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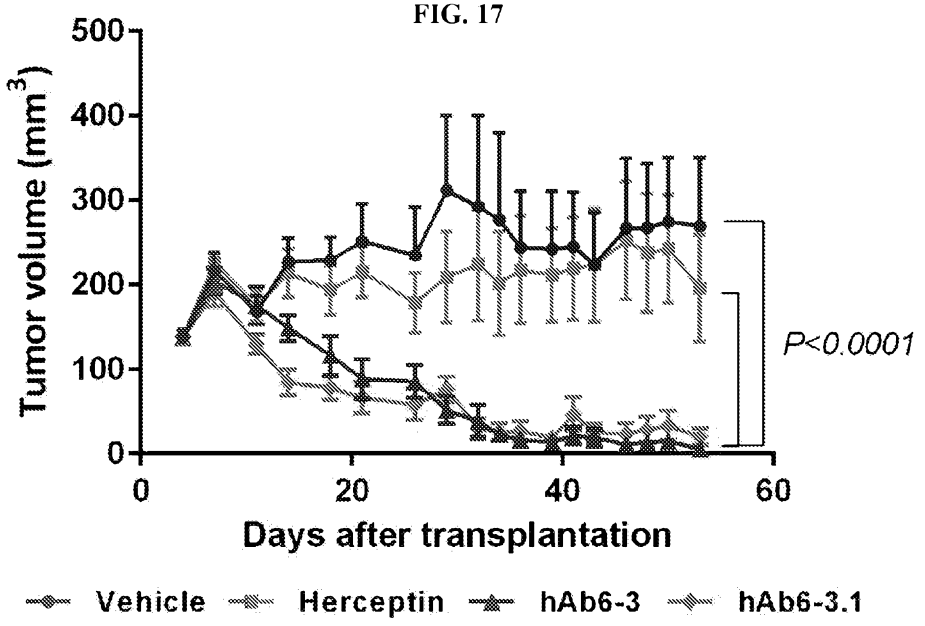
FIG. 17




(57) Abstract: The present disclosure relates to anti-SSEA4 antibodies and bindings fragments thereof comprising specific complementarity determining regions capable of high affinity binding to SSEA4 molecules and SSEA4-associated expressing tumor cells, such as breast cancer, pancreatic cancer, and renal cancer cells. The anti-SSEA4 antibodies and binding fragments induce ADCC or CDC effects in the targeted tumor cells and inhibit and/or reduce the cancer/tumor proliferation. The present disclosure also provides anti-SSEA4 antibodies and binding fragments thereof as a pharmaceutical composition for treating cancer. In addition, the anti-SSEA4 antibodies and binding fragments are useful in the diagnosis of cancers.

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# ANTIBODIES, BINDING FRAGMENTS, AND METHODS OF USE

## RELATED APPLICATIONS

**[0001]** This application claims the benefit of priority to U.S. provisional application serial No. 62/378,102, filed on August 22, 2016, the contents of which are incorporated by reference in their entirety.

## FIELD OF INVENTION

**[0002]** The present disclosure is directed to antibodies and binding fragments for immunotherapy in the treatment of proliferative disorders, including cancer and methods of diagnosis for the same. In particular, the disclosure relates to carbohydrate-related immunotherapy comprising an antibody and/or binding fragment against an immunogenic oligosaccharide SSEA4 and a pharmaceutical composition. Moreover, the disclosure relates to detection and/or diagnosis of hyperproliferative conditions and oncologically-related or specific carbohydrates expressed on abnormal cells.

## BACKGROUND

**[0003]** Stage-specific embryonic antigen 4 (SSEA4) is a hexasaccharide belonged to globo-series glycosphingolipids (GSLs) and comprises the structure of Neu5Ac$\alpha$2→3Gal$\beta$1→3GalNAc$\beta$1→3Gal$\alpha$1→4Gal$\beta$1→4Glc$\beta$1. Since SSEA4 was first isolated from human teratocarcinoma cells in 1983 (Kannagi *R,* et al., 1983), it is widely used as a surface marker to define human embryonic stem cells (hESCs) so far. In past decades, more and more studies indicated that GloboH, a GSL which shares the core structure Gal$\beta$1→3GalNAc$\beta$1→3Gal$\alpha$1→4Gal$\beta$1→4Glc$\beta$1 (SSEA3) with SSEA4, is overexpressed in many epithelial cancers, including ovarian, gastric, prostate, lung, breast, and pancreatic cancers (Zhang S, et al., 1997). And high-level expression of SSEA4 was observed in renal cell carcinoma (Saito S, et al., 1997) and glioblastoma multiforme (Lou YW, et al., 2014). More interestingly, together with SSEA3 and GloboH, the expression of SSEA4 was found not only in breast tumor cells but also in breast cancer stem cells (Chang WW, et al., 2008; Huang YL, et al., 2013).

**[0004]** Carbohydrate antigens, however, are often tolerated by the immune system and consequently induce weak or non-specific immune response (Stein KE, et al., 1992; Snapper CM, et al. 1996.). It is proposed that the carbohydrate antigens are unable to be

internalized and digested by the antigen presenting cells (APC), such as macrophages, B cells or dendritic cells, and therefore cannot be presented to helper T (Th) cells. The lack of simulations from APC to T-cell results in the absent of antibody maturation and isotype switching. Accordingly, low affinity and non-class-switching IgM antibody against carbohydrate antigen is predominately produced (Musher DM, et al. 1990; Lortan JE, et al. 1993). Various approaches have been developed to address the deficiencies. Conjugating carbohydrate antigens with carrier proteins to improve the immunogenicity has been developed since 1950s (Lindberg AA, et al., 1999). Such kind of highly immunogenic proteins include diphtheria toxoid (DT), tetanus toxoid (TT), CRM197 (a non-toxic variant of diphtheria toxin), and a complex outer-membrane protein (OMP) mixture from *N. meningitides* (Ada G. et al., 1999). In addition to the intrinsic immunogenic property of these proteins, a booster effect is expected if the recipient had been immunized with these toxoids before. The carrier protein-carbohydrate antigen conjugates provide peptides conjugated with certain carbohydrate antigen to be processed and presented by APC through MHC II molecules. With the co-simulation from Th cells, T and B cells against certain carbohydrate antigen are then activated. Followed by antibody isotype-switching and maturation, the IgG antibody against certain carbohydrate antigen with high affinity and specificity could be further generated (Bazendale HE, et al., 2000). WO 2016029071 provides a carbohydrate based vaccine comprising synthetic SSEA4 analogs chemically conjugated to the immunogenic carrier diphtheria toxin cross-reacting material 197 (CRM 197) via a linker.

**[0005]** Although the carrier proteins in the carbohydrate vaccination provide a solution to improve the immunogenicity, the strategy poses some new and existing problems (Ingale S, et al., 2007). First, the foreign carrier protein and the attaching linker may elicit strong immune responses, thereby leading to the suppression of an antibody response against the carbohydrate antigen. Second, the chemical conjugation is basically on the lysine of the protein surface. The experiment process is difficult to control, resulting in the heterogeneous composition and final structure. The ambiguous composition probably causes different immune response. Third, the conjugation to mimic the expression of the carbohydrate on the cell surface is not ideal, thereby the induced antibody somehow is failed to recognize the carbohydrate cluster. Alternative approaches, such as carbohydrate PEGylation (Giorgi ME. et al., 2014), are investigated to overcome the remaining problems.

**[0006]** Nevertheless, the active immunization therapy mentioned above is not practice well in cancer patients who is in the status of hypoimmune. Particular those who receive

chemotherapy or radiation therapy, as well as late-stage cancer patients, the efficacy of active immune intervention is often limited.

**[0007]** In view of the foregoing, instead of the vaccination, there exists a need to develop a therapeutic antibody against the cancer carbohydrate epitope to adapt passive immunity.

## SUMMARY

**[0008]** The present disclosure provides exemplary isolated anti-SSEA4 monoclonal antibodies, binding fragments thereof, the nucleic acids encoding them, and the compositions containing such antibodies and fragment thereof, and their methods of use for inhibiting and/or reducing tumor growth and treatment of cancer. The exemplary monoclonal anti-SSEA4 antibodies and binding fragments provided herein can mediate antibody dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC) activities to target and kill the tumor cells expressing SSEA4. In addition, the monoclonal anti-SSEA4 antibodies provided herein can be used to detect the SSEA4 expressing tumor cells within the tumor sample and/or sections in an exemplary diagnostic application.

**[0009]** Accordingly, provided herein are novel recombinant anti-SSEA4 antibodies specifically binding to SSEA4 or its derivatives and fragments, and methods of their use in anti-tumor immunotherapies, such as the treatment of cancer. Once bound to a cancer antigen, antibodies can induce antibody-dependent cell-mediated cytotoxicity, activate the complement system, and inhibit the growth of tumor.

**[0010]** In one embodiment, SSEA4 is highly expressed on various tumor cells, including brain tumor cells, lung tumor cells, breast tumor cells, oral tumor cells, esophageal tumor cells, stomach tumor cells, liver tumor cells, bile duct tumor cells, pancreatic tumor cells, colon tumor cells, renal tumor cells, cervical tumor cells, ovarian tumor cells, prostate tumor cells.

**[0011]** In one embodiment, the monoclonal anti-SSEA4 antibody specifically binds to SSEA4 molecule and derivatives.

**[0012]** In one embodiment, the compositions comprising the anti-SSEA4 antibody described herein are useful in anti-cancer therapies. In particular, the present embodiments provide the complementarity determining region (CDR) sequences of specific anti-SSEA4 antibody, which can be used in a variety of anti-SSEA4 binding portion. In particular, the

present invention provides a humanized or chimeric antibody or an antigen-binding fragment thereof capable of binding to SSEA4 or its derivatives.

**[0013]** In certain embodiments, the CDR sequences are defined by Kabat method.

**[0014]** In certain embodiments, the anti-SSEA4 antibody has the activity of inhibiting tumor growth upon binding to SSEA4-positive or SSEA4 expressing cells.

**[0015]** In certain embodiments, the isolated anti-SSEA4 antibody is a monoclonal antibody. Monoclonal antibodies to SSEA4 can be made according to knowledge and skill in the art. For example, it can be made by injecting test subjects with human embryonic carcinoma cell and then isolating hybridomas expressing antibodies having the desired sequence or functional characteristics.

**[0016]** In one embodiment, the present disclosure provides an isolated monoclonal antibody or an antigen binding portion thereof that binds to SSEA4 wherein upon target binding the antibody has CDC inducing activity.

**[0017]** In one embodiment, the present disclosure provides an isolated monoclonal antibody or an antigen binding portion thereof that binds to SSEA4 wherein upon target binding the antibody has ADCC inducing activity.

**[0018]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising:

(i) H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170, or 80% or more conserved sequence homologs thereof;

(ii) H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, or 80% or more conserved sequence homologs thereof;

(iii) H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172, or 80% or more conserved sequence homologs thereof;

(iv) L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175, or 80% or more conserved sequence homologs thereof;

(v) L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, or 80% or more conserved sequence homologs thereof, and

(vi) L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177 respectively, or 80% or more conserved sequence homologs thereof.

**[0019]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising:

(i) H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170, or a conserved sequence homolog thereof containing less than 5 amino acid substitutions;

(ii) H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, or a conserved sequence homolog thereof containing less than 5 amino acid substitutions;

(iii) H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172, or a conserved sequence homolog thereof containing less than 5 amino acid substitutions; and

(iv) L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175, or a conserved sequence homolog thereof containing less than 5 amino acid substitutions;

(v) L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, or a conserved sequence homolog thereof containing less than 5 amino acid substitutions; and

(vi) L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177 or a conserved sequence homolog thereof containing less than 5 amino acid substitutions.

**[0020]** In certain embodiments, the isolated monoclonal antibody or an antigen-binding fragment thereof further comprising amino acid substitution on the CDR selected from one or more of A100R, N31S, T62A on the heavy chain and/or S52Y on the light chain.

**[0021]** In certain embodiments, the isolated monoclonal antibody or an antigen-binding fragment thereof further comprising amino acid substitution on the CDR selected from one or more of V50A, G53A, S35T on the heavy chain and/or one or more of V30I/A, G91A, Y94F on the light chain.

**[0022]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof, comprising: (i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or 80% or more conserved sequence homologs thereof; and (ii) a light chain variable domain

selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or 80 % or more conserved sequence homologs thereof.

**[0023]** In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof of claim 1, further comprising: (i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or 80% or more conserved sequence homologs thereof further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and (ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or 80 % or more conserved sequence homologs thereof further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177.

**[0024]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof, comprising: (i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions; and (ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions.

**[0025]** In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof, further comprising: (i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and (ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175;

and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177.

**[0026]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof, further comprising: (i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173, or a conserved sequence homologs thereof containing less than 10 amino acid substitutions; and (ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178, or sequence homologs thereof containing less than 10 conserved amino acid substitutions, further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177.

**[0027]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof, further comprising: (i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173, or a conserved sequence homologs thereof containing less than 10 amino acid substitutions, further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively; and (ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178; or a conserved sequence homologs thereof containing less than 10 amino acid substitutions.

**[0028]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof, comprising the respective corresponding VH, VL and respective H-CDRs and L-CDRS as set forth in each variant in Tables 2A-2D.

**[0029]** In certain embodiments, the isolated antibody or antigen-binding fragment is:

a. a chimeric antibody or a fragment thereof; or

b. a humanized antibody or fragment thereof; or

c. a human antibody or fragment thereof; or

d. an antigen-binding fragment selected from the group consisting of Fab, Fab', Fv, scFv, dsFv, $F(ab)_2$, Fd and a diabody.

**[0030]** In certain embodiments, the isolated antibody or antigen-binding fragment is IgG.

**[0031]** In certain embodiments, the isolated antibody or antigen-binding fragment thereof targets the carbohydrate antigen SSEA4 having the structure Neu5Acα 2→3Galβ 1 →3GalNAcβ 1→3Galα 1→4Galβ 1→4Glcβ 1.

**[0032]** In certain embodiments, the isolated antibody or antigen-binding fragment of wherein the antibody has CDC and/or ADCC inducing activity upon binding to the target cells.

**[0033]** In certain embodiments, the pharmaceutical composition, comprising the isolated antibody or antigen-binding fragment thereof and a pharmaceutical acceptable carrier.

**[0034]** In certain embodiments, the pharmaceutical composition further comprising one or more anti-tumor agent.

**[0035]** In certain embodiments, the pharmaceutical composition wherein the anti-tumor agent is a chemotherapeutic agent.

**[0036]** In certain embodiments, the immunoconjugate comprising the antibody and a cytotoxic agent.

**[0037]** In certain embodiments, the immunoconjugate having the formula AB-(L-D)p, wherein: (a) AB is the antibody of anyone of claims 1-10; (b) L is a linker; (c) D is a suitable cytotoxic drug, and (d) p ranges from 1 to 8.

**[0038]** In certain embodiments, the immunoconjugate wherein the drug is MMAE.

**[0039]** In certain embodiments, the immunoconjugate wherein the linker is cleavable linker.

**[0040]** In certain embodiments, the ADC wherein the linker is an alkoxyamine-cleavable linker.

**[0041]** In certain embodiments, the pharmaceutical formulation comprising the immunoconjugate of claims and a pharmaceutically acceptable carrier.

**[0042]** In certain embodiments, the pharmaceutical formulation further comprising an additional therapeutic agent.

**[0043]** In certain embodiments, the isolated nucleic acid (cDNA) encoding the antibody of or a binding fragment disclosed herein.

**[0044]** In certain embodiments, the host cell comprising the nucleic acid encoding the antibody of or a binding fragment disclosed herein.

**[0045]** In certain embodiments, the disclosure provides a method of producing an antibody comprising culturing the host cell so that the antibody is produced.

**[0046]** In certain embodiments, the disclosure provides an antibody produced by steps comprising:

(a) providing a nucleic acid encoding 3 VL domain CDRs having sequences of: L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177, respectively;

(b) combining a repertoire of nucleic acids encoding 3 VH domain CDRs having the sequences of H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively with the nucleic acid encoding the 3 VL domain CDRs, so as to provide a product repertoire of nucleic acids encoding the 3 VL domain CDRs and the repertoire of 3 VH domain CDRs;

(c) expressing the nucleic acids of the product repertoire;

(d) selecting an antigen-binding fragment comprising a variable domain that specifically binds to SSEA4 and that is expressed from the nucleic acids of the product repertoire; and

(e) producing an antibody comprising the antigen-binding fragment.

**[0047]** In certain embodiments, the disclosure provides a method of treating a subject having a SSEA4-positive cancer, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition desclosed herein.

**[0048]** In certain embodiments, the disclosure provides a method wherein the SSEA4-positive cancer is selected from brain, lung, breast, oral, esophageal, stomach, liver, bile duct, pancreatic, colon, kidney, cervical, ovarian, and prostate cancer.

**[0049]** In certain embodiments, the disclosure provides method further comprising administering an additional therapeutic modality or agent in combination to the individual.

**[0050]** In certain embodiments, the disclosure provides a method wherein the combined treatment modality is selected from therapeutic antibodies, cell therapies, radiation, cytokines, and/or chemotherapeutic agents.

**[0051]** In certain embodiments, the disclosure provides a method of inhibiting proliferation of a SSEA4-positive cell, the method comprising exposing the cell to the pharmaceutical formulations as disclosed herein under conditions permissive for binding of the antibodies/fragments/ADCs to SSEA4 on the surface of the cell expressing carbohydrate antigen, thereby inhibiting proliferation of the cell.

**[0052]** In certain embodiments, the method of treating a subject having a SSEA4-positive cancer, wherein the SSEA4-positive cancer is resistant to a first therapeutic agent, the method comprising administering to the individual an effective amount of the pharmaceutical formulation disclosed herein.

**[0053]** In certain embodiments, the method wherein the SSEA4-positive cancer is brain, lung, breast, oral, esophageal, stomach, liver, bile duct, pancreatic, colon, kidney, cervical, ovarian, and/or prostate cancer.

**[0054]** In certain embodiments, the method wherein the first therapeutic agent comprises a first antibody/binding fragment/ADC that binds an antigen other than SSEA4, and/or radiation, and/or chermotherapeutic agents.

**[0055]** In certain embodiments, the method of detecting SSEA4 in a biological sample comprising contacting the biological sample with the anti-SSEA4 antibody as disclosed herein under conditions permissive for binding of the anti-SSEA4 antibody to a naturally occurring SSEA4, and detecting whether a complex is formed between the anti-SSEA4 antibody and a naturally occurring SSEA4 in the biological sample.

**[0056]** In certain embodiments, the method wherein the biological sample is a cancer sample.

**[0057]** In certain aspect, the disclosure provides a method for detecting a SSEA4-positive cancer comprising (i) administering a labeled anti-SSEA4 antibody to a subject having or suspected of having a carbohydrate antigen expressing tumor, wherein the labeled anti-SSEA4 antibody comprises the anti-SSEA4 antibody as disclosed herein, and (ii) detecting the labeled anti-SSEA4 antibody in the subject, wherein detection of the labeled anti-SSEA4 antibody indicates a SSEA4-positive cancer in the subject.

**[0058]** In certain embodiments, the isolated antibody wherein the antibody specifically binds to SSEA4 with an affinity constant less than $10^{-7}$ M.

**[0059]** In certain embodiments, the isolated antibody wherein the antibody is IgG1, IgG2, IgG3, or IgG4.

**[0060]** In certain embodiments, the isolated antibody wherein the antibody is IgG1λ or IgG1κ.

**[0061]** In certain embodiments, the monoclonal antibody or antigen-binding portion thereof wherein the monoclonal antibody or antigen-binding portion thereof binds to SSEA4 with a $K_D$ of $1\times10^{-7}$M or less, and wherein the $K_D$ is measured by surface plasmon resonance (Biacore) analysis.

**[0062]** In certain embodiments, the isolated anti-SSEA4 antibody or binding fragment thereof whererin the binding affinity is < 50 nM.

**[0063]** The present disclosure is directed to antibodies and binding fragments thereof which specifically binds to SSEA4 according to any of the aspect/embodiments of the present invention. In one aspect, the present disclosure provides an isolated monoclonal antibody or a binding fragment thereof that binds to SSEA4 wherein upon target binding the antibody has ADCC inducing activity.

**[0064]** According to certain embodiments, the antibody is a monoclonal antibody.

**[0065]** According to certain embodiments, the antibody is a chimeric or humanized antibody.

**[0066]** According to certain embodiments, the antibody is bispecific antibody.

**[0067]** According to certain embodiment, the invention disclosed a chimeric antigen receptor (CAR) which selectively binds to SSEA4. In this embodiment, the CAR may comprise an antigen-binding domain which has a variable heavy chain ($V_H$) and a variable light chain ($V_L$).

**[0068]** In one aspect the antibody or binding fragment thereof have the half-maximum binding to SSEA4 with an $EC_{50}$ of about 5, 10, 15, 20, 15, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250 nano-gram/mL or a value between any of the two values recited herein by ELISA binding assay.

**[0069]** In one aspect, the isolated anti-SSEA4 antibody or binding fragment thereof whererin the binding affinity is < 50 nM (less than 50nM). In certain embodiments, the binding affinity can range from <5, < 10, < 15, < 20, < 25, < 30, < 35, < 40, < 45, or < 50 nM.

**[0070]** According to one embodiment of the present disclosure, the pharmaceutical composition comprises (1) a therapeutically effective amount of the antibody or antigen-binding fragment according to any of aspects/embodiments of the present disclosure, and optionally (2) a pharmaceutically acceptable carrier.

**[0071]** In one aspect, the present invention is directed to a pharmaceutical composition for treating cancer in a subject in need thereof comprising the isolated antibody, or antigen-binding fragment thereof comprising the exemplary H-CDR1, H-CDR2, H-CDR3, L-CDR1, L-CDR2, and L-CDR3 as disclosed herein and a pharmaceutical acceptable carrier.

**[0072]** In certain embodiments, the pharmaceutical composition is useful in the treatment against a hyperproliferative disease, such as cancer. Exemplary hyperproliferative disease can include, for example, one or more of the tumors listed in Table 4.

**Table 4: Expression of globo-series glycosphingolipids in tumor cells lines.**

Expression of globo-series GSLs was determined by flow cytometry. Cell lines in which more than 15% of total cells were positive in flow cytometry are labeled positive.

| Tumor origin | SSEA-4$^+$ | SSEA-3$^+$ | Globo H$^+$ |
|---|---|---|---|
| Brain | 12/17 | 9/17 | 6/17 |
| Lung | 13/20 | 5/20 | 13/20 |
| Breast | 17/23 | 6/23 | 14/23 |
| Mouth | 8/13 | 2/13 | 11/13 |
| Esophagus | 1/2 | 0/2 | 2/2 |
| Stomach | 4/6 | 3/6 | 6/6 |
| Liver | 6/10 | 4/10 | 9/10 |
| Bile duct | 2/5 | 1/5 | 3/5 |
| Pancreas | 8/8 | 3/8 | 6/8 |
| Colon | 5/7 | 0/7 | 6/7 |
| Kidney | 5/6 | 0/6 | 5/6 |
| Cervix | 3/4 | 2/4 | 1/4 |
| Ovary | 8/9 | 2/9 | 5/9 |
| Prostate | 4/4 | 1/4 | 1/4 |

**[0073]** **Table 4.** The list of globo-series glycosphingolipids expression on tumor cells lines. Various tumor cells expressing high globo-series glycosphingolipids, such as brain tumor cells, lung tumor cells, breast tumor cells, oral tumor cells, esophageal tumor cells, stomach tumor cells, liver tumor cells, bile duct tumor cells, pancreatic tumor cells, colon tumor cells, renal tumor cells, cervical tumor cells, ovarian tumor cells, prostate tumor cells.

**[0074]** In certain aspects, the disclosure provides a method of treating cancer in a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of the representative pharmaceutical composition whereby the administered antibody enhances ADCC or CDC activity in said subject.

**[0075]** In certain embodiments, the method provided treats cancer selected from the group consisting of brain cancer, lung cancer, breast cancer, oral cancer, esophageal cancer, stomach cancer, liver cancer, bile duct cancer, pancreatic cancer, colon cancer, kidney cancer, bone cancer, skin cancer, cervical cancer, ovarian cancer, and prostate cancer.

**[0076]** According to embodiments of the present disclosure, the method includes administering to the subject an effective amount of the pharmaceutical composition comprising the antibody and/or pharmaceutical composition according to any of the aspects/embodiments of the present disclosure.

**[0077]** In certain embodiments, the present disclosure provides methods for diagnosing cancers.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0078]** As used herein, symbolic, graphic, and text nomenclature for describing glycans and related structures are well-established and understood in the art, including, for example, "*Symbols Nomenclatures for Glycan Representation*", Proteomics. 2009 December; 9(24): 5398–5399 by Ajit Varki et al.

**[0079]** **Fig. 1A-1E. 1A: CDR sequences of representative Ab6 antibodies and/or binding fragments.** The CDR sequences are defined by Kabat, AbM, Chothia, Contact, and IMGT methods, respectively. **FIG 1 B**: Demonstration of anti-SSEA4 antibodies with CDRs modifications. **FIG 1C**: Demonstration of anti-SSEA4 antibodies with variable domain modifications. **FIG. 1D**. Demonstration of anti-SSEA4 antibodies with non-conservative CDR modification: Sequence alignment of hAb6-3, hAb6-3.1/2/3/4. **FIG. 1E**. Demonstration of anti-SSEA4 antibodies with conservative CDR modification: Sequence alignment of hAb6-3 and hAb6-3.101/103/105/106/107/108/110.

**[0080]** **Fig. 2. Representative humanized Ab6 sequences with 6 or 10 amino acid substitutes in variable domain.** The CDR regions are underlined and the substituted amino acids are in box.

**[0081]** **Fig. 3.** Kabat number of chAb6 heavy chain variable domain.

**[0082]** **Fig. 4.** Kabat number of chAb6 light chain variable domain.

**[0083]** **Fig. 5. Kinetic binding assay of exemplary chimeric and humanized Ab6 by surface plasmon resonance**. The antigen binding affinity of hAb6-3.1, hAb6-3 and chAb6 were determined using Biacore system. The calculated Kd values for hAb6-3.1, hAb6-3 and chAb6 are 23.1, 17.8 and 10.11 nM, respectively.

**[0084]** **Fig. 6A. Determination of the binding affinity of one exemplary chAb6 to SSEA4 by ELISA.** The exemplary chimeric Ab6 (chAb6) binds to SSEA4 in a dose-dependent manner. The binding $EC_{50}$ of chAb6 to SSEA4 is about 50 ng/mL.

**[0085]** **Fig. 6B. Determination of the binding affinity of other exemplary chimeric and humanized Ab6s to SSEA4 by ELISA.** The exemplary chimeric and humanized Ab6s bound to SSEA4 in a dose-dependent manner. The binding $EC_{50}$ of chAb6, hAb6-3 and hAb6-3.1 to SSEA4 are about 106, 125 and 98 ng/mL, respectively.

**[0086]** **Fig. 6C-6D. Figure 6C** represents a demonstration of the binding affinity of non-conservatively modified amino acid substitutions. **Figure 6D** represents a demonstration of the binding affinity of conservatively modified amino acid substitutions.

**[0087]** **Fig. 7. The binding specificity of an exemplary chAb6 to SSEA4 by glycan array analysis.** The binding septicity of chAb6 against various oligosaccharide was examined and the result indicated chAb6 binds to SSEA4 (spot A) and SSEA4 analog SSEA4 Gc (the Gc substituted sialic acid on amine group of SSEA4, spot B).

**[0088]** **Fig. 8A -8B. The binding of chAb6 and humanized Ab6s to breast cancer cell lines.** Characterization of chAb6, hAb6s (hAb6-2, hAb6-3) binding to (**FIG. 8A**) MDA-MB-231 and (**FIG. 8B**) MCF-7 by flow cytometry analysis.

**[0089]** **Fig. 9A-9B**: **The binding of exemplary chAb6 and humanized Ab6s to breast cancer cell lines.** The binding of chAb6, hAb6-2 and hAb6-3 to (**FIG. 9A**) MDA-MB-231 and (**FIG. 9B**) MCF7 cells were examined by flow cytometry analysis. The antibody concentration used for staining was 1 microgram per milliliter.

**[0090]** **Fig. 10A-10B. Determination of the binding affinity of an exemplary chAb6 to a pancreatic cancer cell line HPAC by flow cytometry analysis.** (**FIG. 10A**) The exemplary chimeric Ab6 (chAb6, 20 μg/mL) binds to HPAC, an exemplary pancreatic tumor cells line with high expression level of SSEA4, in (as shown in **FIG. 10B**) a dose-dependent manner. The binding $EC_{50}$ to HPAC cells is about 4 μg/mL.

**[0091]** **Fig. 11A-11B**: **The binding of exemplary chimeric and humanized Ab6s to breast and pancreatic cancer cell lines.** The binding of chAb6, hAb6-3 and hAb6-3.1 to (11A) MDA-MB-231 and (11B) HPAC cells were examined by flow cytometry analysis. The antibody concentration used for staining was 5 microgram per milliliter.

**[0092]** **Fig. 11C-Fig. 11D. FIG. 11C** is a demonstration of the binding of exemplary humanized Ab6s with non-conservative CDR modifications to MDA-MB-231 cell line. **FIG. 11D** is a demonstration of the binding of exemplary humanized Ab6s with non-conservative CDR modifications to MCF7 cell line.

**[0093]** **Fig. 11E-Fig. 11F.** FIG. **11E** is a demonstration of the binding of exemplary humanized Ab6s with conservative CDR modifications to MDA-MB-231 cell line. **FIG. 11F** is a demonstration of the binding of exemplary humanized Ab6s with conservative CDR modifications to MCF7 cell line

**[0094] Fig. 12. Demonstration of the ADCC activity of an exemplary chAb6 on a pancreatic tumor cells line HPAC.** Representative chAb6 induces ADCC to kill HPAC cells in a dose-dependent manner. The $EC_{50}$ is 5 ng/mL. Human IgG1, kappa (hIgG1, kappa) is used as control.

**[0095] Fig. 13. The ADCC activity of exemplary chAb6 and humanized Ab6s on MDA-MB-231 cells. The exemplary** chAb6, hAb6s mediate ADCC to kill MDA-MB-231 cells in a dose-dependent manner. The $EC_{50}$ are about 5 ng/mL and 10 ng/mL for chAb6 and hAb6s, respectively.

**[0096] Fig. 14A-14B**: **The ADCC activity of exemplary humanized Ab6s on breast cancer cell lines.** Both exemplary hAb6-3 and exemplary hAb6-3.1 mediated ADCC to kill **(FIG. 14A)** MDA-MB-231 and **(FIG. 14B)** MCF7 cells in a dose-dependent manner. In this study, the $EC_{50}$ for hAb6-3-mediated ADCC to kill MDA-MB-231 and MCF7 are 39.2 and 39.5 ng/mL, respectively. For hAb6-3.1-mediated ADCC to kill MDA-MB-231 and MCF7 are 32.6 and 38.9 ng/mL, respectively.

**[0097] Fig. 15A-15B. 15A: Demonstration of the CDC activity of an exemplary chAb6 on HPAC cells.** Representative chAb6 induces CDC to kill HPAC cells in a dose-dependent manner. The $EC_{50}$ is 3 μg/mL. Human IgG1, kappa (hIgG1, k) is used as a negative control in this study. **15B: Demonstration of the CDC activity of exemplary humanized Ab6s on breast cancer cell line.** The exemplary humanized anti-SSEA4 antibodies hAb6-3 and hAb6-3.1 induced CDC to kill MCF7 cells in a dose-dependent manner. The $EC_{50}$ are about 4.4 and about 2.6 μg/mL for hAb6-3 and hAb6-3.1, respectively.

**[0098] Fig. 16A-16B. Demonstration of *in vivo* anti-tumor efficacy of representative anti-SSEA4 antibodies in HPAC xenograft model.** Comparing to vehicle control group, the growth of tumor is significantly suppressed in mice with anti-SSEA4 antibody treatment. Moreover, as shown in figure, the average tumor volume (**FIG. 16A**) and weight (**FIG. 16B**) in mice treated with chAb6 are significantly smaller than those treated with hMC41, demonstrating that this exemplary chAb6 has an unexpectedly surprising *in vivo* anti-tumor activity.

**[0099] FIG. 17. Demonstration of *in vivo* anti-tumor efficacy of exemplary humanized Ab6s in MDA-MB-231 orthotopic model.** The *in vivo* tumor growth was significantly suppressed by treating tumor-bearing mice with exemplary anti-SSEA4 antibodies hAb6-3 and hAb6-3.1, as comparing with the control groups (vehicle and Herceptin). Herceptin was used as a control antibody in this study.

**[00100] FIG. 18: Demonstration of *in vivo* anti-tumor efficacy of an exemplary humanized Ab6 MCF7 orthotopic model.** As comparing with vehicle control treatment, the growth of tumor was significantly suppressed in a dose-dependent manner under the treatment of hAb6-3.1.

**[00101] Fig. 19 Demonstration of diagnostic utility: Detection of SSEA4 expression in tumor tissue using exemplary chAb6.** The result of immune-histochemistry staining showed that chAb6 can be applied to detect SSEA4 expression in tumor samples.

**[00102] Fig. 20. Characterization of glycoengineered hAb6-3.1 by SDS-PAGE.** Lane 1, native antibody produced from mammalian cells; Lane 2, Antibody with mono-GlcNAc; Lane 3-4, Glyo-engineered hAb6-3.1 produced in 30 mins and 60 mins; Lane 5, Purified glyco-engineered antibody.

**[00103] Fig. 21. The binding property of glycoengineered hAb6-3.1 by cell flow cytometry.** The glyco-engineered antibody (red line) exhibits similar binding property with the native antibody (blue line) to SSEA4-expressing cell line MDA-MB-231. The result indicated that glycoengineering does not affect the antigen binding property of hAb6-3.1

**[00104] Fig. 22. Fc gamma receptor IIIA binding**. The binding (EC50) of hAb6-3.1 to Fc gamma receptor IIIA was dramatically enhanced by glycoengineering. The EC50 for native and glyco-engineered antibody are 0.84 and 0.047 μg (microgram)/mL, respectively.

**[00105] Fig. 23. The ADCC activity of native and glyco-engineered hAb6-3.1 on MDA-MB-231 cells.** Both native and glyco-engineered hAb6-3.1 mediated ADCC to kill MDA-MB-231 cells in a dose-dependent manner. The ADCC activity of hAb6-3.1 was significantly improved by glycoengineering. The EC50 for native and glyco-engineered hAb6-3.1 are about 50.29 and about 6.02 ng/ml, respectively.

**[00106] Fig. 24A.** The oxmine ligation of drug onto the Fc glycan for ADC formation. **Fig. 24B.** is the SDS-PAGE profile of ADC complex formation. Lane 1: Marker, Lane 2: ketone tagged of hAb6-3.1, Lane 3: hAb6-3.1-A01.

**[00107] Fig. 25A and 25B**. **Fig. 25A** is The binding ability of hAb6-3.1-A01 to SSEA4-expressing cells by flow cytometry. SSEA4-expressing cell line MCF7 and SKOV3 were washed with PBS and 1x105 of cells were incubated with 10 ug/mL of hAb6-3.1 or hAb6-3.1-A01 in FACS buffer (PBS containing 2 % FBS and 0.1 % NaN3) on ice for 1 hr. After washing with PBS, the cells were stained with Alexa-Fluor 488 labeled anti-human IgG antibody and incubated on ice for 0.5 hr. The signals for cell binding of antibodies were

detected by flow cytometry (Figure XX11AB). The result indicated the binding property of hAb6-3.1-A01 to SSEA4-expressing cell is similar with parental antibody hAb6-3.1. **Fig. 25B** is the comparison of cell binding property of hAb6-3.1 and hAb6-3.1-A01.

**[00108]** **Fig. 26.** Comparing the efficacy of hAb6-3.1-A01 in cell cytotoxicity on a SSEA4-expressing breast cell line MCF7 with antibody hAb6-3.1.

**[00109]** **Fig. 27.** Comparing the efficacy of hAb6-3.1-A01 in cell cytotoxicity on a SSEA4-expressing ovarian cell line SKOV3 with antibody hAb6-3.1.

## DETAILED DESCRIPTIONS

**[00110]** Accordingly, antibody methods and compositions directed to the markers for use in diagnosing and treating a broad spectrum of cancers are provided. Anti-SSEA4 antibodies was developed and disclosed herein. Methods of use include, without limitation, cancer therapies and diagnostics. The antibodies described herein can bind to a broad spectrum of SSEA4-expressing tumor cells, thereby facilitating cancer diagnosis and treatment. Cells that can be targeted by the antibodies include carcinomas, such as those in brain, lung, breast, oral, esophageal, stomach, liver, bile duct, pancreatic, colon, kidney, cervical, ovarian, prostate cancer, etc.

### Definitions

**[00111]** Unless otherwise defined herein, scientific and technical terminologies employed in the present disclosure shall have the meanings that are commonly understood and used by one of ordinary skill in the art. Unless otherwise required by context, it will be understood that singular terms shall include plural forms of the same and plural terms shall include the singular. Specifically, as used herein and in the claims, the singular forms "a" and "an" include the plural reference unless the context clearly indicates otherwise. Also, as used herein and in the claims, the terms "at least one" and "one or more" have the same meaning and include one, two, three, or more.

**[00112]** Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in the respective testing measurements. Also, as used herein, the term "about" generally means within 10%, 5%, 1%, or 0.5% of a given value or range. Alternatively, the term "about" means within an acceptable standard error of the mean when considered by one of ordinary

skill in the art. Other than in the operating/working examples, or unless otherwise expressly specified, all of the numerical ranges, amounts, values and percentages such as those for quantities of materials, durations of times, temperatures, operating conditions, ratios of amounts, and the likes thereof disclosed herein should be understood as modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the present disclosure and attached claims are approximations that can vary as desired. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

**[00113]** Unless specified otherwise, in the polynucleotide notation used herein, the left-hand direction is 5'-terminal and the right-hand direction is 3'-terminal; in the peptide notation used herein, the left-hand direction is the amino-terminal (N-terminal) direction and the right-hand direction is the carboxyl-terminal (C-terminal) direction, in accordance with standard usage and convention.

**[00114]** The term "polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction.

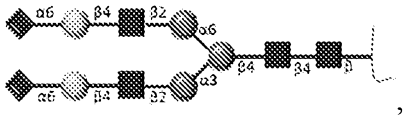
**[00115]** The term "oligonucleotide," as used herein, generally refers to short, generally single-stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

**[00116]** The term "vector" as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are

replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "recombinant vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

**[00117]** The term "glycan" refers to a polysaccharide, or oligosaccharide. Glycan is also used herein to refer to the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, glycopeptide, glycoproteome, peptidoglycan, lipopolysaccharide or a proteoglycan. Glycans usually consist solely of *O*-glycosidic linkages between monosaccharides. Glycans can be homo or heteropolymers of monosaccharide residues, and can be linear or branched. Glycans can be found attached to proteins as in glycoproteins and proteoglycans. They are generally found on the exterior surface of cells. *O*- and *N*-linked glycans are very common in eukaryotes but may also be found, although less commonly, in prokaryotes. *N*-Linked glycans are found attached to the R-group nitrogen (N) of asparagine in the sequon. The sequon is a Asn-X-Ser or Asn-X-Thr sequence, where X is any amino acid except proline.

**[00118]** The term "universal glycan" refers to the glycan sequence $Sia_2(\alpha 2\text{-}6)Gal_2GlcNAc_2Man_3GlcNAc_2$.

The structure is  , wherein ◆ is sialic acid (Sia); ● is galactose (Gal); ■ is N-Acetylglucosamine (GlcNAc); ● Mannose (Man).

**[00119]** The term "antigen" as used herein is defined as a substance capable of eliciting an immune response. Said immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both.

**[00120]** The term "epitope" refers to a unit of structure conventionally bound by an immunoglobulin $V_H/V_L$ pair. An epitope defines the minimum binding site for an antibody, and thus represent the target of specificity of an antibody.

**[00121]** As used herein, the term "immunogen" refers to an antigen capable of inducing the production of an antibody.

**[00122]** As used herein, the term "immunogenicity" generally refers to the ability of an immunogen or antigen to stimulate an immune response.

**[00123]** The term “vaccine” refers to a preparation that contains an antigen, consisting of whole disease-causing organisms (killed or weakened) or components of such organisms, such as proteins, peptides, or polysaccharides, that is used to confer immunity against the disease that the organisms cause. Vaccine preparations can be natural, synthetic or derived by recombinant DNA technology.

**[00124]** As used herein, the term “antigen specific” refers to a property of a cell population such that supply of a particular antigen, or a fragment of the antigen, results in specific cell proliferation.

**[00125]** As used herein, the term "specifically binding" refers to the interaction between binding pairs (e.g., an antibody and an antigen). In various instances, specifically binding can be embodied by an affinity constant of about $10^{-6}$ moles/liter, about $10^{-7}$ moles/liter, or about $10^{-8}$ moles/liter, or less. In an additional or an alternative embodiment, the binding of the antibodies to their respective antigens is termed specific in terms of the antibody specificity. The term "specific" here is generally used to refer to the situation in which one member of a binding pair will not show any significant binding to molecules other than its specific binding partner (s) and e.g. has less than about 30%, preferably 20%, 15%, 10%, 5%, or 1 % cross-reactivity with any other molecule other than those specified herein.

**[00126]** The term “binding affinity” generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant ($K_d$). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention.

**[00127]** As used herein, the term “dissociation constant ($K_d$)” is a specific type of equilibrium constant that measures the propensity of a larger object to dissociate reversibly into smaller components, as when a complex falls apart into its component molecules. For a

reaction $A_xB_y \rightleftharpoons xA + yB$, the dissociation constant is defined $K_d = [A]^x[B]^y / [A_xB_y]$, wherein [A], [B], and $[A_xB_y]$ are the concentration of A, B, and $A_xB_y$, respectively. In particular, the $K_d$ value is determined by Biacore surface plasmon resonance system or enzyme-linked immunosorbent assay (ELISA).

**[00128]** As used herein, an antibody that "specifically binds to SSEA4 is intended to refer to an antibody that binds to SSEA4 with a $K_D$ of $1\times10^{-7}$ M or less, more preferably $5\times10^{-8}$ M or less, more preferably $1\times10^{-8}$ M or less, more preferably $5\times10^{-9}$ M or less or binds to SSEA-4 with a $K_D$ of between $1\times10^{-8}$ M and $1\times10^{-10}$ M or less.

**[00129]** The term K "$K_{assoc}$" or "$K_a$", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "$K_{dis}$" or "$K_d$," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "$K_D$", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of $K_d$ to $K_a$ (i.e., $K_d/K_a$) and is expressed as a molar concentration (M). $K_D$ values for antibodies can be determined using methods well established in the art. A preferred method for determining the $K_D$ of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

**[00130]** As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a $K_D$ of $10^{-8}$ M or less, more preferably $10^{-9}$ M or less and even more preferably $10^{-10}$ M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a $K_D$ of $10^{-7}$ M or less, more preferably $10^{-8}$ M or less, even more preferably $10^{-9}$ M or less.

**[00131]** The term "half maximal effective concentration ($EC_{50}$)" refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. It is used as a measure of drug's potency.

**[00132]** 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) and may also include certain antibody fragments. Most antibodies are glycoproteins having the same structural characteristics: two heavy chains and two light chains linked to each other by disulfide bonds. The light chain includes a variable domain ($V_L$) and a constant domain ($C_L$); while the heavy chain includes a variable domain

($V_H$) and three constant domains ($C_H1$, $C_H2$ and $C_H3$, collectively referred to as $C_H$). The variable regions of both light ($V_L$) and heavy ($V_H$) chains determine binding recognition and specificity to the antigen. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed hypervariable region (HVR), interspersed with regions that are more conserved, termed framework regions (FR). The constant region domains of the light ($C_L$) and heavy ($C_H$) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies 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. An antibody can be chimeric, human, humanized and/or affinity matured.

**[00133]** The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. In one embodiment, the chain is kappa type. In another embodiment, the chain is lamda type.

**[00134]** As used herein, "variable domain" refers to the portions of the light and heavy chains of antibody molecules that include amino acid sequences of hypervariable regions (HVRs), and framework regions (FRs). According to the methods used herein, the amino acid positions assigned to HVRs and FRs can be defined according to Kabat (*Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, Md., 1987 and 1991). Amino acid numbering of antibodies or antigen binding fragments is also according to that of Kabat.

**[00135]** As used herein, the term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th

Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a standard Kabat numbered sequence.

**[00136]** As used herein, the term "framework region" (FR) residues are those variable domain residues other than the hypervariable region residues as herein defined.

**[00137]** As used herein, the term "hypervariable region" (HVR or HV) and "complementarity-determining region" (CDR) are used interchangeably, when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the $V_H$ (H-CDR1, H-CDR2, H-CDR3), and three in the $V_L$ (L-CDR1, L-CDR2, L-CDR3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5$^{th}$ Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "Contact" hypervariable regions are based on an analysis of the available complex crystal structures. "IMGT" (the international ImMunoGeneTics information system) provides unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. The residues from each of these hypervariable regions defined by Kabat, AbM, Chothia, and Contact are noted below; IMGT are predicted on the website: http://www.imgt.org/

| Loop | Kabat | AbM | Chothia | Contact |
|---|---|---|---|---|
| L1 | L24 -- L34 | L24 -- L34 | L24 -- L34 | L30 -- L36 |
| L2 | L50 -- L56 | L50 -- L56 | L50 -- L56 | L46 -- L55 |
| L3 | L89 -- L97 | L89 -- L97 | L89 -- L97 | L89 -- L96 |
| H1 | H31 -- H35B (Kabat Numbering) | H26 -- H35B | H26 -- H32..34 | H30 -- H35B |
| H1 | H31 -- H35 (Chothia Numbering) | H26 -- H35 | H26 -- H32 | H30 -- H35 |
| H2 | H50 -- H65 | H50 -- H58 | H52 -- H56 | H47 -- H58 |
| H3 | H95 -- H102 | H95 -- H102 | H95 -- H102 | H93 -- H101 |

**[00138]** The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably, to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain the Fc region.

**[00139]** The term "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. 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.

**[00140]** As used herein, the term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinant engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a

composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

**[00141]** As used herein, the term "Fv region" 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 $V_H$-$V_L$ 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.

**[00142]** As used herein, the term "Fab fragment" contains the Fv region, the constant domain of the light chain and the first constant domain ($C_H1$) of the heavy chain. Fab′ fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain $C_H1$ domain including one or more cysteines from the antibody hinge region.

**[00143]** The term "antigen-binding fragment", refers to full length or one or more fragments of an antibody that retains the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" include a Fab fragment; a Fv fragment; a single chain Fv (scFv) fragment; a diabody; a Fab'-SH fragment, which is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group; a $F(ab)_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the $V_H$ and $C_H1$ domains; a dAb fragment (Ward et al., 1989 *Nature* 341 :544-546), which consists of a $V_H$ domain; a dsFv fragment, two different disulfide-stabilized Fv antibody fragments connected by flexible linker peptides; and an

isolated complementarity determining region (CDR); or any fusion proteins comprising such antigen-binding fragment.

**[00144]** The term "single-chain Fv" or "scFv" antibody fragments comprise the $V_H$ and $V_L$ domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the $V_H$ and $V_L$ domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Pluckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

**[00145]** The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain ($V_H$) connected to a light-chain variable domain ($V_L$) in the same polypeptide chain ($V_H$-$V_L$). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO93/1161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

**[00146]** 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 epitopes, each monoclonal antibody of a monoclonal antibody is directed against a single epitope 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).

**[00147]** The term "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. In particular, in the present invention the chimeric antibody may be a humanized antibody in which the antigen binding sequences/variable domains of a non-human antibody have been grafted onto human antibody framework regions. Such antibodies are 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)).

**[00148]** As used herein, the term "humanized 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 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).

**[00149]** The term "human antibody" is one 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.

**[00150]** An "affinity matured antibody" is one with one or more alterations in one or more HVRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In one embodiment, an affinity matured antibody has nano-molar or even pico-molar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. *BioTechnology* 10:779-783 (1992) describes affinity maturation by $V_H$ and $V_L$ domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier et al. *Gene* 169:147-155 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):3310-9 (1995); and Hawkins et al, *J. Mol. Biol.* 226:889-896 (1992).

**[00151]** 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 non-proteinaceous solutes. In one embodiment, the antibody will be purified (1) to greater than 90% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments more than 95% 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 non-reducing 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.

**[00152]** A "blocking antibody" or an "antagonist antibody" is one which inhibits or reduces biological activity of the antigen it binds. Certain blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

**[00153]** An "agonist antibody", as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest.

**[00154]** The term "chimeric antigen receptor (CAR)" is an artificially constructed hybrid protein or polypeptide containing the antigen-binding domains of an antibody (e.g., scFv) linked to T- cell signaling domains. Characteristics of CARs include their ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

**[00155]** A "disorder" is any condition that would benefit from treatment with an antibody of the invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancer.

**[00156]** The terms "cell proliferative disorder" or "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

**[00157]** The term "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.

**[00158]** The terms "cancer" or "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 brain cancer, oral cancer, 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, stomach cancer, bile duct cancer, bladder cancer, hepatoma, breast cancer, colon cancer, bone cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulvar cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

**[00159]** As used herein, the term "individual" or "subject" is intended to include human and non-human animals. Preferred subjects include human patients in need of enhancement of an immune and/or anti-proliferative and/or anti-cancer therapeutic response. The methods are particularly suitable for treating human patients suitable for treatment of cancer cells in vivo.

**[00160]** As used herein, the term "theapeutic agent" is characterized by any agent that can reduce and/or inhibit hyperproliferative disease. Exemplary therapeutic agent can include, but no limited to, cytotoxic agent, chemotherapeutic agent, anti-proliferative agent, immune modulatoirs, hormonal modulators, cytokines as well as other anti-cancer substance and/or modalities.

**[00161]** The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., $At^{211}$, $I^{131}$, $I^{125}$, $Y^{90}$, $Re^{186}$, $Re^{188}$, $Sm^{153}$, $Bi^{212}$, $P^{32}$, $Pb^{212}$ and radioactive isotopes of Lu), chemotherapeutic agents (e.g., methotrexate, adriamicin,

vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolyticenzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. As used herein, a tumoricidal agent causes destruction of tumor cells. Cytotoxic agents and chemotherapeutic agents are not mutually exclusive.

**[00162]** Additionally or alternatively, a cytotoxin or cytotoxic agent may include any agent that is detrimental to (e.g., kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

**[00163]** The term “chemotherapeutic agent” is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic

analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., *Agnew, Chem. Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues 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; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2′ ,2″-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-

engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

**[00164]** As used herein, the term "cytokine" includes but not limited to examples listed in Kiefer et al. 2016, Immunol. Revs. 270:178-192. Exemplary suitable cytokines include but not limited to G-CSF, GM-CFS, IFNγ, IFNα, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-9, IL-12, IL-13, IL-15, IL-17, IL-21, IL-23, and TNF.

**[00165]** In one embodiment, cytokine is linked to the binding domain via cross-links between lysine residues.

**[00166]** The term "therapeutic antibody" is an antibody useful in the treatment of disease. Examples of therapeutic antibodies are etaracizumab, atlizumab, tocilizumab, tacatuzumab tetraxetan, ruplizumab, ofatumumab, tefibazumab, bevacizumab, belimumab, tositumomab, blontuvetmab, mepolizumab, labetuzumab, arcitumomab, certolizumab pegol, ramucirumab, TRBS07, cetuximab, biciromab, obinutuzumab, trastuzumab, clivatuzumab tetraxetan, votumumab, zanolimumab, zalutumumab, adalimumab, fontolizumab, altumomab pentetate, canakinumab, igovomab, trastuzumab emtansine, alemtuzumab, rovelizumab, sulesomab, ranibizumab, FBTA05, bectumomab, rituximab, efungumab, gemtuzumab ozogamicin, imciromab, fanolesomab, motavizumab, visilizumab, pertuzumab, nivolumab, muromonab-cd3, oregovomab, edrecolomab, denosumab, capromab pendetide, efalizumab, infliximab, catumaxomab, girentuximab, abciximab, ertumaxomab, besilesomab, golimumab, basiliximab, eculizumab, ustekinumab, palivizumab, tamtuvetmab, nimotuzumab, pemtumomab, natalizumab, panitumumab, nofetumomab merpentan, omalizumab, ipilimumab, daclizumab, ibritumomab tiuxetan.

**[00167]** 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.

**[00168]** The term "mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. In certain embodiments, the mammal is human.

**[00169]** The term "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

**[00170]** As used herein, the term "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.

**[00171]** As used herein, the term "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.

**[00172]** The term "pharmaceutically acceptable carrier" is one that is suitable for use with the subjects without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. Also, each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the

pharmaceutical composition. The carrier can be in the form of a solid, semi-solid, or liquid diluent, cream or a capsule. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, and is selected to minimize any degradation of the active agent and to minimize any adverse side effects in the subject.

**[00173]** The phrase “substantially similar”, “substantially the same”, “equivalent”, or “substantially equivalent”, as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., $K_d$ values, anti-viral effects, etc.). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the value for the reference/comparator molecule.

**[00174]** The phrase “substantially reduced,” or “substantially different”, as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., $K_d$ values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

**[00175]** The term "Percentage (%) amino acid sequence identity" with respect to the amino acid sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percentage sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein,

sequence comparison between two amino acid sequences was carried out by computer program Blastp (protein-protein BLAST) provided online by Nation Center for Biotechnology Information (NCBI). Specifically, the percentage amino acid sequence identity of a given amino acid sequence A to a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has a certain % amino acid sequence identity to a given amino acid sequence B) is calculated by the formula as follows:

$(X \div Y) \times 100\ \%$.

**[00176]** where X is the number of amino acid residues scored as identical matches by the sequence alignment program BLAST in that program's alignment of A and B, and where Y is the total number of amino acid residues in A or B, whichever is shorter.

**[00177]** Sequence Identity or homology with respect to a specified amino acid sequence is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments. As discussed herein, minor variations in the amino acid sequences of proteins/polypeptides are contemplated as being encompassed by the presently disclosed and claimed inventive concept(s), providing that the variations in the amino acid sequence maintain at least 80% such as at least, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99%.

**[00178]** "Conservatively modified amino acid substitution" are contemplated. Conservative modified amino acid substitutions are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families:

(1) acidic: aspartate (D), glutamate (E);

(2) basic: lysine (K), arginine (R), histidine (H);

(3)nonpolar: glycine (G), alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), methionine (M), tryptophan (W); and

(4) uncharged polar: asparagine (N), glutamine (Q), cysteine (C), serine (S), threonine (T), tyrosine (Y).

**[00179]** More preferred families are:

(3-1) aliphatic : alanine, valine, leucine and isoleucine;

(3-2) aromatic : phenylalanine, tryptophan, and tyrosine;

(4-1) aliphatic-hydroxyl : serine and threonine;

(4-2) amide-containing : asparagine and glutamine.

**[00180]** For example, it is reasonable to expect that an isolated substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the substitution does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Fragments or analogs of proteins/polypeptides can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxyl-termini of fragments or analogs occur near boundaries of functional domains. Additional groups of amino acids may also be formulated using the principles described in, e.g., Creighton (1984) Proteins: Structure and Molecular Properties (2d Ed. 1993), W.H. Freeman and Company.

**[00181]** In certain embodiments, the conserved amino acid substitution can include sequence homologs which differs from the reference sequence (e.g. CDRs, VH, VL, Framework, full length etc) by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more substituted amino acid residues.

Derivatives

**[00182]** This disclosure also provides a method for obtaining an antibody specific for SSEA4. CDRs in such antibodies are not limited to the specific sequences of $V_H$ and $V_L$ identified in Table 2 and elsewherer herein and may include variants of these sequences that retain the ability to specifically bind SSEA4. Such variants may be derived from the sequences and concerved substitutions thereof listed below by a skilled artisan using techniques well known in the art.

| Original Residues | Exemplary Substitutions | Typical Substitutions |
|---|---|---|
| Ala (A) | Val, Leu, Ile | Val |
| Arg (R) | Lys, Gln, Asn | Lys |
| Asn (N) | Gln | Gln |

| Original Residues | Exemplary Substitutions | Typical Substitutions |
|---|---|---|
| Asp (D) | Glu | Glu |
| Glu (E) | Asp | Asp |
| Cys (C) | Ser, Ala | Ser |
| Gln (Q) | Asn | Asn |
| Gly (G) | Pro, Ala, Gly | Ala |
| His (H) | Asn, Gln, Lys, Arg | Arg |
| Ile (I) | Leu, Val, Met, Ala, Phe, Norleucine | Leu |
| Leu (L) | Norleucine, Ile, Val, Met, Ala, Phe | Ile |
| Lys (K) | Arg, 1,4-Diamino-butyric Acid, Gln, Asn | Arg |
| Met (M) | Leu, Phe, Ile | Leu |
| Phe (F) | Leu, Val, Ile, Ala, Tyr | Leu |
| Pro (P) | Ala, Gly | Gly |
| Ser (S) | Thr, Ala, Cys | Thr |
| Thr (T) | Ser | Ser |
| Trp (W) | Tyr, Phe | Tyr |
| Tyr (Y) | Trp, Phe, Thr, Ser | Phe |
| Val (V) | Ile, Met, Leu, Phe, Ala, Norleucine | Leu |

**[00183]** In certain embodiments, an antibody of the invention comprises a heavy chain variable region comprising H-CDR1, H-CDR2 and H-CDR3 sequences and a light chain variable region comprising L-CDR1, L-CDR2 and L-CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the preferred antibodies described herein, or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-SSEA4 antibodies of the invention. Accordingly, the invention provides an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising H-CDR1, H-CDR2, and H-CDR3 sequences and a light chain variable region comprising L-CDR1, L-CDR2, and L-CDR3 sequences as well as conserved amino acid substituted variants thereof as well as full length sequences and homologs thereof comprising all 3 heavy chain and/or light chain CDRs.

***Engineered and Modified Antibodies***

**[00184]** An antibody of the invention further can be prepared using an antibody having one or more of the $V_H$ and/or $V_L$ sequences disclosed herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or

both variable regions (i.e., $V_H$ and/or $V_L$), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

**[00185]** One type of variable region engineering that can be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, e.g., Riechmann, L. et al. (1998) *Nature* 332:323-327; Jones, P. et al. (1986) *Nature* 321:522-525; Queen, C. et al. (1989) *Proc. Natl. Acad. See. U.S.A.* 86:10029-10033; U.S. Pat. No. 5,225,539 to Winter, and U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al.).

**[00186]** Accordingly, another embodiment of the invention pertains to an isolated monoclonal antibody, or antigen-binding portion thereof, comprising a heavy chain variable region comprising H-CDR1, H-CDR2, and H-CDR3 sequences and a light chain variable region comprising L-CDR1, L-CDR2, and L-CDR3 sequences. Thus, such antibodies contain the $V_H$ and $V_L$ CDR sequences of monoclonal antibodies described herein yet may contain different framework sequences from these antibodies.

**[00187]** Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I. M., et al. (1992) "The Repertoire of Human Germline $V_H$ Sequences Reveals about Fifty Groups of $V_H$ Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J. P. L. et al. (1994) "A Directory of Human Germ-line $V_H$ Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836;

the contents of each of which are expressly incorporated herein by reference. As another example, the germline DNA sequences for human heavy and light chain variable region genes can be found in the GenBank database. For example, the following heavy chain germline sequences found in the HCo7 HuMAb mouse are available in the accompanying GenBank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 3-33 (NG_0010109 and NT_024637) and 3-7 (NG_0010109 and NT_024637). As another example, the following heavy chain germline sequences found in the HCo12 HuMAb mouse are available in the accompanying GenBank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 5-51 (NG_0010109 and NT_024637), 4-34 (NG_0010109 and NT_024637), 3-30.3 (AJ556644) and 3-23 (AJ406678).

**[00188]** The CDR1, CDR2, and CDR3 sequences can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see e.g., U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al).

**[00189]** Another type of variable region modification is to mutate amino acid residues within each respective heavy or light chain CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (e.g., affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, can be evaluated in in vitro or in vivo assays as described herein and provided in the Examples. Preferably conservative modifications (as discussed above) are introduced. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

**[00190]** Engineered antibodies of the invention include those in which modifications have been made to framework residues within $V_H$ and/or $V_K$, e.g. to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody

that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived.

**[00191]** In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (e.g., one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody.

**[00192]** In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies.

**[00193]** Another modification of the antibodies herein that is contemplated by the invention is PEGylation. An antibody can be pegylated to, for example, increase the biological (e.g., serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term “polyethylene glycol” is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention. See for example, EP 0 154 316 by Nishimura et al. and EP 0 401 384 by Ishikawa et al.

**[00194]** In certain embodiments of the methods of engineering antibodies of the invention, mutations can be introduced randomly or selectively along all or part of an anti-PD-1 antibody coding sequence and the resulting modified anti-SSEA4 antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, PCT Publication WO 02/092780 by Short describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, PCT Publication WO 03/074679 by Lazar et al. describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

***Nucleic Acid Molecules Encoding Antibodies of the Invention***

**[00195]** Another aspect of the invention pertains to nucleic acid molecules that encode the antibodies of the invention. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. See, F. Ausubel, et al., ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. A nucleic acid of the invention can be, for example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA molecule. In certain embodiments, the nucleic acid is expressed by a vector.

**[00196]** Nucleic acids of the invention can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (e.g., hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (e.g., using phage display techniques), nucleic acid encoding the antibody can be recovered from the library.

**[00197]** Exemplary nucleic acids molecules of the invention are those encoding the $V_H$ and $V_L$ amino acid sequences of the exemplary Ab6 monoclonal antibodies (see **Tables 2A-2D**).

**[00198]** Sequences with exemplary amino acid substitution on framework region (FR) are shown in TABLE 2B. Confirmation of the binding activity to SSEA4 and to SSEA4-

expressing cells are demonstrated by binding assays as illustrated in the examples section. We confirmed that binding affinity function is conserved and retained even in exemplary variants with up to 3/5 amino acid substitutions on the framework of respective exemplary light chain and heavy chain.

**[00199]** Sequences with exemplary non-conservatively modified amino acid substitution on CDR are shown in Table 2C. Confirmation of the binding activity to SSEA4 and to SSEA4-expressing cells are demonstrated by binding assays as illustrated in the examples section. We confirmed that binding affinity and function are conserved and retained in exemplary variants with the following non-limiting exemplary amino acid substitutions on CDRs of light chain and heavy chain such as, for example, but not limited to, Heavy chain: **A100R, N31S, T62A.** Light chain: **S52Y.**

**[00200]** Sequences with exemplary conservatively modified amino acid substitution on CDR are shown in Table 2D. Confirmation of the binding activity to SSEA4 and to SSEA4-expressing cells are demonstrated by binding assays as illustrated in the examples section. We confirmed that binding affinity and function are conserved and retained in exemplary variants with non-limiting exemplary amino acid substitutions on CDRs of light chain and heavy chain, such as, for example, but not limited to, Heavy chain: **V50A, G53A, S35T**. Light chain: **V30I/A, G91A, Y94F**

**Table 2A: Exemplary Parental Antibody**

**[00201]** chAb6 sequences (No. 01x)

| **Antibody/SEQUENCE** | **SEQ ID Nos:** | **Amino Acid or nucleic acid sequence** |
|---|---|---|
| H-CDR1 | No.10 | NYGVS |
| H-CDR2 | No.11 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.12 | PGAGYAMDY |
| Heavy chain variable domain | No.13 | QVQLKESGPGLVAPSQSLSITCTVS GFSLKNYGVSWVRQPPGKGLEWL GVIWGDGSTNYHSTLRSRLTISKDN SKSQLFLKLNRLQTDDTATYYCAKP GAGYAMDYWGQGTSVTVSS |
| Nucleotides of heavy chain | No.14 | CAGGTGCAGCTGAAGGAGTCAGGACCT GGCCTGGTGGCGCCCTCACAGAGCCTG TCCATCACATGCACTGTCTCAGGGTTCTCA TTAAAAAACTATGGTGTAAGCTGGGTTCG CCAGCCTCCAGGAAAGGGTCTGGAGTG GCTGGGAGTAATATGGGGTGACGGGAG CACAAATTATCATTCAACTCTCAGATCCA GACTGACCATCAGCAAGGATAATTCCAA |

| | | |
|---|---|---|
| | | GAGCCAACTTTTCTTAAAACTGAACAGAC TGCAAACTGATGACACAGCCACGTACTA CTGTGCCAAACCTGGGGCGGGTTATGCTA TGGACTACTGGGGTCAAGGAACCTCAGTC ACCGTCTCCTCA |
| L-CDR1 | No.15 | SASSSVSYMH |
| L-CDR2 | No.16 | DTSKLTS |
| L-CDR3 | No.17 | FQGSGYPLT |
| Light chain variable domain | No.18 | QIVLTQSPAIMSVYPGEKVTMTC SASSSVSYMHWYQQKSSTSPKL WIYDTSKLTSGVPGRFSGSGSGN SYSLTISSMEAEDVATYYCFQGSG YPLTFGGGTKLEIKR |
| Nucleotides of light chain | No.19 | CAAATTGTTCTCACCCAGTCTCCAGCAA TCATGTCTGTATATCCAGGGGAAAAGGT CACCATGACCTGCAGTGCCAGCTCAAG TGTAAGTTACATGCACTGGTACCAGCAG AAGTCAAGCACCTCCCCCAAACTCTGGA TTTATGACACATCCAAACTGACTTCTGG AGTCCCAGGTCGCTTCAGTGGCAGTGGG TCTGGAAACTCTTACTCTCTCACGATCAG CAGCATGGAGGCTGAAGATGTTGCCACTT ATTACTGTTTTCAGGGGAGTGGGTACCCA CTCACGTTCGGAGGGGGGACCAAGCTG GAAATAAAACGG |

**Table 2B: Exemplary Antibody Embodiments with Modification in framework Region**

**[00202]** hAb6-2 sequence (No.02x)

| | | |
|---|---|---|
| Heavy chain variable domain | No.23 | QVQLKESGPGLVAPSQTLSITCTVS GFSLKNYGVSWVRQPPGKGLEWI GVIWGDGSTNYHSTLRSRVTISKD NSKSQLFLKLNRLQTDDTATYYCAK PGAGYAMDYWGQGTSVTVSS |
| Light chain variable domain | No.28 | EIVLTQSPAIQSVYPGEKVTMTCSA SSSVSYMHWYQQKSSTSPKLWIYD TSKLTSGVPGRFSGSGSGNSYTLTIS SMEAEDVATYYCFQGSGYPLTFGG GTKLEIKR |

**[00203]** hAb6-3 sequence (No.03x)

| | | |
|---|---|---|
| Heavy chain variable domain | No.33 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG AGYAMDYWGQGTLVTVSS |
| Light chain variable domain | No.38 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW |

| | | |
|---|---|---|
| | | IYDTSKLTSGVPGRFSGSGSGNSY<br>TLTISSMEAEDAATYYCFQGSGYP<br>LTFGGGTKVEIKR |

**Table 2C: Exemplary Antibody Embodiment with Non-conservative modification in CDRs**

**[00204]** hAb6-3.1 sequences (No.04x, H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.40 | NYGVS |
| H-CDR2 | No.41 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.42 | PGRGYAMDY |
| Heavy chain variable domain | No.43 | QVQLQESGPGLVAPSQTLSI<br>TCTVSGFSLKNYGVSWVRQ<br>PPGKGLEWIGVIWGDGSTN<br>YHSTLRSRVTISKDNSKSQLF<br>LKLNRLQTDDTATYYCAKPG<br>RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.44 | CAGGTGCAGCTGCAGGAGTCCGGACCAG<br>GACTGGTGGCTCCCAGCCAGACCCTGTCT<br>ATCACCTGCACAGTGTCTGGCTTCTCCCTG<br>AAGAACTACGGCGTGAGCTGGGTGAGAC<br>AGCCACCTGGCAAGGGACTGGAGTGGAT<br>CGGCGTGATCTGGGGCGACGGCTCTACC<br>AATTATCACTCCACACTGAGGAGCCGGG<br>TGACCATCTCCAAGGATAACTCCAAGAG<br>CCAGCTGTTTCTGAAGCTGAATCGCCTG<br>CAGACAGACGATACCGCCACATACTATT<br>GCGCTAAGCCAGGCCGGGGCTACGCTA<br>TGGACTATTGGGGCCAGGGCACCCTGG<br>TGACAGTGTCCAGC |
| L-CDR1 | No.45 | SASSSVSYMH |
| L-CDR2 | No.46 | DTSKLTS |
| L-CDR3 | No.47 | FQGSGYPLT |
| Light chain variable domain | No.48 | EIVLTQSPAIQSVYPGEKVTMTCS<br>ASSSVSYMHWYQQKSSTSPKLW<br>IYDTSKLTSGVPGRFSGSGSGNSY<br>TLTISSMEAEDAATYYCFQGSGYP<br>LTFGGGTKVEIKR |
| Nucleotides of light chain | No.49 | GAGATCGTGCTGACCCAGTCTCCTGCCA<br>TCCAGTCCGTGTACCCAGGCGAGAAGG<br>TGACCATGACATGTTCCGCTTCTTCCAG<br>CGTGAGCTACATGCATTGGTATCAGCAG<br>AAGTCTTCCACATCTCCCAAGCTGTGGA<br>TCTACGACACCTCTAAGCTGACATCCGG<br>AGTGCCTGGCAGGTTCTCTGGATCCGGA<br>AGCGGCAACAGCTATACCCTGACAATCA<br>GCTCTATGGAGGCTGAGGATGCCGCTAC<br>CTACTATTGTTTCCAGGGCTCTGGCTATC |

| | | |
|---|---|---|
| | | CCCTGACCTTTGGCGGCGGCACAAAGG TGGAGATCAAGCGT |

**[00205]** hAb6-3.2 sequences (No.05x, H-CDR1:N31S and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.50 | SYGVS |
| H-CDR2 | No.51 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.52 | PGRGYAMDY |
| Heavy chain variable domain | No.53 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKSYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.54 | |
| L-CDR1 | No.55 | SASSSVSYMH |
| L-CDR2 | No.56 | DTSKLTS |
| L-CDR3 | No.57 | FQGSGYPLT |
| Light chain variable domain | No.58 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.59 | |

**[00206]** hAb6-3.3 sequences (No.06x, H-CDR2:T62A and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.60 | NYGVS |
| H-CDR2 | No.61 | VIWGDGSTNYHSALRS |
| H-CDR3 | No.62 | PGRGYAMDY |
| Heavy chain variable domain | No.63 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSALRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.64 | |
| L-CDR1 | No.65 | SASSSVSYMH |
| L-CDR2 | No.66 | DTSKLTS |
| L-CDR3 | No.67 | FQGSGYPLT |
| Light chain variable domain | No.68 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.69 | |

**[00207]** hAb6-3.4 sequences (No.07x, L-CDR2:S52Y and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.70 | NYGVS |
| H-CDR2 | No.71 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.72 | PGRGYAMDY |
| Heavy chain variable domain | No.73 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.74 | |
| L-CDR1 | No.75 | SASSSVSYMH |
| L-CDR2 | No.76 | DTYKLTS |
| L-CDR3 | No.77 | FQGSGYPLT |
| Light chain variable domain | No.78 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTYKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.79 | |

**Table 2D: Exemplary Antibody embodiment with Conservative modification in CDRs**

**[00208]** hAb6-3.101 sequences (No.08x, H-CDR2:V50A and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.80 | NYGVS |
| H-CDR2 | No.81 | AIWGDGSTNYHSTLRS |
| H-CDR3 | No.82 | PGRGYAMDY |
| Heavy chain variable domain | No.83 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGAIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.84 | |
| L-CDR1 | No.85 | SASSSVSYMH |
| L-CDR2 | No.86 | DTSKLTS |
| L-CDR3 | No.87 | FQGSGYPLT |
| Light chain variable domain | No.88 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.89 | |

**[00209]** hAb6-3.103 sequences (No.10x, H-CDR2:G53A and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.100 | NYGVS |
| H-CDR2 | No.101 | VIWADGSTNYHSTLRS |
| H-CDR3 | No.102 | PGRGYAMDY |
| Heavy chain variable domain | No.103 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWADGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.104 | |
| L-CDR1 | No.105 | SASSSVSYMH |
| L-CDR2 | No.106 | DTSKLTS |
| L-CDR3 | No.107 | FQGSGYPLT |
| Light chain variable domain | No.108 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.109 | |

**[00210]** hAb6-3.105 sequences (No.12x, H-CDR1:S35T and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.120 | NYGVT |
| H-CDR2 | No.121 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.122 | PGRGYAMDY |
| Heavy chain variable domain | No.123 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVTWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.124 | |
| L-CDR1 | No.125 | SASSSVSYMH |
| L-CDR2 | No.126 | DTSKLTS |
| L-CDR3 | No.127 | FQGSGYPLT |
| Light chain variable domain | No.128 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.129 | |

**[00211]** hAb6-3.106 sequences (No.13x, L-CDR1:V30I and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.130 | NYGVS |
| H-CDR2 | No.131 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.132 | PGRGYAMDY |
| Heavy chain variable domain | No.133 | QVQLQESGPGLVAPSQTLSI |

| | | |
|---|---|---|
| | | TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.134 | |
| L-CDR1 | No.135 | SASSSISYMH |
| L-CDR2 | No.136 | DTSKLTS |
| L-CDR3 | No.137 | FQGSGYPLT |
| Light chain variable domain | No.138 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSISYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.139 | |

**[00212]** hAb6-3.107 sequences (No.14x, L-CDR1:V30A and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.140 | NYGVS |
| H-CDR2 | No.141 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.142 | PGRGYAMDY |
| Heavy chain variable domain | No.143 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.144 | |
| L-CDR1 | No.145 | SASSSASYMH |
| L-CDR2 | No.146 | DTSKLTS |
| L-CDR3 | No.147 | FQGSGYPLT |
| Light chain variable domain | No.148 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSASYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.149 | |

**[00213]** hAb6-3.108 sequences (No.15x, L-CDR3:G91A and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.150 | NYGVS |
| H-CDR2 | No.151 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.152 | PGRGYAMDY |
| Heavy chain variable domain | No.153 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG |

| | | |
|---|---|---|
| | | RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.154 | |
| L-CDR1 | No.155 | SASSSVSYMH |
| L-CDR2 | No.156 | DTSKLTS |
| L-CDR3 | No.157 | FQASGYPLT |
| Light chain variable domain | No.158 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQASGYP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.159 | |

**[00214]** hAb6-3.110 sequences (No.17x, L-CDR3:Y94F and H-CDR3:A100R)

| | | |
|---|---|---|
| H-CDR1 | No.170 | NYGVS |
| H-CDR2 | No.171 | VIWGDGSTNYHSTLRS |
| H-CDR3 | No.172 | PGRGYAMDY |
| Heavy chain variable domain | No.173 | QVQLQESGPGLVAPSQTLSI TCTVSGFSLKNYGVSWVRQ PPGKGLEWIGVIWGDGSTN YHSTLRSRVTISKDNSKSQLF LKLNRLQTDDTATYYCAKPG RGYAMDYWGQGTLVTVSS |
| Nucleotides of heavy chain | No.174 | |
| L-CDR1 | No.175 | SASSSVSYMH |
| L-CDR2 | No.176 | DTSKLTS |
| L-CDR3 | No.177 | FQGSGFPLT |
| Light chain variable domain | No.178 | EIVLTQSPAIQSVYPGEKVTMTCS ASSSVSYMHWYQQKSSTSPKLW IYDTSKLTSGVPGRFSGSGSGNSY TLTISSMEAEDAATYYCFQGSGFP LTFGGGTKVEIKR |
| Nucleotides of light chain | No.179 | |

**[00215]** Nucleic acid molecules encoding the antibodies, or antigen-binding portions thereof, of the invention are also encompassed by the invention, as well as expression vectors comprising such nucleic acids and host cells comprising such expression vectors. Moreover, the invention provides a transgenic mouse comprising human immunoglobulin heavy and light chain transgenes, wherein the mouse expresses an antibody of the invention, as well as hybridomas prepared from such a mouse, wherein the hybridoma produces the antibody of the invention.

***Production of Monoclonal Antibodies of the Invention***

**[00216]** Monoclonal antibodies (mAbs) of the present invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256: 495. Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed e.g., viral or oncogenic transformation of B lymphocytes.

**[00217]** The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known.

**[00218]** Chimeric or humanized antibodies of the present invention can be prepared based on the sequence of a murine monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the murine hybridoma of interest and engineered to contain non-murine (e.g., human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (see e.g., U.S. Pat. No. 4,816,567 to Cabilly et al.). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (see e.g., U.S. Pat. No. 5,225,539 to Winter, and U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen et al.).

**[00219]** In an embodiment, the antibodies of the invention are human monoclonal antibodies. Such human monoclonal antibodies directed against SSEA4 can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as HuMAb mice and KM Mice™, respectively, and are collectively referred to herein as "human Ig mice."

**[00220]** The HuMAb mouse (Medarex, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see e.g., Lonberg, et al. (1994) Nature 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or K, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to

generate high affinity human IgGK monoclonal (Lonberg, N. et al. (1994), supra; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation and use of HuMab mice, and the genomic modifications carried by such mice, is further described in Taylor, L. et al. (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. et al. (1993) *International Immunology* 5: 647-656; Tuaillon et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:3720-3724; Choi et al. (1993) *Nature Genetics* 4:117-123; Chen, J. et al. (1993) *EMBO J.* 12: 821-830; Tuaillon et al. (1994) *J. Immunol.* 152:2912-2920; Taylor, L. et al. (1994) *International Immunology* 6: 579-591; and Fishwild, D. et al. (1996) *Nature Biotechnology* 14: 845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Pat. Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Pat. No. 5,545,807 to Surani et al.; PCT Publication Nos. WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman et al.

**[00221]** In another embodiment, human antibodies of the invention can be raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. Such mice, referred to herein as "KM Mice™", are described in detail in PCT Publication WO 02/43478 to Ishida et al.

**[00222]** Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-PD-1 antibodies of the invention. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Pat. Nos. 5,939,598; 6,075,181; 6,114,598; 6,150,584 and 6,162,963 to Kucherlapati et al.

**[00223]** Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-SSEA4 antibodies of the invention. For example, mice carrying both a human heavy chain transchromosome and a human light chain transchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art

(Kuroiwa et al. (2002) *Nature Biotechnology* 20:889-894) and can be used to raise anti-PD-1 antibodies of the invention.

**[00224]** Human monoclonal antibodies of the invention can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such phage display methods for isolating human antibodies are established in the art. See for example: U.S. Pat. Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner et al.; U.S. Pat. Nos. 5,427,908 and 5,580,717 to Dower et al.; U.S. Pat. Nos. 5,969,108 and 6,172,197 to McCafferty et al.; and U.S. Pat. Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to Griffiths et al.

**[00225]** Human monoclonal antibodies of the invention can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Pat. Nos. 5,476,996 and 5,698,767 to Wilson et al.

***Generation of Hybridomas Producing Human Monoclonal Antibodies of the Invention***

**[00226]** To generate hybridomas producing human monoclonal antibodies of the invention, splenocytes and/or lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen-specific antibodies.

***Generation of Transfectomas Producing Monoclonal Antibodies of the Invention***

**[00227]** Antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (e.g., Morrison, S. (1985) *Science* 229:1202).

**[00228]** The term "antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No.

5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al., *PNAS USA* 95:652-656 (1998).

**[00229]** The term "complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202: 163 (1996), may be performed. Antibody variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

**[00230]** The term "inhibits growth" (e.g. referring to cells, such as tumor cells) is intended to include any measurable decrease in the cell growth when contacted with an anti-SSEA4 antibody as compared to the growth of the same cells not in contact with an anti-SSEA4 antibody, e.g., the inhibition of growth of a cell culture by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%. Such a decrease in cell growth can occur by a variety of mechanisms, e.g. effector cell phagocytosis, ADCC, CDC, and/or apoptosis.

**[00231]** In another aspect, the present invention features an anti-SSEA4 antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (e.g., an immunosuppressant) or a radiotoxin.

**[00232]** Other examples of therapeutic cytotoxins that can be conjugated to an antibody of the invention include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof. An example of a calicheamicin antibody conjugate is commercially available (Mylotarg™; Wyeth-Ayerst).

**[00233]** Cytotoxins can be conjugated to antibodies of the invention using linker technology. Examples of linker types that have been used to conjugate a cytotoxin to an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low

pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (e.g., cathepsins B, C, D).

**[00234]** For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. et al. (2003) *Adv. Drug Deliv. Rev.* 55:199-215; Trail, P. A. et al. (2003) *Cancer Immunol. Immunother.* 52:328-337; Payne, G. (2003) *Cancer Cell* 3:207-212; Allen, T. M. (2002) *Nat. Rev. Cancer* 2:750-763; Pastan, I. and Kreitman, R. J. (2002) *Curr. Opin. Investig. Drugs* 3:1089-1091; Senter, P. D. and Springer, C. J. (2001)*Adv. Drug Deliv. Rev.* 53:247-264.

**[00235]** Antibody-drug conjugates (ADC) can include targeted chemotherapeutic molecules which combine properties of both antibodies and cytotoxic drugs by targeting potent cytotoxic drugs to antigen-expressing tumor cells (Teicher, B. A. (2009) *Current Cancer Drug Targets* 9:982-1004), thereby enhancing the therapeutic index by maximizing efficacy and minimizing off-target toxicity (Carter, P. J. and Senter P. D. (2008) *The Cancer Jour.* 14(3):154-169; Chari, R. V. (2008) *Acc. Chem. Res.* 41:98-107.

**[00236]** The ADC compounds of the invention include those with anticancer activity. In some embodiments, the ADC compounds include an antibody conjugated, i.e. covalently attached, to the drug moiety. In some embodiments, the antibody is covalently attached to the drug moiety through a linker. The antibody-drug conjugates (ADC) of the invention selectively deliver an effective dose of a drug to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose, may be achieved while increasing the therapeutic index ("therapeutic window").

**[00237]** In certain embodiments the ADC has the formula AB-(L-D)p, wherein: AB is the antibody or binding fragments described herein, L is a linker; D is a suitable cytotoxic drug, and p can range from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more.

**[00238]** Exemplary linker "L" suitable for ADC construction can include "L" selected from the group consisting of 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio)pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate (SMCC), N-Succinimidyl (4-iodo-acetyl)aminobenzoate (STAB), glucuronidase substrate, and PEG.

**[00239]** The drug moiety (D) of the antibody-drug conjugates (ADC) may include any compound, moiety or group that has a cytotoxic or cytostatic effect. Drug moieties may impart their cytotoxic and cytostatic effects by mechanisms including but not limited to tubulin binding, DNA binding or intercalation, and inhibition of RNA polymerase, protein synthesis, and/or topoisomerase. Exemplary drug moieties include, but are not limited to, a maytansinoid, dolastatin, auristatin, calicheamicin, pyrrolobenzodiazepine (PBD), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), nemorubicin and its derivatives, PNU-159682, anthracycline, duocarmycin, vinca alkaloid, taxane, trichothecene, CC1065, camptothecin, elinafide, and stereoisomers, isosteres, analogs, and derivatives thereof that have cytotoxic activity.

**[00240]** Antibodies of the present invention also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates.

*Bispecific Molecules*

**[00241]** In another aspect, the present invention features bispecific molecules comprising an anti-SSEA4 antibody, or a fragment thereof, of the invention. An antibody of the invention, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, e.g., another peptide or protein (e.g., another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. The antibody of the invention may in fact be derivatized or linked to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules; such multispecific molecules are also intended to be encompassed by the term "bispecific molecule" as used herein. To create a bispecific molecule of the invention, an antibody of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a bispecific molecule results.

*Pharmaceutical Compositions*

**[00242]** In another aspect, the present invention provides a composition, e.g., a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (e.g., two or more different) antibodies, or immunoconjugates or bispecific molecules of the

invention. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

**[00243]** Pharmaceutical compositions of the invention also can be administered in combination therapy, i.e., combined with other agents. For example, the combination therapy can include an anti-SSEA4 antibody of the present invention combined with at least one other anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

**[00244]** As used herein, "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). Depending on the route of administration, the active compound, i.e., antibody, immunoconjuage, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

**[00245]** The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S. M., et al. (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N′-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

**[00246]** A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine

hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

**[00247]** Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

**[00248]** These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

**[00249]** Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

**[00250]** Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a

coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

**[00251]** Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

**[00252]** The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 percent to about ninety-nine percent of active ingredient, preferably from about 0.1 percent to about 70 percent, most preferably from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

*Dosing and Dosage*

**[00253]** Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in

association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

**[00254]** For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-PD-1 antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

**[00255]** In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg/ml and in some methods about 25-300 μg/ml.

**[00256]** Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease

is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

**[00257]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

**[00258]** A "therapeutically effective dosage" of an anti-SSEA4 antibody of the invention preferably results in 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. For example, for the treatment of tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition in vitro by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

**[00259]** In another aspect, the instant disclosure provides a pharmaceutical kit of parts comprising an anti-SSEA4 antibody, as described herein. The kit may also further comprise instructions for use in the treatment of a hyperproliferative disease (such as cancer as

described herein). In another embodiment, the anti-SSEA4 antibodies may be co-packaged in unit dosage form, such as for example, PD-1 modulating and/or CAR-T therapeutic agents.

**[00260]** In another aspect, the present dosclosure provides therapeutic methods and compositions that can be administered in combination with any other "cell therapy" or adoptive immunotherapeutic modalities. Exemplary adoptive immunotherapeutic modalities are described in, for example, Maus et al, Annu. Rev. Immunol. 2014. 32:189–225; and can include Chimeric Antigen Receptor (CAR-T) therapy, anti-PD-1 therapy, anti-PD-L1 therapy, and anti-CTL4 therapy etc.

**[00261]** A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

**[00262]** Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

**[00263]** The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

**[00264]** Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices

disclosed in U.S. Pat. No. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: U.S. Pat. No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Pat. No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Pat. No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Pat. No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Pat. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Pat. No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

**[00265]** In certain embodiments, the human monoclonal antibodies of the invention can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V. V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Pat. 5,416,016 to Low et al.); mannosides (Umezawa et al., (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P. G. Bloeman et al. (1995) *FEBS Lett.* 357:140; M. Owais et al. (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe et al. (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier et al. (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M. L. Laukkanen (1994) *FEBS Lett.* 346:123; J. J. Killion; I. J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods of the Invention

**[00266]** The antibodies, antibody compositions and methods of the present invention have numerous in vitro and in vivo utilities involving. In a preferred embodiment, the antibodies of the present invention are human antibodies. For example, these molecules can be administered to cells in culture, in vitro or ex vivo, or to human subjects, e.g., in vivo, to enhance immunity in a variety of situations. Accordingly, in one aspect, the invention provides a method of modifying ADCC response in a subject comprising administering to the

subject the antibody, or antigen-binding portion thereof, of the invention such that the ADCC response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated.

**[00267]** Accordingly, in one embodiment, the invention provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-SSEA4 antibody, or antigen-binding portion thereof. Preferably, the antibody is a human anti-SSEA4 antibody (such as any of the human anti-SSEA4 antibodies described herein). Additionally or alternatively, the antibody may be a chimeric or humanized anti-SSEA4 antibody.

**[00268]** In certain embodiments, the combination of therapeutic antibodies discussed herein may be administered concurrently as a single composition in a pharmaceutically acceptable carrier, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable carrier.

**Antibodies to inhibit tumor growth**

**[00269]** Provided herein are novel recombinant anti-SSEA4 antibodies specifically binding to SSEA4 or its derivatives, and methods of their use in anti-tumor immunotherapies, such as the treatment of cancer. Once bound to a cancer antigen, antibodies can induce antibody-dependent cell-mediated cytotoxicity, activate the complement system, and inhibit the growth of tumor.

**[00270]** In one embodiment, SSEA4 is highly expressed on various tumor cells, including brain tumor cells, lung tumor cells, breast tumor cells, oral tumor cells, esophageal tumor cells, stomach tumor cells, liver tumor cells, bile duct tumor cells, pancreatic tumor cells, colon tumor cells, renal tumor cells, cervical tumor cells, ovarian tumor cells, prostate tumor cells.

**[00271]** In one embodiment, the monoclonal anti-SSEA4 antibody specifically binds to SSEA4 molecule.

**[00272]** In one embodiment, the compositions comprising the anti-SSEA4 antibody described herein are useful in anti-cancer therapies. In particular, the present embodiments provide the complementarity determining region (CDR) sequences of specific anti-SSEA4 antibody, which can be used in a variety of anti-SSEA4 binding portion. In particular, the present invention provides a humanized or chimeric antibody or an antigen-binding fragment thereof capable of binding to SSEA4 or its derivatives.

**[00273]** In certain embodiments, the CDR sequences are defined by Kabat method.

**[00274]** In certain embodiments, the anti-SSEA4 antibody has the activity of inhibiting of tumor growth.

**[00275]** In certain embodiments, the isolated anti-SSEA4 antibody is a monoclonal antibody. Monoclonal antibodies to SSEA4 can be made according to knowledge and skill in the art. For example, it can be made by injecting test subjects with human embryonic carcinoma cell and then isolating hybridomas expressing antibodies having the desired sequence or functional characteristics.

**[00276]** In one embodiment, the present disclosure provides an isolated monoclonal antibody or an antigen binding portion thereof that binds to SSEA4 wherein upon target binding the antibody has CDC inducing activity.

**[00277]** In one embodiment, the present disclosure provides an isolated monoclonal antibody or an antigen binding portion thereof that binds to SSEA4 wherein upon target binding the antibody has ADCC inducing activity.

**[00278]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising: (i) a heavy chain variable region selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereof; and (ii) a light chain variable region selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereof.

**[00279]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising: (i) a heavy chain variable region selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than or equal to 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions; and (ii) a light chain variable region selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than or equal to 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions.

**[00280]** In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof further comprising: (i) a heavy chain variable region selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or 80% or more conserved sequence homologs thereof further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170 or a conserved sequence homologs thereof containing less than or equal to 5, 4, 3, 2, or 1 amino acid substitutions; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171 or a conserved sequence homologs thereof containing less than or equal to 5, 4, 3, 2, or 1 amino acid substitutions, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172 or a conserved sequence homologs thereof containing less than or equal to 5, 4, 3, 2, or 1 amino acid substitutions ; respectively, and (ii) a light chain variable region selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or 80 % or more conserved sequence homologs thereof further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175 or a conserved sequence homologs thereof containing less than or equal to 5, 4, 3, 2, or 1 amino acid substitutions; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176 or a conserved sequence homologs thereof containing less than or equal to 5, 4, 3, 2, or 1 amino acid substitutions , and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177 or a conserved sequence homologs thereof containing less than or equal to 5, 4, 3, 2, or 1 amino acid substitutions.

**[00281]** In one embodiment, the isolated monoclonal antibody or an antigen-binding fragment thereof comprising: (i) a heavy chain variable region selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and (ii) a light chain variable region selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177.

**[00282]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising: (i) a heavy chain variable region selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173, and (ii) a light chain variable region selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177.

**[00283]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising: (i) a heavy chain variable region selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and (ii) a light chain variable region selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178.

**[00284]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen-binding fragment thereof comprising the respective corresponding VH, VL and respective H-CDRs and L-CDRS as set forth in each variant in Tables 2A-2D.

**[00285]** As used herein, homolog or the conserved sequence homolog can include isolated monoclonal antibody or an antigen-binding fragment thereof targeting SSEA4 comprising the respective corresponding VH, VL and respective H-CDRs and L-CDRS having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity as compared to the reference sequence as disclosed herein AND/OR having less than or equal to 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions as compared to the reference sequence.

**[00286]** In certain embodiments, the anti-SSEA4 antibody is monoclonal antibody.

**[00287]** In certain embodiments, the anti-SSEA4 antibody is chimeric or humanized antibody.

**[00288]** In one aspect, the framework sequences are derived from human consensus framework sequences or human germline framework sequences.

**[00289]** In a further aspect, the heavy chain variable domain, antibody or antigen-binding fragment further comprises at least a $C_H1$ domain.

**[00290]** In a further aspect, the heavy chain variable domain, antibody or antigen-binding fragment further comprises a $C_H1$, a $C_H2$ and a $C_H3$ domain.

**[00291]** In a further aspect, the variable region light chain, antibody or antibody fragment further comprises a $C_L$ domain.

**[00292]** In a further aspect, the antibody further comprises a $C_H1$, a $C_H2$, a $C_H3$ and a $C_L$ domain.

**[00293]** In a further specific aspect, the antibody further comprises a human or murine constant domain.

**[00294]** In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG3, IgG4.

**[00295]** In a further aspect, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-SSEA4 antibodies. In a still further specific aspect, the vector further comprises a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese Hamster Ovary (CHO). In another aspect the cell is yeast cell.

**[00296]** In an embodiment, the invention provides for a process of making an anti-SSEA4 antibody or antigen binding fragment thereof, comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-SSEA4 antibodies or antigen-binding fragment in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

**[00297]** In an embodiment, the invention provides for a composition comprising an anti-SSEA4 antibody or antigen-binding fragment thereof as provided herein and at least one pharmaceutically acceptable carrier.

**[00298]** In one aspect, the present disclosure provides an isolated monoclonal antibody or an antigen binding portion thereof that binds to SSEA4 wherein upon target binding the antibody has ADCC and CDC inducing activity.

**[00299]** Any of the antibodies described herein can be a full length antibody or an antigen-binding fragment thereof. In some examples, the antigen binding fragment is a Fab fragment, a $F(ab')_2$ fragment, or a single-chain Fv fragment. In some examples, the antigen binding fragment is a Fab fragment, a $F(ab')_2$ fragment, or a single-chain Fv fragment (scFv). In some examples, the isolated antibody is a human antibody, a humanized antibody, a chimeric antibody, or a single-chain antibody.

**[00300]** Any of the antibodies described herein has one or more characteristics of:

a) is a recombinant antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, an antibody fragment, a bispecific antibody, a monospecific antibody, a monovalent antibody, an IgG antibody, or derivative of an antibody;

b) is a human, murine, humanized, or chimeric antibody, antigen-binding fragment, or derivative of an antibody;

c) is a single-chain antibody fragment, a multibody, a Fab fragment, and/or an immunoglobulin of the IgG, IgM, IgA, IgE, IgD isotypes and/or subclasses thereof;

d) has one or more of the following characteristics: (i) mediates ADCC and/or CDC of cancer cells; (ii) induces and/or promotes apoptosis of cancer cells; (iii) inhibits proliferation of target cells of cancer cells; (iv) induces and/or promotes phagocytosis of cancer cells; and/or (v) induces and/or promotes the release of cytotoxic agents;

e) specifically binds the tumor-associated carbohydrate antigen, which is a tumor-specific carbohydrate antigen;

f) does not bind an antigen expressed on non-cancer cells, non-tumor cells, benign cancer cells and/or benign tumor cells; and/or

g) specifically binds a tumor-associated carbohydrate antigen expressed on cancer stem cells and on normal cancer cells.

**[00301]** The antibodies are suitable bind to its target epitopes with a high affinity (low $K_D$ value), and preferably $K_D$ is in the nano-molar range or lower. Affinity can be measured by methods known in the art, such as, for example; surface plasmon resonance.

**[00302]** In certain embodiments, the exemplary anti-SSEA4 antibody or antigen-binding fragment inhibits or reduces the tumor growth by combined with one or more cytotoxic agent, chemotherapeutic agent or therapeutic antibody.

**[00303]** In an embodiment, the present invention provides a composition of bispecific antibody comprising an exemplary anti-SSEA4 antibody or antigen-binding fragment thereof fused to or linked by a spacer to a T cell-binding molecule including but not limited to anti-CD3 antibody or antigen-binding fragment thereof.

**[00304]** In another embodiment, the present invention provides a composition of chimeric antigen receptor (CAR) comprising an extracellular domain containing an exemplary anti-SSEA4 antibody or antigen-binding fragment thereof, a transmembrane domain that anchors the CAR to the cell membrane, and an intracellular domain which transmits an activation signal to the immune cells once the CAR engage with SSEA4. The CAR can be genetically/artificially expressed on immune cells including but not limited to T cell, NK cell, and NKT cell to target and kill cancer cells expressing SSEA4.

**[00305]** The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

## EXAMPLES

### Example 1. Detection of globo-series glycosphingolipids expression on various cancer cell lines

**[00306]** Cells ($1 \times 10^5$) of various cancer cell line, including brain tumor cell, lung tumor cells, breast tumor cells, oral tumor cells, esophageal tumor cells, stomach tumor cells, liver tumor cells, bile duct tumor cells, pancreatic tumor cells, colon tumor cells, renal tumor cells, cervical tumor cells, ovarian tumor cells, prostate tumor cells (Table 4) were stained with 0.5 μg Alexa Flour 488-conjugated anti-SSEA3 mAb (MC-631), anti-SSEA4 mAb (MC813-70), or allophycocyanin (APC)-conjugated anti-GloboH mAb (VK9, a gift from Philip O. Livingston, Memorial Sloan–Kettering Cancer Center, New York) in 50 μL FACS buffer (PBS solution with 1% FBS) on ice for 30 min. For lectin staining, cells were incubated for 30 min on ice in lectin-binding buffer [1% BSA, 0.5× Carbo-Free Blocking buffer (Vector Laboratories), 2 mM $MgCl_2$ , 2 mM $CaCl_2$ ] containing biotinylated lectin. After being washed twice with lectin-binding buffer, cells were incubated with streptavidin-APC (1:500 diluted in FACS buffer; Biolegend). After being washed twice with 200 μL FACS buffer, cells were re-suspended in 200 μL FACS buffer containing 1 μg/mL propidium iodide (PI) and subjected to analysis. Data acquisition was performed on a FACSCanto (BD Biosciences) with FACSDiva software (BD Biosciences), and data analyses were performed

using FlowJo software (TreeStar). Live $PI^-$ cells were gated for analysis. For methanol washing, cells were washed and fixed with 4% (wt/vol) paraformaldehyde in PBS for 15 min at room temperature, followed by incubation in methanol for 10 min before staining with specific antibodies.

**Example 2. Representative methodology for generating and producing exemplary monoclonal anti-SSEA4 antibodies**

**[00307]** Hybridoma technology was employed for generating the monoclonal antibodies specific to and/or targeting SSEA4. For example, female Balb/c mice, aged 6 weeks old (Biolasco, Taiwan) were intraperitoneally injected with $10^7$ NCCIT cells three times at 2-week intervals. Sera were collected one week after the 3rd immunization, and the titer of anti-SSEA4 IgG and/or IgM antibodies were measured by ELISA. ELISA was conducted by using 96-well assay plates coated with 0.1 μg of BSA-conjugated SSEA4. One week later, mice which met the fusion criteria were then given a final boost with $10^7$ NCCIT cells. Three days after the final boost, mice were sacrificed and the spleen cells from these mice were used for generating the hybridomas. The hybridoma clones positive to BSA-conjugated SSEA4 and negative to BSA were selected for further sub-cloning to ensure every hybridoma clone was derived from a single cell. In one exemplary run, a total of 10 SSEA4-positive hybridoma clones were selected from over 5,000 clones. Among these clones, only Ab6 is IgG, and the others are IgM antibodies. The subclass of Ab6 was further determined by antibody isotyping kit, and the result showed that the isotype of Ab6 is IgG3, kappa. Following the sequencing, the murine variable regions ($V_H$ and $V_L$) of Ab6 was PCR amplified and sub-cloned into an expression vector containing the constant region ($C_H$ and $C_L$) of human IgG1 to generate chAb6, a human-mouse chimeric antibody.

**Example 3. Binding of exemplary anti-SSEA4 antibodies to SSEA4 by ELISA**

The binding affinity of exemplary chimeric and humanized anti-SSEA4 antibodies to SSEA4 were determined by ELISA. Briefly, antibodies were diluted in PBS at the indicated concentration and then allowed to incubate with BSA-conjugated SSEA4 in 96-well assay plates for 1 hour at room temperature. Following the wash cycles, HRP-conjugated goat anti-human IgG antibody (1:10,000 diluted in PBS, Jackson Immuno Research) was added to wells and incubated at room temperature for another one hour. After the wash cycles, TMB ELISA substrate (Abcam) was added for color development, and the reaction was stopped by adding equal volume of 2N $H_2SO_4$. The absorbance at O.D. 450 nm was read and recorded by M5 ELISA reader (Molecular Device). (Fig. 6A-6D)

**Example 4. The binding affinity of chAb6 to a pancreatic cancer cell line HPAC**

**[00308]** For flow cytometry analysis, 5 x $10^5$ HPAC cells were incubated with chAb6 at the indicated concentration in FACS buffer (1% of FBS in PBS) for 30 minutes at 4°C. After wash by FACS buffer, cells were then incubated with PE-conjugated goat anti-human IgG antibody (1:250 diluted in FACS buffer, Jackson Immuno Research) for 30 minutes at 4°C. The binding of anti-SSEA4 antibody to cells was then analyzed by BD FACSVerse flow cytometer. (Fig. 10A-10B)

**Example 5. Binding specificity of exemplary anti-SSEA4 antibody**

**[00309]** The binding specificity of exemplary anti-SSEA4 antibody was analyzed by using glycan microarray with 152 chemically synthesized glycans (Fig. 7). Briefly, the glycan microarray was incubated with the antibody at the indicated concentration at 37°C for 1 hour. After washed by PBST (0.05% Tween 20 in PBS), the glycan microarray was then incubated with FITC-labeled goat anti-human IgG antibody for 1 hour at room temperature. Followed by another wash cycles, the glycan microarray was air-dried and scanned at 635 nm by a microarray fluorescence chip reader (4000B, Genepix). The data was then analyzed by GenePix Pro-6.0 (Axon Instruments).

**Example 6. Antibody-dependent cellular cytotoxicity (ADCC) activity of chAb6 on a pancreatic tumor cells line HPAC**

**[00310]** The Calcein AM-labeled HPAC cells, a human pancreatic tumor cells line with high SSEA4 expression, were mixed with PBMC first, and the anti-SSEA4 antibody was then added at the indicated concentration and allowed to incubate for 4 hrs at 37°C. After incubation, the culture supernatant was collected and detected at ex.485 nm /em.535 nm, and the percentage of cell cytotoxicity was calculated as: (experimental value - spontaneous lysis)/(maximum lysis - spontaneous lysis) x 100. (Fig. 12)

**Example 7. Complement-dependent cytotoxicity (CDC) activity of chAb6 on a pancreatic tumor cells line HPAC**

**[00311]** For CDC assay, 2x$10^5$ HPAC cells were incubated with 15% of human serum and anti-SSEA4 antibody at the indicated concentration at 37°C for 1 hour. After incubation, the dead cells were stained by propidium iodide (PI) for 5 minutes at room temperature, and then counted and analyzed by BD FACSVerse flow cytometer (Fig. 15A).

**Example 8. *In vivo* anti-tumor efficacy of exemplary anti-SSEA4 antibodies in HPAC xenograft model**

**[00312]** To evaluate the anti-tumor efficacy of exemplary anti-SSEA4 antibodies *in vivo*, male CB17.SCID mice, aged 8 weeks old (Biolasco, Taiwan), were subcutaneously injected with $5x10^6$ HPAC cells. While the tumor formed and the volume reached 50 to 100 $mm^3$, vehicle or exemplary anti-SSEA4 antibodies (20 mpk) was intravenously injected into the tail vein twice per week. Tumor growth was monitored twice weekly by measuring the perpendicular tumor diameters, length (*L*) and width (*W*), with a vernier caliper. The volume of tumor (*V*) was calculated by the formula $V=LW^2/2$. On day 24 (24 days after transplantation), the mice were sacrificed to measure the tumor weight. All the results were showed as mean ± S.D. (n = 3 for each group), and Student's t test was used for statistical analysis. (Fig.16A-16B)

**Example 9. Detecting SSEA4 expressed in tumor tissue by using exemplary chAb6**

**[00313]** Immunohistochemistry (IHC) was employed to detect the presence of SSEA4 in tumor tissue (Fig. 19). Briefly, the frozen sections of HPAC xenograft tumor were fixed with 10% neutral buffered formalin (Sigma-Aldrich) at room temperature for 10 minutes first, and the endogenous peroxidase activity was quenched by immersing sections in 0.3% hydrogen peroxide / 0.1% sodium azide in $ddH_2O$ for 15 min at RT. After wash cycles (PBS, 3 times, 5 minutes for each), the sections were incubated with 2 μg/mL of FITC-labeled chAb6 or human IgG1, kappa at 4°C overnight. After an overnight incubation, the sections were then washed and incubated with HRP-labeled goat anti-FITC antibody (1:200, KPL) for 1 hour at room temperature. After another wash cycles, DAB substrate (Vector laboratories) was used for color development, and hematoxylin (Sigma-Aldrich) was used for counter staining.

**Example 10 Treatment of Disorders**

**[00314]** Subjects at risk for or afflicted with cancer may be in need of immune response augmentation would benefit from treatment with a SSEA4 antibody of the present invention in a soluble form. Most commonly, antibodies are administered in an outpatient setting by weekly administration at about 0.1-10 mg/kg dose by slow intravenous (IV) infusion. The appropriate therapeutically effective dose of an antagonist is selected by a treating clinician and would range approximately from 1 μg/kg to 20 mg/kg, from 1 μg/kg to 10 mg/kg, from 1 μg/kg to 1 mg/kg, from 10 μg/kg to 1 mg/kg, from 10 μg/kg to 100 μg/kg,

from 100 μg to 1 mg/kg, and from 500 μg/kg to 5 mg/kg.It is anticipated that SSEA4 antibodies of the invention would be administered with a frequency of one per month or less. Treatment duration could range between one month and several years.

**[00315]** To test the clinical efficacy of antibodies in humans, individuals with cancer are identified and randomized to a treatment group. Treatment groups include a placebo group and one to three groups treated with a SSEA4 antibody (different doses). Individuals are followed prospectively for one to three years. It is anticipated that individuals receiving treatment would exhibit an improvement.

**Example 11 : Kinetic binding assays of exemplary anti-SSEA4 antibodies by surface plasmon resonance.**

**[00316]** The kinetic binding of exemplary anti-SSEA4 antibodies were analyzed by surface plasmon resonance (SPR) using Biacore T200 system. Firstly, the biotinylated SSEA4 was immobilized on Sensor Chip SA. The representative anti-SSEA4 antibodies hAb6-3.1, hAb6-3 and chAb6 were serially diluted in running buffer (1x PBS buffer containing 0.05 % Tween-20, pH7.4) to concentrations of 100, 33.3, 11.1, 3.7, 1.2 nM, and then injected for 5 min at 30 uL/min using single-cycle mode. The analysis of parameters was performed by BIAevaluation software. (Fig. 5)

**Example 12: Binding of exemplary anti-SSEA4 antibodies to cell by flow cytometry analysis**

**[00317]** For flow cytometry analysis, 3 x $10^5$ cancer cells, such as breast cancer cell line MDA-MB-231, MCF7, were incubated with exemplary anti-SSEA4 antibodies at the indicated concentration in FACS buffer (1% of FBS in PBS) for 30 minutes at 4°C. After wash by FACS buffer, cells were then incubated with PE- or Alexa Fluor488-conjugated goat anti-human IgG antibody (1:250 to 1:400 diluted in FACS buffer, Jackson Immuno Research) for 30 minutes at 4°C. The binding of anti-SSEA4 antibody to cells was then analyzed by BD FACSVerse flow cytometer. (see Fig. 9A-9B and Fig. 11A-11F)

**EXAMPLE 13: Antibody-dependent cellular cytotoxicity (ADCC) activity of exemplary chimeric and humanized Ab6s on breast and pancreatic cancer cell lines.**

**[00318]** The MDA-MB-231, MCF7 and HPAC cells were labeled with 20 uM of Calcein AM for 30 min. After washing, the Calcein-AM labeled target cells (1x$10^4$ cells/well) were co-incubated with fresh isolated human PBMC (2.5x$10^5$ cells/well, E/T ratio = 25/1), and treated with or without serially diluted anti-SSEA4 antibodies for 4 hr. The

release of Calcein-AM was detected by M5 ELISA reader (ex. 485, em. 520) and used for evaluation of relative cytotoxicity. (Fig. 13, Fig. 14A-14B)

**Example 14: Complement-dependent cytotoxicity (CDC) activity of exemplary chimeric and humanized Ab6s on breast and pancreatic cancer cell lines.**

**[00319]** For HPAC, $2x10^5$ cells were incubated with 15% of human serum and anti-SSEA4 antibody at the indicated concentration at 37°C for 1 hour. After incubation, the dead cells were stained by propidium iodide (PI) for 5 minutes at room temperature, and then counted and analyzed by BD FACSVerse flow cytometer. (Fig. 15A)

**[00320]** For MCF7, cells were labeled with 20 uM of Calcein AM for 30 min firstly. After washing, the Calcein-AM labeled target cells ($1x10^4$ cells per assay) were co-incubated with 10% of human serum, and treated with or without anti-SSEA4 antibody at the indicated concentration at 37°C for 2 hour. The release of Calcein-AM was detected by M5 ELISA reader (ex. 485, em. 520) and used for evaluation of relative cytotoxicity. (Fig. 15B)

**Example 15: *In vivo* anti-tumor efficacy of anti-SSEA4 antibodies in MDA-MB-231 xenograft model**

**[00321]** To evaluate the anti-tumor efficacy of anti-SSEA4 antibodies *in vivo*, female Balb/c nude mice, aged 8 weeks old (NLAC, Taiwan), were orthotopically injected with $5x10^6$ MDA-MB-231 cells. While the tumor formed and the volume reached 100 to 150 $mm^3$, vehicle, Herceptin or anti-SSEA4 antibodies (20 mpk) was intravenously injected into the tail vein twice per week. Tumor growth was monitored twice weekly by measuring the perpendicular tumor diameters, length (*L*) and width (*W*), with a vernier caliper. The volume of tumor (*V*) was calculated by the formula $V=LW^2/2$. All the results were showed as mean ± SEM (n = 8 for each group), and Student's t test was used for statistical analysis. (Fig. 17)

**Example 16: *In vivo* anti-tumor efficacy of anti-SSEA4 antibodies in MCF7 xenograft model**

**[00322]** To establish MCF7 xenograft model, female Balb/c nude mice (aged 8 weeks old, purchased from Biolasco, Taiwan) were subcutaneously implanted with 17-beta-estradiol pellet at Day 1. Five million MCF7 cells were mixed with Matrigel, and then orthotopically injected into mammary fat pad. While the tumor formed and the volume reached 150 to 200 $mm^3$, vehicle or hAb6-3.1 (at the indicated dose) was intravenously injected into the tail vein twice per week. Tumor growth was monitored twice weekly by measuring the perpendicular tumor diameters, length (*L*) and width (*W*), with a vernier caliper. The volume of tumor (*V*)

was calculated by the formula $V=LW^2/2$. All the results were showed as mean ± SEM. (n = 7 for each group), and Student's t test was used for statistical analysis. (Fig. 18)

**Example 17**: **Exemplary methodology for the development of glyco-engineered hAb6-3.1**

*Production of glyco-engineered hAb6-3.1*

**[00323]** The glycans of exemplary anti-SSEA4 antibody hAb6-3.1 was hydrolyzed to mono-GlcNAc form via co-incubating with endo-beta-N-acetylglucosaminidase and fucosidase. The glyco-engineered antibody was produced by transglycosylating the universal glycan onto the mono-GlcNAc in the presence of endo-beta-N-acetylglucosaminidase mutant, followed by the purification of rProtein A chromatography. Characterization of glyco-engineered Ab6-3.1 was performed by SDS-PAGE and flow cytometry anlaysis (Figure 20 and 21, respectively).

*In vitro functional assays of glyco-engineered hAb6-3.1*

**[00324]** Glycoengineering was shown to improve the binding affinity of antibody to Fc gamma receptors expressed on immune cells, which contributes the protective function of the immune system. We demonstrated the Fc gamma receptor IIIA binding and ADCC function (antibody-dependent cell-mediated cytotoxicity) of glyco-engineered hAb6-3.1 as below.

Fc *gamma receptor IIIA binding*

**[00325]** Fc gamma receptor IIIA was coated on the ELISA plate, and incubated with native and glyco-engineered antibody at the indicated concentration. The binding activity was then determined by using HRP-conjugated anti-Human IgG H+L and TMB substrate. (Fig. 22)

*ADCC assay*

**[00326]** *The* Calcein AM-labeled MDA-MB-231 cells, a human triple-negative breast cancer cell line with high SSEA-4 expression, were mixed with PBMC first, and the native or glyco-engineered anti-SSEA-4 antibodies were then added at the indicated concentration and allowed to incubate for 4 hours at 37°C. After incubation, the culture supernatant was collected and detected at ex.485/em.535, and the percentage of cell cytotoxicity was calculated as: (experimental value - spontaneous lysis)/(maximum lysis - spontaneous lysis) x 100. (Fig. 23)

**[00327]** Glycoengineering of anti-SSEA-4 antibody significantly enhanced the binding of antibody to Fc gamma receptor IIIA, resulting in the improved antibody-dependent cellular cytotoxicity (ADCC) activity as compared with the native antibody.

**Example 18: Representative methodology for the development/Formation of antibody-drug conjugation complex**

**[00328]** Several chemical approaches were available for the antibody-drug conjugation, such as thiol–melimide formation on the lysine and cysteine residues (Lewis Phillips et al., 2008), selenol-maleimide formation on the selenocysteine residues (Hofer et al., 2009), oxmie ligation to the modified Fc glycans (Zhou et al., 2014), Click chemistry (Axup et al., 2012), Hydrazino-iso-Pictet-Spengler ligation to fomylglycine residue (Drake et al., 2014). We adapted the use of a representative oxime ligation onto the modified Fc glycan as our exemplary ADC formation approach. Oxime ligation between the modified glycan on the antibody and payload compound A1 (cytotoxic drug MMAE with a alkoxyamine-cleavable linker, M.W.: 1348.7265) was carried out in the present of antibody (8 mg/mL) and A01 (3mM) in 100 mM acetate buffer pH 4.5 at 25 °C. The reaction was incubated for 48 hours and the product was purified by rProtein A, Capto S and Capto Q column sequentially. The result of hAb6-3.1-A01 complex formation was analyzed by SDS-PAGE (Fig. 24A-B).

**Example 19: The binding ability of hAb6-3.1-A01 to SSEA4-expressing cells by flow cytometry**

**[00329]** SSEA4-expressing cell line MCF7 and SKOV3 were washed with PBS and $1x10^5$ of cells were incubated with 10 ug/mL of hAb6-3.1 or hAb6-3.1-A01 in FACS buffer (PBS containing 2 % FBS and 0.1 % $NaN_3$) on ice for 1 hr. After washing with PBS, the cells were stained with Alexa-Fluor 488 labeled anti-human IgG antibody and incubated on ice for 0.5 hr. The signals for cell binding of antibodies were detected by flow cytometry (Figure XX11AB). The result indicated the binding property of hAb6-3.1-A01 to SSEA4-expressing cell is similar with parental antibody hAb6-3.1 (Fig. 25A-B).

**Example 20. In vitro cell cytotoxicity assay in breast cancer cell line**

**[00330]** MCF7, a SSEA4-expressing breast cancer cell line, were seeded in 96-well white plate ($1x10^3$ cells/well) and incubated at 37 °C overnight. The cells were treated with serially diluted hAb6-3.1 or hAb6-3.1-A01 and incubated for further 5 days. After treatment, the culture medium was removed and the cells were treated with CellTiter Glo reagent (Promega). The luminescence signals were detected by ELISA reader (M5) after incubation

for 10 mins, and the cell viability was calculated (the signals of untreated cells were set as 100 % of viability).

**[00331]** As shown in Figure 26, hAb6-3.1-A01 performed cytotoxicity in a dose dependent manner. The ADC exhibits a sigmoid curve, indicating specific binding to the target SSEA-4. In contrast, the hAb6-3.1 alone does not have much cytotoxicity. This result indicates that the ADC achieves the advantages of both specificity and cytotoxicity. The preserved cytotoxicity would achieve the expected therapeutic effects, while the specificity would target the cancer cells and spare the normal cells, thereby minimizing adverse effects.

**Example 21. In vitro cell cytotoxicity assay in ovarian cancer cell line**

**[00332]** SKOV3, a SSEA4-expressing ovarian cancer cell line, was applied to demonstrate the efficacy of hAb6-3.1-A01. The method of cell cytotoxicity assay was described in Example 20. hAb6-3.1-A01 exhibited a more potent efficacy of cytotoxicity on SKOV3 in a nano-molar level.

**[00333]** All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

**[00334]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood to one of ordinary skill in the art to which this invention pertains. Although any known methods, devices, and materials may be used in the practice or testing of the invention, the methods, devices, and materials in this regard are described herein.

## CLAIMS

What the claim is:

1. An isolated monoclonal antibody or an antigen-binding fragment thereof comprising:

(i) H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170, or 80% or more conserved sequence homologs of (i);

(ii) H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, or 80% or more conserved sequence homologs of (ii);

(iii) H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172, or 80% or more conserved sequence homologs of (iii);

(iv) L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175, or 80% or more conserved sequence homologs of (iv);

(v) L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, or 80% or more conserved sequence homologs of (v); and

(vi) L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177, or 80% or more conserved sequence homologs of (vi); respectively.

2. An isolated monoclonal antibody or an antigen-binding fragment thereof comprising:

(i) H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170, or conserved sequence homologs of (i) containing less than 5 amino acid substitutions;

(ii) H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, or conserved sequence homologs of (ii) containing less than 5 amino acid substitutions;

(iii) H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172, or conserved sequence homologs of (iii) containing less than 5 amino acid substitutions;

(iv) L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175, or conserved sequence homologs of (iv) containing less than 5 amino acid substitutions;

(v) L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, or conserved sequence homologs of (v) containing less than 5 amino acid substitutions; and

(vi) L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177, or conserved sequence homologs of (vi) containing less than 5 amino acid substitutions; respectively..

3. The isolated monoclonal antibody or an antigen-binding fragment thereof of claims 1 or 2, further comprising amino acid substitution on the CDR selected from one or more of A100R, N31S, T62A on the heavy chain and/or S52Y on the light chain.

4. The isolated monoclonal antibody or an antigen-binding fragment thereof of claims 1 or 2, further comprising amino acid substitution on the CDR selected from one or more of V50A, G53A, S35T on the heavy chain and/or one or more of V30I/A, G91A, Y94F on the light chain.

5. An isolated monoclonal antibody or an antigen-binding fragment thereof, comprising:

(i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or 80% or more conserved sequence homologs thereof; and

(ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or 80 % or more conserved sequence homologs thereof .

6. An isolated monoclonal antibody or an antigen-binding fragment thereof, comprising:

(i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173, or a conserved sequence homolog thereof containing less than 10 amino acid substitutions; and

(ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178, or a conserved sequence homolog thereof containing less than 10 amino acid substitutions.

7. An isolated monoclonal antibody or an antigen-binding fragment thereof comprising:

(i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or 80% or more conserved sequence homologs thereof further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171; H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and

(ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or 80 % or more conserved sequence homologs thereof further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176; and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177; respectively.

8. An isolated monoclonal antibody or an antigen-binding fragment thereof comprising:

(i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171; H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and

(ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176; and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177; respectively.

9. The isolated monoclonal antibody or an antigen-binding fragment thereof, further comprising:

(i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173; and

(ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising L-CDR1 selected from

SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176; and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177; respectively.

10. The isolated monoclonal antibody or an antigen-binding fragment thereof, further comprising:

(i) a heavy chain variable domain selected from SEQ ID Nos. 13, 23, 33, 43, 53, 63, 73, 83, 103, 123, 133, 143, 153, and 173 or a conserved sequence homologs thereof containing less than 10 amino acid substitutions further comprising H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171; H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172; respectively, and

(ii) a light chain variable domain selected from SEQ ID Nos. 18, 28, 38, 48, 58, 68, 78, 88, 108, 128, 138, 148, 158, and 178.

11. An isolated monoclonal antibody or an antigen-binding fragment thereof, comprising the respective corresponding $V_H$, $V_L$ and respective H-CDRs and L-CDRS as set forth in each variant in Tables 2A-2D.

12. The isolated antibody or antigen-binding fragment of any one of claims 1-11, wherein the antibody or antigen-binding fragment is:

a) a chimeric antibody or a fragment thereof; or

b) a humanized antibody or fragment thereof; or

c) a human antibody or fragment thereof; or

d) an antigen-binding fragment selected from the group consisting of Fab, Fab', Fv, scFv, dsFv, $F(ab)_2$, Fd and a diabody.

13. The isolated antibody or antigen-binding fragment of any one of claims 1-12, wherein the antibody is IgG.

14. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-13 wherein the antibody binding or the binding fragment target is carbohydrate antigen SSEA4 having the structure Neu5Acα 2→3Galβ 1→3GalNAcβ 1→3Galα 1→4Gal β 1→4Glcβ 1.

15. The isolated antibody or antigen-binding fragment of any one of claims 1-14, wherein the antibody has CDC and/or ADCC inducing activity upon binding to the target cells.

16. A pharmaceutical composition, comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1-14 and a pharmaceutical acceptable carrier.

17. A pharmaceutical composition of claim 16, further comprising one or more therapeutic agent.

18. A pharmaceutical composition of claim 17, wherein the therapeutic agent is selected from therapeutic antibodies, chemotherapeutic agents, or cytokines.

19. An immunoconjugate comprising the antibody of any one of claims 1 to 14 and a cytotoxic agent.

20. The immunoconjugate of claim 19, having the formula AB-(L-D)p, wherein:

(a) AB is the antibody of anyone of claims 1-14;

(b) L is a linker;

(c) D is a suitable cytotoxic drug, and

(d) p ranges from 1 to 8.

21. The immunoconjugate (ADC) of claim 20, wherein the drug is MMAE or MMAF.

22. The immunoconjugate of claim 20, wherein the linker is cleavable linker.

23. The ADC of claim 22, wherein the cleavable linker is an alkoxyamine-cleavable linker.

24. A pharmaceutical formulation comprising the immunoconjugate of claims and a pharmaceutically acceptable carrier.

25. The pharmaceutical formulation of claim 24, further comprising an additional therapeutic agent.

26. Isolated nucleic acid (cDNA) encoding the antibody of any one of claims 1-14.

27. A host cell comprising the nucleic acid of claim 26.

28. A method of producing an antibody comprising culturing the host cell of claim 27 so that the antibody is produced.

29. An antibody produced by steps comprising:

(a) providing a nucleic acid encoding 3 VL domain CDRs having sequences of: L-CDR1 selected from SEQ ID Nos. 15, 45, 55, 65, 75, 85, 105, 125, 135, 145, 155 and 175; and L-CDR2 selected from SEQ ID Nos. 16, 46, 56, 66, 76, 86, 106, 126, 136, 146, 156 and 176, and L-CDR3 selected from SEQ ID Nos: 17, 47, 57, 67, 77, 87, 107, 127, 137, 147, 157, and 177, or conserved sequence homologs of each respective L-CDRs having 5 or less conserved amino acid substitutions;

(b) combining a repertoire of nucleic acids encoding 3 VH domain CDRs having the sequences of H-CDR1 selected from SEQ ID Nos. 10, 40, 50, 60, 70, 80, 100, 120, 130, 140, 150 and 170; H-CDR2 selected from SEQ ID Nos. 11, 41, 51, 61, 71, 81, 101, 121, 131, 141, 151, and 171, H-CDR3 selected from SEQ ID Nos: 12, 42, 52, 62, 72, 82, 102, 122, 132, 142, 152 and 172, or conserved sequence homologs of each respective H-CDRs having 5 or less conserved amino acid substitutions;

with the nucleic acid encoding the 3 VL domain CDRs, so as to provide a product repertoire of nucleic acids encoding the 3 VL domain CDRs and the repertoire of 3 VH domain CDRs

(c) expressing the nucleic acids of the product repertoire;

(d) selecting an antigen-binding fragment comprising a variable domain that specifically binds to SSEA4 and that is expressed from the nucleic acids of the product repertoire; and

(e) producing an antibody comprising the antigen-binding fragment.

30. A method of treating a subject having a SSEA4-positive cancer, the method comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of any one of claims 16, 17, 18, 24, and 25.

31. The method of claim 30, wherein the SSEA4-positive cancer is selected from brain, lung, breast, oral, esophageal, stomach, liver, bile duct, pancreatic, colon, kidney, cervical, ovarian, and prostate cancer.

32. The method of claim 30, further comprising administering one or more additional therapeutic modality or agent in combination to the individual.

33. The method of claim 32, wherein the combined treatment modality is selected from therapeutic antibodies, cell therapies, radiation, cytokines, or chemotherapeutic agents.

34. A method of inhibiting proliferation of a SSEA4-positive cell, the method comprising exposing the cell to the pharmaceutical formulations of any one of claims 16, 17, 18, 24, and 25 under conditions permissive for binding of the antibodies/fragments/ADCs to SSEA4 on the surface of the cell expressing carbohydrate antigen, thereby inhibiting proliferation of the cell.

35. A method of treating a subject having a SSEA4-positive cancer, wherein the SSEA4-positive cancer is resistant to a first therapeutic agent, the method comprising administering to the individual an effective amount of the pharmaceutical formulation of any one of claims claims 16, 17, 18, 24, and 25.

36. The method of claim 35, wherein the SSEA4-positive cancer is brain, lung, breast, oral, esophageal, stomach, liver, bile duct, pancreatic, colon, kidney, cervical, ovarian, and/or prostate cancer.

37. The method of claim 35, wherein the first therapeutic agent comprises a first antibody/binding fragment/ADC that binds an antigen other than SSEA4, and/or radiation, and/or chermotherapeutic agent.

38. A method of detecting SSEA4 in a biological sample comprising contacting the biological sample with the anti-SSEA4 antibody of any one of claims 1-14 under conditions permissive for binding of the anti-SSEA4 antibody to a naturally occurring SSEA4, and detecting whether a complex is formed between the anti-SSEA4 antibody and a naturally occurring SSEA4 in the biological sample.

39. The method of claim 38, wherein the biological sample is a cancer sample.

40. A method for detecting a SSEA4-positive cancer comprising (i) administering a labeled anti-SSEA4 antibody to a subject having or suspected of having a carbohydrate antigen expressing tumor, wherein the labeled anti-SSEA4 antibody comprises the anti-SSEA4 antibody of any one of claims 1-14, and (ii) detecting the labeled anti-SSEA4 antibody in the subject, wherein detection of the labeled anti-SSEA4 antibody indicates a SSEA4-positive cancer in the subject.

41. A method for detecting a SSEA4-positive cancer comprising (i) contacting a labeled anti-SSEA4 antibody with a sample from a subject having or suspected of having a carbohydrate antigen expressing tumor, wherein the labeled anti-SSEA4 antibody comprises the anti-SSEA4 antibody of any one of claims 1-14, and (ii) detecting the labeled anti-SSEA4 antibody in the sample, wherein detection of the labeled anti-SSEA4 antibody indicates a SSEA4-positive cancer in the sample.

42. The isolated antibody of any one of claims 1-14, wherein the antibody specifically binds to SSEA4 with an affinity constant less than $10^{-7}$ M.

43. The isolated antibody of any one of claims 1-14, wherein the antibody is $IgG_1$, $IgG_2$, $IgG_3$, or $IgG_4$.

44. The isolated antibody of any one of claims 1-14, wherein the antibody is $IgG_{1\lambda}$ or $IgG_{1\kappa}$.

45. The monoclonal antibody or antigen-binding portion thereof of any one of claims 1-14, wherein the monoclonal antibody or antigen-binding portion thereof binds to SSEA4 with a $K_D$ of $1\times10^{-7}$M or less, and wherein the $K_D$ is measured by surface plasmon resonance (Biacore) analysis.

46. The isolated anti-SSEA4 antibody or binding fragment thereof of claim 45 whererin the binding affinity is < 50 nM.

# FIG. 1A

The CDRs of representative anti-SSEA4 antibody chAb6 in Kabat, AbM, Chothia, Contact, and IMGT method, respectively

**H-CDR1**

| *Method* | *Definition* | *Sequence* |
|---|---|---|
| Kabat | H31-H35B | NYGVS |
| AbM | H26-H35 | GFSLKNYGVS |
| Chothia | H26-H32...H34 | GFSLKNY[GV] |
| Contact | H30-H35 | KNYGVS |
| IMGT | Online prediction | GFSLKNYG |

**H-CDR2**

| *Method* | *Definition* | *Sequence* |
|---|---|---|
| Kabat | H50-H65 | VIWGDGSTNYHSTLRS |
| AbM | H50-H58 | VIWGDGSTN |
| Chothia | H52-H56 | WGDGS |
| Contact | H47-H58 | WLGVIWGDGSTN |
| IMGT | Online prediction | IWGDGST |

**H-CDR3**

| *Method* | *Definition* | *Sequence* |
|---|---|---|
| Kabat | H95-H102 | PGAGYAMDY |
| AbM | H95-H102 | PGAGYAMDY |
| Chothia | H95-H102 | PGAGYAMDY |
| Contact | H93-H101 | AKPGAGYAMD |
| IMGT | Online prediction | AKPGAGYAMDY |

**L-CDR1**

| *Method* | *Definition* | *Sequence* |
|---|---|---|
| Kabat | L24-L34 | SASSSVSYMH |
| AbM | L24-L34 | SASSSVSYMH |
| Chothia | L24-L34 | SASSSVSYMH |
| Contact | L30-L36 | VSYMHWY |
| IMGT | Online prediction | SSVSY |

**L-CDR2**

| *Method* | *Definition* | *Sequence* |
|---|---|---|
| Kabat | L50-L56 | DTSKLTS |
| AbM | L50-L56 | DTSKLTS |
| Chothia | L50-L56 | DTSKLTS |
| Contact | L46-L55 | LWIYDTSKLT |
| IMGT | Online prediction | DTS |

**L-CDR3**

| *Method* | *Definition* | *Sequence* |
|---|---|---|
| Kabat | L89-L97 | FQGSGYPLT |
| AbM | L89-L97 | FQGSGYPLT |
| Chothia | L89-L97 | FQGSGYPLT |
| Contact | L89-L96 | FQGSGYPL |
| IMGT | Online prediction | FQGSGYPLT |

FIG. 1B

**Heavy Chain**

| | | |
|---|---|---|
| | | **H-CDR1** **H-CDR2** |
| chAb6 | QVQLKESGPGLVAPSQSLSITCTVSGFSLKNYGVSWVRQPPGKGLEWLGVIWGDGSTNYH | |
| hAb6–3.1 | QVQLQESGPGLVAPSQSLSITCTVSGFSLKNYGVSWVRQPPGKGLEWLGVIWGDGSTNYH | |
| | | **H-CDR3** |
| chAb6 | STLRSRLTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTSVTVSS | |
| hAb6–3.1 | STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS | |

**Light Chain**

| | | |
|---|---|---|
| | | **L-CDR1** **L-CDR2** |
| chAb6 | QIVLTQSPAIMSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR | |
| hAb6–3.1 | EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR | |
| | | **L-CDR3** |
| chAb6 | FSGSGSGNSYSLTISSMEAEDVATYYCFQGSGYPLTFGGGTKLEIKR | |
| hAb6–3.1 | FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKLEIKR | |

FIG. 1C

**Heavy Chain**

chAb6 QVQLKESGPGLVAPSQSLSITCTVSGFSLKNYGVSWVRQPPGKGLEWLGVIWGDGSTNYH
hAb6–2 QVQLKESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH
hAb6–3 QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH

chAb6 STLRSRLTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTSVTVSS
hAb6–2 STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTSVTVSS
hAb6–3 STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTLVTVSS

**Light Chain**

chAb6 QIVLTQSPAIMSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–2 EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3 EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR

chAb6 FSGSGSGNSYSLTISSMEAEDVATYYCFQGSGYPLTFGGGTKLEIKR
hAb6–2 FSGSGSGNSYTLTISSMEAEDVATYYCFQGSGYPLTFGGGTKLEIKR
hAb6–3 FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR

FIG. 1D

**Heavy Chain**

| | |
|---|---|
| hAb6–3 | QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH |
| hAb6–3.1 | QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH |
| hAb6–3.2 | QVQLQESGPGLVAPSQTLSITCTVSGFSLKSYGVSWVRQPPGKGLEWIGVIWGDGSTNYH |
| hAb6–3.3 | QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH |
| hAb6–3.4 | QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH |

| | |
|---|---|
| hAb 6–3 | STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTLVTVSS |
| hAb6–3.1 | STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS |
| hAb6–3.2 | STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS |
| hAb6–3.3 | SALRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS |
| hAb6–3.4 | STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS |

**Light chain**

| | |
|---|---|
| hAb6–3 | EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR |
| hAb6–3.1 | EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR |
| hAb6–3.2 | EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR |
| hAb6–3.3 | EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR |
| hAb6–3.4 | EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTYKLTSGVPGR |

| | |
|---|---|
| hAb6–3 | FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR |
| hAb6–3.1 | FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR |
| hAb6–3.2 | FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR |
| hAb6–3.3 | FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR |
| hAb6–3.4 | FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR |

FIG. 1E

**Heavy Chain**

```
hAb6–3      QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH
hAb6–3.101  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGAIWGDGSTNYH
hAb6–3.103  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWADGSTNYH
hAb6–3.105  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVTWVRQPPGKGLEWIGVIWGDGSTNYH
hAb6–3.106  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH
hAb6–3.107  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH
hAb6–3.108  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH
hAb6–3.110  QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYH

hAb6–3      STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTLVTVSS
hAb6–3.101  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
hAb6–3.103  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
hAb6–3.105  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
hAb6–3.106  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
hAb6–3.107  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
hAb6–3.108  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
hAb6–3.110  STLRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGRGYAMDYWGQGTLVTVSS
```

**Light Chain**

```
hAb6–3      EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.101  EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.103  EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.105  EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.106  EIVLTQSPAIQSVYPGEKVTMTCSASSSISYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.107  EIVLTQSPAIQSVYPGEKVTMTCSASSSASYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.108  EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR
hAb6–3.110  EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGR

hAb6–3      FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR
hAb6–3.101  FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR
hAb6–3.103  FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR
hAb6–3.105  FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR
hAb6–3.106  FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR
hAb6–3.107  FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR
hAb6–3.108  FSGSGSGNSYTLTISSMEAEDAATYYCFQASGYPLTFGGGTKVEIKR
hAb6–3.110  FSGSGSGNSYTLTISSMEAEDAATYYCFQGSGFPLTFGGGTKVEIKR
```

# FIG. 2

## chAb6

**Heavy chain**

QVQLKESGPGLVAPSQSLSITCTVSGFSLKNYGVSWVRQPPGKGLEWLGVIWGDGSTNYHST
LRSRLTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTSVTVSS

**Light chain**

QIVLTQSPAIMSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGRFS
GSGSGNSYSLTISSMEAEDVATYYCFQGSGYPLTFGGGTKLEIKR

## humanized Ab6-2

**Heavy chain**

QVQLKESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYHST
LRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTSVTVSS

**Light chain**

EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGRFS
GSGSGNSYTLTISSMEAEDVATYYCFQGSGYPLTFGGGTKLEIKR

## humanized Ab6-3

**Heavy chain**

QVQLQESGPGLVAPSQTLSITCTVSGFSLKNYGVSWVRQPPGKGLEWIGVIWGDGSTNYHST
LRSRVTISKDNSKSQLFLKLNRLQTDDTATYYCAKPGAGYAMDYWGQGTLVTVSS

**Light chain**

EIVLTQSPAIQSVYPGEKVTMTCSASSSVSYMHWYQQKSSTSPKLWIYDTSKLTSGVPGRFS
GSGSGNSYTLTISSMEAEDAATYYCFQGSGYPLTFGGGTKVEIKR

FIG. 3

| H01 | H02 | H03 | H04 | H05 | H06 | H07 | H08 | H09 | H10 | H11 | H12 | H13 | H14 | H15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Q** | **V** | **Q** | **L** | **K** | **E** | **S** | **G** | **P** | **G** | **L** | **V** | **A** | **P** | **S** |

| H16 | H17 | H18 | H19 | H20 | H21 | H22 | H23 | H24 | H25 | H26 | H27 | H28 | H29 | H30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Q** | **S** | **L** | **S** | **I** | **T** | **C** | **T** | **V** | **S** | **G** | **F** | **S** | **L** | **K** |

| H31 | H32 | H33 | H34 | H35 | H36 | H37 | H38 | H39 | H40 | H41 | H42 | H43 | H44 | H45 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | **Y** | **G** | **V** | **S** | **W** | **V** | **R** | **Q** | **P** | **P** | **G** | **K** | **G** | **L** |

| H46 | H47 | H48 | H49 | H50 | H51 | H52 | H53 | H54 | H55 | H56 | H57 | H58 | H59 | H60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E** | **W** | **L** | **G** | **V** | **I** | **W** | **G** | **D** | **G** | **S** | **T** | **N** | **Y** | **H** |

| H61 | H62 | H63 | H64 | H65 | H66 | H67 | H68 | H69 | H70 | H71 | H72 | H73 | H74 | H75 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S** | **T** | **L** | **R** | **S** | **R** | **L** | **T** | **I** | **S** | **K** | **D** | **N** | **S** | **K** |

| H76 | H77 | H78 | H79 | H80 | H81 | H82 | H82A | H82B | H82C | H83 | H84 | H85 | H86 | H87 | H88 | H89 | H90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S** | **Q** | **L** | **F** | **L** | **K** | **L** | **N** | **R** | **L** | **Q** | **T** | **D** | **D** | **T** | **A** | **T** | **Y** |

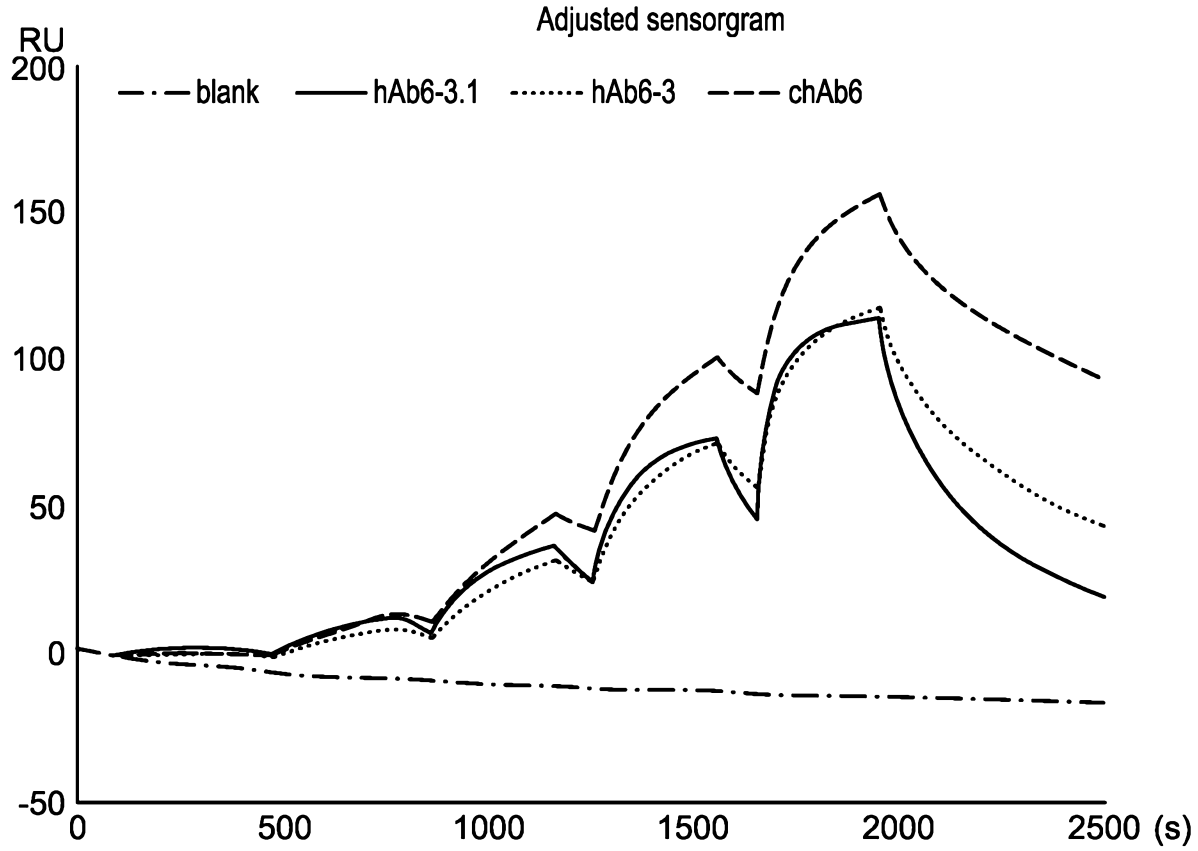
| H91 | H92 | H93 | H94 | H95 | H96 | H97 | H98 | H99 | H100 | H100A | H101 | H102 | H103 | H104 | H105 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Y** | **C** | **A** | **K** | **P** | **G** | **A** | **G** | **Y** | **A** | **M** | **D** | **Y** | **W** | **G** | **Q** |

| H106 | H107 | H108 | H109 | H110 | H111 | H112 | H113 | H114 |
|---|---|---|---|---|---|---|---|---|
| **G** | **T** | **S** | **V** | **T** | **V** | **S** | **S** | **-** |

FIG. 4

| L01 | L02 | L03 | L04 | L05 | L06 | L07 | L08 | L09 | L10 | L11 | L12 | L13 | L14 | L15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Q | I | V | L | T | Q | S | P | A | I | M | S | V | Y | P |
| L16 | L17 | L18 | L19 | L20 | L21 | L22 | L23 | L24 | L25 | L26 | L27 | L28 | L29 | L30 |
| G | E | K | V | T | M | T | C | S | A | S | S | - | S | V |
| L31 | L32 | L33 | L34 | L35 | L36 | L37 | L38 | L39 | L40 | L41 | L42 | L43 | L44 | L45 |
| S | Y | M | H | W | Y | Q | Q | K | S | S | T | S | P | K |
| L46 | L47 | L48 | L49 | L50 | L51 | L52 | L53 | L54 | L55 | L56 | L57 | L58 | L59 | L60 |
| L | W | I | Y | D | T | S | K | L | T | S | G | V | P | G |
| L61 | L62 | L63 | L64 | L65 | L66 | L67 | L68 | L69 | L70 | L71 | L72 | L73 | L74 | L75 |
| R | F | S | G | S | G | S | G | N | S | Y | S | L | T | I |
| L76 | L77 | L78 | L79 | L80 | L81 | L82 | L83 | L84 | L85 | L86 | L87 | L88 | L89 | L90 |
| S | S | M | E | A | E | D | V | A | T | Y | Y | C | F | Q |
| L91 | L92 | L93 | L94 | L95 | L96 | L97 | L98 | L99 | L100 | L101 | L102 | L103 | L104 | L105 |
| G | S | G | Y | P | L | T | F | G | G | G | T | K | L | E |
| L106 | L107 | L108 | L109 | L110 | L111 | | | | | | | | | |
| I | K | R | - | - | - | | | | | | | | | |

# FIG. 5




| Mode | 1:1 Binding | | | | |
|---|---|---|---|---|---|
| Parameter | ka (1/Ms) | kd (1/s) | KD (nM) | Rmax (RU) | $Chi^2$ ($RU^2$) |
| hAb6-3.1 | $2.4x10^5$ | $5.6x10^{-3}$ | 23.1 | 128.16 | 2.74 |
| hAb6-3 | $1.7x10^5$ | $3.0x10^{-3}$ | 17.8 | 131.08 | 1.99 |
| chAb6 | $0.9x10^5$ | $0.9x10^{-3}$ | 10.11 | 164.90 | 3.43 |

FIG. 6A

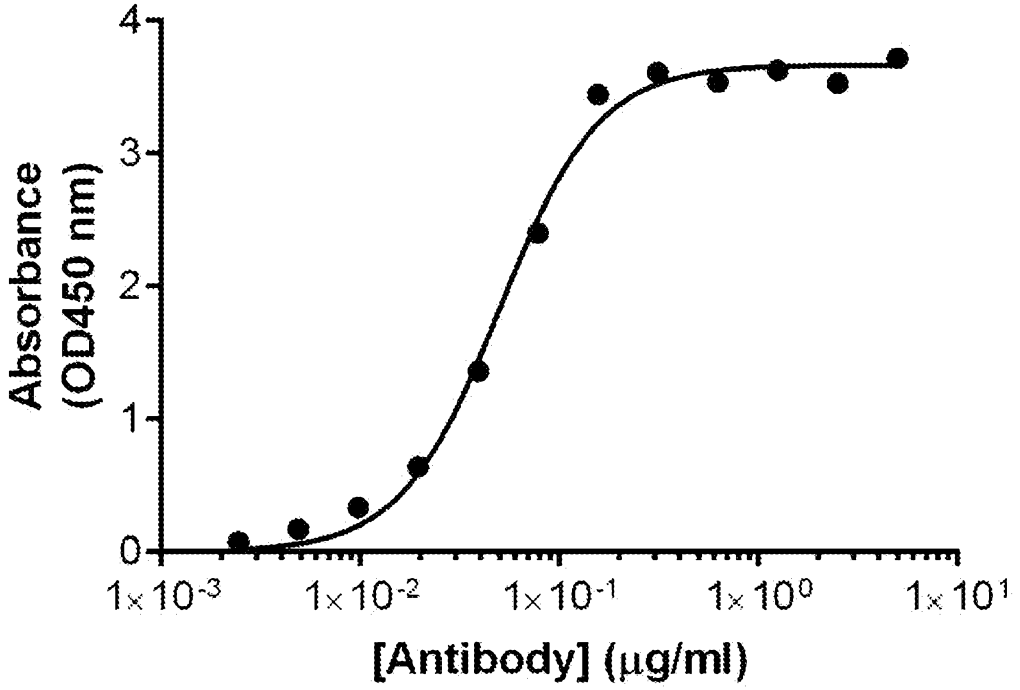

Absorbance (OD450 nm)
4
3
2
1
0
1×10-3
1×10-2
1×10-1
1×100
1×101
[Antibody] (μg/ml)

FIG. 6B

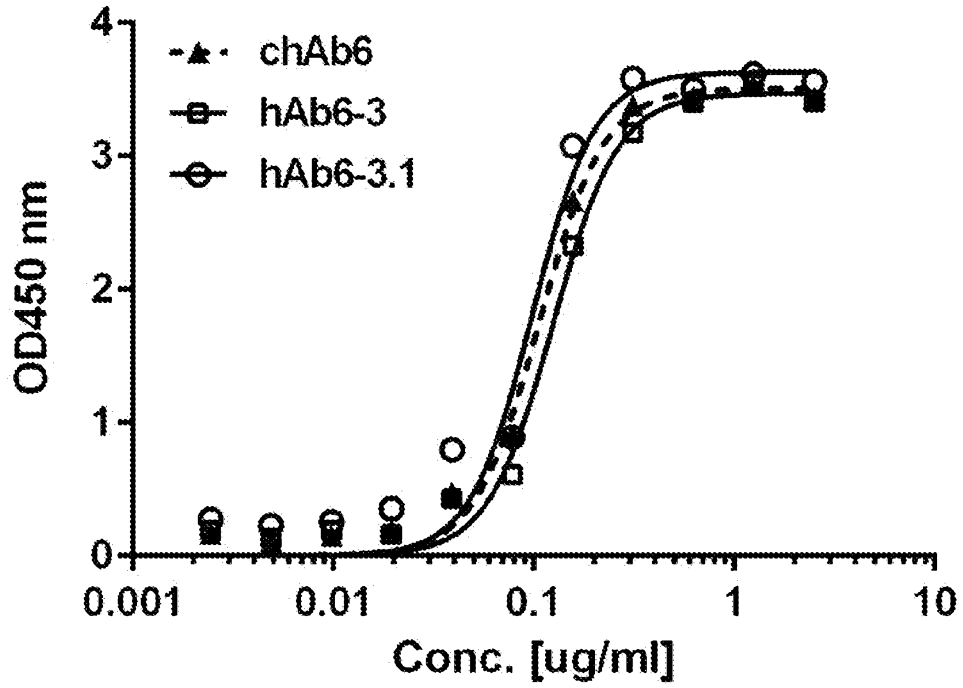

chAb6
hAb6-3
hAb6-3.1
OD450 nm
4
3
2
1
0
0.001
0.01
0.1
1
10
Conc. [ug/ml]

FIG. 6C

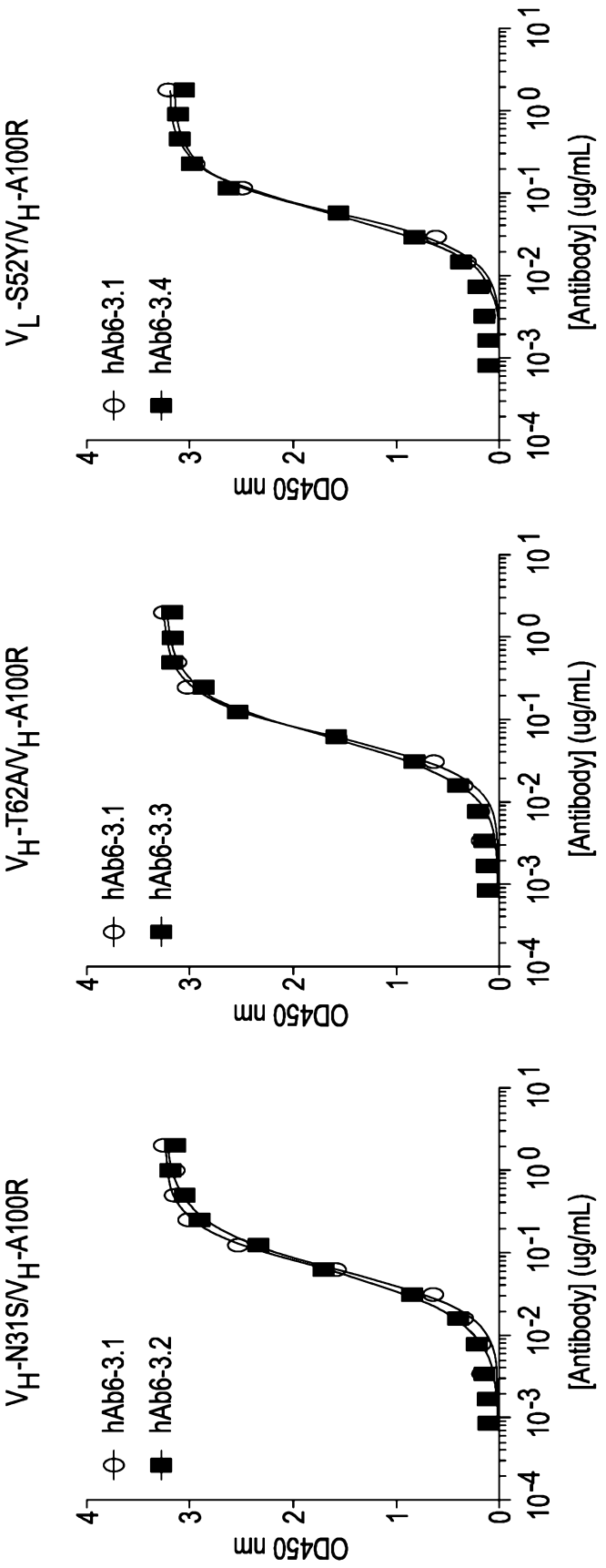
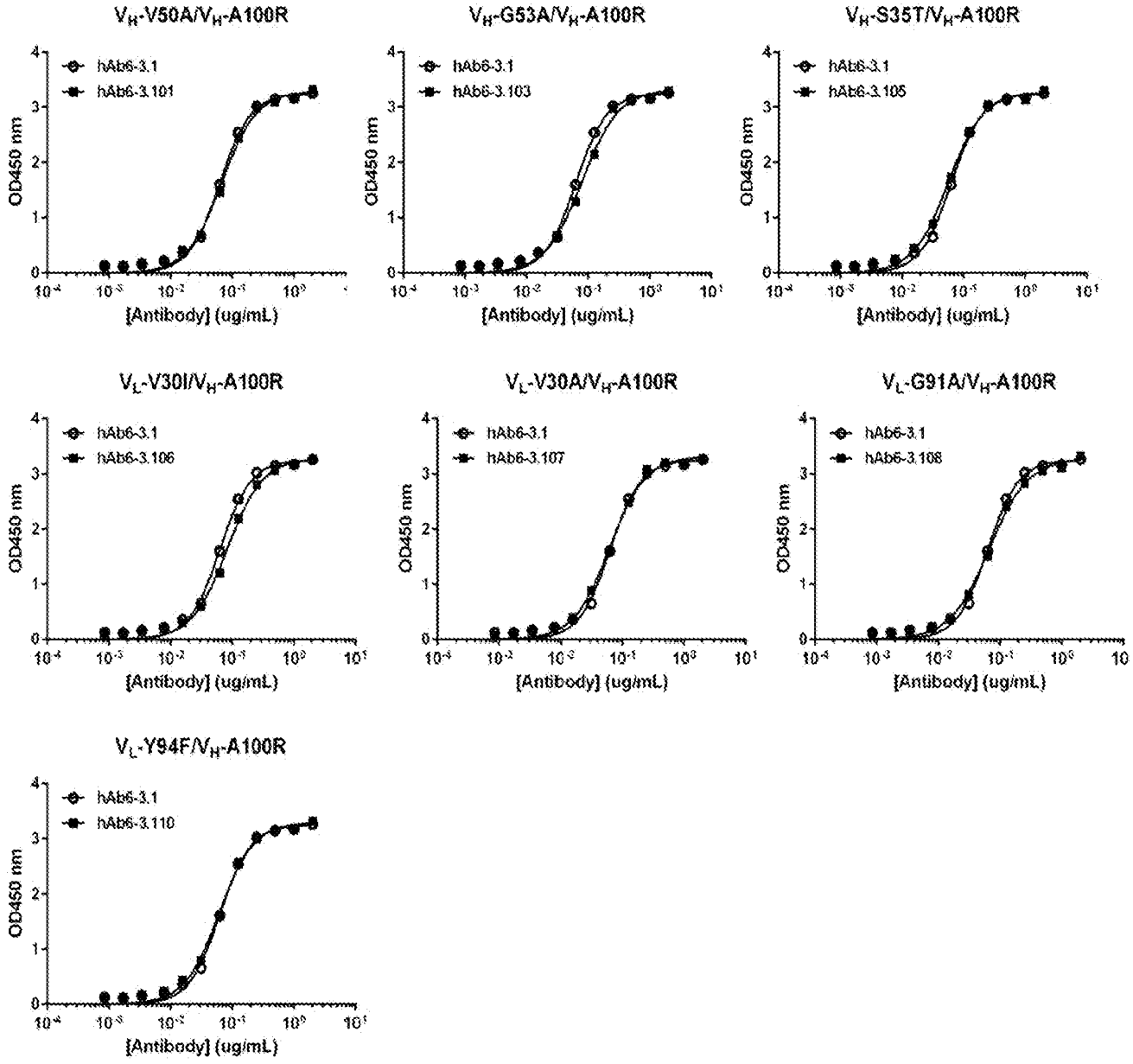

$V_H$-N31S/$V_H$-A100R
hAb6-3.1
hAb6-3.2
OD450 nm
[Antibody] (ug/mL)
$V_H$-T62A/$V_H$-A100R
hAb6-3.1
hAb6-3.3
OD450 nm
[Antibody] (ug/mL)
$V_L$-S52Y/$V_H$-A100R
hAb6-3.1
hAb6-3.4
OD450 nm
[Antibody] (ug/mL)

FIG. 6D


$V_H$-V50A/$V_H$-A100R
hAb6-3.1
hAb6-3.101
$V_H$-G53A/$V_H$-A100R
hAb6-3.1
hAb6-3.103
$V_H$-S35T/$V_H$-A100R
hAb6-3.1
hAb6-3.105
$V_L$-V30I/$V_H$-A100R
hAb6-3.1
hAb6-3.106
$V_L$-V30A/$V_H$-A100R
hAb6-3.1
hAb6-3.107
$V_L$-G91A/$V_H$-A100R
hAb6-3.1
hAb6-3.108
$V_L$-Y94F/$V_H$-A100R
hAb6-3.1
hAb6-3.110
OD450 nm
[Antibody] (ug/mL)

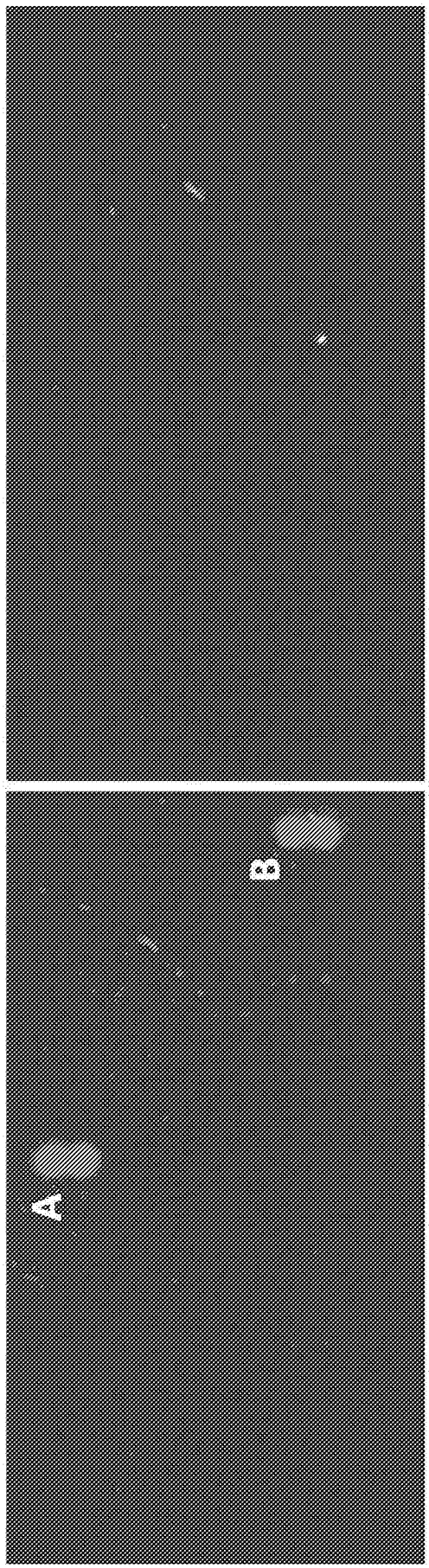



FIG. 7

FIG. 8A

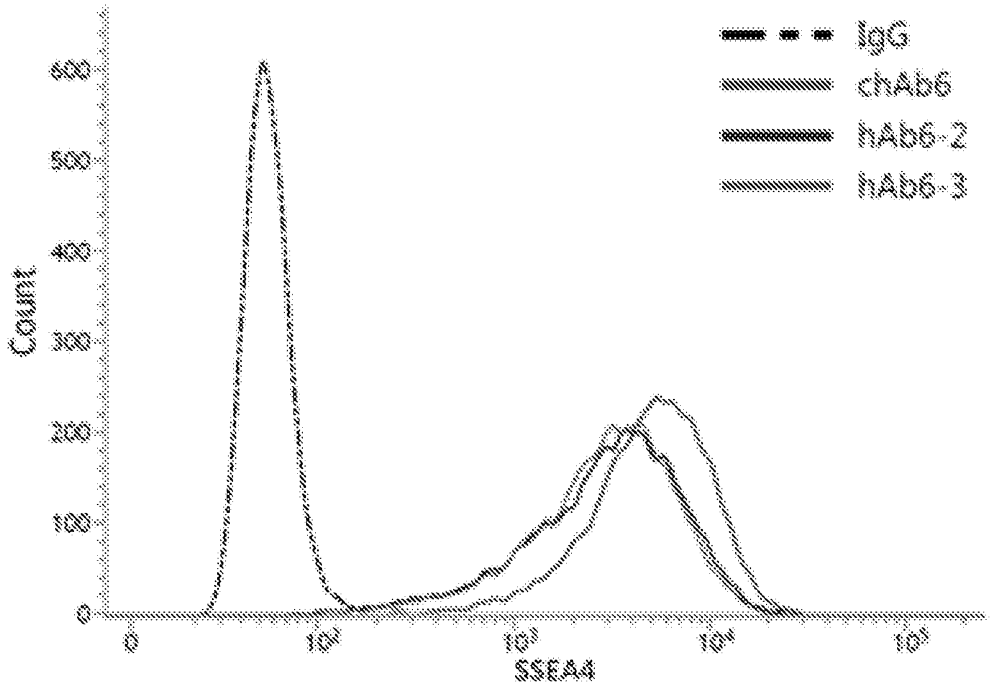

IgG
chAb6
hAb6-2
hAb6-3
Count
SSEA4

FIG. 8B

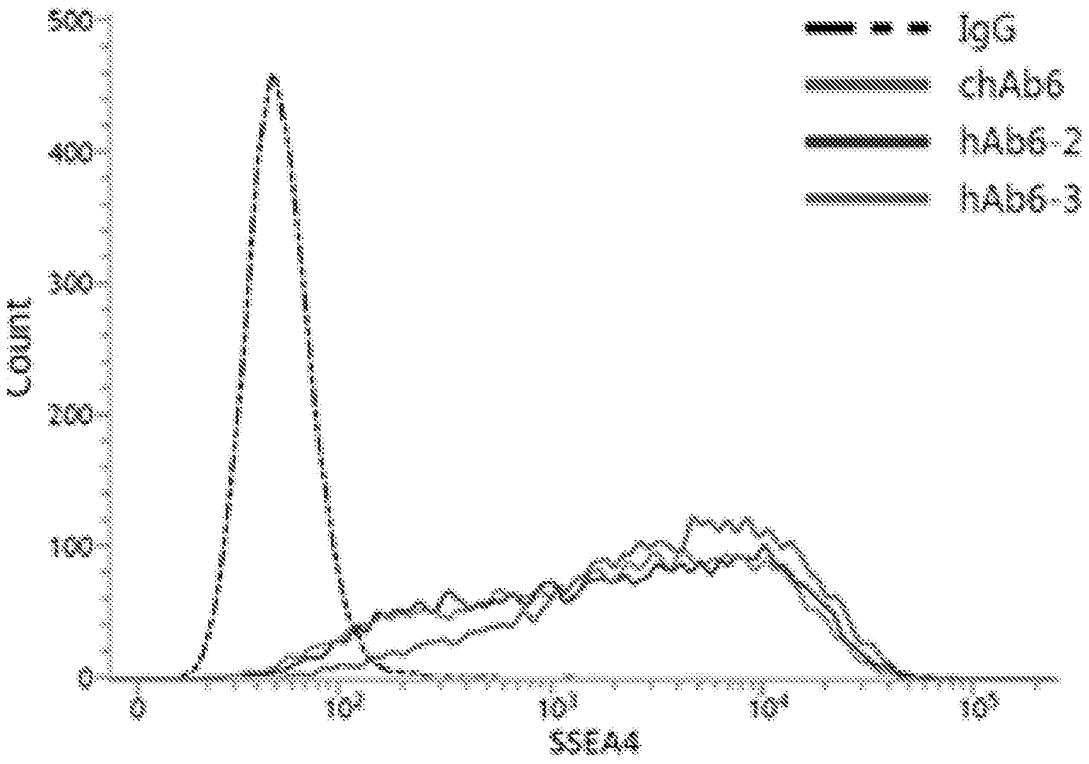

IgG
chAb6
hAb6-2
hAb6-3
Count
SSEA4

**FIG. 9A**

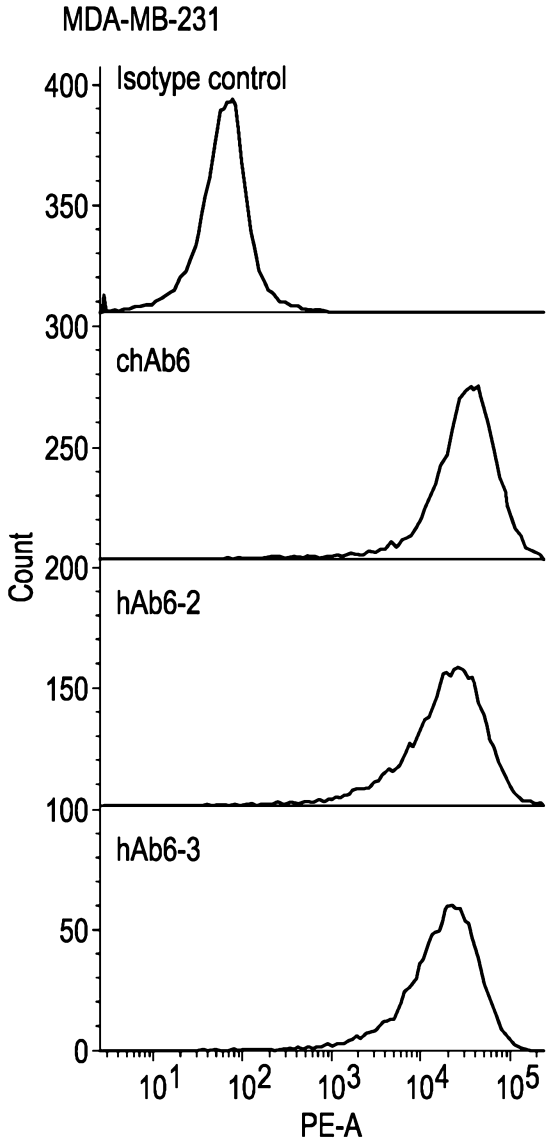

MDA-MB-231
Isotype control
chAb6
hAb6-2
hAb6-3
Count
PE-A
0
50
100
150
200
250
300
350
400
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$

**FIG. 9B**

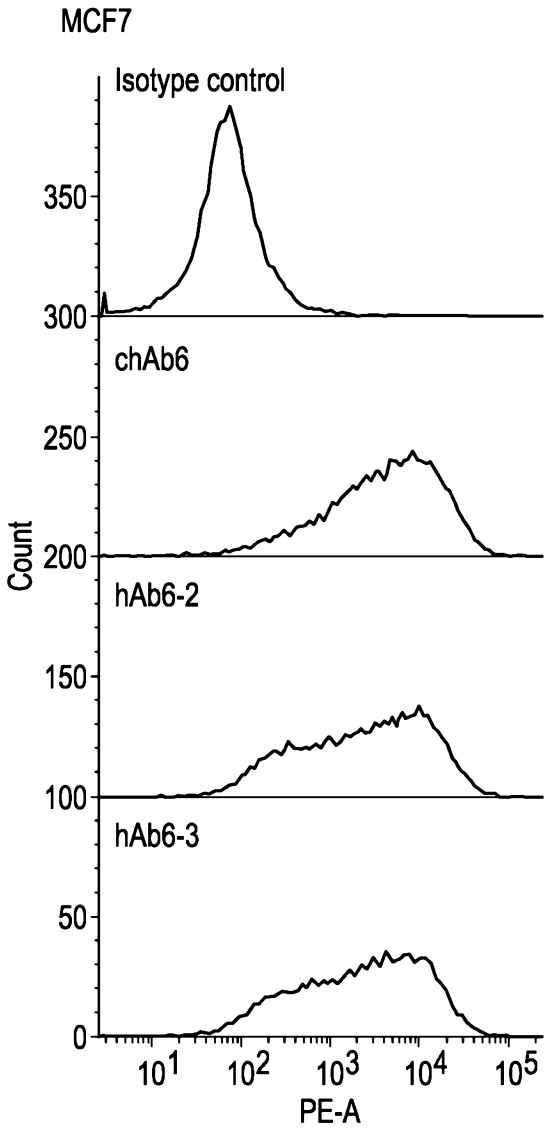

MCF7
Isotype control
chAb6
hAb6-2
hAb6-3
Count
PE-A
0
50
100
150
200
250
300
350
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$

FIG. 10A

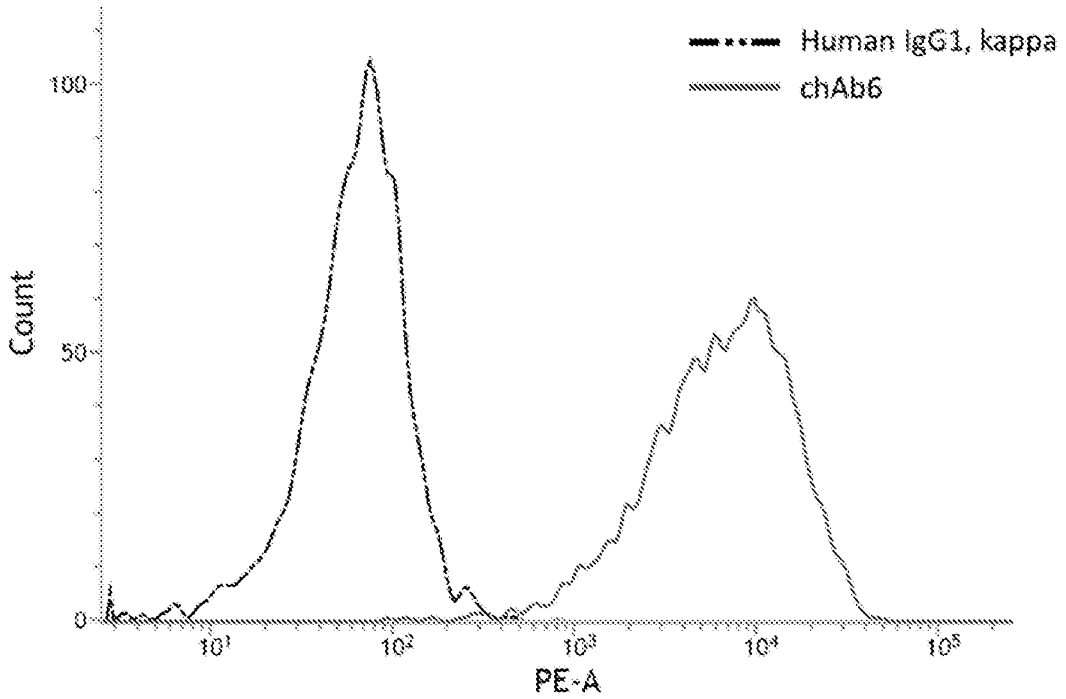



FIG. 10B

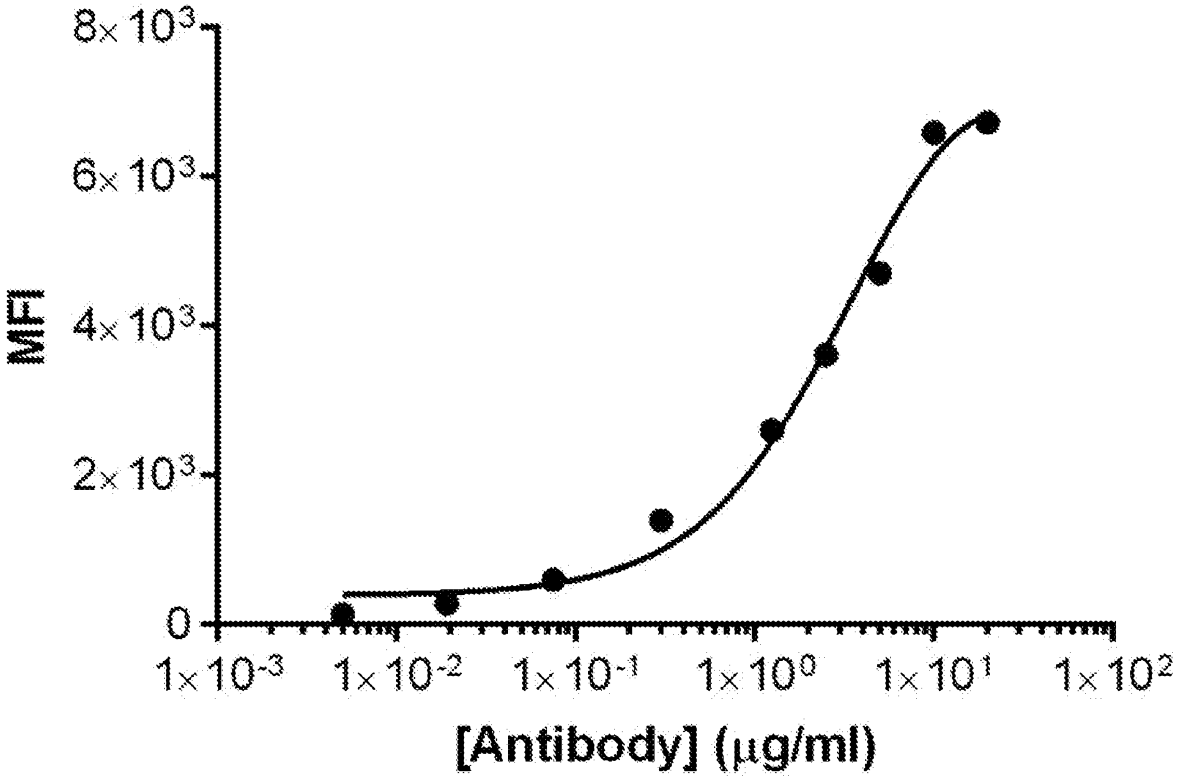


**FIG. 11A**

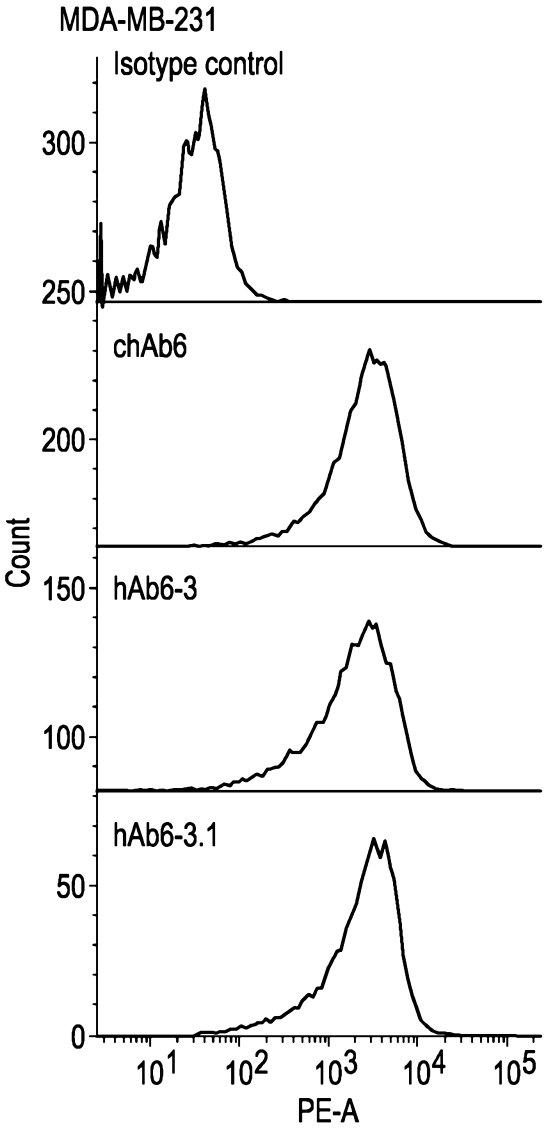



**FIG. 11B**

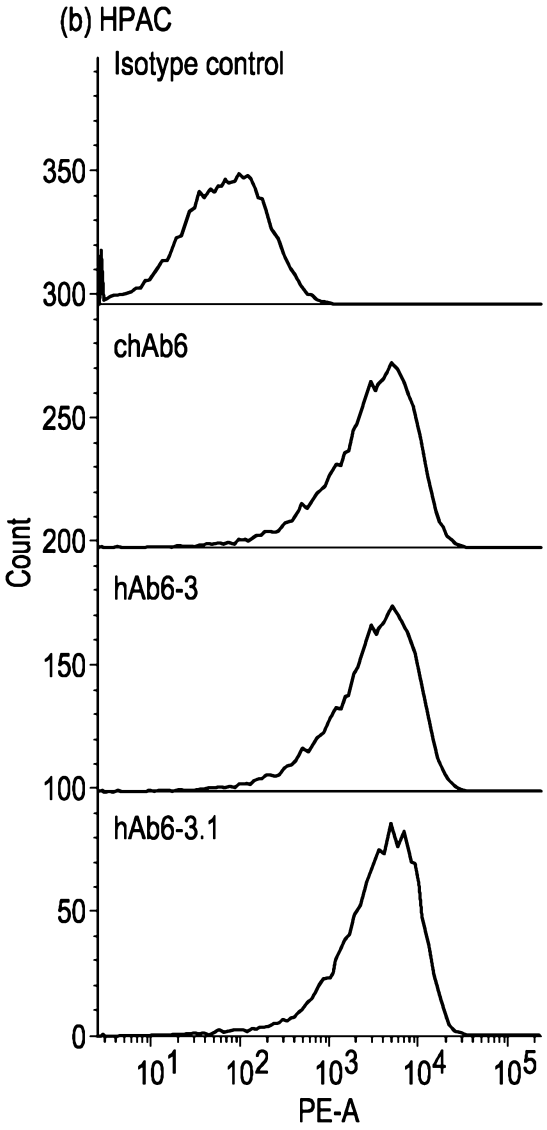


**FIG. 11C**

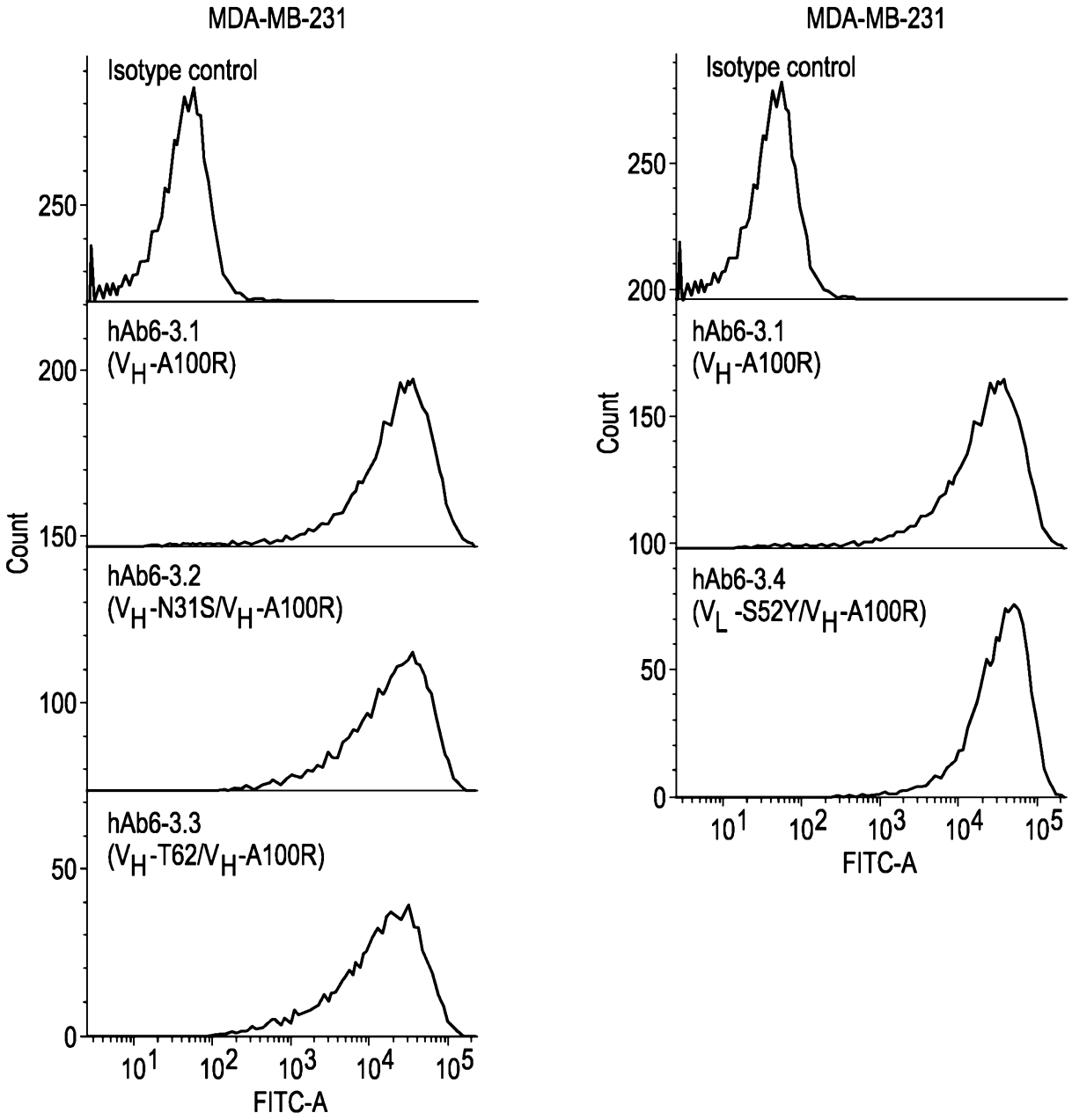
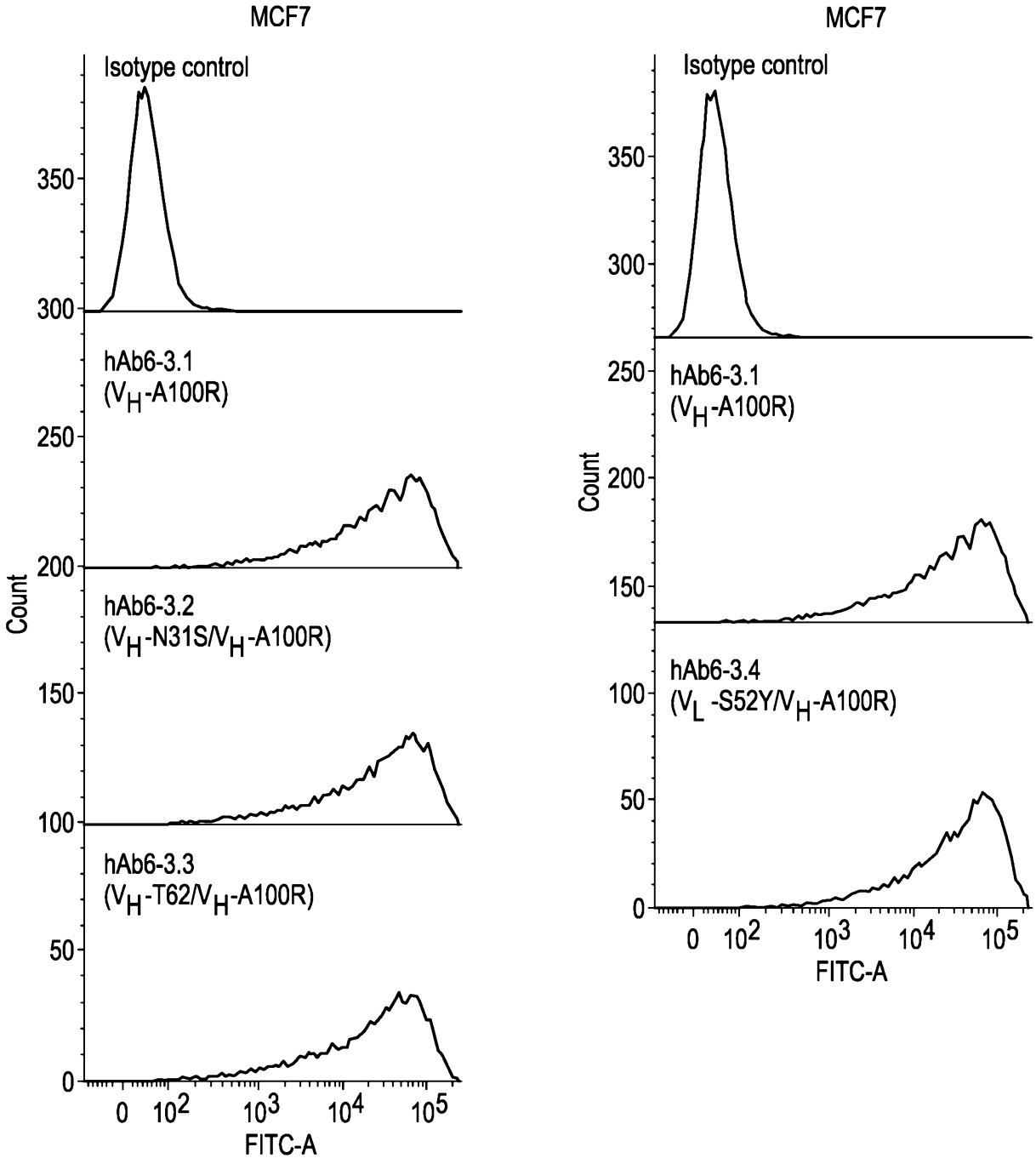
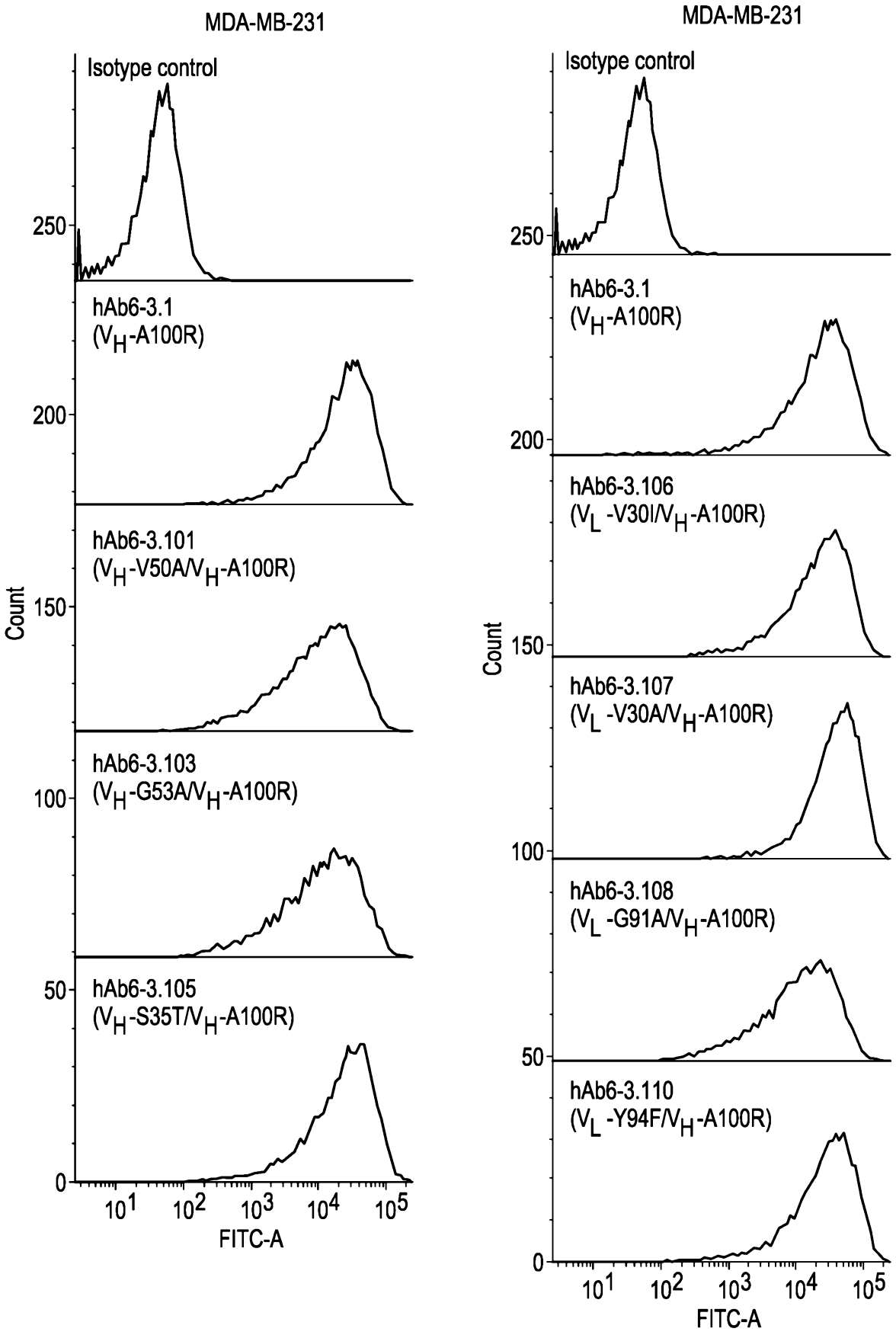

MDA-MB-231
Isotype control
hAb6-3.1
($V_H$-A100R)
hAb6-3.2
($V_H$-N31S/$V_H$-A100R)
hAb6-3.3
($V_H$-T62/$V_H$-A100R)
Count
FITC-A
MDA-MB-231
Isotype control
hAb6-3.1
($V_H$-A100R)
hAb6-3.4
($V_L$-S52Y/$V_H$-A100R)
Count
FITC-A

**FIG. 11D**


MCF7
Isotype control
hAb6-3.1
($V_H$-A100R)
hAb6-3.2
($V_H$-N31S/$V_H$-A100R)
hAb6-3.3
($V_H$-T62/$V_H$-A100R)
Count
FITC-A
MCF7
Isotype control
hAb6-3.1
($V_H$-A100R)
hAb6-3.4
($V_L$ -S52Y/$V_H$-A100R)
Count
FITC-A

# FIG. 11E


MDA-MB-231
Isotype control
hAb6-3.1
($V_H$-A100R)
hAb6-3.101
($V_H$-V50A/$V_H$-A100R)
hAb6-3.103
($V_H$-G53A/$V_H$-A100R)
hAb6-3.105
($V_H$-S35T/$V_H$-A100R)
Count
FITC-A
MDA-MB-231
Isotype control
hAb6-3.1
($V_H$-A100R)
hAb6-3.106
($V_L$ -V30I/$V_H$-A100R)
hAb6-3.107
($V_L$ -V30A/$V_H$-A100R)
hAb6-3.108
($V_L$ -G91A/$V_H$-A100R)
hAb6-3.110
($V_L$ -Y94F/$V_H$-A100R)
Count
FITC-A

# FIG. 11F

Demonstration of the binding of exemplary humanized Ab6s with conservative CDR modifications to MCF7 cell line.

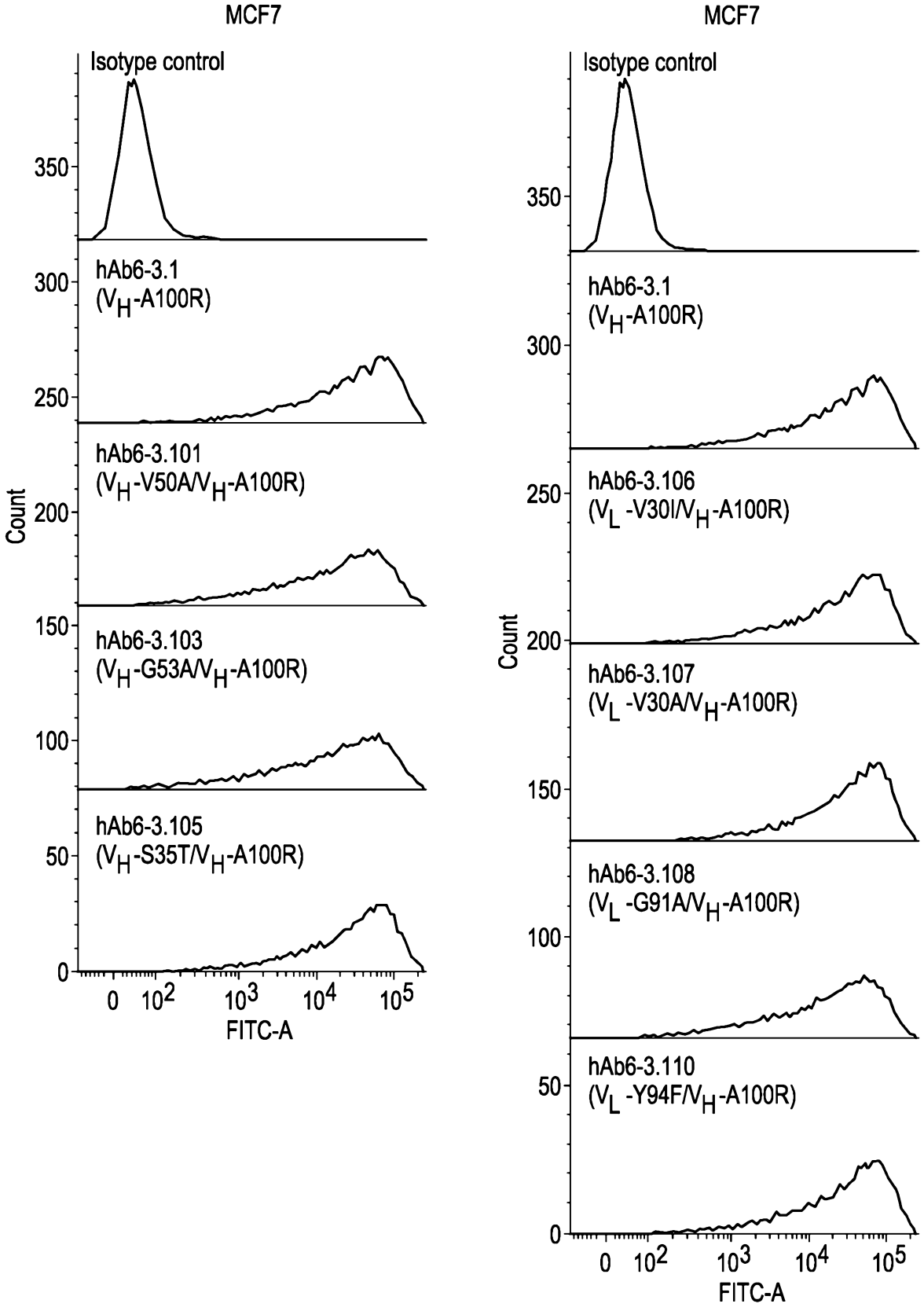


**FIG. 12**

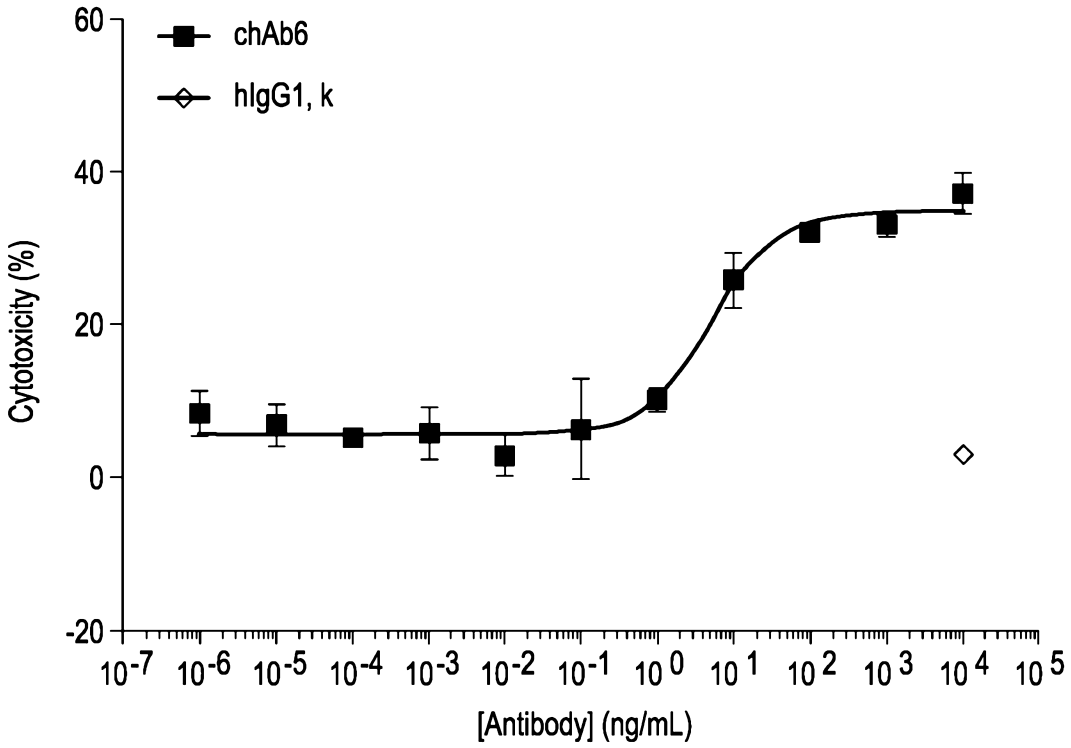

chAb6
hIgG1, k
Cytotoxicity (%)
60
40
20
0
-20
10-7 10-6 10-5 10-4 10-3 10-2 10-1 10 0 10 1 10 2 10 3 10 4 10 5
[Antibody] (ng/mL)

FIG. 13

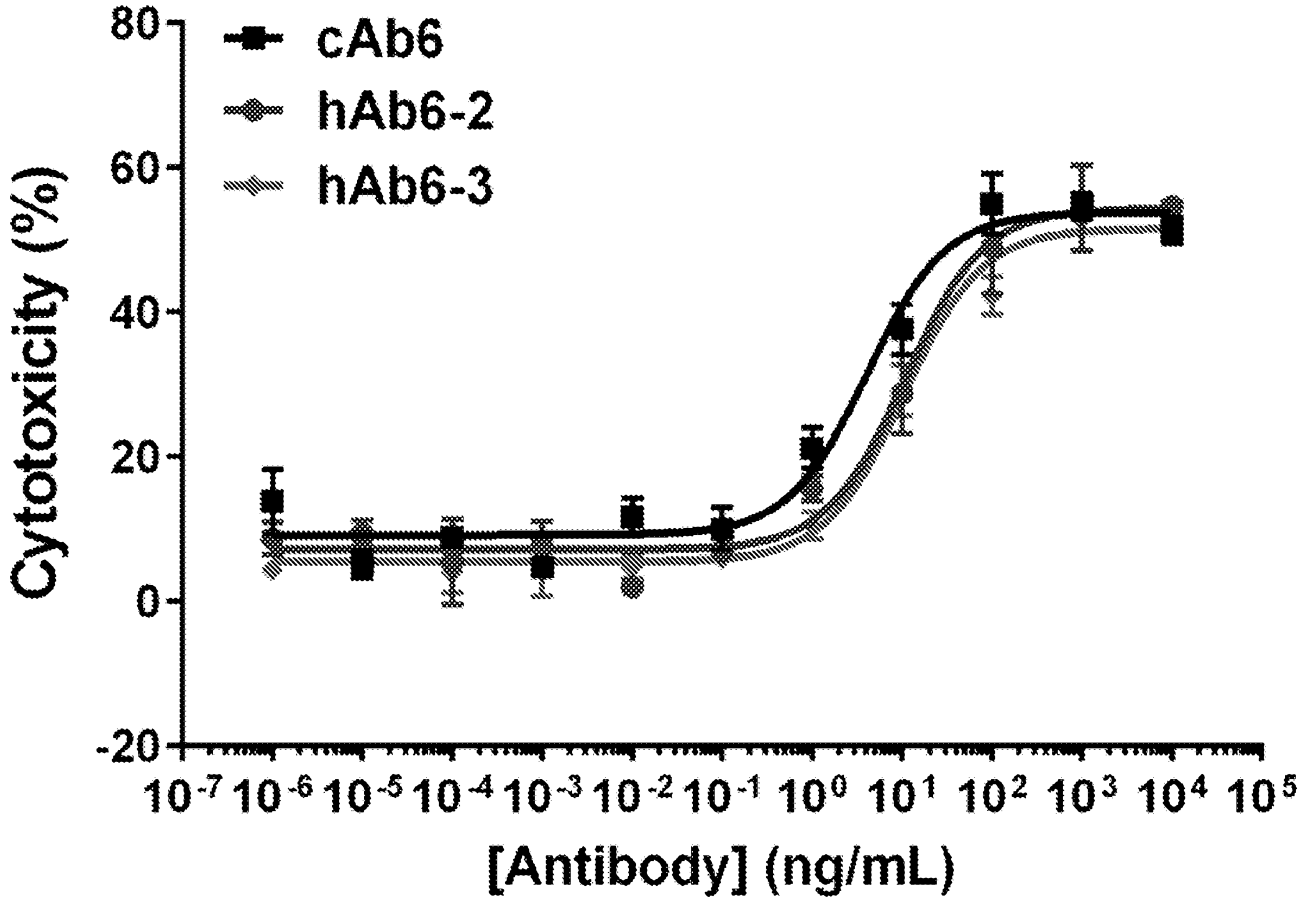

cAb6
hAb6-2
hAb6-3
Cytotoxicity (%)
80
60
40
20
0
-20
10-7 10-6 10-5 10-4 10-3 10-2 10-1 100 101 102 103 104 105
[Antibody] (ng/mL)

**FIG. 14A**

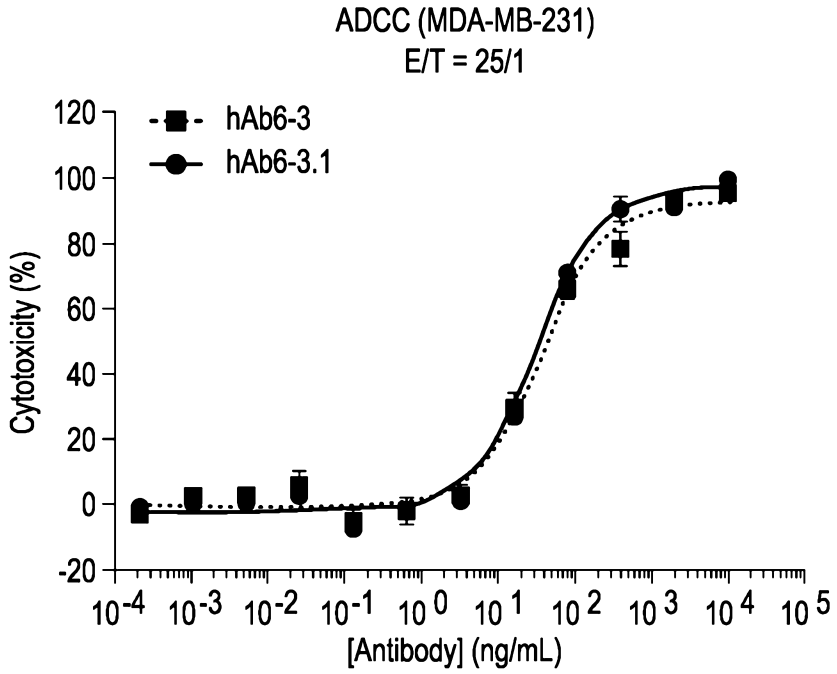



**FIG. 14B**

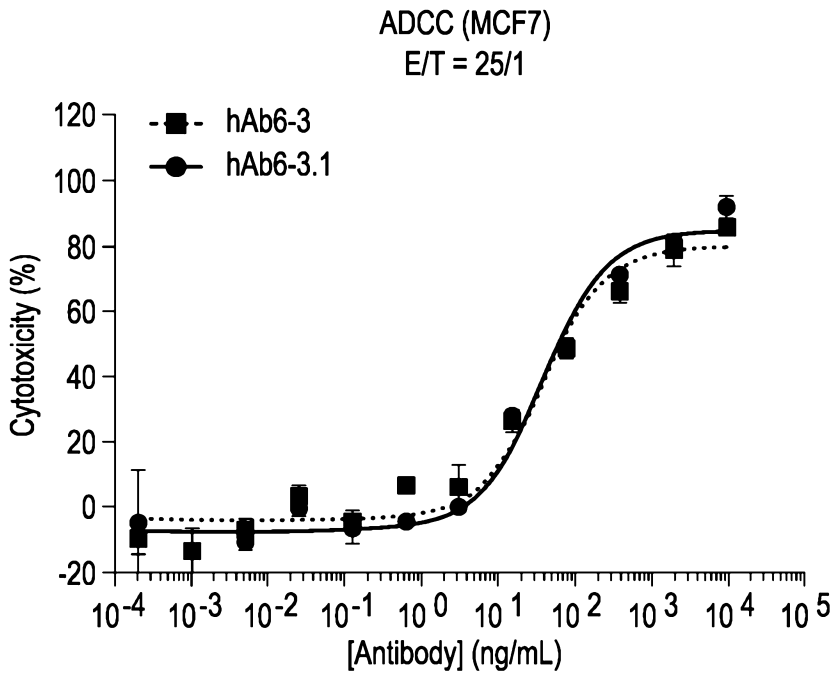


FIG. 15A

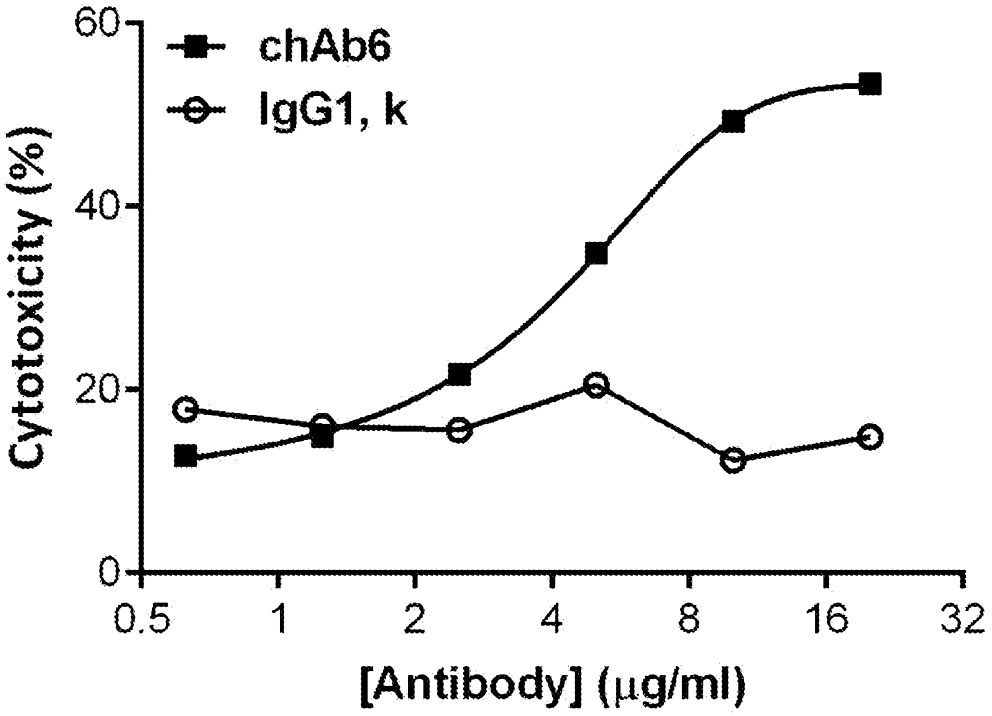



FIG. 15B

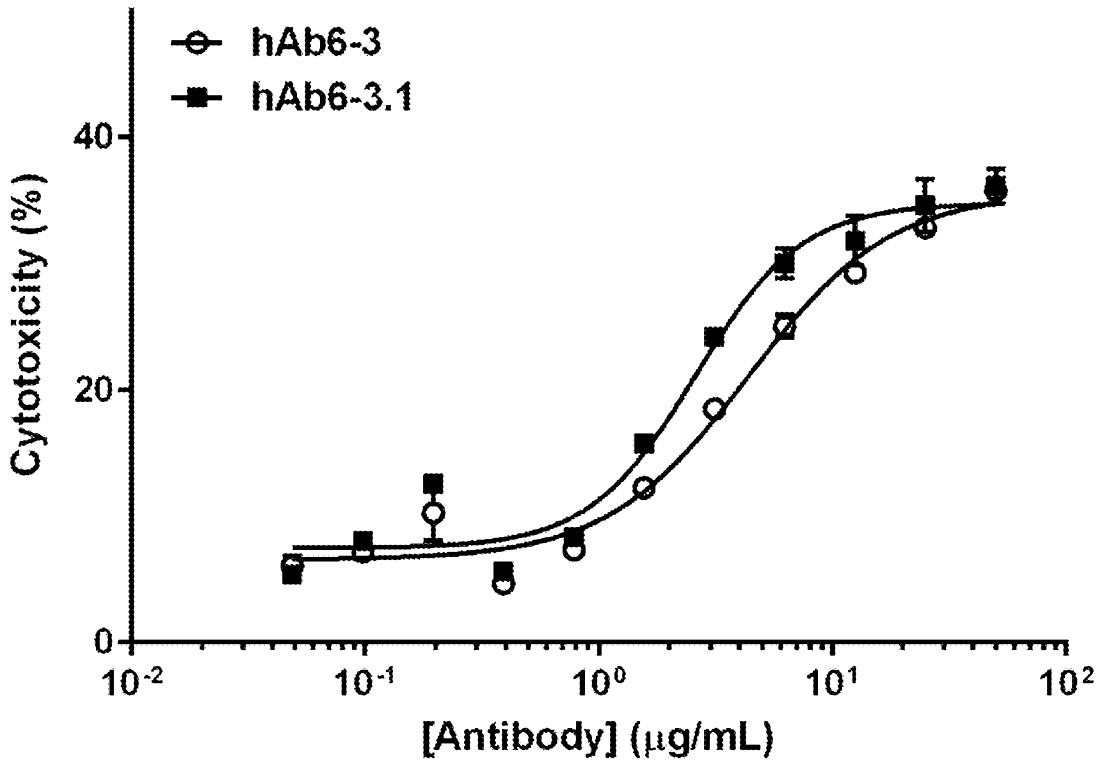


FIG. 16A

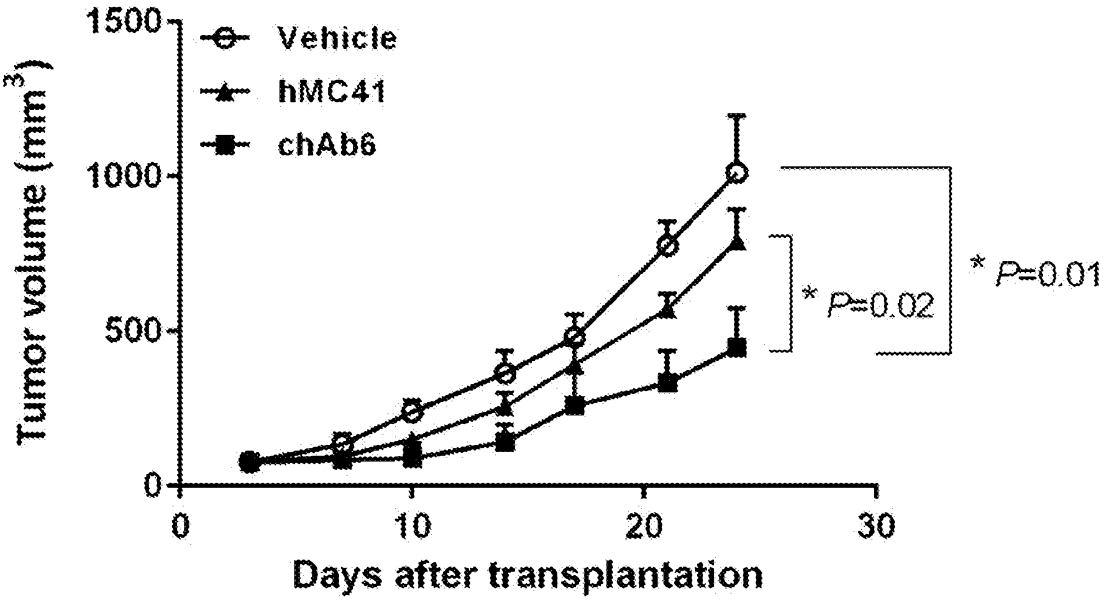



FIG. 16B

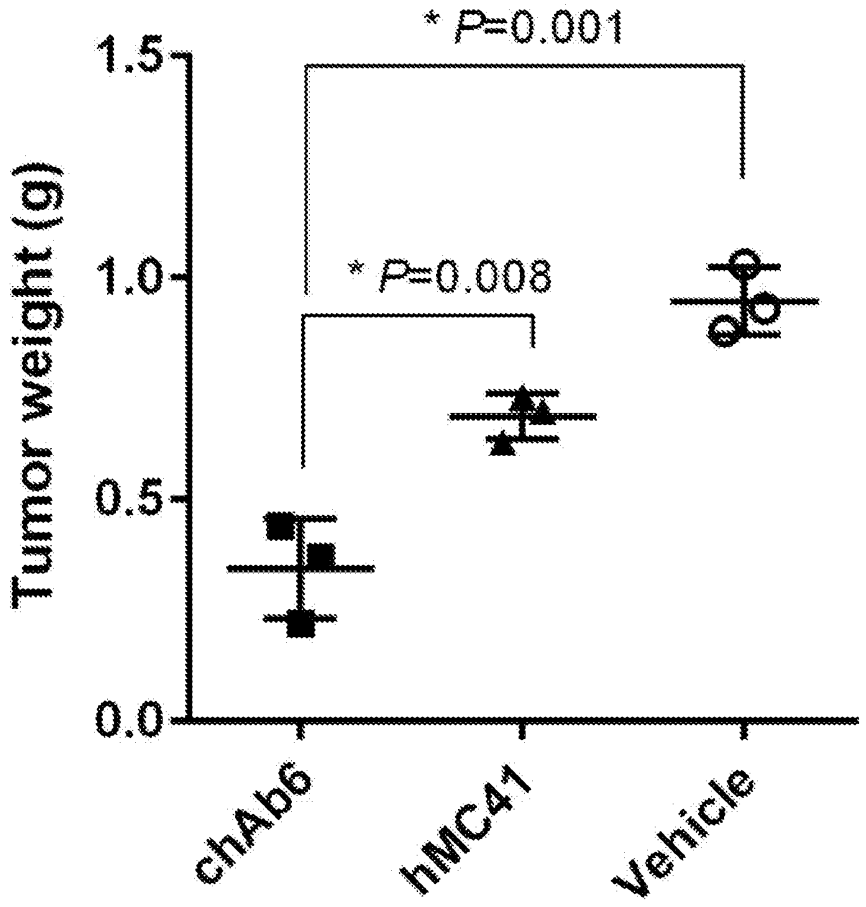


FIG. 17

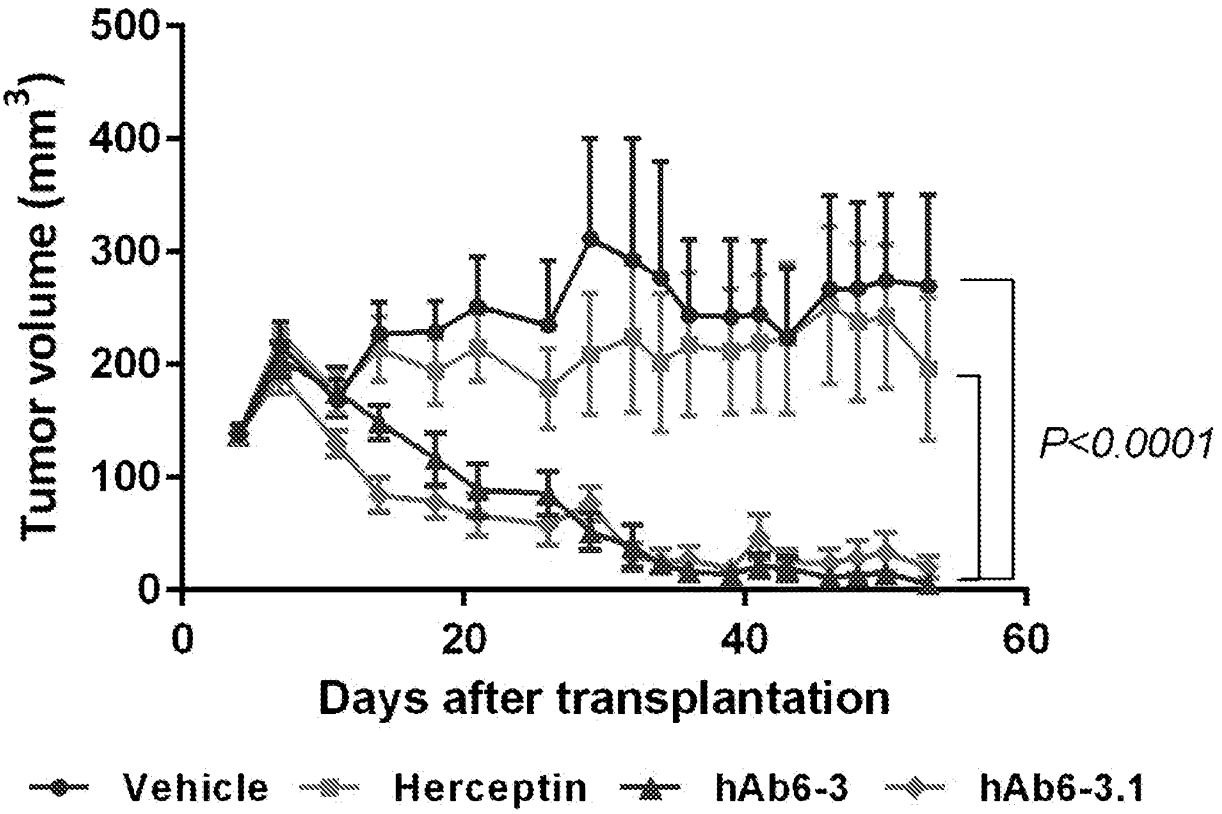

500
400
300
200
100
0
Tumor volume (mm3)
0
20
40
60
Days after transplantation
P<0.0001
Vehicle
Herceptin
hAb6-3
hAb6-3.1

FIG. 18

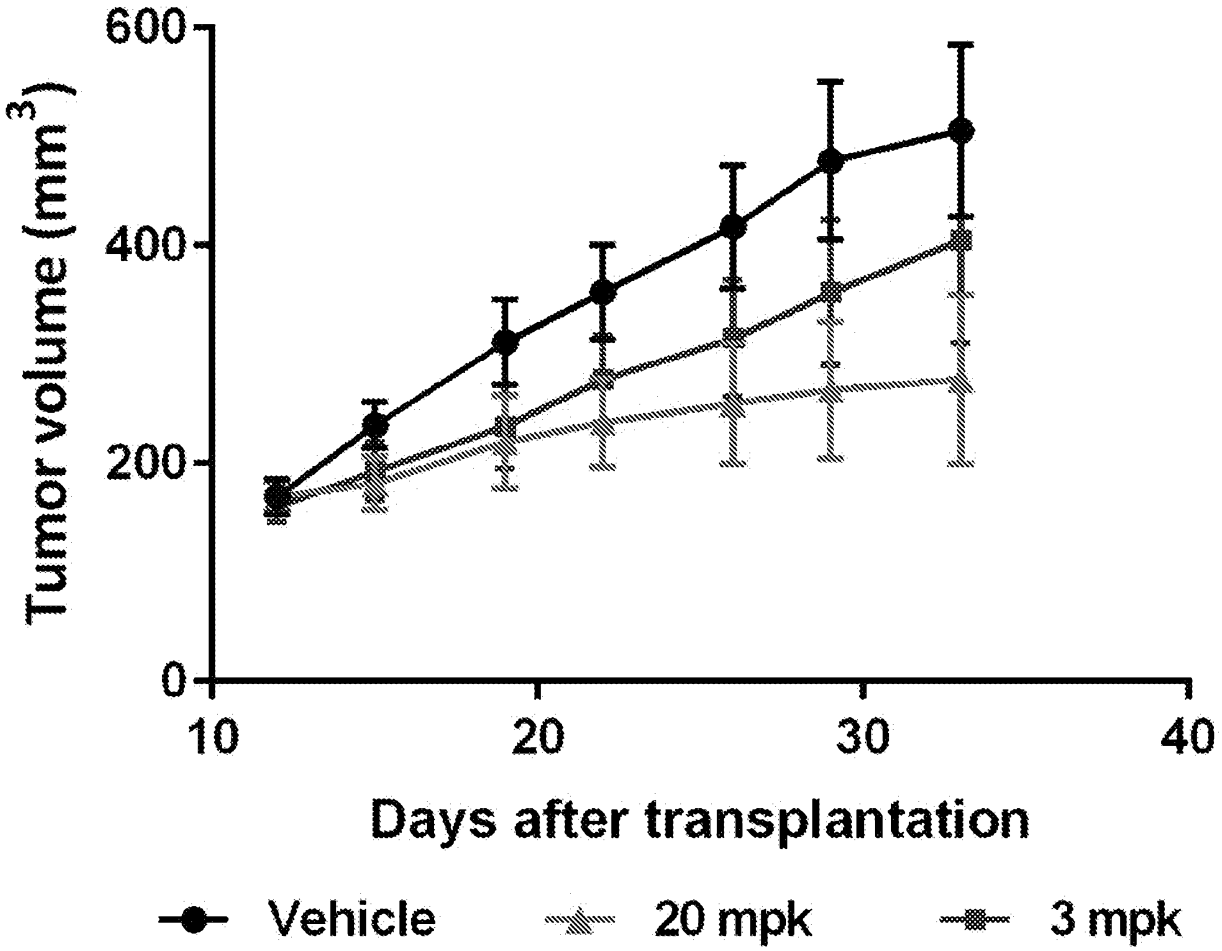

600
400
200
0
Tumor volume (mm3)
10
20
30
40
Days after transplantation
Vehicle
20 mpk
3 mpk

FIG. 19

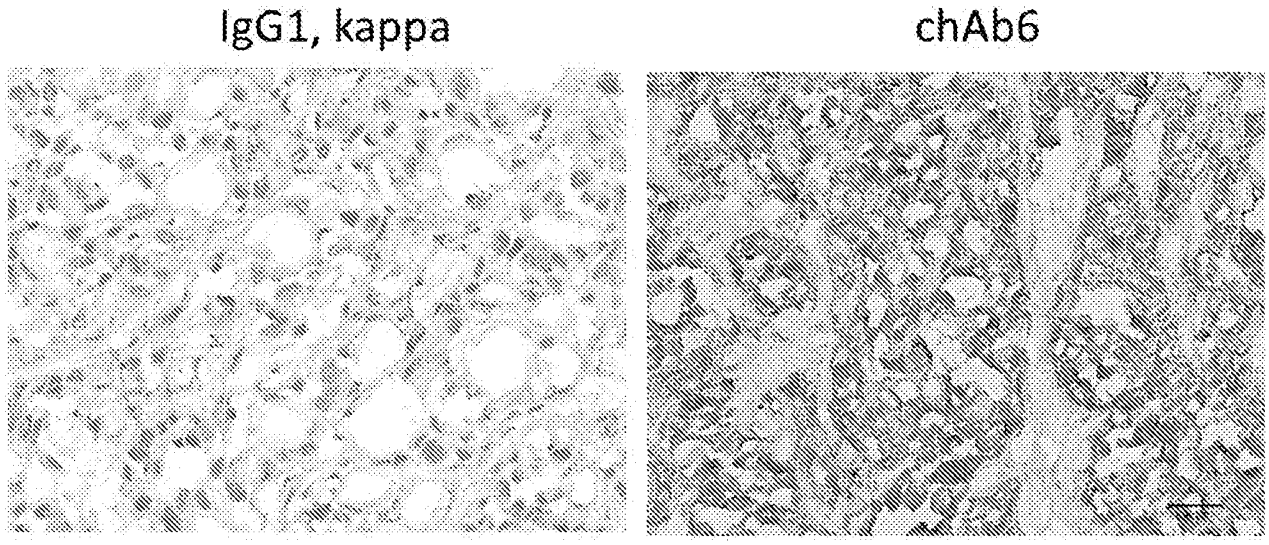

IgG1, kappa
chAb6

FIG. 20

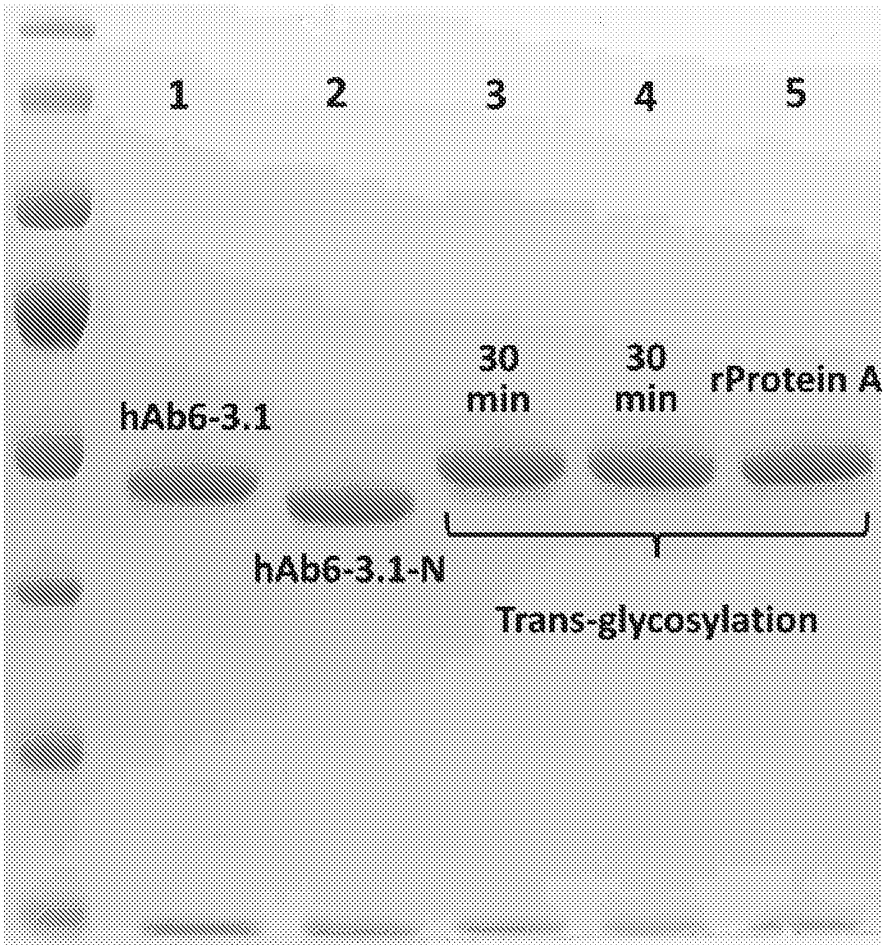

1
2
3
4
5
hAb6-3.1
30 min
30 min
rProtein A
hAb6-3.1-N
Trans-glycosylation

FIG. 21

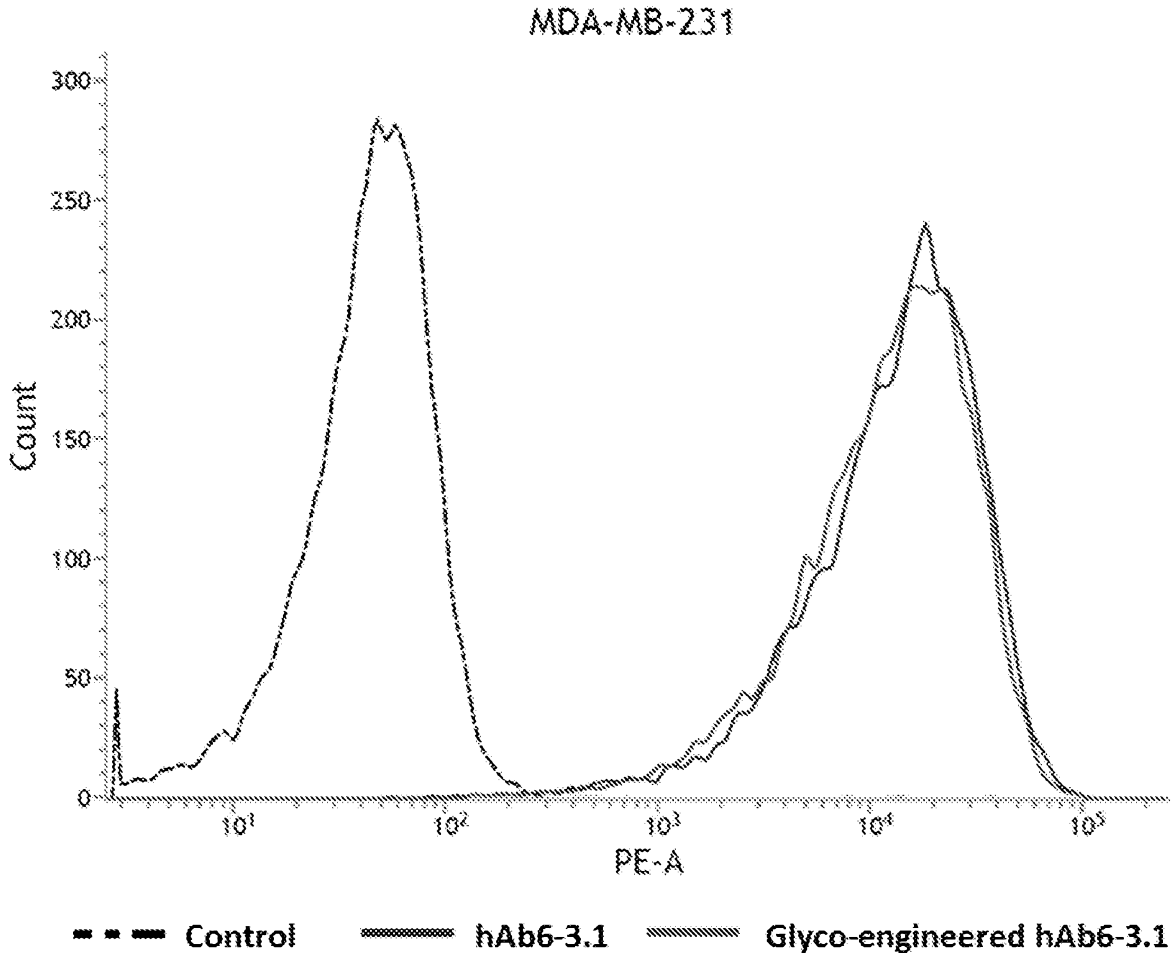

MDA-MB-231
Count
300
250
200
150
100
50
0
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
PE-A
Control
hAb6-3.1
Glyco-engineered hAb6-3.1

FIG. 22

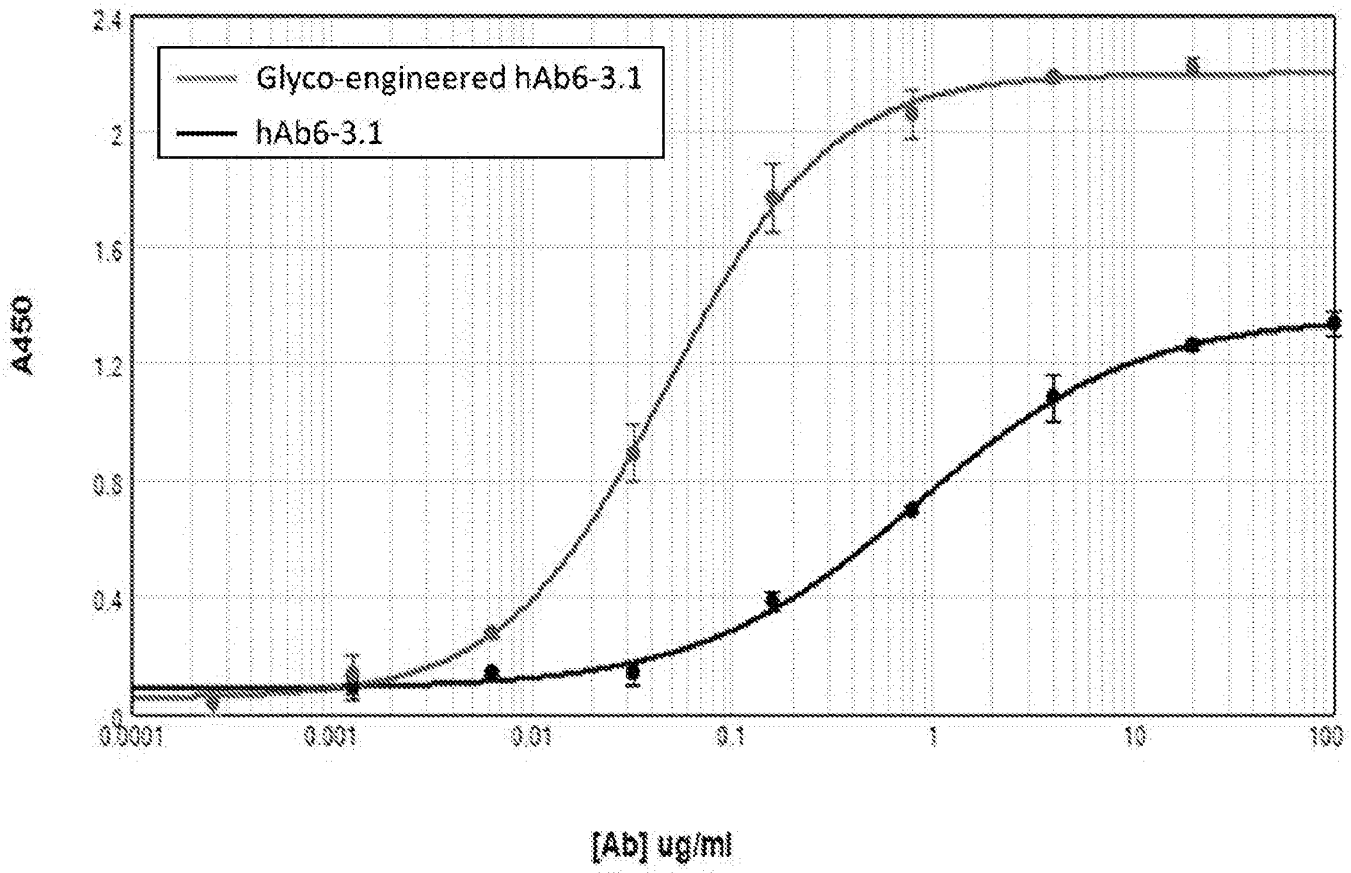
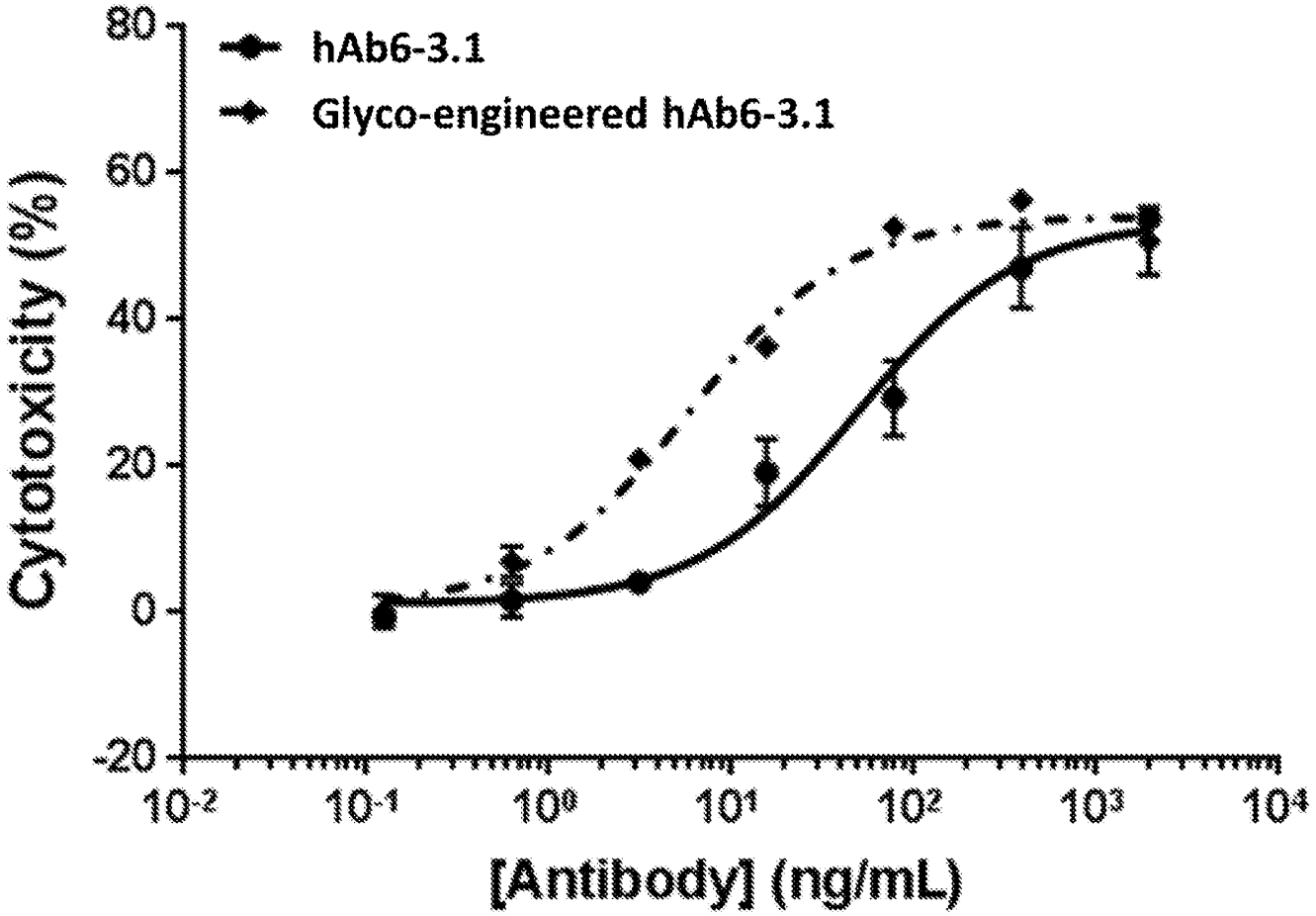

Glyco-engineered hAb6-3.1
hAb6-3.1
A450
2.4
2
1.6
1.2
0.8
0.4
0
0.0001
0.001
0.01
0.1
1
10
100
[Ab] ug/ml

FIG. 23


hAb6-3.1
Glyco-engineered hAb6-3.1
Cytotoxicity (%)
80
60
40
20
0
-20
10-2
10-1
100
101
102
103
104
[Antibody] (ng/mL)

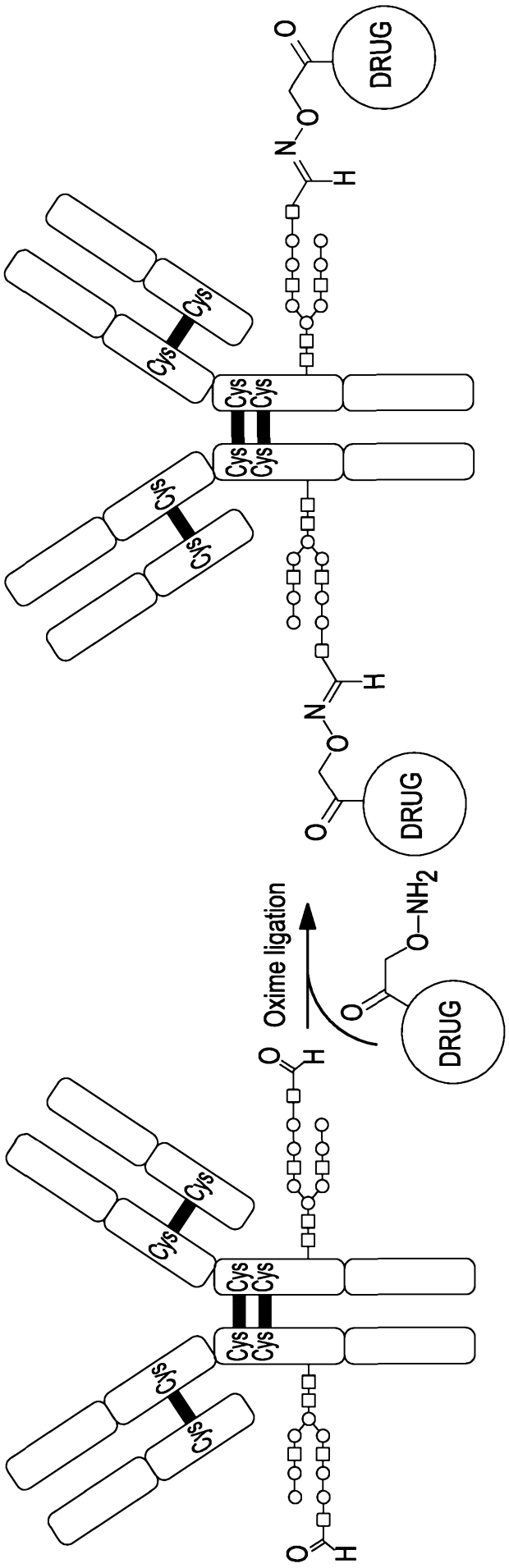

DRUG
O
O
N
H
Cys
Cys
Cys
Cys
Cys
Cys
Cys
Cys
Oxime ligation
O
H
O
O
NH2
DRUG
DRUG
O
O
N
H
O
H

FIG. 24A

FIG. 24B

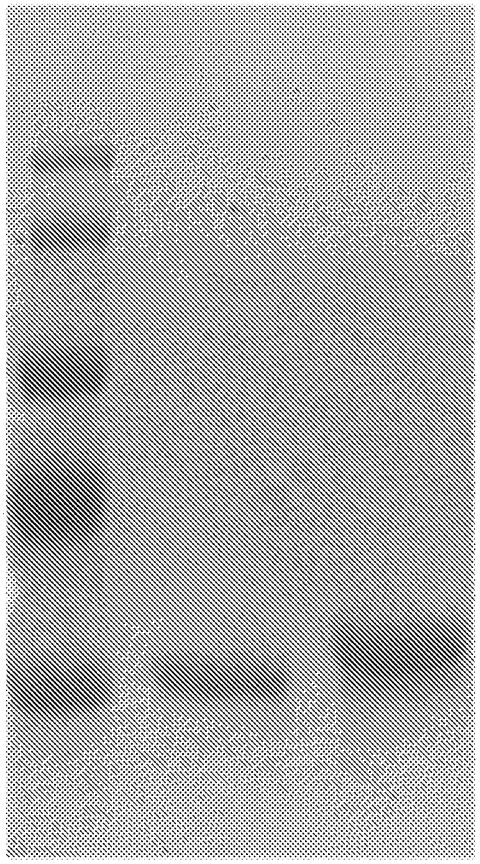


FIG. 25A

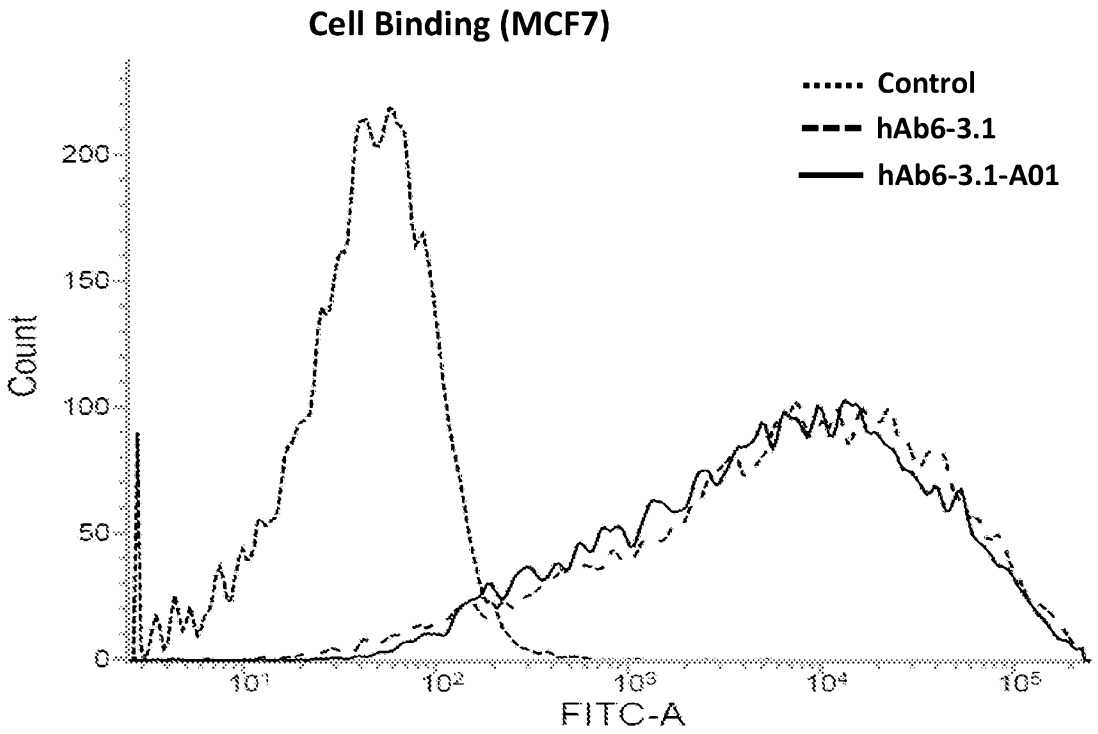



FIG. 25B

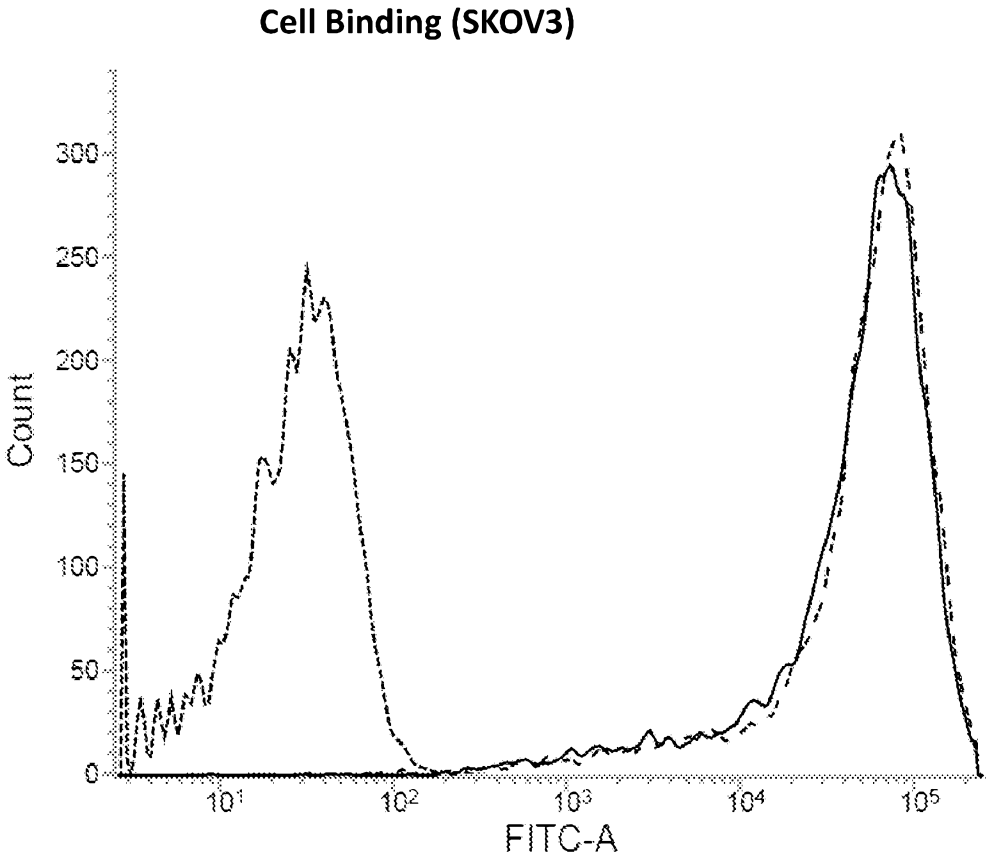
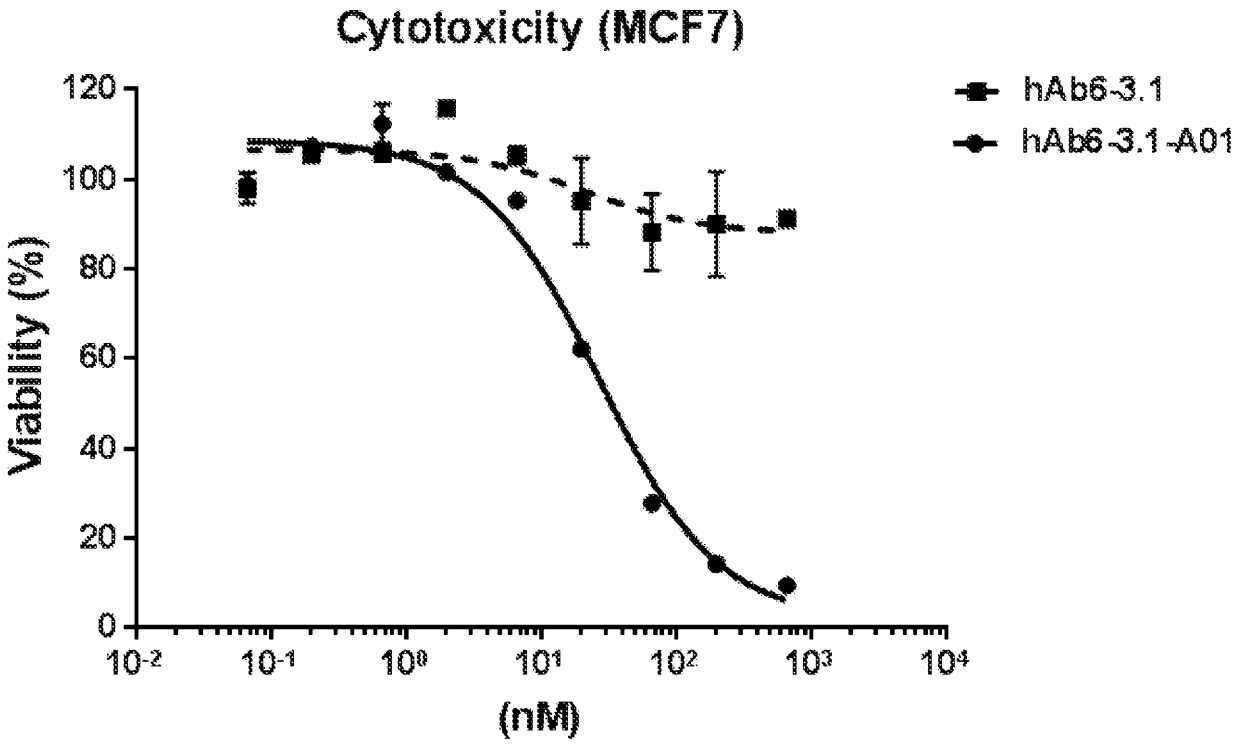
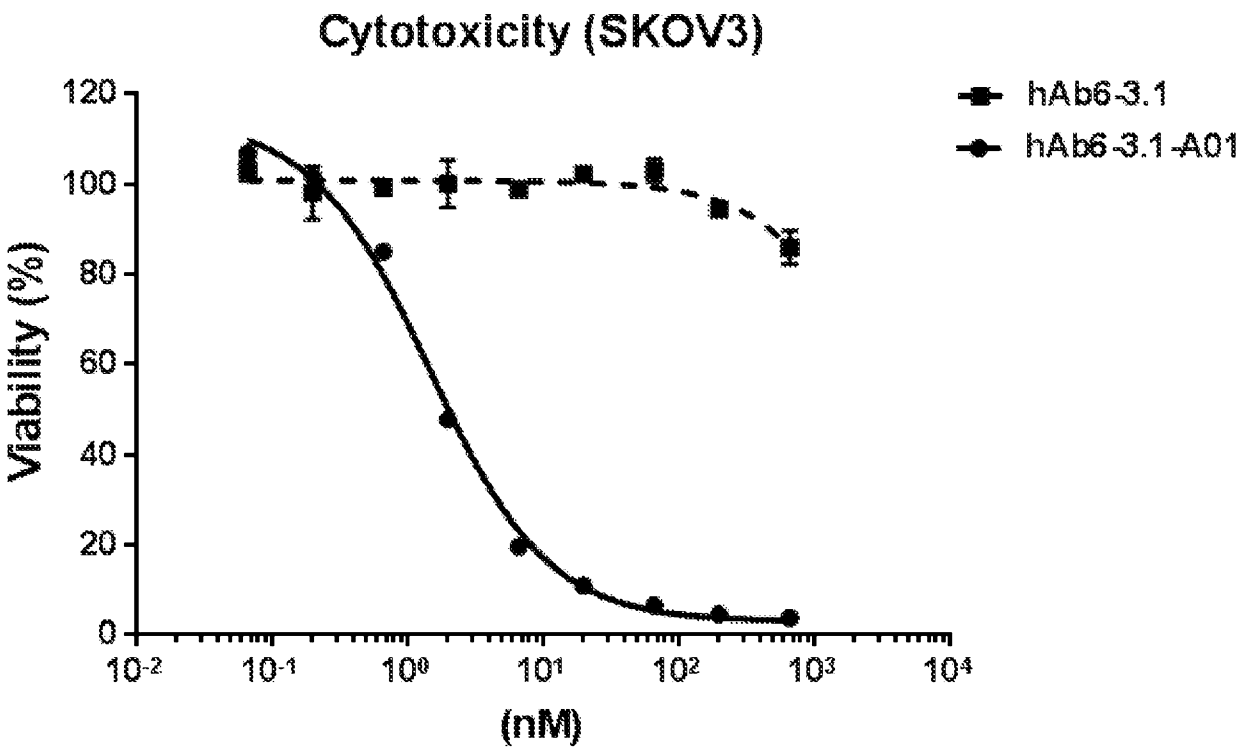


FIG. 26




| Antibody | EC50 (nM) | Max. Killing (%) |
|---|---|---|
| hAb6 | - | 8.8 |
| hAb6-A01 | 27.42 | 90.5 |

FIG. 27




| Antibody | EC50 (nM) | Max. Killing (%) |
|---|---|---|
| hAb6 | - | 14.07 |
| hAb6-A01 | 1.48 | 96.21 |