His Ser Trp Asn Ala Leu His Glu Thr Asp Ser Ala Val Ala Leu Gly
 Arg Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val Leu Ala Gly Gly
 Gly Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile Lys Ser Ser Ser
 Glu Ala Thr Asn Ile Thr Pro Lys His Asn Lys Lys Ala Phe Leu Asp
 Glu Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Arg
 Ile Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln Leu Ala Lys Gln
 Ile Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser Val Glu Leu Thr
 His Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr
 Ile Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu
 Phe Glu Pro Pro Pro Pro Gly Tyr Glu Asn Val Ser Asp Val Val Pro
 Pro Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val
 Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp
 Met Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys
 Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala Thr
 Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val
 Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly Val Gln Arg
 Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly
 Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val
 Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln
 Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp
 Lys Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly
 Asn Phe Ser Thr Gln Lys Val Lys Met His Ile His Ser Thr Ser Glu
 Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu
 Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe
 Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val
 Arg Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr
 Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser
 Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val
 Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg
 Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val Tyr Asn Leu Thr Lys
 Glu Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu
 Ser Trp Thr Glu Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg
 Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg
 Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr
 Asn Lys Phe Ser Ser Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr
 Glu Leu Val Glu Lys Phe Tyr Asp Pro Thr Phe Lys Tyr His Leu Thr
 Val Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile
 Val Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr
 Ala Asp Lys Ile Tyr Asn Ile Ser Met Lys His Pro Gln Glu Met Lys
 Ala Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe
 Thr Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Leu Asp Lys
 Ser Asn Pro Ile Leu Leu Arg Ile Met Asn Asp Gln Leu Met Phe Leu
 Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr

Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu
 Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val
 Asp Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Ser Ile Ala
 Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu ValAla

SEQ ID NO: 15 Consensus WT1-L with modified Zinc Fingers nucleic acid
 sequence plus IgE leader

ggatccgccca ccatggactg gacctggatt ctgttcctgg tcgccgccgc aacacgggtg 60
catagtggga gtgatgtgag agacctgaac gccctgctgc cagcagtgcc atccctgcct 120
 ggccgggggag gctgcgctct gccagtctct ggagcagctc agtgggctcc cgtgctggac 180
 tttgcacccc ctgcagcccc ttacggaagt ctggcgccgc cacactcatt catcaaacag 240
 gagccaagct gggcgggggc agatcctcat gaggaacagt gcctgtcagc cttcacagtc 300
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 ccaccccctt cccaggcacc ttctggacag gcacgcatgt tcccaaacgc tccctatctg 420
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 ctgctgtgct gaactccata caacagtgat aatctgtatc agatgacctc acagctggag 720
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 tgataactcg ag

SEQ ID NO: 16 Con WT1-L with modified Zinc Fingers protein sequence plus
 IgE leader (underlined)

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
His Ser Gly Ser Asp Val Arg Asp Leu Asn Ala Leu Leu Pro Ala Val
 Pro Ser Leu Pro Gly Gly Gly Gly Cys Ala Leu Pro Val Ser Gly Ala
 Ala Gln Trp Ala Pro Val Leu Asp Phe Ala Pro Pro Ala Ala Pro Tyr
 Gly Ser Leu Gly Gly Pro His Ser Phe Ile Lys Gln Glu Pro Ser Trp
 Gly Gly Ala Asp Pro His Glu Glu Gln Cys Leu Ser Ala Phe Thr Val
 His Phe Ser Gly Gln Phe Thr Gly Thr Ala Gly Ala Cys Arg Tyr Gly
 Pro Phe Gly Ala Pro Pro Pro Ser Gln Ala Pro Ser Gly Gln Ala Arg
 Met Phe Pro Asn Ala Pro Tyr Leu Pro Asn Cys Leu Glu Ser Gln Pro
 Ala Ile Arg Asn Gln Gly Tyr Ser Thr Val Ala Phe Asp Gly Thr Pro
 Ser Tyr Gly His Thr Pro Ser His His Ala Ala Gln Phe Pro Asn His
 Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly Ser Leu Gly Glu
 Gln Gln Tyr Ser Val Pro Pro Pro Val Tyr Gly Cys His Thr Pro Thr
 Asp Ser Cys Thr Gly Ser Gln Ala Leu Leu Leu Arg Thr Pro Tyr Asn
 Ser Asp Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp
 Asn Gln Met Asn Leu Gly Ser Thr Leu Lys Gly His Ala Thr Gly Tyr
 Glu Ser Asp Asn His Thr Thr Pro Met Leu Tyr Ser Cys Gly Ala Gln
 Tyr Arg Ile His Thr His Gly Val Phe Arg Gly Ile Gln Asp Val Arg
 Arg Val Pro Gly Val Ala Pro Thr Ile Val Arg Ser Ala Ser Glu Thr
 Asn Glu Lys Arg Pro Phe Met Gly Ala Tyr Pro Gly Gly Asn Lys Arg
 Tyr Phe Lys Leu Ser His Leu Gln Met Gly Ser Arg Lys Gly Thr Gly
 Glu Lys Pro Tyr Gln Gly Asp Phe Lys Asp Gly Glu Arg Arg Phe Ser
 Arg Ser Asp Gln Leu Lys Arg Gly Gln Arg Arg Gly Thr Gly Val Lys
 Pro Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His
 Leu Lys Thr His Thr Arg Thr His Thr Gly Lys Thr Ser Glu Lys Pro
 Phe Ser Cys Arg Trp Pro Ser Cys Gln Lys Lys Phe Ala Arg Ser Asp

Glu Leu Val Arg His His Asn Met His Gln Arg Asn Met Thr Lys Leu
Gln Leu Ala Leu

SEQ ID NO: 17 Synthetic Consensus hTERT nucleic acid sequence operably
linked to IgE (underlined)

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 tgcagaaagc tgcccggggc cactctggct gccctggaag ccgctgccga ccctgccctg 3420
 acctccgatt tcaagactat tctggac

SEQ ID NO: 18 Synthetic consensus hTERT amino acid sequence operably linked to IgE (underlined)

Met Asp Trp Thr Trp Ile Leu Phe Leu Val Ala Ala Ala Thr Arg Val
His Ser Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg
 Ser Arg Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu
 Gly Pro Gln Gly Arg Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe
 Arg Ala Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg
 Pro Pro Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu
 Leu Val Ala Arg Val Val Gln Arg Leu Cys Glu Arg Gly Ala Arg Asn
 Val Leu Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro
 Pro Glu Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val
 Thr Asp Thr Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg
 Val Gly Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Tyr
 Val Leu Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu
 Tyr Asp Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser
 Gly Thr Arg Arg Gly Leu Gly Thr Glu Gln Ala Trp Asn His Ser Val
 Arg Glu Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg
 Arg Arg Gly Ser Ala Gly Arg Ser Leu Pro Leu Ala Lys Arg Pro Arg
 Arg Gly Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser
 Trp Ala His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys
 Val Val Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly
 Ala Leu Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His
 His Ala Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr
 Pro Cys Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser
 Gly Asp Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg
 Pro Ser Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly
 Ser Arg Pro Trp Met Pro Gly Thr Pro Arg Arg Thr Pro Arg Leu Pro
 Gln Arg Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Gly Asn
 His Ala Gln Cys Pro Tyr Gly Ala Leu Leu Arg Thr His Cys Pro Leu
 Arg Ala Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro
 Gln Gly Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg
 Leu Val Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly
 Phe Leu Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly
 Ser Arg His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile
 Ser Leu Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys
 Met Ser Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly
 Cys Val Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys
 Phe Leu His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser
 Phe Phe Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Tyr Leu Phe Phe
 Tyr Arg Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln
 His Leu Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg
 Gln His Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe
 Leu Pro Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val
 Val Gly Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr
 Ser Arg Val Lys Thr Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg
 Arg Pro Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His
 Arg Ala Trp Arg Ala Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro
 Pro Glu Leu Tyr Phe Val Lys Val Ala Val Thr Gly Ala Tyr Asp Thr
 Ile Pro Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro
 Gln Asn Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Arg Arg Ala Ala
 His Gly His Val Arg Lys Ser Phe Lys Arg His Val Ser Thr Leu Thr
 Asp Leu Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr
 Ser Pro Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn
 Glu Ala Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Val Cys His

His Ala Val Arg Ile Gly Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile
 Pro Gln Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly
 Asp Met Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu
 Leu Arg Leu Val Ala Ala Phe Leu Leu Val Thr Pro His Leu Thr His
 Ala Lys Ala Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly
 Cys Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp
 Glu Ala Leu Gly Gly Thr Ala Phe Val Gln Leu Pro Ala His Gly Leu
 Phe Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln
 Ser Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr
 Phe Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe
 Gly Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Tyr Leu Gln Val
 Asn Ser Leu Gln Thr Val Cys Thr Asn Val Tyr Lys Ile Phe Leu Leu
 Gln Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln
 Gln Val Arg Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr
 Ala Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser
 Leu Gly Ala Lys Gly Ala Ala Gly Pro Phe Pro Ser Glu Ala Ala Gln
 Trp Leu Cys His Gln Ala Phe Leu Leu Lys Leu Ala Arg His Arg Val
 Thr Tyr Arg Cys Leu Leu Gly Ala Leu Arg Thr Ala Gln Thr Gln Leu
 Cys Arg Lys Leu Pro Gly Ala Thr Leu Ala Ala Leu Glu Ala Ala Ala
 Asp Pro Ala Leu Thr Ser Asp Phe Lys Thr Ile Leu Asp

SEQ ID NO: 19 Synthetic Consensus hTERT nucleic acid sequence
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SEQ ID NO: 20 Synthetic Consensus hTERT amino acid sequence

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 KTVVNFVVEDEALGGTAFVQLPAHGLFPWCGLLLDTRTLEVQSDYSSYARTSIRASLT FNRGFKAGRNMRRLKLF
 VLRLKCHSLFLYLQVNSLQTVCTNVYKIFLLQAYRFHACVLQLPFHQQVRKNPTFFLRVISDTASLCYSILKAKN
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 FKTI LD**

SEQ ID NO: 21 nucleic acid sequence encoding PSMA with IgE leader sequence

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SEQ ID NO: 22 nucleic acid sequence encoding IL12 p35 (pGX6001)
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SEQ ID NO: 24 nucleic acid sequence encoding IL12 p40 (pGX6001)
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SEQ ID NO: 25 IL12 p40 amino acid sequence (pGX6001)
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SEQ ID NO: 26 Con WT1-L with modified Zinc Fingers protein
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KRYFKLSHLQMSRKGTGEKPYQGF KDGERRFSRSDQLKRGQRRTGVKPFQCKTCQRKFSRSDHLKTHTRHT
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SEQ ID NO: 27 Consensus WT1-L with modified Zinc Fingers nucleic acid
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SEQ ID NO: 28 PSMA

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*

SEQ ID NO: 29 nucleic acid sequence encoding PSMA

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