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(54) **Title:** CANCER IMMUNOTHERAPY BY IMMUNE ACTIVATION OR IMMUNE MODULATION VIA GLOBO SERIES ANTIGENS

(57) **Abstract:**

CANCER IMMUNOTHERAPY BY IMMUNE ACTIVATION OR IMMUNE MODULATION VIA GLOBO SERIES ANTIGENS

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the priority of U.S. Provisional Patent Applications No. 62/326,623 (filed April 22, 2016), 62/343,530 (filed May 31, 2016), 62/345,755 (filed June 4, 2016) and 62/381,875 (filed August 31, 2016). The entirety of the aforementioned application is incorporated herein by reference.

FIELD OF THE INVENTION

[2] This invention relates to methods for immunotherapy of a cancer patient comprising administering to the patient vaccines against Globo series antigens.

BACKGROUND OF THE INVENTION

[3] The carbohydrate antigen Globo H (Fucα1→2 Galβ1→3 GalNAcβ1→3 Galα1→4 Galβ1→4 Glc) was first isolated as a ceramide-linked Glycolipid and identified in 1984 by Hakomori et al. from breast cancer MCF-7 cells. (Bremer E G, et al. (1984) J Biol Chem 259:14773-14777). Further studies with anti-Globo H monoclonal antibodies showed that Globo H was present on many other cancers, including prostate, gastric, pancreatic, lung, ovarian and colon cancers and only minimal expression on luminal surface of normal secretory tissue which is not readily accessible to immune system. (Ragupathi G, et al. (1997) Angew Chem Int Ed 36:125-128). In addition, it has been established that the serum of breast cancer patient contains high level of anti-Globo H antibody. (Gilewski T et al. (2001) Proc Natl Acad Sci USA 98:3270-3275; Huang C- Y, et al. (2006) Proc Natl Acad Sci USA 103:15-20; Wang C-C, et al. (2008) Proc Natl Acad Sci USA 105(33):11661-11666). Patients with Globo H-positive tumors showed a shorter survival in comparison to patients with Globo H-negative tumors. (Chang, Y-J, et al. (2007) Proc Natl Acad Sci USA 104(25):10299-10304). These findings render Globo H, a hexasaccharide epitope, an attractive tumor marker and a feasible target for cancer vaccine development.

SUMMARY OF THE INVENTION

[4] Aspects and embodiments of the present disclosure provides methods for treating a subject afflicted with cancer by immunotherapy comprising administering to the subject in need thereof Globo series antigens targeting immunogenic agent (e.g., OBI-822) useful for inducing/modulating an immune response (IgG and/or IgM), said method comprising

improving the survival (including overall survival and/or progression free survival) by modulating Globo series antigens interaction, such that survival of the subject is improved. In one aspect the Globo series antigens targeting immunogenic agent is, for example, OBI-822. Please referred to our previous PCT patent application (publication number: WO2015159118A2 and WO2016044326A1). These applications disclosure encompasses immunogenic/therapeutic compositions including Globo H-KLH glycoconjugates (OBI-822) and/or therapeutic adjuvants (OBI-821/OBI-834) as well as methods of making and using the same to treat proliferative diseases such as cancer. The therapeutic compositions are in part envisaged to act as cancer vaccines for boosting the body's natural ability to protect itself, through the immune system from dangers posed by damaged or abnormal cells such as cancer cells.

[5] In one embodiment, the Immunogenic agent can include OBI-822 and related variants.

[6] In certain embodiments, the immune response can include: IgG (including subclasses IgG1, IgG2, IgG3, IgG4), IgM, CTLs (cytotoxic lymphocyte) directed to Globo H series antigens/tumor.

[7] In certain embodiments, clinically meaningful benefits can include modulations in: Progression free survival; overall survival; (well tolerated and/or no major safety concerns); objective response rate; time to progression; disease free survival; tumor response; improvements in quality of life; reduction in size of solid tumors, and/or reduction in tumor associated antigens (primarily or including Globo H).

[8] In certain aspects, administration regimen can include: Administer vaccine two or more times (e.g., 3, 4, 5, 6, 7, 8, 9, 10 or more times); adjusting time interval and/or dosing amount regimen between two successive administrations; adjusting routes of administration and/or altering/alternating injection sites/locations of administration or ANY combination of any of the above, whereby each administration increases the immune response [e.g. titer - IgG and/or IgM Ab amount, and/or increases affinity/avidity; induction of Abs to less immunogenic sites of Globo H portion of the Globo H antigen-conjugate (e.g., portions of Globo H antigen that may be less accessible in the conjugate)]. In certain aspects the injections can be altered and/or supplemented by the addition of immune response booster agents.

[9] In certain aspects, modulation of Globo series antigens interaction can include: Induction of anti-Globo H antibodies with increased affinity with multiple rounds of

vaccination; expansion of germinal centers of B cells to Globo H series tumor antigens; preferential expansion of germinal centers containing high affinity anti-Globo H antibodies; induction of low frequency B cells normally not present in sufficient numbers to produce meaningful responses with single (or low repetition of exposure to antigen) (e.g., they may bind to epitopes to which few antibodies bind); expansion of antibody secreting plasma cells ("antibody secreting plasma cells" are what B cells differentiate into) and memory B cells which may be important for long term maintenance of the anti-tumor response; increase kinetics of Ab class switching [need good T cell helper function or B cells will not switch to IgG]; increase in kinetics of antibody response (e.g., successive vaccination may lead to more rapid expansion of antibody than obtained without either conjugation to KLH, without co-administration of cyclophosphamide, and/or without repeated vaccinations; reduction of Treg activity that interferes with the development and maintenance of the above anti-tumor response; induction of Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC) for tumor killing; induction of anti-Globo series antigens IgM/IgG immune response to elicit CDC and ADCC mediated tumor cell killing; induction of anti-Globo series antigens antibodies trap Globo series antigens-ceramide shedding from tumor cells to block translin-associated factor X (TRAX)-dependent angiogenesis; induction of anti-Globo series antigens antibodies to block Globo series antigens-ceramide induced Notch 1-dependent immunosuppression and thereby enhancing T cell proliferation and cytokine production; induction of anti-Globo series antigens antibodies to lead to apoptosis; Inhibition of Globo series antigens induced angiogenesis; OBI-822 vaccination induces apoptosis; Induction of CTLs (cytotoxic lymphocyte).

BRIEF DESCRIPTION OF THE DRAWINGS

[10] **Error! Reference source not found.**. OBI-822: Active cancer immunotherapy induces ADCC and CDC for tumor killing. Globo H-ceramide depletion by OBI-822 effectively blocks TRAX-dependent angiogenesis and achieves tumor regression. Tumor-secreted soluble factors bind to their respective receptors on endothelial cells, trigger phospholipase C (PLC) activation and intracellular calcium release, and promote proliferation, migration, and tube formation of endothelial cells. PLC serves as an "early generator" of second messengers, driving early stages of angiogenesis. PLC activity is regulated by several binding partners, including translin-associated factor X (TRAX), which blocks PLCβ1 activity. The molecular mechanism of this event involves the binding of Globo-H ceramide to TRAX with consequent release and activation of PLCβ1.

[11] **Error! Reference source not found.**. OBI-822 depletes Globo H-ceramide, which in turns increases Notch 1 degradation, blocking the tumor immunosuppression effect, leading to tumor regression. The Notch signaling pathway is an evolutionarily conserved cell signaling system in most organisms, and it can regulate cell proliferation, differentiation, apoptosis, and survival. The regulation of Notch 1 is controlled by the E-protein transcription factor E2A and its natural inhibitor ID3. It is degraded by ubiquitination though E3 ubiquitin ligase, ITCH. Addition of Globo-H ceramide to immune cells could inhibit their proliferation and cytokine or immunoglobulin secretion. Globo-H ceramide could induce immunosuppression which involved inhibition of Notch 1 signaling through the induction of ID3 and EGR2/3 accompanied ITCH expression.

[12] **Error! Reference source not found.**. OBI-822 leads to apoptosis. Apoptosis (programmed cell death) contributes to the normal development and tissue remodeling of multicellular organisms. Focal adhesion kinase (FAK) has been implicated in the integration of signals from integrins, oncogenes, and neuropeptides. Proteolytic cleavage of FAK by caspase-3 has been reported during growth factor deprivation-induced apoptosis in human umbilical vein endothelial cells, which implies an association between FAK and apoptosis.

[13] **Error! Reference source not found.** illustrated the Progression-Free Survival (mITT) after received 9 Injections of Study Drugs (Stratified Log-rank Test, p=0.0542).

[14] **Error! Reference source not found.** illustrated the Progression-Free Survival (mITT) after received 9 Injections of Study Drugs and with Disease at Baseline (Stratified Log-rank Test, P=0.0362).

[15] **Error! Reference source not found.** illustrated the Progression-Free Survival (mITT) of Study Drugs Treatment with/without IgG immune response (IgG Criteria 1:640) vs placebo.

[16] **Error! Reference source not found.** illustrated the Overall Survival (mITT) of Study Drugs Treatment with/without IgG immune response (IgG Criteria 1:640) vs placebo.

[17] **Error! Reference source not found.** illustrated the Progression-Free Survival (mITT) after received one injection of Study Drug on Week 2 with/without early IgM immune response (IgM Criteria) vs placebo.

[18] Figure 9 showed there was a high anti-KLH IgG immune response after patients received the injections of OBI-822 vaccine.

[19] Figure 10 illustrated the Negative Control (Patient No: 065) with ovarian cancer clinical trial of OBI-822 Treatment.

[20] Figure 11 illustrated the Stage III Ovarian Cancer (Patient No: 035) with ovarian cancer clinical trial of OBI-822 Treatment.

[21] Figure 12 illustrated the Stage IV Fallopian Tube Cancer (Patient No: 041) with ovarian cancer clinical trial of OBI-822 Treatment.

[22] Figure 13 illustrated the Stage III Ovarian Cancer (Patient No: 060) with ovarian cancer clinical trial of OBI-822 Treatment.

[23] Figure 14 illustrated the procedure of Investigational Drugs Mixing.

DETAILED DESCRIPTION OF THE INVENTION

[24] The present invention relates to methods for immunotherapy of a subject afflicted with diseases such as cancer or an infectious disease, which methods comprise administering to the subject a composition comprising a therapeutically effective amount of a compound or agent that potentiates an endogenous immune response, either stimulating the activation of the endogenous response or inhibiting the suppression of the endogenous response. More specifically, this disclosure provides methods for potentiating an endogenous immune response in a subject afflicted with cancer so as to thereby treat the patient, which method comprises administering to the subject a therapeutically effective amount of an immunogenic agent.

[25] Definitions:

[26] "Administering" As used herein, embodiments of Administration regimen can include the following features: 1) Administer vaccine two or more times (e.g., 3, 4, 5, 6, 7, 8, 9, 10 or more times); 2) Each administration increases Immune Response (see above) [titer - IgG and/or IgM Ab amount, and/or increases affinity/avidity; induction of Abs to less immunogenic sites of Globo H portion of the Globo H antigen-conjugate (e.g., portions of Globo H antigen that may be less accessible in the conjugate)].

[27] As used herein, "treatment" refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing or decreasing inflammation and/or tissue/organ damage, decreasing the rate of disease progression,

amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or disorder.

[28] An “effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[29] A “therapeutically effective amount” of a substance/molecule of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount.

[30] "Adverse Event" (AE) Toxicity will be measured according to US NCI Common Toxicity Criteria, Version 4, developed by the Cancer Therapy Evaluation Program at the National Cancer Institute. The criteria for unacceptable toxicities should include any ≥ Grade 4 toxicity, with the exception of local skin reactions, fever, chilling, sweats, urticaria, and/or pruritis since these are common side effects of antibody/adjuvant administration, are reversible, and controlled by supportive management. Theoretically, immune complex disease as manifested by skin, joint, renal, or other manifestations could occur, but these should be rare in the absence of prior exposure to mouse protein. These will be an indication to stop therapy in the affected subjects, but accrual of new subjects may continue. An adverse event is any physical or clinical change or disease experienced by the subject from the date of randomization and up to two years from randomization for subjects continuing in the follow up period, whether or not considered related to the use of the investigational drug. This includes the onset of new illness and the exacerbation of the preexisting condition. For subjects who withdraw treatment during the treatment period, adverse events should be recorded through 28 days after the last administration of study treatment (OBI-822/OBI-821 or Control).

[31] "Antibody" (Ab), “Antibodies” (Abs) and “immunoglobulins” (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-

like molecules which generally lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full length or intact monoclonal antibodies), polyclonal antibodies, monovalent, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be chimeric, human, humanized and/or affinity matured.

[32] "Variable" and "Complementarity Determining Regions" (CDRs)

[33] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab′)2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[34] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by a

flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[35] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab′ fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab′ -SH is the designation herein for Fab′ in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab′)2 antibody fragments originally were produced as pairs of Fab′ fragments which have hinge cysteines between them.

[36] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[37] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[38] As used herein, "isolated antibody" can include an "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would

interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In one embodiment, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[39] The term "monoclonal antibody" (mAB) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, the monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a

variety of techniques, including, for example, the hybridoma method (e.g., Kohler et al., Nature, 256: 495 (1975); Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567), phage display technologies (See, e.g., Clackson et al., Nature, 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO98/24893; WO96/34096; WO96/33735; WO91/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks et al., Bio. Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996) and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

[40] "human monoclonal antibody" (HuMAb)

A "human monoclonal antibody" is a mAb which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[41] "humanized antibody"

Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

[42] "chimeric antibody"

The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)).

[43] "antigen-binding portion" ‘or “antibody fragment”

“Antibody fragments” comprise only a portion of an intact antibody, wherein the portion retains at least one, and as many as most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen.

[44] In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half-life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an in vivo half-life substantially similar to an intact antibody.

For example, such an antibody fragment may comprise an antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment.

[45] The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are **NOT limited to**, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer. “Tumor,” as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms “cancer,” “cancerous,” “cell proliferative disorder,” “proliferative disorder” and “tumor” are not mutually exclusive as referred to herein.

[46] "Immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[47] "Immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

[48] "Immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

[49] "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

[50] "Potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

[51] "Subject" includes any human or nonhuman animal.

[52] "Therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent, such as an Ab of the invention, is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom- free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[53] "Promoting cancer regression" means that administering an effective amount of the drug, alone or in combination with an anti-neoplastic agent, results in a reduction in tumor growth or size, necrosis of the tumor, a decrease in severity of at least one disease symptom, an increase in frequency and duration of disease symptom- free periods, or a prevention of impairment or disability due to the disease affliction. In addition, the terms "effective" and "effectiveness" with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote cancer regression in the patient. Physiological safety refers to the level of toxicity, or other

adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug.

[54] "Immune-related" response pattern refers to a clinical response pattern often observed in cancer patients treated with immunotherapeutic agents that produce antitumor effects by inducing cancer-specific immune responses or by modifying native immune processes. This response pattern is characterized by a beneficial therapeutic effect that follows an initial increase in tumor burden or the appearance of new lesions, which in the evaluation of traditional chemotherapeutic agents would be classified as disease progression and would be synonymous with drug failure. Accordingly, proper evaluation of immunotherapeutic agents may require long-term monitoring of the effects of these agents on the target disease.

[55] A therapeutically effective amount of a drug includes a "prophylactically effective amount," which is any amount of the drug that, when administered alone or in combination with an anti-neoplastic agent to a subject at risk of developing a cancer (e.g., a subject having a pre-malignant condition) or of suffering a recurrence of cancer, inhibits the development or recurrence of the cancer. In preferred embodiments, the prophylactically effective amount prevents the development or recurrence of the cancer entirely. "Inhibiting" the development or recurrence of a cancer means either lessening the likelihood of the cancer's development or recurrence, or preventing the development or recurrence of the cancer entirely.

[56] "Tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

[57] Immunogenic agent and Antibodies generated of the present invention may be constituted in a composition, e.g., a pharmaceutical composition, containing one Ab or a combination of Abs, or an antigen- binding portion(s) thereof, and a pharmaceutically acceptable carrier. As used herein, a "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). A pharmaceutical composition of the invention may include one or more pharmaceutically acceptable salts, anti-oxidant, aqueous and

nonaqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

[58] Preferred subjects include human patients in need of enhancement of an immune response. The immunotherapeutic methods disclosed herein are particularly suitable for treating human patients having a disorder that can be treated by potentiating an immune response. In certain embodiments, the methods are employed for treatment of subjects afflicted with a disease caused by an infectious agent. In preferred embodiments, the methods are employed for treatment of subjects afflicted with, or at risk of being afflicted with, a cancer.

[59] "Cancer Immunotherapy"

As used herein, cancer immunotherapy can include, but not limited to, immune-based therapies capable of reducing tumor size in patients with metastatic cancer. Currently, there are three main approaches to cancer immunotherapy, a non-specific stimulation of immune reactions by stimulating effector cells and/or inhibiting regulatory cells, an active immunization to enhance specific anti-tumor reactions, known as cancer vaccines, and a passive transfer of anti-tumor antibodies or activated immune cells with antitumor activity, also known as adoptive immunotherapy (DeVita et al., 2008).

[60] "Combination therapy" In certain embodiments, the immunomodulatory agents discussed herein may be used in combination with one or more anti-proliferative / chemotherapeutic agent that are effective for reducing tumor burden without significant systemic toxicity and may act to improve the effectiveness of the immune response. The agents can be combined as co-administration combination therapy and/or co-formulated combination therapy.

[61] Combination therapy in which two or more drugs are used together in some dosing regimen or administration form, typically has one or more goals of: (i) reducing the frequency at which acquired resistance arises by combining drugs with minimal cross-resistance, (ii) lowering the doses of drugs with non-overlapping toxicity and similar therapeutic profile so as to achieve efficacy with fewer side effects, i.e., increase therapeutic index, (iii) sensitizing cells to the action of one drug through use of another drug, such as altering cell-cycle stage or growth properties, and (iv) achieving enhanced potency by exploiting additivity, or greater than additivity, effects in the biological activity of two drugs (Pegram, M., et al (1999) Oncogene 18:2241-2251; Konecny, G., et al (2001) Breast Cancer Res. and Treatment 67:223-233; Pegram, M., et al (2004) J. of the Nat. Cancer Inst. 96(10):739-

749; Fitzgerald et al (2006) Nature Chem. Biol. 2(9):458-466; Borisy et al (2003) Proc. Natl. Acad. Sci. 100(13):7977-7982). Loewe additivity (Chou, T. C. and Talalay, P. (1977) J. Biol. Chem. 252:6438-6442; Chou, T. C. and Talalay, P. (1984) Adv. Enzyme Regul. 22:27-55; Berenbaum, M. C. (1989) Pharmacol. Rev. 41:93-141) and Bliss independence/synergy (Bliss, C.I. (1956) Bacteriol. Rev. 20:243-258; Greco et al (1995) Pharmacol. Rev. 47:331-385) are methods used for calculating the expected dose-response relationship for combination therapy compared to monotherapy based on parameters such as IC50, the dose of drug needed to achieve 50% target inhibition and equal to Ki in the simplest case.

[62] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic/antitumor antibiotics, topoisomerase inhibitors, antibodies, photosensitizers, and kinase inhibitors. Chemotherapeutic agents include compounds used in "targeted therapy" and conventional chemotherapy. Examples of chemotherapeutic agents include: erlotinib (TARCEVA®, Genentech/OSI Pharm.), docetaxel (TAXOTERE®, Sanofi-Aventis), 5-FU (fluorouracil, 5-fluorouracil, CAS No. 51-21-8), gemcitabine (GEMZAR®, Lilly), PD-0325901 (CAS No. 391210-10-9, Pfizer), cisplatin (cis-diamine,dichloroplatinum(II), CAS No. 15663-27-1), carboplatin (CAS No. 41575-94-4), paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.), trastuzumab (HERCEPTIN®, Genentech), temozolomide (4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo[4.3.0]nona-2,7,9-triene-9-carboxamide, CAS No. 85622-93-1, TEMODAR®, TEMODAL®, Schering Plough), tamoxifen ((Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethyl-ethanamine, NOLVADEX®, ISTUBAL®, VALODEX®), and doxorubicin (ADRIAMYCIN®), Akti-1/2, HPPD, and rapamycin.

[63] More examples of chemotherapeutic agents include: oxaliplatin (ELOXATIN®, Sanofi), bortezomib (VELCADE®, Millennium Pharm.), sutent (SUNITINIB®, SU11248, Pfizer), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), XL-518 (MEK inhibitor, Exelixis, WO 2007/044515), ARRY-886 (Mek inhibitor, AZD6244, Array BioPharma, Astra Zeneca), SF-1126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis), PTK787/ZK 222584 (Novartis), fulvestrant (FASLODEX®, AstraZeneca), leucovorin (folinic acid), rapamycin (sirolimus, RAPAMUNE®, Wyeth), lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), lonafarnib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer),

tipifarnib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Il), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chloranmbucil, AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosfamide (TELCYTA®, Telik), thiotepa and cyclosphosphamide (CYTOXAN®, NEOSAR®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, calicheamicin gamma1I, calicheamicin omegaI1 (Angew Chem. Intl. Ed. Engl. (1994) 33:183-186); dynemicin, dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2′,2″-trichlorotriethylamine; trichothecenes (T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); cyclophosphamide; thiotepa; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®, Roche); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[64] Also included in the definition of “chemotherapeutic agent” are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors such as MEK inhibitors (WO 2007/044515); (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, for example, PKC-alpha, Raf and H-Ras, such as oblimersen (GENASENSE®, Genta Inc.); (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN® rIL-

2; topoisomerase 1 inhibitors such as LURTOTECAN®; ABARELIX® rmRH; (ix) anti-angiogenic agents such as bevacizumab (AVASTIN®, Genentech); and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[65] Also included in the definition of "chemotherapeutic agent" are therapeutic antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG™, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug-conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth).

[66] Humanized monoclonal antibodies with therapeutic potential as chemotherapeutic agents in combination with the immunogenic/therapeutic agents of the present invention can include or exclude one or more of: alemtuzumab, apolizumab, aselizumab, atlizumab, bapineuzumab, bevacizumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pertuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, trastuzumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, and visilizumab.

[67] "Standard-of-care therapeutic" is a treatment process, including a drug or combination of drugs, radiation therapy (RT), surgery or other medical intervention that is recognized by medical practitioners as appropriate, accepted, and/or widely used for a certain type of patient, disease or clinical circumstance. Standard-of-care therapies for different types of cancer are well known by persons of skill in the art. For example, the National Comprehensive Cancer Network (NCCN), an alliance of 21 major cancer centers in the USA, publishes the NCCN Clinical Practice Guidelines in Oncology (NCCN GUIDELINES®) that provide detailed up-to-date information on the standard-of-care treatments for a wide variety of cancers (see NCCN GUIDELINES®, 2013).

[68] Kits: Also within the scope of the present invention are kits, including pharmaceutical kits, for therapeutic uses, and diagnostic kits

[69] As used herein, additional aspects of the present disclosure includes aspects and factors relating to Dose escalation; patient Cohort; Safety; and Pharmacokinetics / Pharmacodynamics Analyses.

[70] In broad terms, the immune system can be divided into innate and adaptive immunity. Innate immunity, is the more primitive of the two and is comprised of non-specific defenses, such physical barriers (e.g., the skin), non-specific defensive cells (e.g., macrophages) and variety of cytokines (e.g., IL-1). In general, vaccines will not upregulate innate system to a specific pathogen or disease, but adjuvants added to the vaccines may non-specific activate the innate immunity, which in turn may improve the adaptive-immune response. Adaptive immune can be further divided into humor (i.e., antibody) and cellular (e.g, cytotoxic T cells) immune responses. The effector cells of the humor immune response are comprised of cells that specialized solely in adaptive immunity (e.g., T and B lymphocytes); however, cells of innate immunity provide essential functions (e.g., antigen presentation). Thus, for example, the induction of antibody production to a virus would require a series of complex interactions of several cell types. Simplified, these would include capture and processing of viral components (e.g., virus' envelope proteins) by dendritic cells, which would in turn be *presented* to T cells specific to the presented antigen. Once activated by presented antigen, the T cells would "help" virus-specific B cells to generate antibodies to the invading pathogen.

[71] Tolerance

[72] It has long been recognized that while the immune system has the capability to recognize host antigens, normally, such responses are not observed (*i.e.*, the immune system exhibits tolerance to self). This tolerance to self includes both "normal" as well as tumor antigens.

[73] In one aspect, the current disclosure features a vaccine capable of disrupting the immune system's tolerance to the tumor antigen Globo H.

[74] Types of tolerance

[75] Tolerance can result from either central and/or peripheral tolerance. Central tolerance prevents maturation of T and B lymphocytes which recognize self. Self-tolerance is not absolute, and some B cells producing anti-self antibodies may be found in normal

individuals. However, because of a lack of anti-self T-cell help to self-antigens--an essential component of B cell activation--antibodies to self are rarely found. Peripheral tolerance is the ongoing active suppression of the immune response to self, and is thought to be primarily maintained by Treg cells. Treg's are thought to prevent the induction of T cell help to self antigens, which include both normal and tumor antigens.

[76] Strategy to break tolerance to self

[77] In one aspect, the current disclosure features compositions and methods to overcome both central and peripheral tolerance to Globo H series tumor antigens. For example, in some aspects, the compositions and methods are useful in reducing Treg suppression and stimulating T-cell help to B cells producing anti-Globo H antibodies.

[78] In some aspects, the current disclosure features co-administration of a Treg downmodulating agent to overcome peripheral tolerance. In aspects the Treg downmodulating agent may be cyclophosphamide, anti-Treg antibody or other agent that [selectively] inhibits Treg activity (more than other cells of the adaptive immune system).

[79] Thus, in some aspects, cyclophosphamide can be administered to the patient concomitantly with the Globo H-KLH conjugate, thereby inhibiting Treg suppression of anti-Globo H antibody production. In some aspects, the Treg downregulating agent may also stimulate expansion of cytotoxic T lymphocytes to Globo H antigen positive cells, allowing the direct killing of Globo-H expressing tumor cells.

[80] It is recognized that B cell antibody production requires help from a T cell that recognizes the antigen. However, central tolerance and/or antigen presenting deficiencies may result in a lack of T cell recognition of the Globo H (self) antigen. Accordingly, in some embodiments, this disclosure features compositions and methods for stimulating T-cell help to B cells producing anti-Globo H antibodies through conjugation of a Globo H series antigen to a strongly immunogenic agent, such as KLH. KLH is a phylogenetically distant organism and has a large molecular weight (over 390,000), both attributes known to increase immunogenicity. When the Globo H conjugate is administered to a patient, dendritic cells and/or other antigen-presenting cells process the Globo H conjugate, for example, the Globo H –KLH conjugate into Globo H and KLH components. T cells recognize the KLH antigen, which then helps B cells to produce the desired anti-Globo H antibodies.

[81] Other effects of tumor vaccines

[82] Increased antibody titer: In some embodiments the compositions and methods of this invention features a clinical benefit by producing an antibody response above a threshold titer. Below the threshold titer, the antitumor response may be insufficient to produce a meaningful clinical benefit.

[83] Increased affinity of anti-Globo H antibodies: In some embodiments the methods of this disclosure feature administering the Globo H conjugate to the patient two, three, four, five, six, seven, eight, nine, or ten or more times.

[84] In some embodiments, repeated administration increases the affinity of the resulting antibody response as B cells expressing surface antibody with the highest affinity to Globo H are preferentially stimulated by injected antigen, because they bind the antigen better that B cells expressing lower affinity antibodies. In some embodiments, each cycle of administration increases the antibody response, increases the affinity and/or avidity of the antibodies, and/or induces production of antibodies to less immunogenic sites of the Globo H portion of the Globo H conjugate.

[85] In some embodiments, repeated administration further induces low frequency B cells normally not present in sufficient numbers to produce meaningful responses with single (or low repetition of exposure to antigen) (*e.g.*, they may bind to epitopes to which few antibodies bind), expansion of antibody secreting plasma cells ("antibody secreting plasma cells" are what B cells differentiate into) and memory B cells which may be important for long term maintenance of the anti-tumor response, and increase kinetics of Ab class switching.

[86] In some embodiments repeated administrations also results in expansion of germinal centers of B cells to Globo H series tumor antigens and preferential expansion of germinal centers containing high affinity anti-Globo H antibodies

[87] Expansion of IgG subclasses: T cell help is able to induce B cells to switch their expression of heavy chain class and sub-classes. In humans, there are four IgG subclasses—IgG1, IgG2, IgG3 and IgG4. Each IgG subclass has biological effector function that differentiates it from the other subclasses. The expression of all four subclasses may maximize the tumor killing activity of the anti- Globo H response.

[88] Increased kinetics in antibody response: In some embodiments, repeated inoculations of the Globo H-KLH conjugate, cyclophosphamide and adjuvant result in a more rapid expansion of antibody than obtained without conjugation to KLH, cyclophosphamide or repeated injections with adjuvant.

[89] In certain embodiments, OBI-822 can block cancer immunosuppression by reducing Globo-H induced Notch signaling.

[90] In certain aspects, OBI-822 can counteract certain negative traits of Globo-H in cancer treatment.

[91] In certain embodiments, OBI-822 depletes Globo H, which in turns increases Notch 1 degradation, blocking the tumor immunosuppression effect, leading to tumor regression.

[92] In certain embodiments, OBI-822 can counteract Globo-H associated reduction of T cell proliferation and cytokine secretion.

[93] In certain embodiments, OBI-822 can counteract Globo-H associated inhibition of B cell differentiation and Ig secretion.

[94] The roles of Globo Series antigens in cancer development is important. In certain embodiments, they can influence tumor survival by inhibiting the caspase 3 activation. In certain embodiments, revert caspase 3 inactivation, leading to apoptosis.

[95] In certain embodiments, Globo-series clustering enhances tumor survival by inhibiting the caspase 3 cascade.

EXAMPLES

[96] Until Applicants' present disclosure and exemplary data in support of affirmative immunogenic response and therapeutic efficacy, there is no prior conclusive demonstration/report of the effective use of the immunomodulatory agents as disclosed herein related to successful use of OBI-821 as an Adjuvant as evidenced in the examples section, including Pre-clinical Studies of Globo H Vaccine; OBI-822 with QS-21 in Phase I trials; and OBI-822 with OBI-821 in Cancer Clinical Trials.

[97] <u>Trial Overview</u>: Globo H is a glycolipid found to be highly expressed in breast cancer. Active immunotherapy with OBI-822, a Globo H–KLH conjugate, and OBI-821, an adjuvant in two Phase I trials, induced Globo H specific antibodies which can mediate *in vitro* binding and cytotoxicity to Globo H expressing breast cancer cells.

[98] <u>Methods</u>: In the international, randomized, double-blind, and placebo-controlled Phase II/III trial (NCT01516307), patients with metastatic breast cancer who had ≤ 2 events of progressive disease (PD), and who achieved at least stable disease (SD) after ≥1 anticancer regimen are randomized 2:1 to receive subcutaneous OBI-822 (30 μg Globo H)/OBI-821 (100

μg) or control (PBS), in combination with low-dose cyclophosphamide (300 mg/m^2) on Weeks 1, 2, 3, 5, 9, 13, 17, 25, 37, or until PD. Hormone therapy is allowed. The primary and secondary efficacy end points are progression-free survival (PFS) and overall survival (OS), correlated with humoral antibody response.

[99] Results: 349 patients were randomized, 348 received study drug (ITT), 168 (48%) received all 9 injections. 70% had hormone receptor positive breast cancer, 13% were triple negative, and 62% received hormone therapy. No difference was observed in PFS (HR, 0.96 [95% CI, 0.74-1.25] P=0.77) or in interim OS (HR, 0.79 [95% CI, 0.51-1.22] P=0.29) across all patients, including patients who developed a Globo H specific IgG response, and those that did not develop a Globo H specific IgG response. However, PFS and OS were significantly improved in the 50% of patients who developed a Globo H specific IgG response to OBI-822/OBI-821 with a titer ≥ 1:160 at any time during treatment vs. control (HR, 0.71 [95% CI, 0.52-0.97] P=0.029 for PFS; HR, 0.57 [95% CI, 0.33-0.97] P=0.04 for OS) and vs non-responders (HR, 0.52 [95% CI, 0.37-0.71] P< .0001 for PFS; HR, 0.52 [95% CI, 0.29-0.92] P=0.025 for OS), adjusted for baseline disease status and/or hormone use. In a time-dependent Cox model, PFS was improved in patients who received all 9 injections of OBI vs control (HR, 0.66 [95% CI, 0.42-1.01] P=0.057). OBI-822/OBI-821 was well tolerated; the most common drug-related adverse event was grade 1/2 injection reaction.

[100] Conclusion: Vaccination with OBI-822/OBI-821 did not improve PFS in the ITT. However, PFS and interim OS were significantly improved in patients who developed an immune response to the vaccine. These sub-group data are used to design a definitive Phase III trial.

[101] Rationale for Trial Design: OBI-822 is a new, investigational anti-cancer treatment that belongs to a novel class of active immunotherapies. It is a synthetic glycoprotein comprised of a tumor-associated carbohydrate antigen (TACA), Globo H, covalent bounded to a carrier protein, Keyhole Limpet Hemocyanin (KLH). OBI-821 is a saponin-based adjuvant. Globo H is expressed in high levels on the surface of malignant tumors in many epithelial cancers, such as breast, prostate, gastric, lung, colon, pancreatic, and ovarian cancer, etc. The immunogenicity of the antigen is enhanced by conjugating Globo H to the KLH carrier protein to form OBI-822 (Globo H-KLH), and co-administered with an adjuvant, OBI-821.

[102] Study Design: The international, randomized, double-blind, and placebo-controlled Phase II/III trial (NCT01516307) consists of 9 injections of OBI-822 in a 41-week

treatment period, a disease progression follow-up period of up to 2 years from randomization, and a survival follow-up period of up to 5 years. A total of 349 previously treated women with histologically or cytologically confirmed metastatic BC are randomly assigned (2:1) to treatment with subcutaneous OBI-822 (30 μg Globo H)/OBI-821 (100 μg) or control (PBS), in combination with low-dose cyclophosphamide (300 mg/m^2).

[103] Eligibility Criteria: (Inclusion and exclusion criteria were established)

1. Inclusion Criteria
2. Exclusion Criteria

[104] Study Procedure Schedule:

1. Screening Phase – 3 weeks prior to Randomization (Visit 1)
2. Treatment Period: Week 1 - Week 3 (Visit 2 - Visit 5)
3. Treatment Period: Week 5 - Week 41 (Visit 6 - Visit 19)
4. Follow-up Period (Every 8 weeks)
5. Early Termination criteria was established
6. Survival Follow Up Period (Every 12 weeks)
7. Other study procedure were considered for completeness

[105] Treatment Plan: (items considered)

1. Randomization and Blinding
2. Cyclophosphamide Administration Schedule
3. OBI-822/OBI-821 and Placebo Administration Schedule

[106] Management of Toxicity and Treatment Discontinuation: (exemplary factors that were considered as part of the design)

1. General Management
2. Management of Drug-Induced Toxicities
3. Guidelines for Individual Subject Study Treatment (OBI-822/OBI-821, Placebo) Discontinuation
4. Guidelines for Cyclophosphamide Treatment Discontinuation

[107] Treatments Permitted and Prohibited During Study: (exemplary factors considered as part of the design)

1. Permitted treatments during study
2. Prohibited treatments during study

[108] Drug Information: (exemplary factors considered as part of the design)

1. Cyclophosphamide
2. OBI-822 (Globo H-KLH)
3. OBI-821
4. Clinical Trial Material (CTM) Supply, Packaging, Labeling, and Storage

[109] Study Endpoint:

1. Efficacy Assessment
2. Safety Assessment
3. Safety Variables

[110] Response Criteria:

1. Definitions for Measurability of Tumor Lesions
2. Recording Tumor Lesions
3. Response Evaluation
4. Criteria for Removal from Protocol Therapy
5. Off Study Criteria

[111] Statistical Consideration:

1. Objectives and Hypotheses
2. Target Sample Size
3. Evaluation of Study Endpoint
4. Statistical Methods
5. Safety Analysis

[112] Adverse Event:

1. Definition of Adverse Event:
2. Assessment of Relationship to Treatment

[113] <u>Demonstration of efficacy</u>: In certain embodiments, OBI-822/OBI-821 with cyclophosphamide improved the “Responders” (with increasing Globo H specific IgG/IgM) compared to “Non-Responders” (with no IgG/IgM response) with Metastatic breast cancer.

[114] Frequency and magnitude of humoral immune responses (Globo H specific IgG/IgM) after immunization and their correlation with PFS and OS in patients with Metastatic breast cancer.

[115] Safety and Toxicity profile of OBI-822/OBI-821 with cyclophosphamide relative to PBS with cyclophosphamide.

[116] Clinical trial as a Specific Example: A Double-blind, Randomized Phase II/III trial of Active Immunotherapy with Globo H-KLH (OBI-822) and OBI-821 adjuvant in Subjects with metastatic breast cancer.

[117] Treatment Plan: This is a double-blind, randomized, two-arm, Phase II/III trial in subjects with metastatic breast cancer.

[118] Tumor Assessment at screening: Full body CT scans (chest, abdomen, and pelvis) are performed at screening and used as the baseline scan. If full-body CT scan has been performed within 2 weeks of the screening scan, then this performed scan can be used as the baseline scan. If full body CT scans prior to screening is not available, sections with lesions after diagnosis of metastatic breast cancer must have imaging from CT or MRI scan to confirm the tumor status.

[119] Tumor response status (SD, PR, CR) is based on RECIST 1.1 criteria. Confirmatory CT scan at screening (baseline) was compared with previous full body CT scan prior to screening. The disease status of the scans previous to the screening scans must be the same disease status as the screening scans. An interval of at least 6 weeks is required for SD, and an interval of at least 4 weeks is required for PR and CR.

[120] If a new lesion is detected at screening (i.e., tumor not evident on previous imaging and/or tumor not previously documented on imaging), the case is considered PD, and thus not eligible for inclusion.

[121] Disease status for stratification is classified into either with evidence of disease (PR/SD) or without evidence of disease (CR).

[122] For subjects with no evidence of disease (CR) due to excision of a metastatic lesion prior to screening, the investigator needs to ensure that the tumor response status is documented by imaging prior to surgical removal.

[123] The tumor status is recorded as the evidence of disease (PR/SD) if enrolled.

[124] Imaging is assessed by site radiologists and a copy is sent to central radiology laboratory for an independent review, which is not interfered with the sites' interpretation and decision.

[125] Subjects are randomized to receive either OBI-822/OBI-821 (Treatment Group) or PBS (Control Group) in a 2:1 allocation.

[126] Subjects are stratified according to their usage of hormone therapy while on study and their disease status at entry.

[127] When enrolled as a hormone therapy non-user, patients are not allowed to start hormone therapy while on study.

[128] If enrolled as a hormone therapy user, patient should be on hormone therapy.

[129] Change to another regimen of hormone therapy due to intolerance of toxicity from previous hormone therapy is allowed.

[130] There are four strata:

1. Hormone Therapy User – With Evidence of Disease (PR/SD)
2. Hormone Therapy User – Without Evidence of Disease (CR)
3. Hormone Therapy Non-User – With Evidence of Disease (PR/SD)
4. Hormone Therapy Non-User – Without Evidence on Disease (CR)

[131] Collect subject tumor biopsy/tissue samples to test for tumor Globo H Expression and to correlate with treatment response.

[132] In certain combination therapy embodiments, treatment subjects are given cyclophosphamide (300 mg/m^2) intravenously at Weeks 1, 5, 9, 13, 17, 25 and 37 (Visits 2, 6, 8, 10, 12, 14, and 17) or until disease progression.

[133] OBI-822/OBI-821 or Control (PBS) is given subcutaneously on Weeks 1, 2, 3, 5, 9, 13, 17, 25, and 37 (Visits 3, 4, 5, 7, 9, 11, 13, 15, and 18). Subjects are followed until disease progression or up to 2 years from randomization.

[134] Blood samples are collected for evaluation of immune responses to Globo H-KLH during the treatment and follow up period for up to 2 years from the start of treatment or up to disease progression.

[135] Tumor Assessment during study

1. Full body CT scans (chest, abdomen, and pelvis) are performed at every 8 weeks until end of study or until disease progression.

2. CT scans from each time point are compared against the baseline scan (at screening) and previous time points for Progression Free Survival (PFS) and tumor response status (PD, SD, PR, CR), based on the RECIST 1.1 criteria.

[136] All surviving subjects are followed-up at 12-week intervals up to 5 years from randomization for Overall Survival (OS).

[137] For subjects with PD, blood and urine samples are drawn at the time of progression or before the subjects are taken off from the study.

Example 1: Clinical Trial Data of Progression-Free Survival (PFS) For Modified Intent-to-treat (Mitt) Population of OBI-822

[138] The Kaplan-Meier plots progression-free survival (PFS) and overall survival (OS) for modified intent-to-treat (mITT) population of OBI-822 (original name OPT-822) indicated that there was a higher PFS and OS after patients completed 9 injections of OBI-822 compared with placebo.

[139] Clinical trial data of human Anti-Globo H IgG titer determination by ELISA

Reagent/Buffer Preparation

[140] Coating antigen: 1 mg/mL Globo H-ceramide was dissolved in ethanol (OBI Pharma Inc.); Secondary antibody for human serum: Goat anti-human IgG-AP (Jackson Immunoresearch, Cat #109-055-008); 10X PBS, pH 7.4 (Gibco, Cat #70011-044); Tween-20 (Sigma-Aldrich, Cat #P2287); Substrate Solution: Alkaline Phosphatase Yellow (pNPP) Liquid Substrate (Sigma-Aldrich, Cat #P7998); Blocking Buffer (Sigma-Aldrich, Cat #B6429); PBST: 0.05% tween-20 in PBS; Stop Solution: Alkaline Phosphatase Stop Solution (Sigma-Aldrich, Cat #A5852).

Assay Procedure

[141] Globo H-ceramide was diluted to 4 μg/mL in ethanol. 50 μL of diluted Globo H-ceramide solution was added into each well of a standard reaction plate. The plate with the reaction mixture was incubated at room temperature for overnight. The plate was decanted and washed with PBST at 200 μL/per well for three times. 100 μL of Blocking Buffer was added to each well and incubated at room temperature for 30 minutes. The plate was decanted and washed with PBST 200 μL/well for three times.

[142] Serum sample dilution: samples were two-fold serial diluted in Blocking Buffer ranging from 20, 40, 80, 160, 320, 640, 1280 to 2560 folds (40 μL of serum sample was added to 760 μL Blocking Buffer to make 20 fold dilution). 50 μL of serum sample was added into each well in coated and un-coated plates, and incubated at room temperature for 60 minutes. The plates were decanted and washed with PBST 200 μL/well for three times. 40 μL of 0.3 mg/mL stock anti-human IgG-AP secondary antibody was added to 7960 μL of Blocking Buffer for 1:200 dilution. 50 μL of the diluted anti-human IgG-AP was added into each well in coated and un-coated plates, and incubated at room temperature for 45 minutes. The plates were decanted and washed with PBST 200 μL/well for three times. 100 μL of Substrate Solution was added into each well of coated and un-coated plates, and incubated at 37 °C for 20 minutes. 50 μL of Stop Solution was added to each well. The plates were read at optical density 405nm by ELISA reader.

Data Analysis

[143] The cut-off value was obtained by the difference of mean OD value of secondary antibody only from coated plate and mean OD value of secondary Ab only from un-coated plate plus 0.1.

[144] Titer was defined as the difference of the OD value of coated plate and OD value of un-coated plate at each dilution. The highest dilution above the cut-off value was the anti-Globo H IgG titer. Statistical analysis was performed using GraphPad Prism 6 software.

[145] The characteristics of the clinical patients were listed in Table 1.

[146] Table 1. The baseline Characteristic

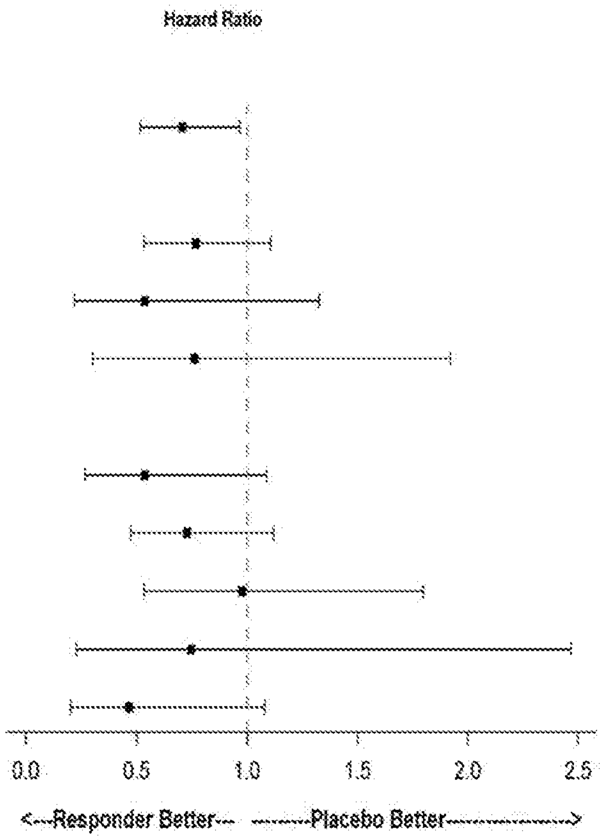
Characteristic	OPT-822/821 N=224	Placebo N=124	P-value
Age, years			0.4826
Median (range)	53 (30-87)	52 (30-82)	
Ethnicity n (%)			0.4065
Asian	185 (82.6%)	97 (78.2%)	
Caucasian	39 (17.4%)	27 (21.8%)	
ECOG Performance Status, n (%)			0.9671
0	165 (74.0%)	92 (74.2%)	
1	58 (26.0%)	32 (25.8%)	
Stage at initial diagnosis, n (%)			0.7292
Stage I-III	141 (63%)	80 (64.6%)	
Stage IV	72 (32.1%)	37 (29.8%)	
Unknown	11 (4.9%)	7 (5.6%)	
Time from first diagnosis to Day 1, months			0.9786

Median (range)	50 (3-307)	41 (5-390)	
Time from first metastatic diagnosis to Day 1, months			0.5246
Median (range)	12 (1-87)	14 (3-151)	
Number of Progression After Metastatic Diagnosis, n (%)			0.8260
0	137 (61.2%)	77 (62.1%)	
1	67 (29.9%)	37 (29.8%)	
2	20 (8.9%)	10 (8.1%)	
Biologic subtypes, n (%)			0.7078
ER(+) and/or PR(+) , HER2(-)	161 (71.9%)	84 (67.8%)	
Triple negative	28 (12.5%)	17 (13.7%)	
HER2(+)	35 (15.6%)	23 (18.5%)	
Use of hormone therapy during the study, n (%)			
Yes	140 (62.5%)	76 (61.3%)	
No	84 (37.5%)	48 (38.7%)	
GLOBO H Expression, IHC, n (%)			0.8704
0	44 (27.7%)	25 (29.8%)	
1+	57 (35.8%)	30 (35.7%)	
2+	27 (17.0%)	10 (11.9%)	
3+	31 (19.5%)	19 (22.6%)	
Any expression	115 (51.3%)	59 (47.6%)	

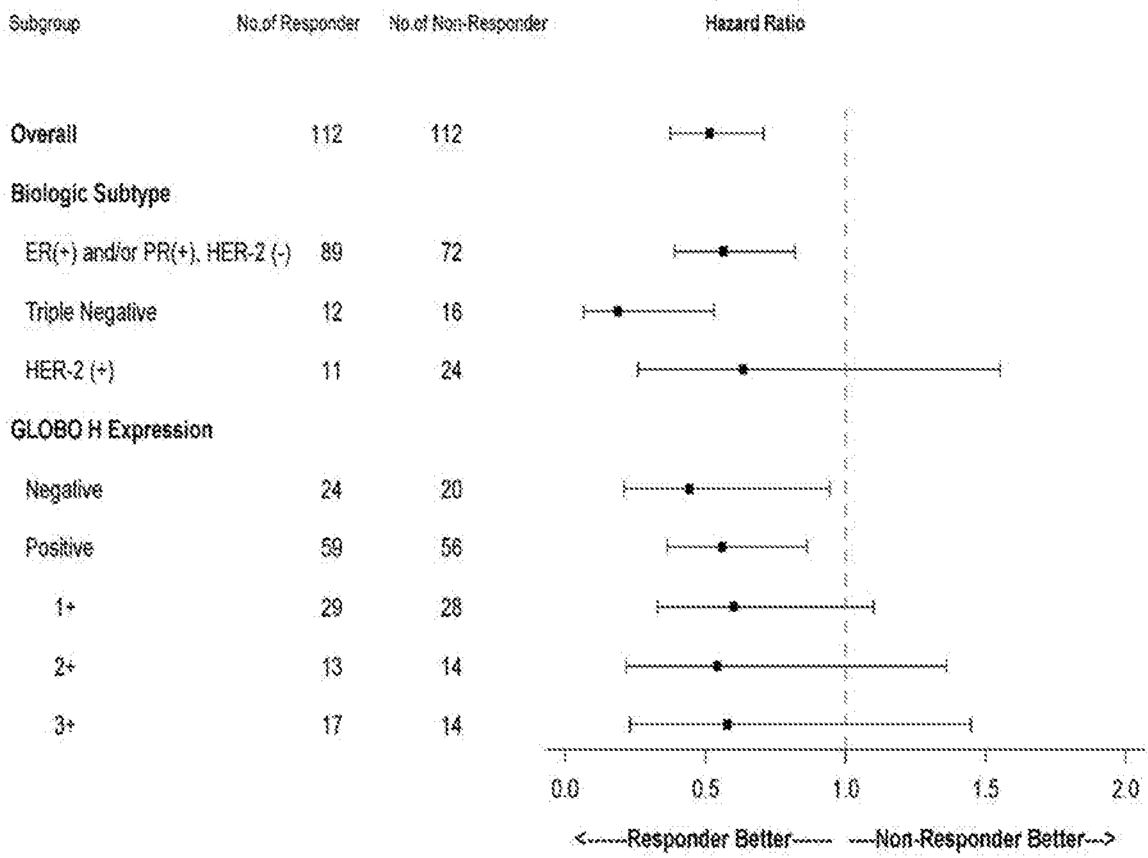
[147] The progression-free survival (PFS) indicated that OBI-822 was reactive in several biological sub-type of breast cancer (Stage I, II, III, ER(+), PR(+), HER2(+) or triple negative). The profiles of Globo H expressions between different tumor subtypes were listed in Table 2.

[148] Table 2A. PFS OBI-822/821 Immune Responder vs Placebo

Subgroup	No.of Responder	No.of Placebo
Overall	112	124
Biologic Subtype		
ER(+) and/or PR(+), HER-2 (-)	69	84
Triple Negative	12	17
HER-2 (+)	11	23
GLOBO H Expression		
Negative	24	25
Positive	59	59
1+	29	30
2+	13	10
3+	17	19



[149] Table 2B. PFS OBI-822/821 Immune Responder vs OBI-822/821 Non-Responder



[150] **Error! Reference source not found.** and 5 showed the Progression Free Survival (PFS) after received 9 injections of Study Drugs. It indicated that OBI-822 vaccine could extend the survival rate of breast cancer patients with a longer time.

[151] **Error! Reference source not found.** and 7 showed the Progression Free Survival (PFS)/Overall Survival (OS) after received of Study Drugs with/without IgG immune response (IgG Criteria 1:640). It indicated that OBI-822 vaccine could induce IgG immune response and extend the survival rate of breast cancer patients with a longer time.

[152] Clinical trial data of human Anti-Globo H IgM titer determination by Glycan Array

<u>Background</u>

[153] Glycan Array platform utilizes automated Agnitio BioIC system in which ELISA is performed within the microfluidic cartridge. The microfluidic cartridge contains an array of microfluidic pumps and valves, a channel network, reagent storage reservoirs, a glycan array reaction zone, and a waste storage reservoir. Automated Agnitio BioIC system pumps reagents and samples from their respected reservoirs into a reaction zone for multiplexed ELISA reaction with chemical luminescence. The collected data are analyzed by the LabIT software provided by Agnitio Science and Technology Inc. The specification of equipment of Agnitio BioIC system was disclosed in a previous PCT patent application (WO2017041027A1).

Reagent/Buffer Preparation

[154] Sixty-six microliter of Normal Human Serum (NHS) or serum from 220 breast cancer patient samples and 124 placebo samples were added in 594 μL Sample Diluent Buffer (BioCheck Inc., Cat #MB10175) to form ten-fold dilution. The Secondary Antibody Solution was prepared by mixing 2 μL Horseradish peroxidase (HRP)-conjugated goat anti-human IgM (KPL Inc., Cat #474-1003) in 98 μL Conjugate Buffer (SuperBlock (TBS) Blocking Buffer plus 0.2% Tween 20, Thermo Fisher Scientific Inc., Cat #37535) to form 50-fold dilution. 40 μL of diluted secondary antibody solution was pumped into 2360 μL Conjugate Buffer to form the Secondary Antibody Solution (3000x diluted).

Assay Procedure

[155] Six hundred and twenty microliter Wash Buffer (Phosphate-buffered saline (Thermo Fisher Scientific Inc., Cat #70011) in 0.2% (v/v) Tween 20 (J.T. Baker, Cat #JTB-X251-07)) was added to the "Wash" reservoir. 120 μL Blocking Buffer (Protein-Free Blocking Buffers, Thermo Fisher Scientific Inc., Cat #37571) was added to the "Blocking" reservoir. 120 μL Secondary Antibody Solution was added to the "Conjugate" reservoir. 100 μL serum was added to the "Serum" reservoir. 120 μL Substrate Buffer (SuperSignal ELISA Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific Inc., Cat #37074) was added to the "Substrate" reservoir in ten minutes.

Data Analysis

[156] The glycan array was pressurized by Agnitio BioIC Pumping Machine for 30 minutes. The bound serum was visually monitored using Agnitio BioIC Analyzer. The absorbance intensity of array was converted into "Ab level (μg/mL)" relative to anti-human Globo H IgG. The internal curve was performed using 0.0625, 0.125, 0.25, 0.5, 0.75, and 1

μg/mL of human IgM. The linear regression of the internal curve of each chip was used to calculate the slope and the intercepts. In certain examples, the Ab level (μg/mL) = [(raw data-intercept)/slope]×0.1.

[157] **Error! Reference source not found.** showed the Progression-Free Survival (PFS) after received one injection on Week 2 of Study Drug with/without early IgM immune response. It indicated that OBI-822 vaccine could induce early IgM immune response and extend the survival rate of breast cancer patients with a longer time.

[158] Demonstration of Efficacy using Clinical trial data Based on Human Anti-Keyhole Limpet Hemocyanin (KLH) IgG Titer Assay

[159] Human Anti-Keyhole limpet hemocyanin (KLH) IgG titer assay was used to show that the immunological-reactions generated by the administration of Globo series antigens vaccine could be induced by the Globo series antigens vaccine, the Globo series antigen and/or the carrier protein.

Reagent/Buffer Preparation

[160] KLH (Sigma-Aldrich, Cat #H1158, ocean harvest, stock concentration 5 mg/mL); Coating buffer: carbonate-bicarbonate buffer pH 9.2 (Sigma-Aldrich, Cat #C3041-50CAP); Secondary antibody: Goat anti-human IgG-HRP (KPL, Cat #474-1002); 10X PBS, pH 7.4 (Gibco, Cat #70011-044); Tween-20 (Sigma-Aldrich, Cat #P2287); TMB Substrate Solution (Clinical, Cat #01016-1-500); Blocking buffer (Sigma-Aldrich, Cat #B6429); PBST: 0.05% tween-20 in PBS; Stop solution: 1N H_2SO_4..

Assay Procedure

[161] KLH was diluted to 4 μg/mL with coating buffer. Fifty microliter of diluted KLH solution was added into each well of a standard well. The plate was incubated at 4 °C overnight. The plates was decanted and washed with PBST at 200 μL/per well for three times. 100 μL blocking buffer was added to each well and incubate at room temperature for 30 minutes. The plate was decanted and washed with PBST 200 μL/per well for three times.

[162] Serum sample dilution: samples were two-fold serial diluted with blocking buffer ranging from about 1000, 2000, 4000, 8000, 16000, 32000, 64000 to 128000 folds (1 μL of serum sample was added to 999 μL blocking buffer to make 1000 fold dilution). 50 μL of samples were added into each well in coated and un-coated plates, then incubated at room temperature for 1.5 hours. The plates were decanted and washed with PBST 200 μL/well for

three times. Anti-human IgG-HRP secondary antibody was diluted to 1:20000 with blocking buffer. 1 μL was taken from 1 mg/mL stock and added to 20 mL of blocking buffer to make 1:20000 dilution. 50 μL of anti-human IgG-HRP was added into each well in coated and un-coated plates and incubated at room temperature for 45 minutes. The plates were decanted and washed with PBST 200 μL/well for three times. 100 μL of TMB substrate solution was added into each well of coated and un-coated plates, then incubated at room temperature for 5 minutes. 100 μL of stop solution was added to each well. The plate was read at optical density 450nm by ELISA reader.

[163] Figure 9 exhibited high anti-KLH IgG immune response after patients received the injections of OBI-822 vaccine. A total of 30 clinical serum samples were divided into three groups (Responder: Receiving OBI-822 vaccine injections with increasing anti-Globo H IgG/IgM level; Non-Responder: Receiving OBI-822 vaccine injections with no increasing anti-Globo H IgG/IgM level). It demonstrated that the administration of OBI-822 vaccine can indeed induce anti-KLH immune response. Therefore, the immunological-reactions generated by the administration of Globo series antigens vaccine can be induced by the Globo series antigens vaccine, the Globo series antigen or the carrier protein.

Example 2: Demonstration of Efficacy using Representative Ovarian Cancer Model

[164] Clinical trial as a Specific Example: An Open Labeled Phase II trial of Active Immunotherapy with Globo H-KLH (OBI-822/821) in Women Who Have Non-Progressive Epithelial Ovarian or Fallopian Tube Cancer.

[165] Method: This was an open labeled, Phase II trial in women who had not progressed after having received cytoreductive surgery followed by platinum-based chemotherapy for newly diagnosed ≥ stage II epithelial ovarian or fallopian tube cancer.

[166] For subjects who participate to receive the treatment, OBI-822 (30 mcg Globo H)/OBI-821 (100 mcg), were given subcutaneously on the Weeks 1, 2, 3, 4, 12, 20, 28, 36, 44, and 52 (Visits 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10). Disease status was determined according to the results of evaluation incorporating RECIST 1.1 criteria. A subject was evaluated to have PD on the basis of objective RECIST 1.1 criteria. The CT Scan/MRI for RECIST criteria were performed at screening week 28 and 52. Whole abdominal (abdomen and pelvis) CT scan were performed at designated times during treatment with an interval of about 24 weeks. The window period for CT scan was +/– 14 days from the planned visit per protocol schedule.

Unscheduled tumor assessment was performed under investigator's discretion and at the time of assessment was consistent with the examination performed at baseline. For subjects that were CT-contraindicated, MRI was performed instead.

[167] Subjects with no lesions, measurable and non-measurable disease were eligible for inclusion in this study. Measurable disease was defined by the presence of ≥1 measurable lesion (longest diameter [LD] ≥10 mm with spiral computed tomography [CT] scan or ≥20 mm with conventional CT, magnetic resonance imaging [MRI], or X-Ray). A maximum of 5 measurable lesions (with a maximum of 2 lesions per organ), representative of all lesions involved, were to be identified as target lesions (TL) at baseline. The RECIST 1.1 criteria used to determine objective tumor response for TLs are summarized in Table 3.

[168] Table 3. Overall tumor response for target lesions

Complete response (CR)	Disappearance of all TLs since baseline (Any pathological lymph nodes must have reduction in short axis to <10 mm.)
Partial response (PR)	>=30% decrease in the sum of the LDs of TLs since baseline
Stable disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD
Progressive disease (PD)	>=20% increase in the sum of the LDs of TLs taking as reference the smallest sum LD recorded since the start of treatment

[169] All other lesions (or sites of disease) not recorded as TLs were identified as non-target lesions (NTLs). The RECIST criteria used to determine objective tumor response for NTLs are summarized in Table 4.

[170] Table 4. Overall tumor response for non-target lesions.

Complete response (CR)	Disappearance of all NTLs since baseline (Any pathological lymph nodes must have reduction in short axis to <10 mm.)
Incomplete response (IR)/ Stable disease (SD)	Persistence of >=1 NTLs
Progressive disease (PD)	Unequivocal progression could be due to progression in one lesion only, or in several lesions and must be clinically significant, as assessed by the study physician

[171] Details of any new lesions were also recorded; the presence of ≥1 new lesions was assessed as progression. RECIST evaluation for overall response is summarized in Table 5.

[172] Table 5A. Time point response: subjects with target (+/- non-target) lesion at baseline.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR, NA	No	CR
CR	SD, NE	No	PR
PR	CR, SD, NE, NA	No	PR
SD	CR, SD, NE, NA	No	SD
NE	CR, SD, NE, NA	No	NE
PD	CR, SD, PD, NE, NA	Yes, No	PD
CR, PR, SD, NE	PD	Yes, No	PD
CR, PR, SD, NE	CR, SD, PD, NE, NA	Yes	PD

[173] Table 5B. Time point response: subjects with non-target lesion only at baseline

Target lesions	Non-target lesions	New lesions	Overall response
NA	CR	No	CR
NA	SD	No	SD
NA	NE	No	NE
NA	PD	No	PD
NA	CR, SD, NE	Yes	PD

[174] Table 5C. Time point response: subjects without target and non-target lesion at baseline

Target lesions	Non-target lesions	New lesions	Overall response
NA	NA	No	NE
NA	NA	Yes	PD

[175] Table 5D. List of Abbreviations

AE	Adverse Event
CR	Complete Response
CT	Computed Tomography
EVA	Evaluable Population
FAS	Full Analysis Set
IR	Incomplete Response
ITT	Intent-to-treat
LD	Longest Diameters
MRI	Magnetic Resonance Imaging
NA	Not Applicable
NE	Not Evaluable
NEJM	New England Journal of Medicine
NCI	National Cancer Institute
NTL	Non-Target Lesion
PD	Progressive Disease
PFS	Progression Free Survival
PP	Per-protocol
PR	Partial Response
PT	Preferred Term
RECIST	Response Evaluation Criteria In Solid Tumors
SAEs	Serious Adverse Event(s)
SD	Stable Disease
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
TL	Target Lesion

[176] Study Population:

[177] Disposition of Patients: The following patient data was summarized and presented for all recruited subjects: Number and percentage of patients screened, received study drug, completed and terminated during treatment period, completed and discontinued during follow-up period.

[178] Number and percentage of patients in each analysis set and reason for exclusion, by study group for all recruited patients.

[179] Protocol violations are reviewed by the study team prior to database lock to determine which violations disqualify the patient from the EVA population.

[180] Clinical Result: Negative Control (Patient No: 065) (Figure 10): The date of assessment was initiated on or before March 18, 2015. It indicated that treating OBI-822 does not lead to any tumor production within 28 weeks (Visit 7). Therefore, the safety of OBI-822 was confirmed.

[181] Stage III Ovarian Cancer (Patient No: 035) (Figure 11): The date of assessment was initiated on or before April 24, 2014. The original symptom of patient was "Sub-centimeter Mesenteric Nodes tumor" (Lesion Category: Non-target tumor) through CT scan. It indicated that there was not any tumor progression/metastasis (SD) after treating OBI-822 within 28 weeks (Visit 7). Therefore, the tumor inhibition ability of OBI-822 is confirmed within 28 weeks.

[182] Stage IV Fallopian Tube Cancer (Patient No: 041) (Figure 12): The date of assessment initiated on or before May 22, 2014. The original symptom of patient was "Lung tumor" (Lesion Category: Non-target tumor) through CT scan. It indicated that there was not any tumor progression/metastasis (SD) after treating OBI-822 within 28 weeks (Visit 7). Therefore, the tumor inhibition ability of OBI-822 is confirmed within 28 weeks.

[183] Stage III Ovarian Cancer (Patient No: 060) (Figure 13): The date of assessment initiated on or before January 6, 2015. The original symptom of patient was "Peritoneum tumor" (Lesion Category: Non-target tumor) through CT scan. It indicated that there was not any tumor progression/metastasis (SD) after treating OBI-822 within 28 weeks (Visit 7). Therefore, the tumor inhibition ability of OBI-822 is confirmed within 28 weeks.

Example 3: Mixing Instruction for OBI-822 and OBI-821

[184] Storage Condition: OBI-822 and its placebo PBS vials are stored at 2-8 °C. OBI-821 and its placebo PBS vials are stored at -15 to -25 °C.

[185] Study Groups:

[186] Group A: Active treatment group; Dosage Per Injection: OBI-822 (Equivalent to 30 μg of Globo H) / 100 μg of OBI-821. Total final injection volume: 0.8 mL.

[187] Group B: Control group; Dosage Per Injection: PBS. Total final injection volume: 0.8 mL.

[188] Investigational Drugs:

[189] Vial 1a (PBS: OBI-821 control test article) Fill Volume - 0.5 mL. Contents: 10 mM sodium phosphate, 150 mM NaCl, pH 6.8;

[190] Vial 1b (OBI-821) Fill Volume - 0.5 mL. Contents: 250 μg/mL OBI-821 in 10 mM sodium phosphate, 150 mM NaCl, pH 6.8;

[191] Vial 2a (PBS: OBI-822 control test article) Fill Volume - 0.75 mL. Contents: 100 mM sodium phosphate, 150 mM NaCl, pH 7.2;

[192] Vial 2b (OBI-822) Fill Volume - 0.75 mL. Contents: Equivalent to 75 µg of Globo H/mL OBI-822 in 100 mM sodium phosphate, 150 mM NaCl, pH 7.2.

[193] Mixing Instruction for OBI-822 and OBI-821:

[194] At time of treatment, with syringe, withdraw 0.5 mL of Vial 2a (PBS only) and place in Vial 1a (PBS only) or withdraw 0.5 mL of Vial 2b (OBI-822 in PBS) and place in Vial 1b (OBI-821 in PBS). Gently mix the contents of the Vial (either Vial 1a or Vial 1b) by gently inverting the vial 4-5 times. Do not shake the vial vigorously. At this time, this vial contains either Placebo (PBS only) or Treatment (OBI-822 plus OBI-821) and is ready for injection. Then withdraw 0.8 mL of from this vial for subject injection. The final administration volume of each study group is summarized in Table 6.

[195] Table 6. The calculation of final administration volume of study groups

Group	**Volume (mL) Added/Used**					**Total Volume Administered**
	Vial 1a PBS (OBI-821 Control Test)	Vial 1b OBI-821	Vial 2a PBS (OBI-822 Control Test)	Vial 2b OBI-822	Total Volume after Reconstitution (mL)	Volume per Dose (mL)
A	0	0.5	0	0.5	1.0	0.8
B	0.5	0	0.5	0	1.0	0.8

[196] The stability of the combined OBI-822 and OBI-821 is stable up to 10 hours from the time of reconstitution at room temperature. The administration of the combined product should occur within 2 hours from reconstitution to minimize potential microbial growth. If administration is not possible within 2 hours from reconstitution, the combined product should be destroyed according to the institutional pharmacy Standard Operating Procedure and documented in the drug accountability records. Figure 14 illustrates the mixing procedure of investigational drugs

Example 4: Permitted and Prohibited Concomitant Medication

[197] Permitted Concomitant Medications:

[198] Opiates: It may be used for pain control, and preventive treatment for constipation is also allowed.

[199] GCSF: Will be allowed if deemed necessary by doctors.

[200] Bisphosphonate.

[201] Hormone therapies (for subjects who have been stratified as a hormone therapy user).

[202] If used, the selected form, dose, or usage of hormone therapy needs to be consistent i.e., no switching of treatments, no adding of more therapies, or increasing doses, unless due to intolerance of toxicity. All kind of hormone therapies can be used in this study, the common used hormone therapies are listed below.

[203] Estrogen inhibitors (e.g., tamoxifen, Fareston).

[204] Aromatase inhibitors (e.g., anastrozole [Arimidex], exemestane [Aromasin] and letrozole [Femara]).

[205] Pituitary downregulators (e.g., goserelin [Zoladex], leuprolide [Lupron]) -These are also called LHRH analogues.

[206] Novaldex® (tamoxifen selective estrogen-receptor modulator, SERM).

[207] Evista® (rolaxifene, another SERM).

[208] Faslodex® (fulvestran, estrogen receptor down-regulator).

[209] Anticoagulant (lepirudin [Refludan]).

[210] Enzyme (rasburicase [Elitek]).

[211] Hematopoietic growth factors.

[212] The method of administrations is preferably conducted in the absence of the concomitant medications listed in Table 7.

[213] Table 7. Concomitant Medications

Anti-neoplastic Agents:	**Radiotherapy**
• Antimetabolites • Vinca alkaloid • Epipodophyllotoxins • Taxanes • Camptothecins • Miscellaneous cytotoxic agents • Alkylating agents • Antitumor antibiotics • Nitrosoureas	**Surgery for the metastatic breast cancer**
Biologic Agents: • HER1/EGFR tyrosine kinase inhibitor: (erlotinib [Tarceva]) • VEGF protein inhibitor: bevacizumab (Avastin) • HER-2/ErbB2 inhibitors: lapatinib (Tyverb/Tykerb) • Interferons • Interleukins • Monoclonal antibodies	**Glucocorticoid steroids** Should not be used, however, topical or temporary systemic steroid use may be allowed, if clinically indicated. In the event of mild to moderate autoimmune disorders, temporary use of systemic steroids is allowed. **Hormone therapies (for subjects who have been stratified as a hormone therapy non-user)**
Immunosuppressive therapy	

[214] **Prohibited Concomitant Medications** (e.g., cyclosporin, rapamycin, tacrolimus, rituximab, etc.).

[215] Eastern Cooperative Oncology Group (ECOG) Performance.

[216] These scales and criteria are used by doctors and researchers to assess how a subject's disease is progressing, assess how the disease affects the daily living abilities of the subject, and determine appropriate treatment and prognosis. They are included in table 8 for health care professionals to access.

[217] Table 8. Eastern Cooperative Oncology Group (ECOG) Performance

ECOG Performance Status	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

[218] While specific aspects of the invention have been described and illustrated, such aspects should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims. All publications and patent

applications cited in this specification are herein incorporated by reference in their entirety for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treating a breast cancer comprising administering to a subject in need thereof a therapeutically effective dose of Globo series antigens vaccine and/or cross-reacting with Globo series antigens vaccine.

2. The method of claim 1, wherein the Globo series antigens vaccine comprising Globo series antigens conjugated with a carrier protein.

3. The method of claim 2, wherein the Globo series antigens comprise antigens selected from Globo H, Stage-specific embryonic antigen 3 (SSEA3) and Stage-specific embryonic antigen 4 (SSEA4).

4. The method of claim 2, wherein the carrier protein comprising KLH (Keyhole limpet hemocyanin), DT-CRM 197 (diphtheria toxin cross-reacting material 197), diphtheria toxoid or tetanus toxoid.

5. The method of claim 1, wherein the Globo series antigens vaccine is administered as a pharmaceutical composition.

6. The method of claim 5, wherein the pharmaceutical composition comprises Globo H-KLH and an adjuvant.

7. The method of claim 6, wherein the adjuvant is selected from saponin, Freund's adjuvant or α-galactosyl-ceramide (α-GalCer) adjuvant.

8. The method of claim 5, wherein the pharmaceutical composition comprising OBI-822/OBI-821.

9. The method of claim 1, wherein the immunological-reactions generated by said administration is characterized by the immune response induced by the Globo series antigens vaccine, the Globo series antigen and/or the carrier protein.

10. The method of claim 1, wherein the subject is human.

11. The method of claim 1, wherein the therapeutically effective dose less than 100 μg.

12. The method of claim 1, wherein the administration procedure comprising intramuscular injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intra-arterial injection, intrasynovial injection, intrathecal injection, epidural injection or intra-pleural

injection.

13. The method of claim 1, wherein the vaccine is administered intermittently at a time interval of one week to five years or more.

14. The method of claim 1, wherein the vaccine is administered once every one week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, once every eleven weeks or once every twelve weeks.

15. A method for treatment of a tumor in a patient, comprising administering to the patient a pharmaceutically effective amount of Globo series antigens vaccine and/or cross-reacting with Globo series antigens vaccine.

16. The method of claim 15, wherein the Globo series antigens vaccine comprising Globo series antigens conjugated with a carrier protein.

17. The method of claim 16, wherein the Globo series antigens comprising Globo H, Stage-specific embryonic antigen 3 (SSEA3) or Stage-specific embryonic antigen 4 (SSEA4).

18. The method of claim 16, wherein the carrier protein comprising KLH (Keyhole limpet hemocyanin), DT-CRM 197 (diphtheria toxin cross-reacting material 197), diphtheria toxoid or tetanus toxoid.

19. The method of claim 15, wherein the Globo series antigens vaccine comprising Globo H-KLH and an adjuvant.

20. The method of claim 19, wherein the adjuvant is selected from saponin, Freund's adjuvant or α-galactosyl-ceramide (α-GalCer) adjuvant.

21. The method of claim 15, wherein the Globo series antigens vaccine comprising OBI-822/OBI-821

22. The method of claim 15, wherein the Globo series antigens vaccine comprising OBI-822 and a pharmaceutically acceptable excipient.

23. The method of claim 15, wherein the immunological-reactions generated by said administration is characterized by the immune response induced by the Globo series antigens

vaccine, the Globo series antigen and/or the carrier protein.

24. The method of claim 15, wherein the Globo series antigens vaccine or cross-reacting with Globo series antigens vaccine induce an immunogenic production of monoclonal antibody directed to Globo series antigens.

25. The method of claim 24, wherein the antibody produced is an IgG, an IgM, or both.

26. The method of claim 15, wherein the tumor is breast cancer, lung cancer, esophageal cancer, rectal cancer, biliary cancer, liver cancer, buccal cancer, gastric cancer, intestinal cancer, colon cancer, nasopharyngeal cancer, kidney cancer, prostate cancer, ovarian cancer, cervical cancer, endometrial cancer, pancreatic cancer, testicular cancer, bladder cancer, head and neck cancer, oral cancer, neuroendocrine cancer, adrenal cancer, thyroid cancer, bone cancer, gallbladder cancer, oropharyngeal cancer, laryngeal cancer, dermal cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, or brain tumor.

27. The method of claim 15, wherein the tumor is metastatic or non-metastatic.

28. The method of claims 15 to 23, wherein the Globo series antigens vaccine or cross-reacting with Globo series antigens vaccine is administered to the patient in combination with one or more anti-proliferative agent.

29. The method of claims 28, wherein the anti-proliferative agent is selected from cyclophosphamide, opiate, granulocyte colony-stimulating factor (GCSF), estrogen inhibitors, aromatase inhibitors, pituitary downregulators, tamoxifen selective estrogen-receptor modulator, rolaxifene, estrogen receptor down-regulator, anticoagulant, enzyme, hematopoietic growth factor, anti-neoplastic agent, antimetabolites, miscellaneous cytotoxic agents, vinca alkaloid, epipodophyllotoxins, alkylating agents, taxanes, antitumor antibiotics, camptothecins, nitrosoureas, HER1/EGFR tyrosine kinase inhibitor, VEGF protein inhibitor, HER-2/ErbB2 inhibitor, interferon, interleukin, monoclonal antibody, or glucocorticoid steroid.

30. The method of claims 28, wherein the anti-proliferative agent is selected from erlotinib, docetaxel, gemcitabine, cisplatin; carboplatin; paclitaxel, trastuzumab, temozolomide, tamoxifen, doxorubicin, oxaliplatin, bortezomib, sutent, letrozole, imatinib mesylate, MEK inhibitor, fulvestrant, leucovorin (folinic acid); rapamycin, lapatinib, lonafarnib, sorafenib,

gefitinib, irinotecan, tipifarnib, Cremophor-free, paclitaxel, vandetanib, chloranmbucil, temsirolimus, pazopanib, canfosfamide, thiotepa, cyclosphosphamide, 5-fluorouracil (5-FU), vinorelbine, novantrone, teniposide, edatrexate, daunomycin, aminopterin, capecitabine, ibandronate, topoisomerase inhibitor RFS 2000, difluoromethylornithine (DMFO), tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, onapristone, toremifine citrate, 4(5)-imidazoles, aminoglutethimide, megestrol acetate,exemestane, formestanie, fadrozole, vorozole, letrozole, anastrozole, flutamide, nilutamide, bicalutamide, leuprolide, goserelin, troxacitabine (α-1,3- dioxolane nucleoside cytosine analog), lipid kinase inhibitor, oblimersen, angiozyme, allovectin, leuvectin, vaxid, aldesleukin, lurtotecan, abarelix, bevacizumab, alemtuzumab, bevacizumab, cetuximab, panitumumab, rituximab, pertuzumab, trastuzumab, tositumomab, gemtuzumab or ozogamicin.

31. A method for treating a subject with afflicted with cancer by immunotherapy comprising administering to subject in need thereof a therapeutically effective amount of an immunogenic agent and/or cross-reacting with the immunogenic agent useful for inducing or modulating an immune response.

32. The method of claim 31, wherein the immunogenic agent comprising Globo series antigens conjugated with a carrier protein.

33. The method of claim 32, wherein the Globo series antigens comprising Globo H, Stage-specific embryonic antigen 3 (SSEA3) or Stage-specific embryonic antigen 4 (SSEA4).

34. The method of claim 32, wherein the carrier protein comprising KLH (Keyhole limpet hemocyanin), DT-CRM 197 (diphtheria toxin cross-reacting material 197), diphtheria toxoid or tetanus toxoid.

35. The method of claim 31, wherein the immunogenic agent is OBI-822 and related variants.

36. The method of claim 31, wherein the immunological-reactions generated by said administration is characterized by the immune response induced by the Globo series antigens vaccine, the Globo series antigen and/or the carrier protein..

37. The method of claim 31, wherein the subject is human.

38. The method of claim 31, wherein the immune response comprising IgG, IgM or cell-

mediated response.

39. The method of claim 38, wherein the cell is B cell or T cell.

40. The method of claim 31, wherein the said method further comprising improving a survival of the subject by modulating Globo series antigens interaction.

41. The method of claim 40, wherein the survival comprising overall survival and/or progression free survival.

42. The method of claim 40, wherein the modulation of Globo series antigens interaction further comprises one or more of the following:

(a) Induction of Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC) for Tumor Killing;

(b) Induction of anti-Globo series antigens IgM/IgG immune response to elicit Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC) mediated tumor cell killing;

(c) Induction of anti-Globo series antigens antibodies trap Globo series antigens-ceramide shedding from tumor cells to block translin-associated factor X (TRAX)-dependent angiogenesis;

(d) Induction of anti-Globo series antigens antibodies to block Globo series antigens-ceramide induced Notch 1-dependent immunosuppression and thereby enhancing T cell proliferation and cytokine production;

(e) Induction of anti-Globo series antigens antibodies to lead to apoptosis;

(f) Inhibition of Globo series antigens induced angiogenesis; or

(g) OBI-822 vaccination induces apoptosis.

43. A method for inducing/enhancing an immune response in a subject, comprising administering to the subject in an immunogenic agent and/or cross-reacting with the immunogenic agent.

44. The method of claim 43, wherein the immunogenic agent is Globo series antigen conjugated with carrier protein.

45. The method of claim 44, wherein the Globo series antigens comprising Globo H, Stage-specific embryonic antigen 3 (SSEA3) or Stage-specific embryonic antigen 4 (SSEA4).

46. The method of claim 44, wherein the carrier protein comprising KLH (Keyhole limpet hemocyanin), DT-CRM 197 (diphtheria toxin cross-reacting material 197), diphtheria toxoid or tetanus toxoid.

47. The method of claim 43, wherein the immunogenic agent is OBI-822 and related variants.

48. The method of claim 43, wherein the immunological-reactions generated by said administration is characterized by the immune response induced by the Globo series antigens vaccine, the Globo series antigen and/or the carrier protein.

49. The method of claim 43, wherein the subject is human.

50. The method of claim 43, wherein the immune response is cellular immunity or humoral immunity.

51. A method of improving OBI-822 vaccine induced immune-responsiveness in a subject in need thereof comprising: Administering an immunogenic effective amount of a pharmaceutically acceptable excipient comprising OBI-822 and one or more of the following selected from:

(a) Administering vaccine two or more times (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more times);

(b) Adjusting time interval and/or dosing amount regimen between two successive administrations;

(c) Adjusting routes of administration and/or altering injection locations of administration; or

(d) Any combination of the above thereby each administration increases antibody immune response and/or increases antigen-antibody binding affinity.

52. The method of claim 51, wherein the injection can be altered and/or supplemented by the addition of immune response booster agents.

53. The method of claim 51, wherein the immune response is cellular immunity or humoral

immunity.

54. The method of claim 51, wherein the immune response comprising IgG, IgM or cell-mediated response.

55. The method of claim 54, wherein the cell is B cell or T cell.

56. The method of claim 51, wherein the subject is human.

57. A method for identifying a suitable patient for cancer therapy in need thereof comprising:

(a) Administering an effective dose of Globo series antigens vaccine to the said patient;

(b) Assessing immune response of the said patient;

(c) Determining the expression of Globo series antigens in each patient; and

(d) Classifying suitability of the patient based on an index of immune response and the expression of Globo series antigens; wherein the index is indicative of the said patient having a good prognosis of recovery.

58. The method of claim 57, wherein the Globo series antigens vaccine comprising Globo series antigens conjugated with a carrier protein.

59. The method of claim 58, wherein the Globo series antigens comprising Globo H, Stage-specific embryonic antigen 3 (SSEA3) or Stage-specific embryonic antigen 4 (SSEA4).

60. The method of claim 58, wherein the carrier protein comprising KLH (Keyhole limpet hemocyanin), DT-CRM 197 (diphtheria toxin cross-reacting material 197), diphtheria toxoid or tetanus toxoid.

61. The method of claim 57, wherein the Globo series antigens vaccine is adminstered as a pharmaceutical composition.

62. The method of claim 61, wherein the pharmaceutical composition comprising Globo H-KLH and an adjuvant.

63. The method of claim 62, wherein the adjuvant is selected from saponin, Freund's adjuvant or α-galactosyl-ceramide (α-GalCer) adjuvant.

64. The method of claim 61, wherein the pharmaceutical composition comprising OBI-822/OBI-821.

65. The method of claim 57, wherein the cancer is breast cancer, lung cancer, esophageal cancer, rectal cancer, biliary cancer, liver cancer, buccal cancer, gastric cancer, intestinal cancer, colon cancer, nasopharyngeal cancer, kidney cancer, prostate cancer, ovarian cancer, cervical cancer, endometrial cancer, pancreatic cancer, testicular cancer, bladder cancer, head and neck cancer, oral cancer, neuroendocrine cancer, adrenal cancer, thyroid cancer, bone cancer, gallbladder cancer, oropharyngeal cancer, laryngeal cancer, dermal cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, or brain tumor.

66. The method of claim 57, wherein the cancer is breast cancer.

67. The method of claim 66, wherein the breast cancer is stage I, II, III, ER(+), PR(+), HER2(+), triple negative, metastatic or non-metastatic.

68. The method of claim 57, wherein the index of immune response comprising the assessment of IgG titer, IgM titer, progression-free survival (PFS) and/or overall survival (OS).

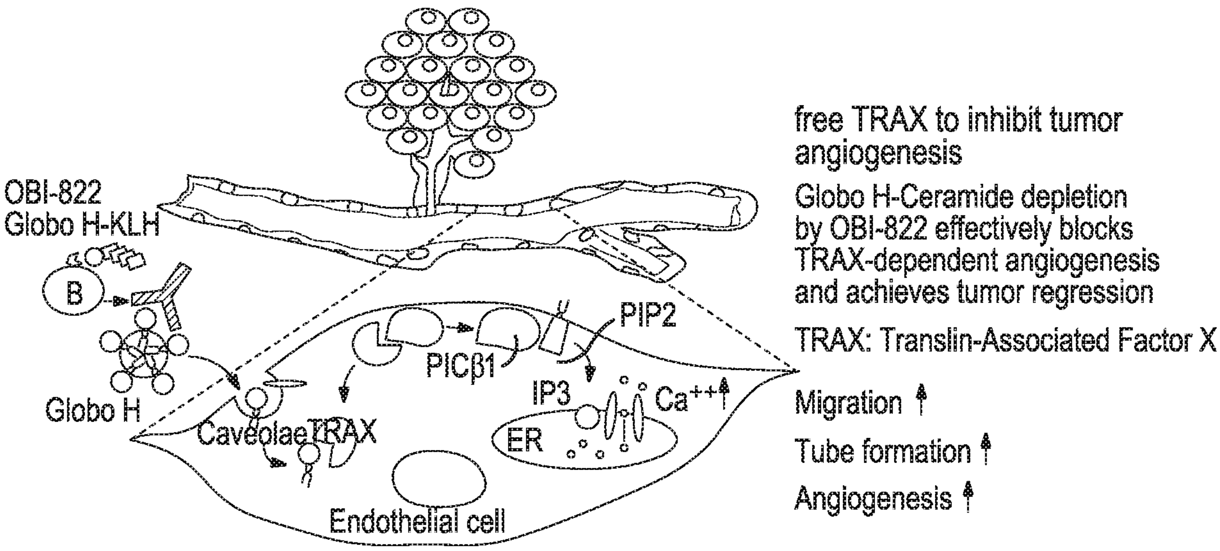
69. A method for determining the prognosis of cancer treatment and/or drug response of a patient in need thereof comprising:

(a) Identifying a patient who has been diagnosed with cancer;

(b) Determining the level of immune response by determining suitability of the said patient after receiving the treatment of claim 1;

(c) Analyzing the data obtained claim 57.

70. The method of claim 69, wherein the cancer is breast cancer, lung cancer, esophageal cancer, rectal cancer, biliary cancer, liver cancer, buccal cancer, gastric cancer, intestinal cancer, colon cancer, nasopharyngeal cancer, kidney cancer, prostate cancer, ovarian cancer, cervical cancer, endometrial cancer, pancreatic cancer, testicular cancer, bladder cancer, head and neck cancer, oral cancer, neuroendocrine cancer, adrenal cancer, thyroid cancer, bone cancer, gallbladder cancer, oropharyngeal cancer, laryngeal cancer, dermal cancer, basal cell carcinoma, squamous cell carcinoma, melanoma, or brain tumor.



OBI-822
Globo H-KLH
B
Globo H
Caveolae
TRAX
PIP2
PICβ1
IP3
Ca++
ER
Endothelial cell
free TRAX to inhibit tumor angiogenesis
Globo H-Ceramide depletion by OBI-822 effectively blocks TRAX-dependent angiogenesis and achieves tumor regression
TRAX: Translin-Associated Factor X
Migration ↑
Tube formation ↑
Angiogenesis ↑

FIG. 1

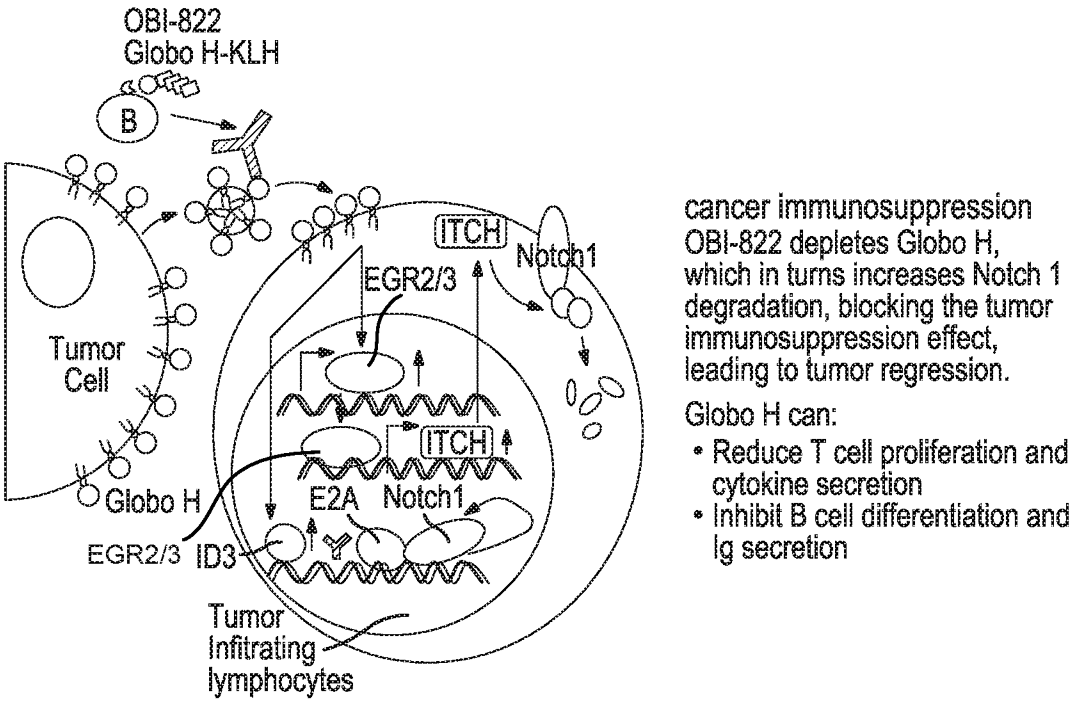
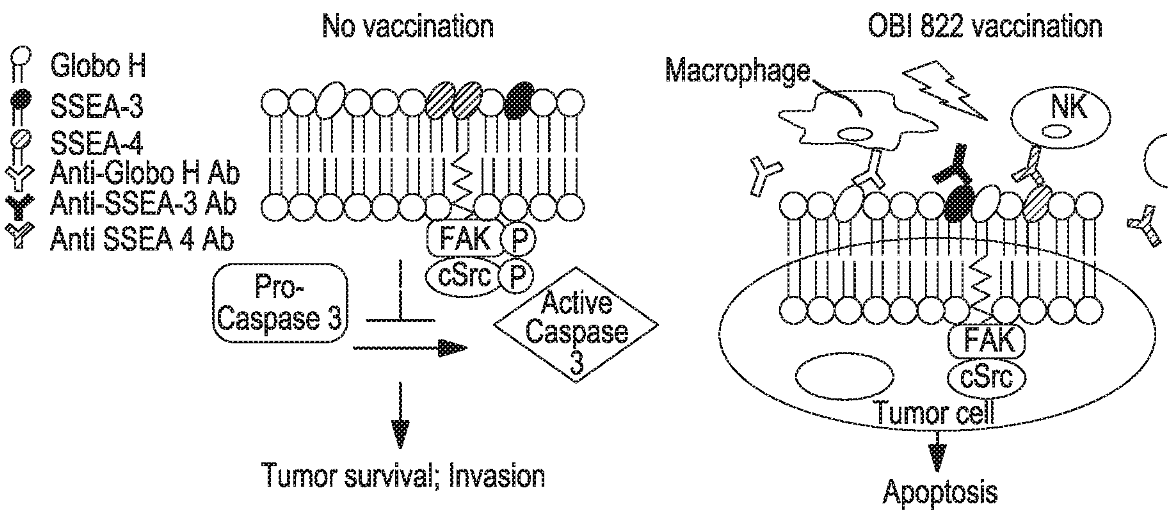

OBI-822
Globo H-KLH
B
Tumor
Cell
Globo H
EGR2/3
ITCH
Notch1
EGR2/3
ITCH
E2A
Notch1
ID3
Tumor
Infitrating
lymphocytes
cancer immunosuppression
OBI-822 depletes Globo H, which in turns increases Notch 1 degradation, blocking the tumor immunosuppression effect, leading to tumor regression.
Globo H can:
• Reduce T cell proliferation and cytokine secretion
• Inhibit B cell differentiation and Ig secretion

FIG. 2



No vaccination
OBI 822 vaccination
Globo H
SSEA-3
SSEA-4
Anti-Globo H Ab
Anti-SSEA-3 Ab
Anti SSEA 4 Ab
FAK
P
cSrc
P
Pro-Caspase 3
Active Caspase 3
Tumor survival; Invasion
Macrophage
NK
FAK
cSrc
Tumor cell
Apoptosis

FIG. 3

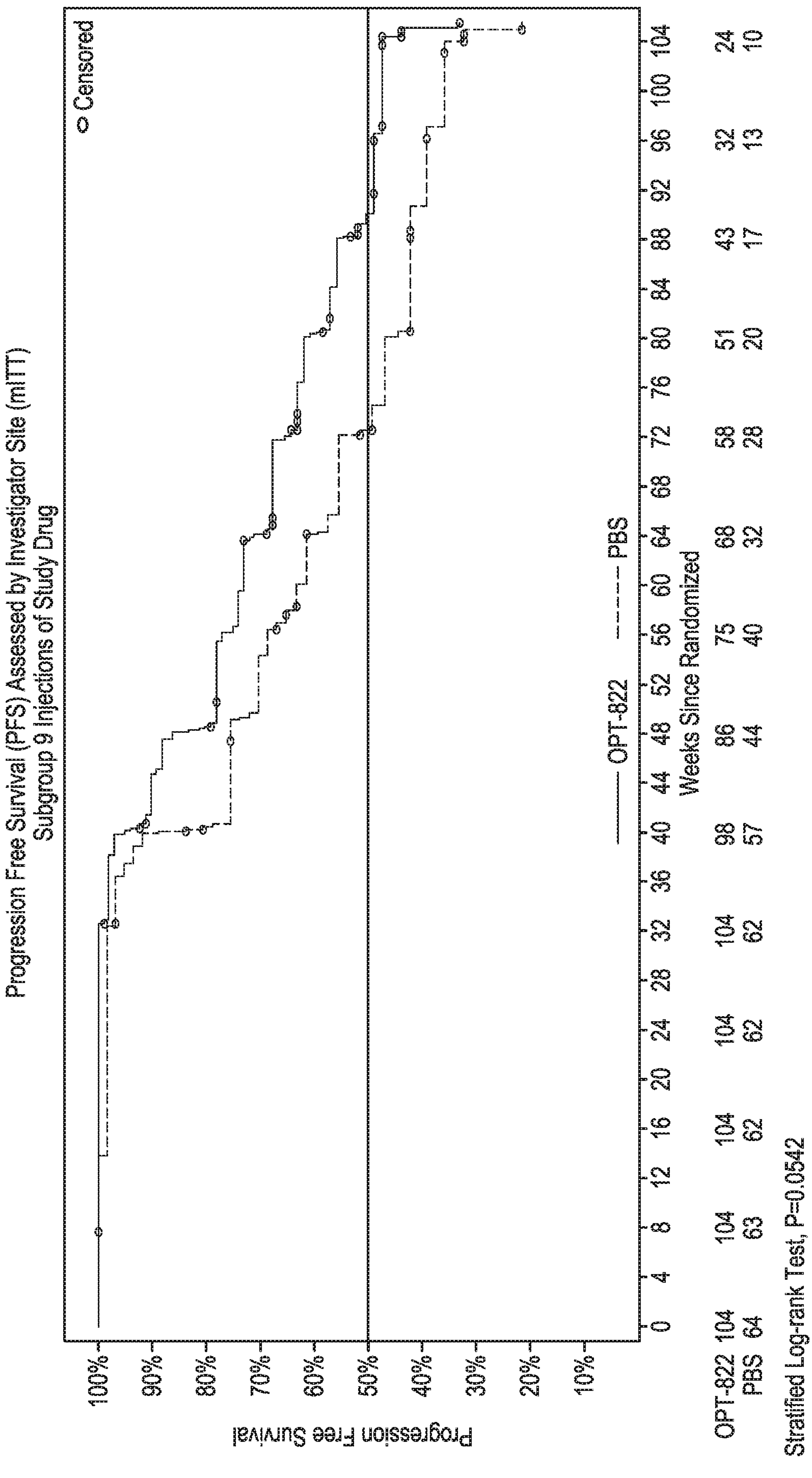

Progression Free Survival (PFS) Assessed by Investigator Site (mITT)
Subgroup 9 Injections of Study Drug
o Censored
Progression Free Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100 104
Weeks Since Randomized
OPT-822
PBS
OPT-822 104 104 104 104 104 98 86 75 68 58 51 43 32 24
PBS 64 63 62 62 62 57 44 40 32 28 20 17 13 10
Stratified Log-rank Test, P=0.0542

FIG. 4

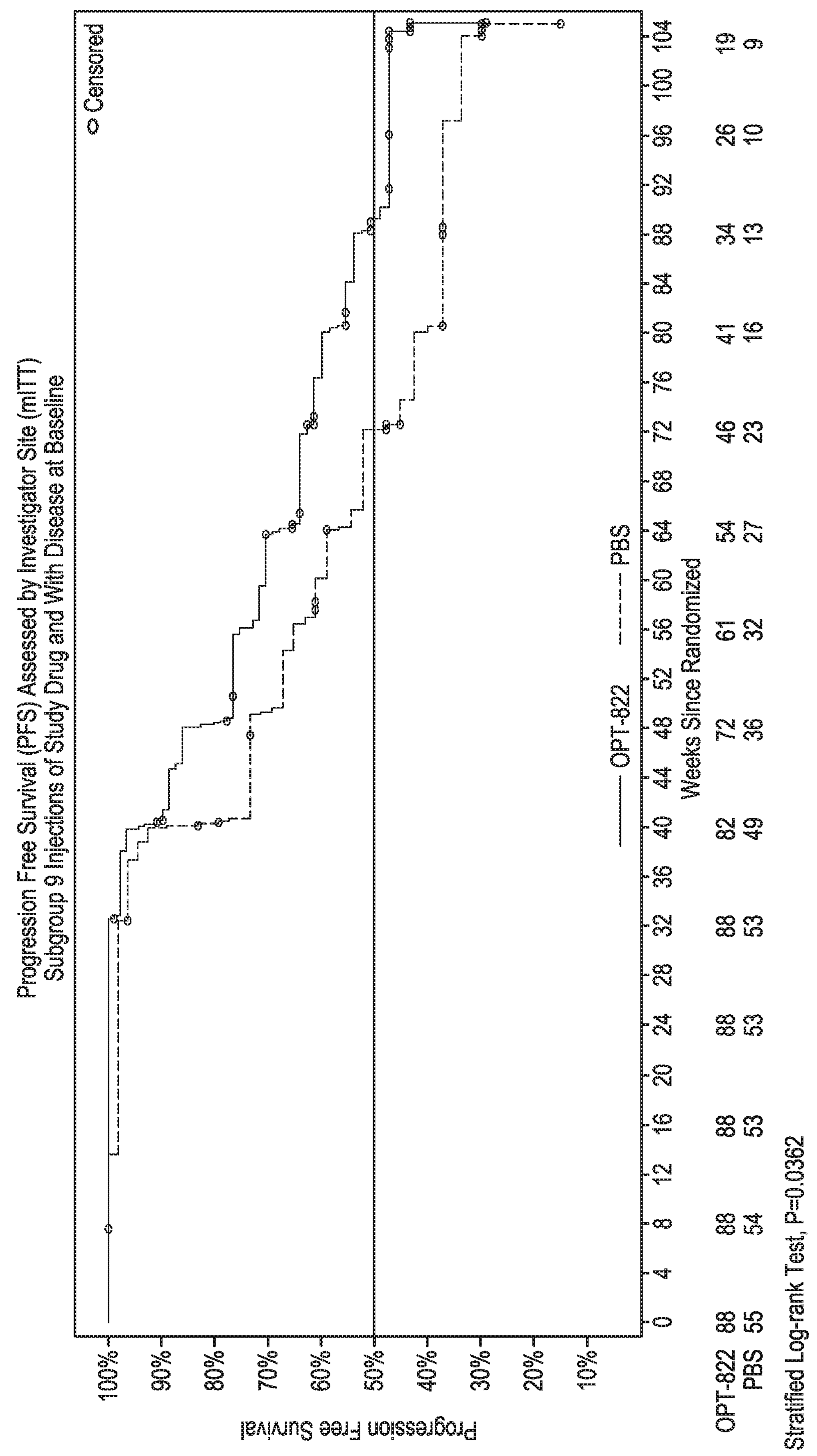

Progression Free Survival (PFS) Assessed by Investigator Site (mITT)
Subgroup 9 Injections of Study Drug and With Disease at Baseline
○ Censored
Progression Free Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80
84
88
92
96
100
104
Weeks Since Randomized
OPT-822
PBS
OPT-822 88 88 88 88 88 82 72 61 54 46 41 34 26 19
PBS 55 54 53 53 53 49 36 32 27 23 16 13 10 9
Stratified Log-rank Test, P=0.0362

FIG. 5

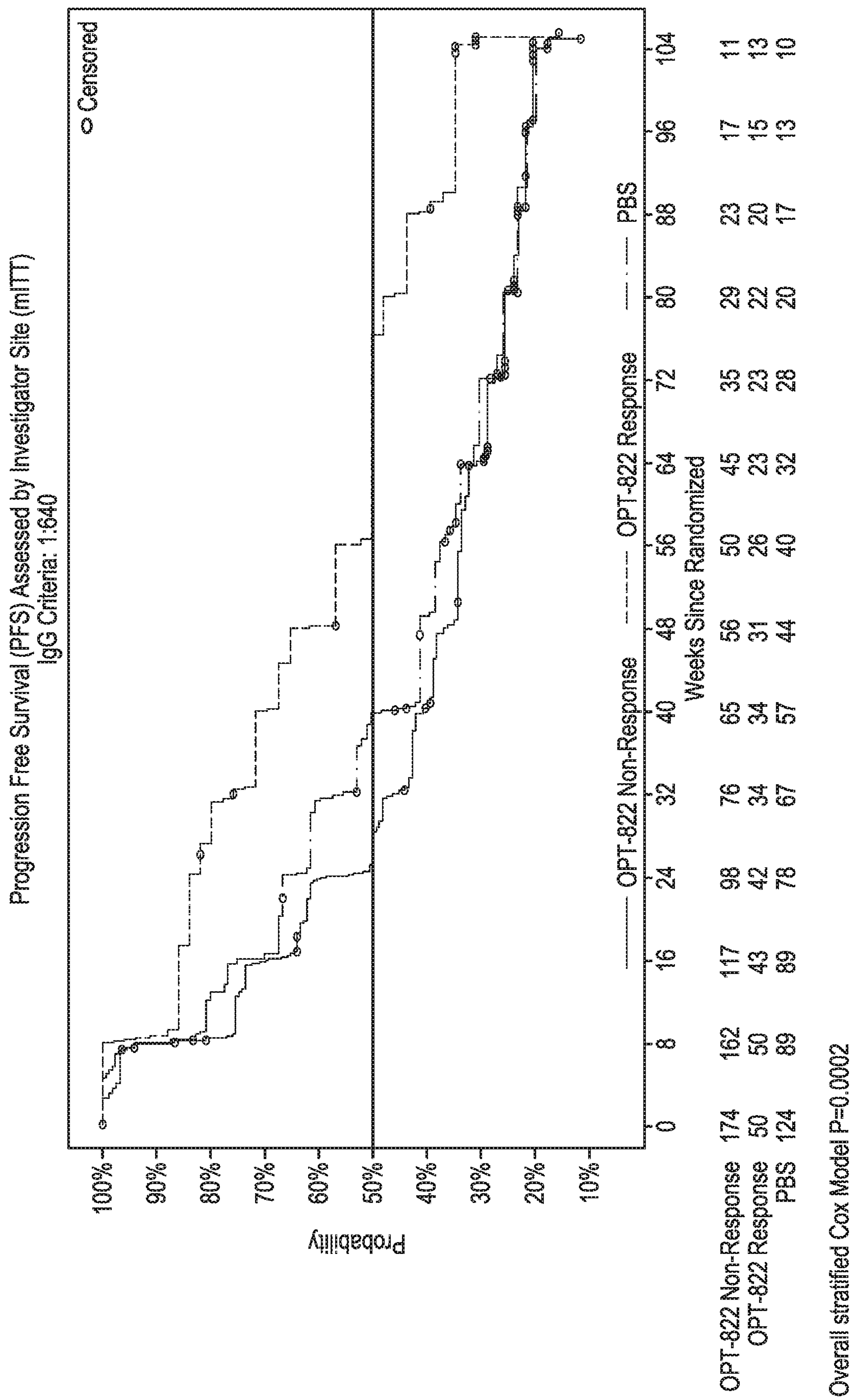

Progression Free Survival (PFS) Assessed by Investigator Site (mITT)
IgG Criteria: 1:640
Censored
Probability
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0
8
16
24
32
40
48
56
64
72
80
88
96
104
Weeks Since Randomized
OPT-822 Non-Response
OPT-822 Response
PBS
OPT-822 Non-Response 174 162 117 98 76 65 56 50 45 35 29 23 17 11
OPT-822 Response 50 50 43 42 34 34 31 26 23 23 22 20 15 13
PBS 124 89 89 78 67 57 44 40 32 28 20 17 13 10
Overall stratified Cox Model P=0.0002

FIG. 6

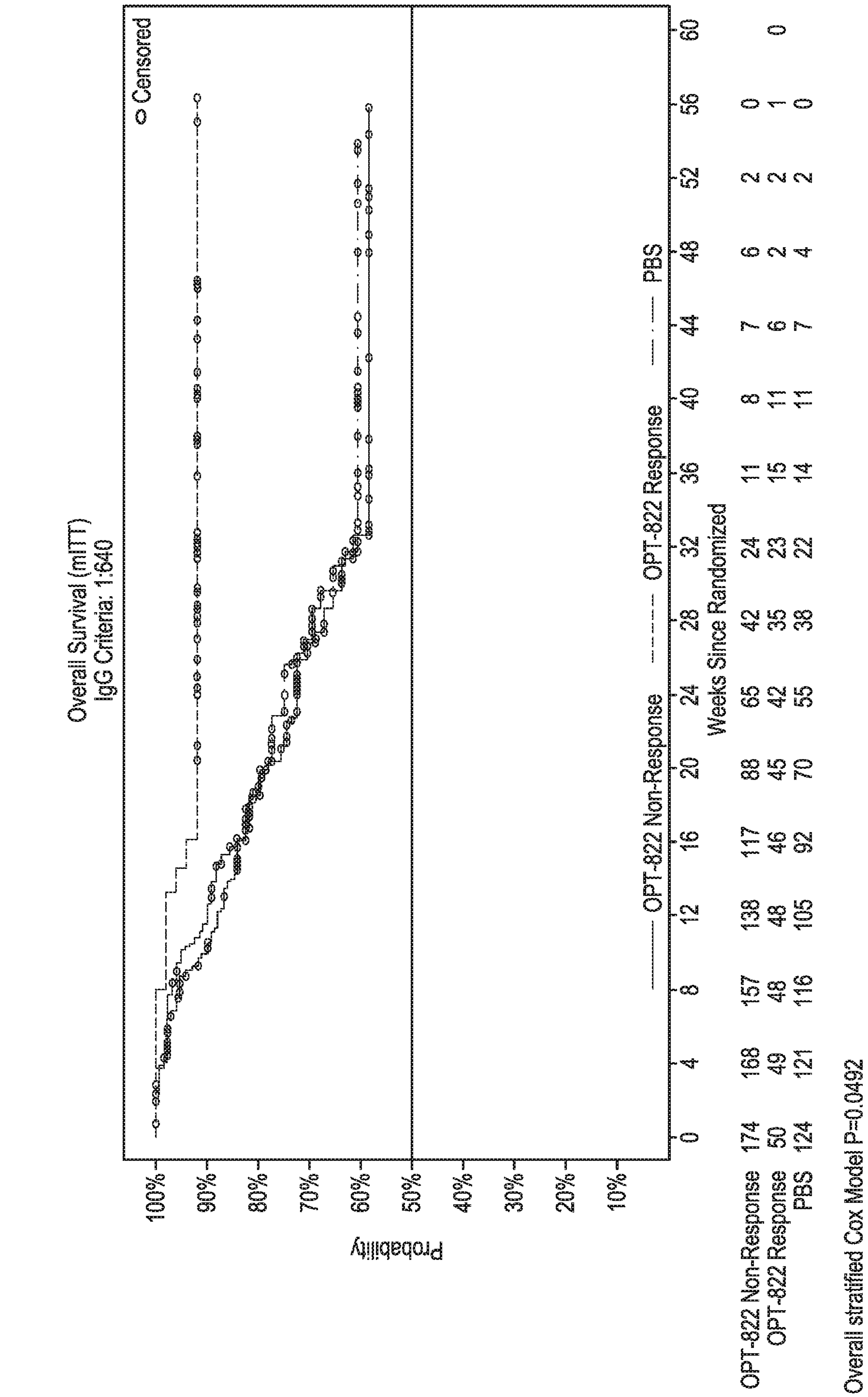

Overall Survival (mITT)
IgG Criteria: 1:640
o Censored
Probability
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Weeks Since Randomized
OPT-822 Non-Response
OPT-822 Response
PBS
OPT-822 Non-Response 174 168 157 138 117 88 65 42 24 11 8 7 6 2 0
OPT-822 Response 50 49 48 48 46 45 42 35 23 15 11 6 2 2 1 0
PBS 124 121 116 105 92 70 55 38 22 14 11 7 4 2 0
Overall stratified Cox Model P=0.0492

FIG. 7

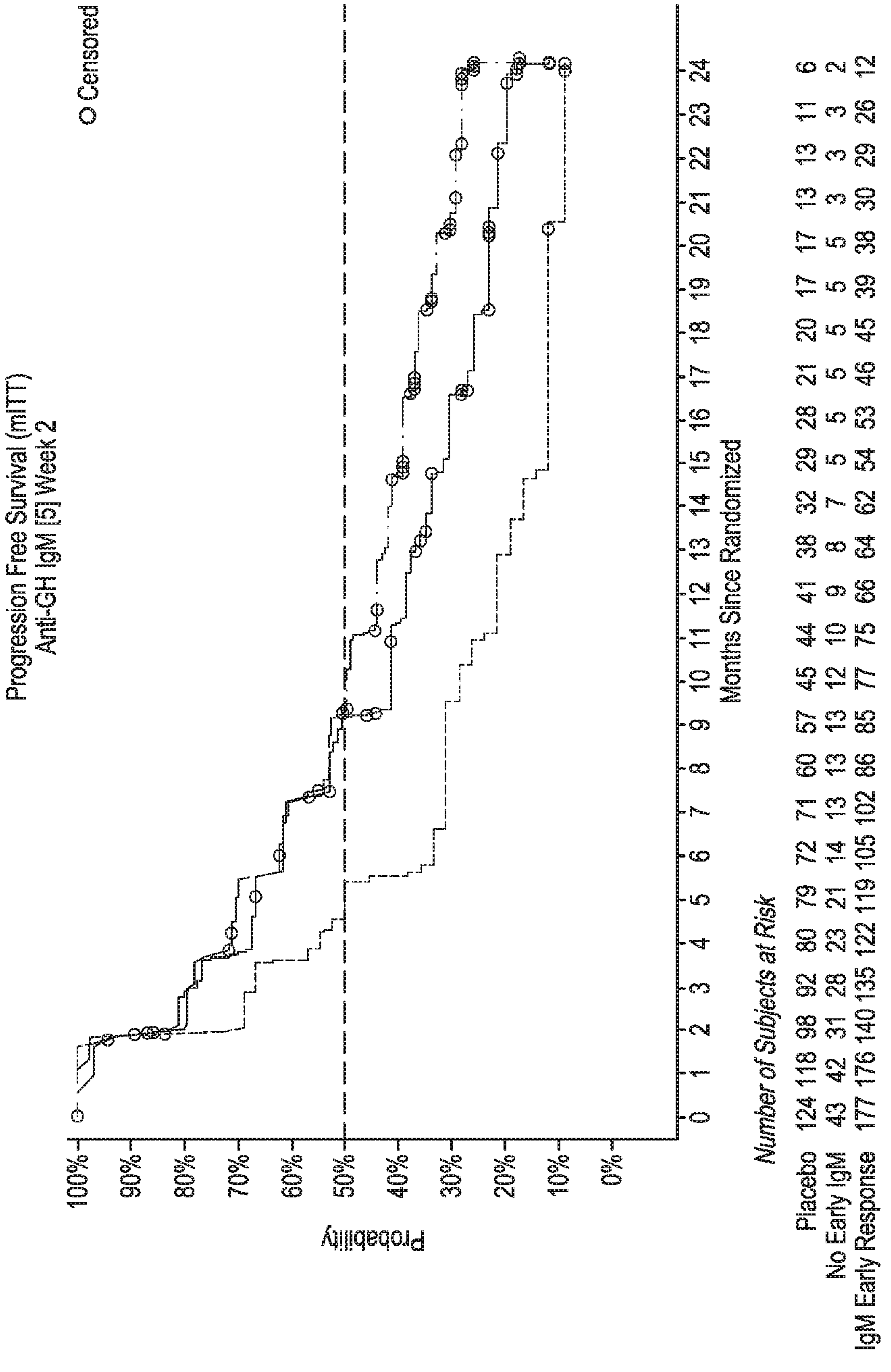

Progression Free Survival (mITT)
Anti-GH IgM [5] Week 2
O Censored
Probability
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Months Since Randomized
Number of Subjects at Risk
Placebo 124 118 98 92 80 79 72 71 60 57 45 44 41 38 32 29 28 21 20 17 17 13 13 11 6
No Early IgM 43 42 31 28 23 21 14 13 13 13 12 10 9 8 7 5 5 5 5 5 5 3 3 3 2
IgM Early Response 177 176 140 135 122 119 105 102 86 85 77 75 66 64 62 54 53 46 45 39 38 30 29 26 12

FIG. 8

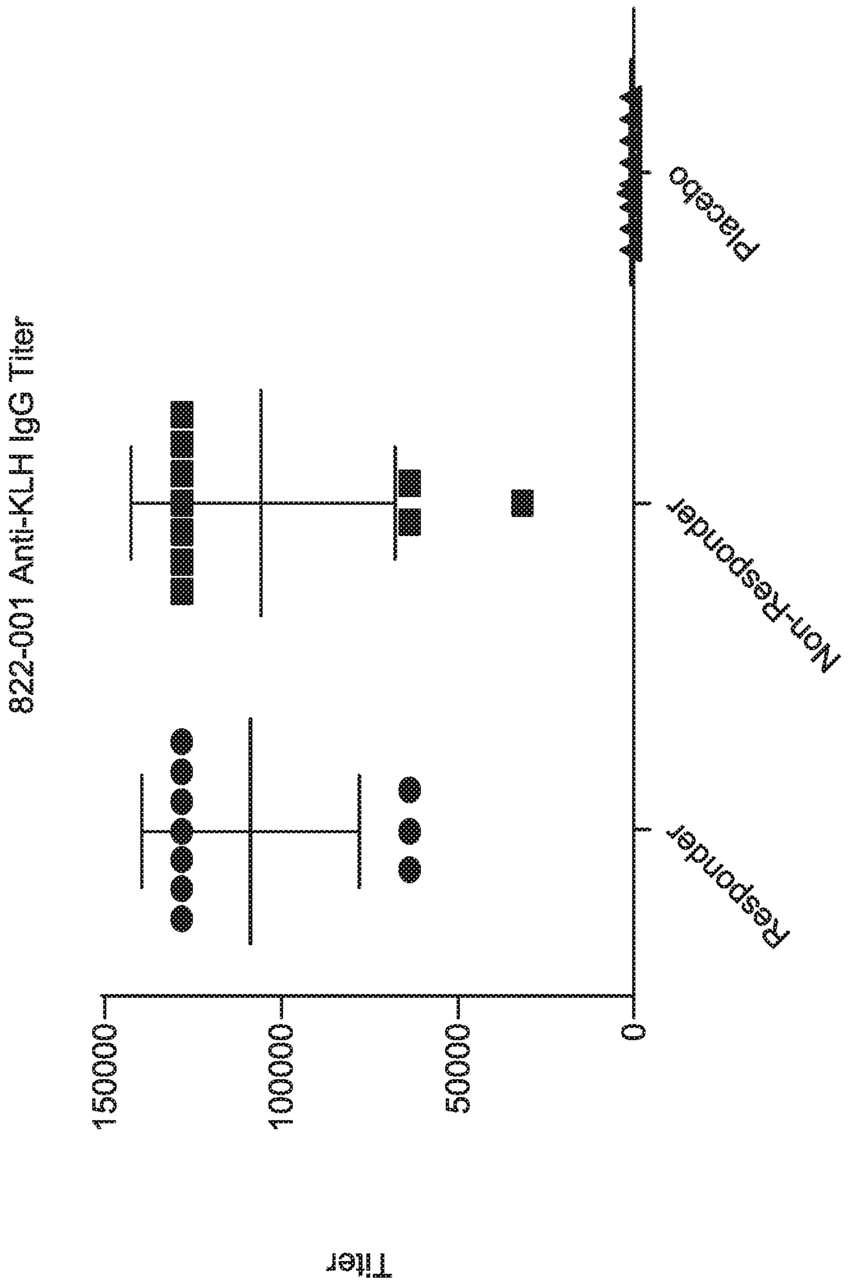

822-001 Anti-KLH IgG Titer
Titer
150000
100000
50000
0
Responder
Non-Responder
Placebo

FIG. 9

Subject No.	Visit	Lesion Assessment	Date of Assessment	Status	Method	Organ	Response
065	Screening	TARGET	2015-03-18	None			
		NON-TARGET	2015-03-18	None			
	Visit 7	TARGET	2015-10-01	None			NA
		NON-TARGET	2015-10-01	None			NA
		NEW	2015-10-01				No
		Overall	2015-10-01				UE

FIG. 10

Subject No.	Visit	Lesion Assessment	Date of Assessment	Status	Method	Organ	Response
035	Screening	TARGET	2014-04-24	None			
		NON-TARGET	2014-04-24	PRESENT	CT SCAN	SUBCENTIMETER MESENTERIC NODES	
	Visit 7	TARGET	2014-11-25	None			NA
		NON-TARGET	2014-11-25	PRESENT	CT SCAN	SUBCENTIMETER MESENTERIC NODES	SD
		NEW	2014-11-25				No
		Overall	2014-11-25				SD
	Visit 10	TARGET	2015-05-12	None			NA
		NON-TARGET	2015-05-12	PD	CT SCAN	SUBCENTIMETER MESENTERIC NODES	PD
		NEW	2015-05-12 2015-05-12		CT SCAN CT SCAN	PERITONEUM PERITONEUM	Yes
		Overall	2015-05-12				FD

FIG. 11

Subject No.	Visit	Lesion Assessment	Date of Assessment	Status	Method	Organ	Response
041	Screening	TARGET	2014-05-22	None			
		NON-TARGET	2014-05-22	PRESENT	CT SCAN	LUNG	
	Visit 7	TARGET	2015-01-08	None			NA
		NON-TARGET	2015-01-08	PRESENT	CT SCAN	LUNG	SD
		NEW	2015-01-08				No
		Overall	2015-01-08				SD
	Visit 10	TARGET	2015-06-25	None			NA
		NON-TARGET	2015-06-25	PRESENT	CT SCAN	LUNG	SD
		NEW	2015-06-25		CT SCAN	SMALL MESENTENIC NODULE	Yes
		Overall	2015-06-25				FD

FIG. 12

Subject No.	Visit	Lesion Assessment	Date of Assessment	Status	Method	Organ	Response
060	Screening	TARGET	2015-01-06	None			
		NON-TARGET	2015-01-06	PRESENT	CT SCAN	PERITONEUM	
	Visit 7	TARGET	2015-08-17	None			NA
		NON-TARGET	2015-08-17	PRESENT	CT SCAN	PERITONEUM	SD
		NEW	2015-08-17				No
		Overall	2015-08-17				SD

FIG. 13

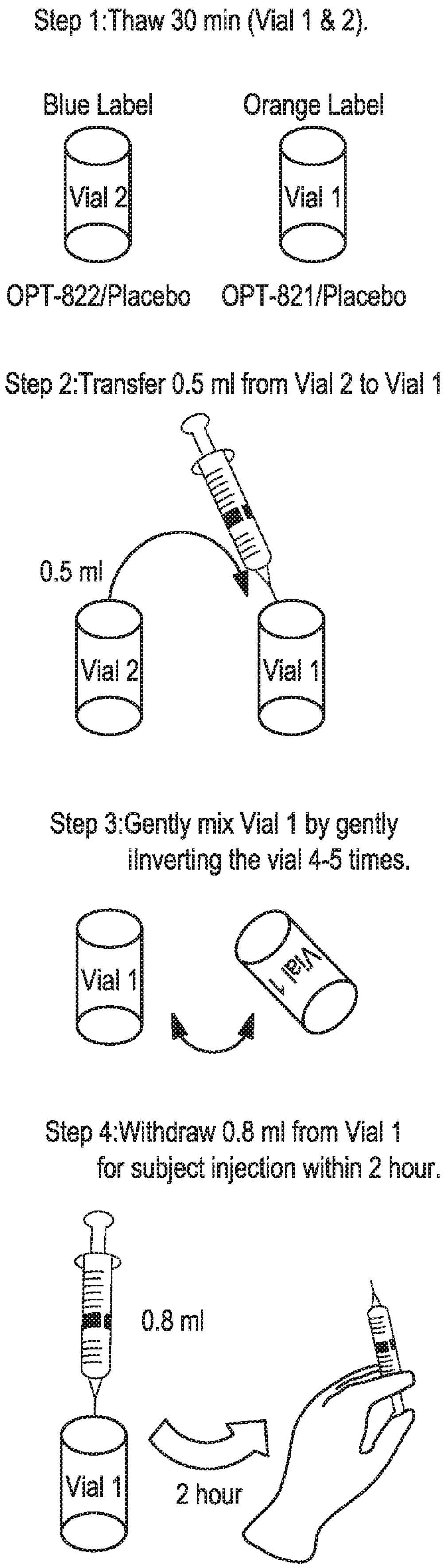

Step 1:Thaw 30 min (Vial 1 & 2).
Blue Label
Orange Label
Vial 2
Vial 1
OPT-822/Placebo
OPT-821/Placebo
Step 2:Transfer 0.5 ml from Vial 2 to Vial 1
0.5 ml
Vial 2
Vial 1
Step 3:Gently mix Vial 1 by gently
iInverting the vial 4-5 times.
Vial 1
Vial 1
Step 4:Withdraw 0.8 ml from Vial 1
for subject injection within 2 hour.
0.8 ml
Vial 1
2 hour

FIG. 14

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13*ter*.1(c) and (d) and 39)

Applicant's or agent's file reference G3004-00605	**IMPORTANT DECLARATION**	Date of mailing (*day/month/year*) 11 July 2017 (11.07.2017)
International application No. **PCT/US2017/029138**	International filing date (*day/month/year*) **24 April 2017 (24.04.2017)**	(Earliest) Priority date (*day/month/year*) 22 April 2016 (22.04.2016)
International Patent Classification (IPC) or both national classification and IPC ***A61K 39/00(2006.01)i, A61K 39/39(2006.01)i, A61K 45/06(2006.01)i***		
Applicant **OBI PHARMA, INC. et al.**		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☒ The subject matter of the international application relates to:
   - a. ☐ scientific theories
   - b. ☐ mathematical theories
   - c. ☐ plant varieties
   - d. ☐ animal varieties
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
   - f. ☐ schemes, rules or methods of doing business
   - g. ☐ schemes, rules or methods of performing purely mental acts
   - h. ☐ schemes, rules or methods of playing games
   - i. ☒ methods for treatment of the human body by surgery or therapy
   - j. ☐ methods for treatment of the animal body by surgery or therapy
   - k. ☐ diagnostic methods practised on the human or animal body
   - l. ☐ mere presentation of information
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art
2. ☒ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
   ☐ the description ☒ the claims ☐ the drawings
3. ☐ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:
   - ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
   - ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
   - ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).
4. Further coments:

Name and mailing address of ISA/KR International Application Division Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea Facsimile No. +82-42-481-8578	Authorized officer CHO, Ki Yun Telephone No. +82-42-481-5655