

ENGINEERED IMMUNOGLOBULINS WITH ALTERED FcRn BINDING

FIELD OF THE INVENTION

The present invention relates to compositions and methods for antibody-mediated
5 therapy. In particular, provided herein are engineered immunoglobulins with altered half-life.

BACKGROUND OF THE INVENTION

Antibodies are immunological proteins that bind a specific antigen. In most mammals,
including humans and mice, antibodies are constructed from paired heavy and light
10 polypeptide chains. Each chain is made up of individual immunoglobulin (Ig) domains, and
thus the generic term immunoglobulin is used for such proteins. Each chain is made up of
two distinct regions, referred to as the variable and constant regions. The light and heavy
chain variable regions show significant sequence diversity between antibodies, and are
responsible for binding the target antigen. The constant regions show less sequence diversity,
15 and are responsible for binding a number of natural proteins to elicit important biochemical
events. In humans there are five different classes of antibodies including IgA (which includes
subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and
IgG4), and IgM. The distinguishing feature between these antibody classes is their constant
regions, although subtler differences may exist in the V region. FIG. 1 shows an IgG1
20 antibody, used here as an example to describe the general structural features of
immunoglobulins. IgG antibodies are tetrameric proteins composed of two heavy chains and
two light chains. The IgG heavy chain is composed of four immunoglobulin domains linked
from N- to C-terminus in the order VH-CH1-CH2-CH3, referring to the heavy chain variable
domain, heavy chain constant domain 1, heavy chain constant domain 2, and heavy chain
25 constant domain 3 respectively (also referred to as VH-C γ 1-C γ 2-C γ 3, referring to the heavy
chain variable domain, constant gamma 1 domain, constant gamma 2 domain, and constant
gamma 3 domain respectively). The IgG light chain is composed of two immunoglobulin
domains linked from N- to C-terminus in the order VL-CL, referring to the light chain
variable domain and the light chain constant domain respectively.

30 The variable region of an antibody contains the antigen binding determinants of the
molecule, and thus determines the specificity of an antibody for its target antigen. The
variable region is so named because it is the most distinct in sequence from other antibodies
within the same class. The majority of sequence variability occurs in the complementarity
determining regions (CDRs). There are 6 CDRs total, three each per heavy and light chain,

designated VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3. The variable region outside of the CDRs is referred to as the framework (FR) region. Although not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. The sequence and structural features of antibody variable regions are well characterized (Morea et al., 1997, *Biophys Chem* 68:9-16; Morea et al., 2000, *Methods* 20:267-279, entirely incorporated by reference), and the conserved features of antibodies have enabled the development of a wealth of antibody engineering techniques (Maynard et al., 2000, *Annu Rev Biomed Eng* 2:339-376, entirely incorporated by reference). For example, it is possible to graft the CDRs from one antibody, for example a murine antibody, onto the framework region of another antibody, for example a human antibody. This process, referred to in the art as “humanization”, enables generation of less immunogenic antibody therapeutics from nonhuman antibodies. Fragments including the variable region can exist in the absence of other regions of the antibody, including for example the antigen binding fragment (Fab) including VH-C γ 1 and VH-CL, the variable fragment (Fv) including VH and VL, the single chain variable fragment (scFv) including VH and VL linked together in the same chain, as well as a variety of other variable region fragments (Little et al., 2000, *Immunol Today* 21:364-370, entirely incorporated by reference).

The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions.

In IgG, a site on Fc including amino acids from both the CH2 and CH3 domains mediates interaction with the neonatal Fc receptor (FcRn), a receptor which recycles endocytosed antibody from the endosome back to the bloodstream (Raghavan et al., 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie et al., 2000, *Annu Rev Immunol* 18:739-766, both entirely incorporated by reference). This process, coupled with preclusion of kidney filtration due to the large size of the full-length molecule, results in favorable antibody serum half-lives ranging from one to three weeks. Binding of Fc to FcRn also plays a key role in antibody transport within and across cells. The binding site on Fc for FcRn is also the site at which the bacterial proteins A and G bind. The tight binding by these proteins is typically exploited as a means to purify antibodies by employing protein A or protein G affinity

chromatography during protein purification. Thus the fidelity of this region on Fc is important for both the clinical properties of antibodies and their purification.

FcRn is a key player in several immunological and non-immunological processes, as it mediates maternal-fetal transfer of IgG, regulates the serum persistence of IgG and albumin, and transports both ligands between different cellular compartments. In addition, FcRn enhances antigen presentation and cross presentation. In contrast to TRIM21 that is found in the cytosol, FcRn is a transmembrane receptor that resides predominantly within acidified endosomes and transports its ligands either via a recycling pathway or a transcytotic pathway across polarized cell layers. However, FcRn may also enhance processing of immune complexes followed by presentation of antigenic peptides to T cells. A hallmark of the FcRn-IgG interaction is that FcRn binds the IgG Fc in a strictly pH dependent fashion, binding at acidic pH and no binding or release at neutral pH, which is a prerequisite for FcRn-mediated transport in and out of cells. Engineering of the FcRn-IgG Fc interaction has given rise to antibodies with shorter or longer serum half-life or altered capacity to be transported across cell layers. FcRn is broadly expressed and as such is found on distinct cellular locations in both hematopoietic and non-hematopoietic cells.

Improved therapeutic IgGs that exhibit enhanced pH dependent binding to FcRn are needed.

20 SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for antibody-mediated therapy. In particular, provided herein are engineered immunoglobulins with altered half-life.

For example, in some embodiments, the present invention provides a composition, comprising: a therapeutic immunoglobulin with altered binding affinity for FcRn, wherein the immunoglobulin comprises at least one mutation in the Fc region of the immunoglobulin. In some embodiments, the immunoglobulin is an IgG1, IgG2, IgG3, or IgG4 subclass. In some embodiments, the immunoglobulin is an Fc fusion of one of the variant immunoglobulins described herein. In some embodiments, the immunoglobulin has mutations at one or more of positions selected from, for example, 311, 434, 428, 438, or 435. In some embodiments, the mutation is, for example, IgG1-Q311R/N434W/M428E, IgG1-Q311R/N434W, IgG1-Q311R, IgG1-N434W, IgG3(b)-Q311R/N434W/M428E, IgG3(b)-Q311R/N434W/M438E/R435H, IgG1-M252S/Q311R/N434W/M428E, IgG1-Q311R/N434P/M428E, IgG1-Q311R/N434W/M428D, IgG1-Q311R/N434W/M428E/H433K, IgG1-L309K/Q311R/N434W/M428E, IgG1-

L309R/Q311R/N434W/M428E, IgG1-L309S/Q311R/N434W/M428E, or IgG3(b)-Q311R/N434W/M428E/R435H (e.g., the constant region of the immunoglobulin has an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 or sequences at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16)).

Further embodiments provide a composition, comprising: an immunoglobulin comprising a at least one mutation in the Fc region, wherein the immunoglobulin has altered binding to FcRn, and wherein the mutation is selected from, for example IgG1-Q311R/N434W/M428E, IgG1-Q311R/N434W, IgG1-Q311R, IgG1-N434W, IgG3(b)-Q311R/N434W/M428E, IgG3(b)-Q311R/N434W/M438E/R435H, IgG1-M252S/Q311R/N434W/M428E, IgG1-Q311R/N434P/M428E, IgG1-Q311R/N434W/M428D, IgG1-Q311R/N434W/M428E/H433K, IgG1-L309K/Q311R/N434W/M428E, IgG1-L309R/Q311R/N434W/M428E, IgG1-L309S/Q311R/N434W/M428E, or IgG3(b)-Q311R/N434W/M428E/R435H.

Still other embodiments provide an IgG1 immunoglobulin with altered binding to FcRn, wherein the immunoglobulin has Q311R, N434W, and M428E mutations.

In some embodiments, the antibody binds to a target selected from, for example, a cancer marker, a cytokine, an infectious disease marker, or a growth factor.

Yet other embodiments provide the use of the immunoglobulins described herein to treat or prevent a disease in a subject in need thereof. In some embodiments, disease is, for example, cancer, autoimmune disease, inflammatory disease, transplant rejection, or infectious disease, although other diseases are specifically contemplated. In some embodiments, the antibody binds to a target selected from, for example, a cancer marker, a cytokine, an infectious disease marker, or a growth factor, although other targets are specifically contemplated.

Still further embodiments provide a method of treating or treating or preventing a disease in a subject, comprising: providing the immunoglobulins described herein to a subject in need thereof.

Further embodiments provide vaccine compositions comprising the variant immunoglobulins or Fc fusions thereof described herein (e.g., fused to an immunogen) and uses thereof to generate an immune response in a subject.

Additional embodiments are described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. ELISA showing binding of a panel of Fc-engineered h9C12 IgG1 variants to human FcRn at pH 6.0 (top panel) and 7.4 (lower panel).

FIG. 2. SPR sensorgrams showing binding of a panel of Fc-engineered h9C12 IgG1 variants to human FcRn at pH 6.0 (7.8-4000.0 nM) and pH 7.4 (4000.0 nM). IgG1-M252Y/S254T/T256E was included as a reference.

FIG. 3 shows alignment showing the amino acid sequence of IgG1 variants. Amino acid sequence of the constant domain of Fc-engineered IgG1 variants.

FIG. 4 shows sequences from Figure 3.

FIG. 5 shows an ELISA showing binding of a panel of Fc-engineered h9C12 IgG1 and IgG3 variants to human FcRn at pH 6.0 and 7.4.

FIG. 6 shows SPR sensorgrams showing binding of a panel of Fc-engineered h9C12 IgG1 variants to human FcRn at pH 6.0 (1000-15.6 nM) (A-D) and pH 7.4 (1000 nM) (E-F).

FIG. 7 shows in vivo half-life (% remaining) of (A) h9C12 and (B) NIP IgG1 variants in human FcRn Tg32-Alb-/- transgenic mice. NIP = 4-hydroxy-3-iodo-5-nitrophenylacetic acid.

FIG. 8 shows sequences of IgG variants.

DEFINITIONS

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. Also included are antibody fragments having an Fc region, and fusion proteins that comprise a region equivalent to the Fc region of an immunoglobulin.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂, single-chain antibody molecules (e.g. scFv), diabodies, and multispecific antibodies formed from antibody fragments.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the

heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

"Effector functions" are used herein refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; cytokine secretion; immune-complex-mediated antigen uptake by antigen presenting cells; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation. The term "wildtype" when used in reference to a protein refers to proteins encoded by the genome of a cell, tissue, or organism, other than one manipulated to produce synthetic proteins.

The term "antigen binding domain" refers to the part of an antigen binding molecule that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species. For chimeric antibodies, for example, the non-antigen binding components may be derived from a wide variety of species, including primates such as chimpanzees and humans. Humanized antibodies are a particularly preferred form of chimeric antibodies.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: 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 , β , δ , ϵ , γ , and μ .

A "region equivalent to the Fc region of an immunoglobulin" is intended to include naturally occurring allelic variants of the Fc region of an immunoglobulin as well as variants having alterations which produce substitutions, additions, or deletions but which do not

decrease substantially the ability of the immunoglobulin to mediate effector functions (such as antibody-dependent cellular cytotoxicity). For example, one or more amino acids can be deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general
5 rules known in the art so as to have minimal effect on activity (see, e.g., Bowie, J. U. et al., Science 247:1306-10 (1990)).

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) (or CDR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally
10 appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

15 A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest,
20 Fifth Edition, NIH Publication 91-3242, Bethesda Md. (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a
25 humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a
30 non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs

generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as complementarity determining regions (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., J. Mol. Biol. 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

The term "variant" and "mutant" when used in reference to a polypeptide refer to an amino acid sequence that differs by one or more amino acids from another, usually related polypeptide. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties. One type of conservative amino acid substitutions refers to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; unnatural amino acids like p-aminophenylalanine, a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. More rarely, a variant may have "non-conservative" changes (e.g., replacement of a glycine with a tryptophan). Similar minor variations may also include amino acid deletions or insertions (i.e., additions), or both. Guidance in determining which and how many amino acid residues may be

substituted, inserted or deleted without abolishing biological activity may be found using computer programs well known in the art, for example, DNASTar software. Variants can be tested in functional assays. Preferred variants have less than 10%, and preferably less than 5%, and still more preferably less than 2% changes (whether substitutions, deletions, and so on). For an amino acid substitution, the following nomenclature is used: Original amino acid, position, substituted amino acid. Accordingly, the substitution of lysine with alanine at position 573 is designated as “K573A” and the substitution of lysine with proline at position 573 is designated as K573P. Multiple mutations are separated by addition marks (“+”) or “/”, e.g., “Gly205Arg + Ser411Phe” or “G205R/S411F”, representing mutations at positions 205 and 411 substituting glycine (G) with arginine (R), and serine (S) with phenylalanine (F), respectively.

The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter “identity”. For purposes of the present invention, the degree of identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends in Genetics 16: 276-277), preferably version 3.0.0 or later. The optional parameters 11644.000-EP7 used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled “longest identity” (obtained using the –nobrief option) is used as the percent identity and is calculated as follows: $(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$.

The expression “amino acid position corresponding to” a position in a reference sequence and similar expression is intended to identify the amino acid residue that in the primary or spatial structure corresponds to the particular position in the reference sequence. The skilled person will appreciate that this can be done by aligning a given sequence with the reference sequence and identifying the amino acid residue that aligns with the particular position in the reference sequence.

The expression Xnnn is intended to mean an amino acid residue X located in a position corresponding to position nnn in HSA and the expression XnnnY is intended to mean a substitution of any amino acid X located in a position corresponding to position nnn in HSA with the amino acid residue Y.

As used herein, the term “affinity” refers to a measure of the strength of binding between two members of a binding pair, for example, an immunoglobulin and FcRn. K_d is

the dissociation constant and has units of molarity. The affinity constant is the inverse of the dissociation constant. An affinity constant is sometimes used as a generic term to describe this chemical entity. It is a direct measure of the energy of binding. The natural logarithm of K is linearly related to the Gibbs free energy of binding through the equation $\Delta G_0 = -RT \ln(K)$ where R= gas constant and temperature is in degrees Kelvin. Affinity may be determined experimentally, for example by surface plasmon resonance (SPR) using commercially available Biacore SPR units (GE Healthcare).

As used herein, the term "under conditions such that said subject generates an immune response" refers to any qualitative or quantitative induction, generation, and/or stimulation of an immune response (e.g., innate or acquired).

As used herein, the term "immune response" refers to a response by the immune system of a subject. For example, immune responses include, but are not limited to, a detectable alteration (e.g., increase) in Toll receptor activation, lymphokine (e.g., cytokine (e.g., Th1 or Th2 type cytokines) or chemokine) expression and/or secretion, macrophage activation, dendritic cell activation, T cell activation (e.g., CD4+ or CD8+ T cells), NK cell activation, and/or B cell activation (e.g., antibody generation and/or secretion). Additional examples of immune responses include binding of an immunogen (e.g., antigen (e.g., immunogenic polypeptide)) to an MHC molecule and inducing a cytotoxic T lymphocyte ("CTL") response, inducing a B cell response (e.g., antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide is derived, expansion (e.g., growth of a population of cells) of cells of the immune system (e.g., T cells, B cells (e.g., of any stage of development (e.g., plasma cells), and increased processing and presentation of antigen by antigen presenting cells. An immune response may be to immunogens that the subject's immune system recognizes as foreign (e.g., non-self antigens from microorganisms (e.g., pathogens), or self-antigens recognized as foreign). Thus, it is to be understood that, as used herein, "immune response" refers to any type of immune response, including, but not limited to, innate immune responses (e.g., activation of Toll receptor signaling cascade) cell-mediated immune responses (e.g., responses mediated by T cells (e.g., antigen-specific T cells) and non-specific cells of the immune system) and humoral immune responses (e.g., responses mediated by B cells (e.g., via generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids). The term "immune response" is meant to encompass all aspects of the capability of a subject's immune system to respond to antigens and/or

immunogens (e.g., both the initial response to an immunogen (e.g., a pathogen) as well as acquired (e.g., memory) responses that are a result of an adaptive immune response).

As used herein, the term "immunity" refers to protection from disease (e.g., preventing or attenuating (e.g., suppression) of a sign, symptom or condition of the disease) upon exposure to a microorganism (e.g., pathogen) capable of causing the disease. Immunity can be innate (e.g., non-adaptive (e.g., non-acquired) immune responses that exist in the absence of a previous exposure to an antigen) and/or acquired (e.g., immune responses that are mediated by B and T cells following a previous exposure to antigen (e.g., that exhibit increased specificity and reactivity to the antigen)).

As used herein, the term "immunogen" refers to an agent (e.g., a microorganism (e.g., bacterium, virus or fungus) and/or portion or component thereof (e.g., a protein antigen)) that is capable of eliciting an immune response in a subject. In some embodiments, immunogens elicit immunity against the immunogen (e.g., microorganism (e.g., pathogen or a pathogen product)).

The term "test compound" refers to any chemical entity, pharmaceutical, drug, and the like that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample. Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (e.g., through animal trials or prior experience with administration to humans) to be effective in such treatment or prevention.

The term "sample" as used herein is used in its broadest sense. As used herein, the term "sample" is used in its broadest sense. In one sense it can refer to a tissue sample. In another sense, it is meant to include a specimen or culture obtained from any source, as well as biological. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include, but are not limited to blood products, such as plasma, serum and the like. These examples are not to be construed as limiting the sample types applicable to the present invention. A sample suspected of containing a human chromosome or sequences associated with a human chromosome may comprise a cell, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern blot analysis), RNA (in solution or bound to a solid support such as for Northern blot analysis), cDNA (in solution or bound to a solid support) and the like. A sample

suspected of containing a protein may comprise a cell, a portion of a tissue, an extract containing one or more proteins and the like.

As used herein, the term "affinity" refers to a measure of the strength of binding between two members of a binding pair, for example, an immunoglobulin and FcRn. K_d is the dissociation constant and has units of molarity. The affinity constant is the inverse of the dissociation constant. An affinity constant is sometimes used as a generic term to describe this chemical entity. It is a direct measure of the energy of binding. The natural logarithm of K is linearly related to the Gibbs free energy of binding through the equation $\Delta G_0 = -RT \ln(K)$ where R = gas constant and temperature is in degrees Kelvin. Affinity may be determined experimentally, for example by surface plasmon resonance (SPR) using commercially available Biacore SPR units (GE Healthcare).

As used herein, the term "under conditions such that said subject generates an immune response" refers to any qualitative or quantitative induction, generation, and/or stimulation of an immune response (e.g., innate or acquired).

As used herein, the term "immune response" refers to a response by the immune system of a subject. For example, immune responses include, but are not limited to, a detectable alteration (e.g., increase) in Toll receptor activation, lymphokine (e.g., cytokine (e.g., Th1 or Th2 type cytokines) or chemokine) expression and/or secretion, macrophage activation, dendritic cell activation, T cell activation (e.g., CD4+ or CD8+ T cells), NK cell activation, and/or B cell activation (e.g., antibody generation and/or secretion). Additional examples of immune responses include binding of an immunogen (e.g., antigen (e.g., immunogenic polypeptide)) to an MHC molecule and inducing a cytotoxic T lymphocyte ("CTL") response, inducing a B cell response (e.g., antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide is derived, expansion (e.g., growth of a population of cells) of cells of the immune system (e.g., T cells, B cells (e.g., of any stage of development (e.g., plasma cells), and increased processing and presentation of antigen by antigen presenting cells. An immune response may be to immunogens that the subject's immune system recognizes as foreign (e.g., non-self antigens from microorganisms (e.g., pathogens), or self-antigens recognized as foreign). Thus, it is to be understood that, as used herein, "immune response" refers to any type of immune response, including, but not limited to, innate immune responses (e.g., activation of Toll receptor signaling cascade) cell-mediated immune responses (e.g., responses mediated by T cells (e.g., antigen-specific T cells) and non-specific cells of the immune system) and humoral immune responses (e.g.,

responses mediated by B cells (e.g., via generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids). The term "immune response" is meant to encompass all aspects of the capability of a subject's immune system to respond to antigens and/or immunogens (e.g., both the initial response to an immunogen (e.g., a pathogen) as well as
5 acquired (e.g., memory) responses that are a result of an adaptive immune response).

As used herein, the term "immunity" refers to protection from disease (e.g., preventing or attenuating (e.g., suppression) of a sign, symptom or condition of the disease) upon exposure to a microorganism (e.g., pathogen) capable of causing the disease. Immunity can be innate (e.g., non-adaptive (e.g., non-acquired) immune responses that exist in the
10 absence of a previous exposure to an antigen) and/or acquired (e.g., immune responses that are mediated by B and T cells following a previous exposure to antigen (e.g., that exhibit increased specificity and reactivity to the antigen)).

As used herein, the term "immunogen" refers to an agent (e.g., a microorganism (e.g., bacterium, virus or fungus) and/or portion or component thereof (e.g., a protein antigen)) that
15 is capable of eliciting an immune response in a subject. In some embodiments, immunogens elicit immunity against the immunogen (e.g., microorganism (e.g., pathogen or a pathogen product)).

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention relates to compositions and methods for antibody-mediated therapy. In particular, provided herein are engineered immunoglobulins with altered half-life.

The immunoglobulin molecule is composed of two identical heavy and two identical light polypeptide chains, held together by interchain disulfide bonds. Each individual light and heavy chain folds into regions of about 110 amino acids, assuming a conserved three-
25 dimensional conformation. The light chain comprises one variable region (termed VL) and one constant region (CL), while the heavy chain comprises one variable region (VH) and three constant regions (CH1, CH2 and CH3). Pairs of regions associate to form discrete structures. In particular, the light and heavy chain variable regions, VL and VH, associate to form an "FV " area that contains the antigen-binding site.

30 The variable regions of both heavy and light chains show considerable variability in structure and amino acid composition from one antibody molecule to another, whereas the constant regions show little variability. Each antibody recognizes and binds an antigen through the binding site defined by the association of the heavy and light chain, variable regions into an FV area. The light-chain variable region VL and the heavy-chain variable

region VH of a particular antibody molecule have specific amino acid sequences that allow the antigen-binding site to assume a conformation that binds to the antigen epitope recognized by that particular antibody.

Within the variable regions are found regions in which the amino acid sequence is extremely variable from one antibody to another. Three of these so-called "hypervariable" regions or "complementarity-determining regions" (CDR's) are found in each of the light and heavy chains. The three CDRs from a light chain and the three CDRs from a corresponding heavy chain form the antigen-binding site.

The amino acid sequences of many immunoglobulin heavy and light chains have been determined and reveal two important features of antibody molecules. First, each chain consists of a series of similar, although not identical, sequences, each about 110 amino acids long. Each of these repeats corresponds to a discrete, compactly folded region of protein structure known as a protein domain. The light chain is made up of two such immunoglobulin domains, whereas the heavy chain of the IgG antibody contains four.

The second important feature revealed by comparisons of amino acid sequences is that the amino-terminal sequences of both the heavy and light chains vary greatly between different antibodies. The variability in sequence is limited to approximately the first 110 amino acids, corresponding to the first domain, whereas the remaining domains are constant between immunoglobulin chains of the same isotype. The amino-terminal variable or V domains of the heavy and light chains (V_H and V_L , respectively) together make up the V region of the antibody and confer on it the ability to bind specific antigen, while the constant domains (C domains) of the heavy and light chains (C_H and C_L , respectively) make up the C region. The multiple heavy-chain C domains are numbered from the amino-terminal end to the carboxy terminus, for example C_{H1} , C_{H2} , and so on.

The protein domains described above associate to form larger globular domains. Thus, when fully folded and assembled, an antibody molecule comprises three equal-sized globular portions joined by a flexible stretch of polypeptide chain known as the hinge region. Each arm of this Y-shaped structure is formed by the association of a light chain with the amino-terminal half of a heavy chain, whereas the trunk of the Y is formed by the pairing of the carboxy-terminal halves of the two heavy chains. The association of the heavy and light chains is such that the V_H and V_L domains are paired, as are the C_{H1} and C_L domains. The C_{H3} domains pair with each other but the C_{H2} domains do not interact; carbohydrate side chains attached to the C_{H2} domains lie between the two heavy chains. The two antigen-

binding sites are formed by the paired V_H and V_L domains at the ends of the two arms of the Y.

Proteolytic enzymes (proteases) that cleave polypeptide sequences have been used to dissect the structure of antibody molecules and to determine which parts of the molecule are responsible for its various functions. Limited digestion with the protease papain cleaves antibody molecules into three fragments. Two fragments are identical and contain the antigen-binding activity. These are termed the Fab fragments, for **F**ragment **a**ntigen **b**inding. The Fab fragments correspond to the two identical arms of the antibody molecule, which contain the complete light chains paired with the V_H and C_{H1} domains of the heavy chains. The other fragment contains no antigen-binding activity but was originally observed to crystallize readily, and for this reason was named the Fc fragment, for **F**ragment **c**rystallizable. This fragment corresponds to the paired C_{H2} and C_{H3} domains and is the part of the antibody molecule that interacts with effector molecules and cells. The functional differences between heavy-chain isotypes lie mainly in the Fc fragment. The hinge region that links the Fc and Fab portions of the antibody molecule is in reality a flexible tether, allowing independent movement of the two Fab arms, rather than a rigid hinge. The present invention relates to compositions and methods for antibody-mediated immunity against viral targets. In particular, provided herein are engineered immunoglobulins with anti-viral activity.

The present invention provides mutant immunoglobulins comprising one or more mutations, preferably in the Fc region or hinge region of the immunoglobulin. In preferred embodiments, the mutation alters the function of the immunoglobulin in a desired way. For example, in some embodiments, the present invention provides the use of a variant immunoglobulin comprising at least one mutation in the Fc region to modulate FcRN binding in order to increase the in vivo half-life of the antibody. Increased half-life is advantageous for therapeutic antibodies.

In some embodiments, the immunoglobulin exhibits altered binding (e.g., increased or decreased binding affinity) to FcRN relative to an immunoglobulin without the mutation. In some embodiments, the immunoglobulin is an IgG1, IgG2, IgG3, or IgG4 subclass. In some embodiments, the immunoglobulin has mutations at one or more of positions selected from, for example, 311, 428, 434, 435, or 438 as numbered by the Kabat system. Throughout this disclosure, reference is made to the numbering system from Kabat, E. A., et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991). In these compendiums, Kabat lists many amino acid sequences for antibodies for

each subclass, and lists the most commonly occurring amino acid for each residue position in that subclass. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. The Kabat numbering scheme is followed in this description. For purposes of this invention, to assign residue numbers to a candidate antibody amino acid sequence which is not included in the Kabat compendium, one follows the following steps. Generally, the candidate sequence is aligned with any immunoglobulin sequence or any consensus sequence in Kabat. Alignment may be done by hand, or by computer using commonly accepted computer programs; an example of such a program is the Align 2 program. Alignment may be facilitated by using some amino acid residues which are common to most Fab sequences. For example, the light and heavy chains each typically have two cysteines which have the same residue numbers; in V_L domain the two cysteines are typically at residue numbers 23 and 88, and in the V_H domain the two cysteine residues are typically numbered 22 and 92. Framework residues generally, but not always, have approximately the same number of residues, however the CDRs will vary in size. For example, in the case of a CDR from a candidate sequence which is longer than the CDR in the sequence in Kabat to which it is aligned, typically suffixes are added to the residue number to indicate the insertion of additional residues. For candidate sequences which, for example, align with a Kabat sequence for residues 34 and 36 but have no residue between them to align with residue 35, the number 35 is simply not assigned to a residue.

In some embodiments, described herein are constant chain (e.g., Fc) variants. The immunoglobulins described herein are not limited to particular CDR or variable region sequences and can be generated or engineered to recognize any antigen or epitope desired. Thus, in some embodiments, the mutation is selected from, for example, IgG1-Q311R/N434W/M428E, IgG1-Q311R/N434W, IgG1-Q311R, IgG1-N434W, IgG3(b)-Q311R/N434W/M428E, IgG3(b)-Q311R/N434W/M438E/R435H, IgG1-M252S/Q311R/N434W/M428E, IgG1-Q311R/N434P/M428E, IgG1-Q311R/N434W/M428D, IgG1-Q311R/N434W/M428E/H433K, IgG1-L309K/Q311R/N434W/M428E, IgG1-L309R/Q311R/N434W/M428E, IgG1-L309S/Q311R/N434W/M428E, or IgG3(b)-Q311R/N434W/M428E/R435H (e.g., the constant region of the immunoglobulin has an amino acid sequence selected from SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 or sequences at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9,

10, 11, 12, 13, 14, 15, or 16)). In some specific embodiments, the immunoglobulin has Q311R, N434W, and M428E mutations.

The mutations described above may be introduced into any suitable immunoglobulin molecule. In some embodiments, the immunoglobulin is a monoclonal antibody and is
5 preferably produced by recombinant techniques.

Monoclonal antibodies against target antigens (e.g., a cell surface protein, such as receptors) are produced by a variety of techniques including conventional monoclonal antibody methodologies such as the somatic cell hybridization techniques of Kohler and Milstein, *Nature*, 256:495 (1975). Although in some embodiments, somatic cell
10 hybridization procedures are preferred, other techniques for producing monoclonal antibodies are contemplated as well (e.g., viral or oncogenic transformation of B lymphocytes). The preferred animal system for preparing hybridomas is the murine system.

Hybridoma production in the mouse is a well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the
15 art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known. Human monoclonal antibodies (mAbs) directed against human proteins can be generated using transgenic mice carrying the complete human immune system rather than the mouse system. Splenocytes from the transgenic mice are immunized with the antigen of interest, which are used to produce hybridomas that secrete human mAbs with specific affinities for
20 epitopes from a human protein. (See e.g., Wood et al., WO 91/00906, Kucheralapati et al., WO 91/10741; Lonberg et al., WO 92/03918; Kay et al., WO 92/03917 [each of which is herein incorporated by reference in its entirety]; N. Lonberg et al., *Nature*, 368:856-859 [1994]; L.L. Green et al., *Nature Genet.*, 7:13-21 [1994]; S.L. Morrison et al., *Proc. Nat. Acad. Sci. USA*, 81:6851-6855 [1994]; Bruggeman et al., *Immunol.*, 7:33-40 [1993];
25 Tuaillon et al., *Proc. Nat. Acad. Sci. USA*, 90:3720-3724 [1993]; and Bruggeman et al. *Eur. J. Immunol.*, 21:1323-1326 [1991]).

Monoclonal antibodies can also be generated by other methods known to those skilled in the art of recombinant DNA technology. An alternative method, referred to as the "combinatorial antibody display" method, has been developed to identify and isolate antibody
30 fragments having a particular antigen specificity, and can be utilized to produce monoclonal antibodies. (See e.g., Sastry et al., *Proc. Nat. Acad. Sci. USA*, 86:5728 [1989]; Huse et al., *Science*, 246:1275 [1989]; and Orlandi et al., *Proc. Nat. Acad. Sci. USA*, 86:3833 [1989]). After immunizing an animal with an immunogen as described above, the antibody repertoire of the resulting B-cell pool is cloned. Methods are generally known for obtaining the DNA

sequence of the variable regions of a diverse population of immunoglobulin molecules by using a mixture of oligomer primers and the PCR. For instance, mixed oligonucleotide primers corresponding to the 5' leader (signal peptide) sequences and/or framework 1 (FR1) sequences, as well as primer to a conserved 3' constant region primer can be used for PCR

5 amplification of the heavy and light chain variable regions from a number of murine antibodies. (See e.g., Larrick et al., *Biotechniques*, 11:152-156 [1991]). A similar strategy can also be used to amplify human heavy and light chain variable regions from human antibodies (See e.g., Larrick et al., *Methods: Companion to Methods in Enzymology*, 2:106-110 [1991]).

10 Chimeric mouse-human monoclonal antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. (See e.g., Robinson et al., PCT/US86/02269;

15 European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; WO 86/01533; US 4,816,567; European Patent Application 125,023 [each of which is herein incorporated by reference in its entirety]; Better et al., *Science*, 240:1041-1043 [1988]; Liu et al., *Proc. Nat. Acad. Sci. USA*, 84:3439-3443 [1987]; Liu et al., *J. Immunol.*, 139:3521-3526 [1987]; Sun et al., *Proc. Nat. Acad. Sci. USA*, 84:214-

20 218 [1987]; Nishimura et al., *Canc. Res.*, 47:999-1005 [1987]; Wood et al., *Nature*, 314:446-449 [1985]; and Shaw et al., *J. Natl. Cancer Inst.*, 80:1553-1559 [1988]).

The chimeric antibody can be further humanized by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General reviews of humanized chimeric antibodies are provided

25 by S.L. Morrison, *Science*, 229:1202-1207 (1985) and by Oi et al., *Bio. Techniques*, 4:214 (1986). Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from 7E3, an anti-GPIIb/IIIa antibody producing

30 hybridoma. The recombinant DNA encoding the chimeric antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Suitable humanized antibodies can alternatively be produced by CDR substitution (e.g., US 5,225,539 (incorporated herein by reference in its entirety); Jones et al., *Nature*, 321:552-525 [1986]; Verhoeyan et al., *Science*, 239:1534 [1988]; and Beidler et al., *J.*

Immunol., 141:4053 [1988]). All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to the Fc receptor.

- 5 An antibody can be humanized by any method that is capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a non-human antibody. The human CDRs may be replaced with non-human CDRs; using oligonucleotide site-directed mutagenesis.

- 10 Also within the scope of the invention are chimeric and humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, in a humanized antibody having mouse CDRs, amino acids located in the human framework region can be replaced with the amino acids located at the corresponding positions in the mouse antibody. Such substitutions are known to
15 improve binding of humanized antibodies to the antigen in some instances.

- In some embodiments, the monoclonal antibody is a murine antibody or a fragment thereof. In other preferred embodiments, the monoclonal antibody is a bovine antibody or a fragment thereof. For example, the murine antibody can be produced by a hybridoma that includes a B cell obtained from a transgenic mouse having a genome comprising a heavy chain transgene
20 and a light chain transgene fused to an immortalized cell. The antibodies can be of various isotypes, including, but not limited to: IgG (e.g., IgG1, IgG2, IgG2a, IgG2b, IgG2c, IgG3, IgG4); IgM; IgA1; IgA2; IgAsec; IgD; and IgE. In some preferred embodiments, the antibody is an IgG isotype. In other preferred embodiments, the antibody is an IgM isotype. The antibodies can be full-length (e.g., an IgG1, IgG2, IgG3, or IgG4 antibody) or can
25 include only an antigen-binding portion (e.g., a Fab, F(ab')₂, Fv or a single chain Fv fragment).

- In preferred embodiments, the immunoglobulin is a recombinant antibody (e.g., a chimeric or a humanized antibody), a subunit, or an antigen binding fragment thereof (e.g., has a variable region, or at least a complementarity determining region (CDR)).
30 In some embodiments, the immunoglobulin is monovalent (e.g., includes one pair of heavy and light chains, or antigen binding portions thereof). In other embodiments, the immunoglobulin is a divalent (e.g., includes two pairs of heavy and light chains, or antigen binding portions thereof).

In some embodiments, the present invention provides vaccine compositions comprising a variant immunoglobulin or Fc fusion thereof and an immunogen. The present invention is not limited by the particular formulation of a vaccine composition. Indeed, a vaccine composition of the present invention may comprise one or more different agents in addition to the fusion protein. These agents or cofactors include, but are not limited to, 5 adjuvants, surfactants, additives, buffers, solubilizers, chelators, oils, salts, therapeutic agents, drugs, bioactive agents, antibacterials, and antimicrobial agents (e.g., antibiotics, antivirals, etc.). In some embodiments, a vaccine composition comprising a fusion protein comprises an agent and/or co-factor that enhance the ability of the immunogen to induce an immune response (e.g., an adjuvant). In some preferred embodiments, the presence of one or more 10 co-factors or agents reduces the amount of immunogen required for induction of an immune response (e.g., a protective immune response (e.g., protective immunization)). In some embodiments, the presence of one or more co-factors or agents can be used to skew the immune response towards a cellular (e.g., T cell mediated) or humoral (e.g., antibody 15 mediated) immune response. The present invention is not limited by the type of co-factor or agent used in a therapeutic agent of the present invention.

Adjuvants are described in general in Vaccine Design--the Subunit and Adjuvant Approach, edited by Powell and Newman, Plenum Press, New York, 1995. The present invention is not limited by the type of adjuvant utilized (e.g., for use in a composition (e.g., 20 pharmaceutical composition). For example, in some embodiments, suitable adjuvants include an aluminium salt such as aluminium hydroxide gel (alum) or aluminium phosphate. In some embodiments, an adjuvant may be a salt of calcium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes.

25 In general, an immune response is generated to an antigen through the interaction of the antigen with the cells of the immune system. Immune responses may be broadly categorized into two categories: humoral and cell mediated immune responses (e.g., traditionally characterized by antibody and cellular effector mechanisms of protection, respectively). These categories of response have been termed Th1-type responses (cell- 30 mediated response), and Th2-type immune responses (humoral response).

Stimulation of an immune response can result from a direct or indirect response of a cell or component of the immune system to an intervention (e.g., exposure to an immunogen). Immune responses can be measured in many ways including activation, proliferation or differentiation of cells of the immune system (e.g., B cells, T cells, dendritic cells, APCs,

macrophages, NK cells, NKT cells etc.); up-regulated or down-regulated expression of markers and cytokines; stimulation of IgA, IgM, or IgG titer; splenomegaly (including increased spleen cellularity); hyperplasia and mixed cellular infiltrates in various organs. Other responses, cells, and components of the immune system that can be assessed with
5 respect to immune stimulation are known in the art.

Although an understanding of the mechanism is not necessary to practice the present invention and the present invention is not limited to any particular mechanism of action, in some embodiments, compositions and methods of the present invention induce expression and secretion of cytokines (e.g., by macrophages, dendritic cells and CD4⁺ T cells).
10 Modulation of expression of a particular cytokine can occur locally or systemically. It is known that cytokine profiles can determine T cell regulatory and effector functions in immune responses. In some embodiments, Th1-type cytokines can be induced, and thus, the immunostimulatory compositions of the present invention can promote a Th1 type antigen-specific immune response including cytotoxic T-cells (e.g., thereby avoiding unwanted Th2
15 type immune responses (e.g., generation of Th2 type cytokines (e.g., IL-13) involved in enhancing the severity of disease (e.g., IL-13 induction of mucus formation))).

Cytokines play a role in directing the T cell response. Helper (CD4⁺) T cells orchestrate the immune response of mammals through production of soluble factors that act on other immune system cells, including B and other T cells. Most mature CD4⁺ T helper
20 cells express one of two cytokine profiles: Th1 or Th2. Th1-type CD4⁺ T cells secrete IL-2, IL-3, IFN- γ , GM-CSF and high levels of TNF- α . Th2 cells express IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, GM-CSF and low levels of TNF- α . Th1 type cytokines promote both cell-mediated immunity, and humoral immunity that is characterized by immunoglobulin class switching to IgG2a in mice and IgG1 in humans. Th1 responses may also be associated with
25 delayed-type hypersensitivity and autoimmune disease. Th2 type cytokines induce primarily humoral immunity and induce class switching to IgG1 and IgE. The antibody isotypes associated with Th1 responses generally have neutralizing and opsonizing capabilities whereas those associated with Th2 responses are associated more with allergic responses.

Several factors have been shown to influence skewing of an immune response
30 towards either a Th1 or Th2 type response. The best characterized regulators are cytokines. IL-12 and IFN- γ are positive Th1 and negative Th2 regulators. IL-12 promotes IFN- γ production, and IFN- γ provides positive feedback for IL-12. IL-4 and IL-10 appear important for the establishment of the Th2 cytokine profile and to down-regulate Th1 cytokine production.

Thus, in preferred embodiments, the present invention provides a method of stimulating a Th1-type immune response in a subject comprising administering to a subject a composition comprising an immunogen. However, in other embodiments, the present invention provides a method of stimulating a Th2-type immune response in a subject (e.g., if balancing of a T cell mediated response is desired) comprising administering to a subject a composition comprising an immunogen. In further preferred embodiments, adjuvants can be used (e.g., can be co-administered with a composition of the present invention) to skew an immune response toward either a Th1 or Th2 type immune response. For example, adjuvants that induce Th2 or weak Th1 responses include, but are not limited to, alum, saponins, and SB-As4. Adjuvants that induce Th1 responses include but are not limited to MPL, MDP, ISCOMS, IL-12, IFN- γ , and SB-AS2.

Several other types of Th1-type immunogens can be used (e.g., as an adjuvant) in compositions and methods of the present invention. These include, but are not limited to, the following. In some embodiments, monophosphoryl lipid A (e.g., in particular 3-de-O-acylated monophosphoryl lipid A (3D-MPL)), is used. 3D-MPL is a well known adjuvant manufactured by Ribi Immunochem, Montana. Chemically it is often supplied as a mixture of 3-de-O-acylated monophosphoryl lipid A with either 4, 5, or 6 acylated chains. In some embodiments, diphosphoryl lipid A, and 3-O-deacylated variants thereof are used. Each of these immunogens can be purified and prepared by methods described in GB 2122204B, hereby incorporated by reference in its entirety. Other purified and synthetic lipopolysaccharides have been described (See, e.g., U.S. Pat. No. 6,005,099 and EP 0 729 473; Hilgers et al., 1986, *Int. Arch. Allergy. Immunol.*, 79(4):392-6; Hilgers et al., 1987, *Immunology*, 60(1):141-6; and EP 0 549 074, each of which is hereby incorporated by reference in its entirety). In some embodiments, 3D-MPL is used in the form of a particulate formulation (e.g., having a small particle size less than 0.2 μm in diameter, described in EP 0 689 454, hereby incorporated by reference in its entirety).

In some embodiments, saponins are used as an immunogen (e.g., Th1-type adjuvant) in a composition of the present invention. Saponins are well known adjuvants (See, e.g., Lacaille-Dubois and Wagner (1996) *Phytomedicine* vol 2 pp 363-386). Examples of saponins include Quil A (derived from the bark of the South American tree *Quillaja Saponaria* Molina), and fractions thereof (See, e.g., U.S. Pat. No. 5,057,540; Kensil, *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279, each of which is hereby incorporated by reference in its entirety). Also contemplated to be useful in the present invention are the haemolytic saponins QS7, QS17, and QS21 (HPLC purified fractions of

Quil A; See, e.g., Kensil et al. (1991). J. Immunology 146,431-437, U.S. Pat. No. 5,057,540; WO 96/33739; WO 96/11711 and EP 0 362 279, each of which is hereby incorporated by reference in its entirety). Also contemplated to be useful are combinations of QS21 and polysorbate or cyclodextrin (See, e.g., WO 99/10008, hereby incorporated by reference in its
5 entirety).

In some embodiments, an immunogenic oligonucleotide containing unmethylated CpG dinucleotides ("CpG") is used as an adjuvant. CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. CpG is known in the art as being an adjuvant when administered by both systemic and mucosal routes (See, e.g., WO 96/02555, EP
10 468520, Davis et al., J.Immunol, 1998, 160(2):870-876; McCluskie and Davis, J.Immunol., 1998, 161(9):4463-6; and U.S. Pat. App. No. 20050238660, each of which is hereby incorporated by reference in its entirety). For example, in some embodiments, the immunostimulatory sequence is Purine-Purine-C-G-pyrimidine-pyrimidine; wherein the CG motif is not methylated.

15 Although an understanding of the mechanism is not necessary to practice the present invention and the present invention is not limited to any particular mechanism of action, in some embodiments, the presence of one or more CpG oligonucleotides activate various immune subsets including natural killer cells (which produce IFN- γ) and macrophages. In some embodiments, CpG oligonucleotides are formulated into a composition of the present
20 invention for inducing an immune response. In some embodiments, a free solution of CpG is co-administered together with an antigen (e.g., present within a solution (See, e.g., WO 96/02555; hereby incorporated by reference). In some embodiments, a CpG oligonucleotide is covalently conjugated to an antigen (See, e.g., WO 98/16247, hereby incorporated by reference), or formulated with a carrier such as aluminium hydroxide (See, e.g., Brazolot-
25 Millan et al., Proc.Natl.AcadSci., USA, 1998, 95(26), 15553-8).

In some embodiments, adjuvants such as Complete Freund's Adjuvant and Incomplete Freund's Adjuvant, cytokines (e.g., interleukins (e.g., IL-2, IFN- γ , IL-4, etc.), macrophage colony stimulating factor, tumor necrosis factor, etc.), detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an E. Coli heat-
30 labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (See, e.g., WO93/13202 and WO92/19265, each of

which is hereby incorporated by reference), and other immunogenic substances (e.g., that enhance the effectiveness of a composition of the present invention) are used with a composition comprising an immunogen of the present invention.

Additional examples of adjuvants that find use in the present invention include
5 poly(di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA);
derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPL; Ribi
ImmunoChem Research, Inc., Hamilton, Mont.), muramyl dipeptide (MDP; Ribi) and
threonyl-muramyl dipeptide (t-MDP; Ribi); OM-174 (a glucosamine disaccharide related to
lipid A; OM Pharma SA, Meyrin, Switzerland); and Leishmania elongation factor (a purified
10 Leishmania protein; Corixa Corporation, Seattle, Wash.).

Adjuvants may be added to a composition comprising an immunogen, or, the adjuvant may be formulated with carriers, for example liposomes, or metallic salts (e.g., aluminium salts (e.g., aluminium hydroxide)) prior to combining with or co-administration with a composition.

15 In some embodiments, a composition comprising an immunogen comprises a single adjuvant. In other embodiments, a composition comprises two or more adjuvants (See, e.g., WO 94/00153; WO 95/17210; WO 96/33739; WO 98/56414; WO 99/12565; WO 99/11241; and WO 94/00153, each of which is hereby incorporated by reference in its entirety).

In some embodiments, a composition comprising an immunogen comprises one or
20 more mucoadhesives (See, e.g., U.S. Pat. App. No. 20050281843, hereby incorporated by reference in its entirety). The present invention is not limited by the type of mucoadhesive utilized. Indeed, a variety of mucoadhesives are contemplated to be useful in the present invention including, but not limited to, cross-linked derivatives of poly(acrylic acid) (e.g., carbopol and polycarbophil), polyvinyl alcohol, polyvinyl pyrrolidone, polysaccharides (e.g.,
25 alginate and chitosan), hydroxypropyl methylcellulose, lectins, fimbrial proteins, and carboxymethylcellulose. Although an understanding of the mechanism is not necessary to practice the present invention and the present invention is not limited to any particular mechanism of action, in some embodiments, use of a mucoadhesive (e.g., in a composition comprising an immunogen) enhances induction of an immune response in a subject (e.g.,
30 administered a composition of the present invention) due to an increase in duration and/or amount of exposure to an immunogen that a subject experiences when a mucoadhesive is used compared to the duration and/or amount of exposure to an immunogen in the absence of using the mucoadhesive.

In some embodiments, the immunoglobulin and/or vaccine compositions are used in conjunction with appropriate salts and buffers to render delivery of the compositions to a subject. Buffers also are employed when the compositions are introduced into a patient. Aqueous compositions comprise an effective amount of composition dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula.

The phrase "pharmaceutically or pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. 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. Except insofar as any conventional media or agent is incompatible with the vectors or cells of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients may also be incorporated into the compositions.

In some embodiments of the present invention, the active compositions include classic pharmaceutical preparations. Administration of these compositions according to the present invention is via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection.

The compositions may also be administered parenterally or intraperitoneally. Solutions of the active compounds as free base or pharmacologically acceptable salts are prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. 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. The prevention of the action of microorganisms can be brought about by various

antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the 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 techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Upon formulation, compositions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution is suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). In some embodiments of the present invention, the active particles or agents are formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses may be administered.

Additional formulations that are suitable for other modes of administration include vaginal suppositories and pessaries. A rectal pessary or suppository may also be used. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum, vagina or the urethra. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed

from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Vaginal suppositories or pessaries are usually globular or oviform and weighing about 5 g each. Vaginal medications are available in a variety of physical forms, e.g., creams, gels or liquids, which depart from the classical concept of suppositories.

5 "Treating" within the context of the instant invention, means an alleviation, in whole or in part, of symptoms associated with a disorder or disease, or slowing, inhibiting or halting of further progression or worsening of those symptoms, or prevention or prophylaxis of the disease or disorder in a subject at risk for developing the disease or disorder. Thus, e.g., treating a viral infection may include inhibiting or preventing replication of the virus in a
10 subject or decreasing symptoms of viral infection in the subject. As used herein, a "therapeutically effective amount" of a compound of the invention refers to an amount of the compound that alleviates, in whole or in part, symptoms associated with a disorder or disease, or slows, inhibits or halts further progression or worsening of those symptoms, or prevents or provides prophylaxis for the disease or disorder in a subject at risk for developing the disease
15 or disorder.

A subject is any animal that can benefit from the administration of a compound as described herein. In some embodiments, the subject is a mammal, for example, a human, a primate, a dog, a cat, a horse, a cow, a pig, a rodent, such as for example a rat or mouse. Typically, the subject is a human.

20 A therapeutically effective amount of a compound as described herein used in the present invention may vary depending upon the route of administration and dosage form. Effective amounts of invention compounds typically fall in the range of about 0.001 up to 100 mg/kg/day, and more typically in the range of about 0.05 up to 10 mg/kg/day. Typically, the compound or compounds used in the instant invention are selected to provide a
25 formulation that exhibits a high therapeutic index. The therapeutic index is the dose ratio between toxic and therapeutic effects which can be expressed as the ratio between LD50 and ED50. The LD50 is the dose lethal to 50% of the population and the ED50 is the dose therapeutically effective in 50% of the population. The LD50 and ED50 are determined by standard pharmaceutical procedures in animal cell cultures or experimental animals.
30 The instant invention also provides for pharmaceutical compositions and medicaments which may be prepared by combining one or more compounds described herein, pharmaceutically acceptable salts thereof, stereoisomers thereof, tautomers thereof, or solvates thereof, with pharmaceutically acceptable carriers, excipients, binders, diluents or the like to inhibit or treat primary and/or metastatic prostate cancers. Such compositions can be in the form of, for

example, granules, powders, tablets, capsules, syrup, suppositories, injections, emulsions, elixirs, suspensions or solutions. The instant compositions can be formulated for various routes of administration, for example, by oral, parenteral, topical, rectal, nasal, or via implanted reservoir. Parenteral or systemic administration includes, but is not limited to, subcutaneous, intravenous, intraperitoneal, and intramuscular injections. The following dosage forms are given by way of example and should not be construed as limiting the instant invention.

For oral, buccal, and sublingual administration, powders, suspensions, granules, tablets, pills, capsules, gelcaps, and caplets are acceptable as solid dosage forms. These can be prepared, for example, by mixing one or more compounds of the instant invention, or pharmaceutically acceptable salts or tautomers thereof, with at least one additive such as a starch or other additive. Suitable additives are sucrose, lactose, cellulose sugar, mannitol, maltitol, dextran, starch, agar, alginates, chitins, chitosans, pectins, tragacanth gum, gum arabic, gelatins, collagens, casein, albumin, synthetic or semi-synthetic polymers or glycerides. Optionally, oral dosage forms can contain other ingredients to aid in administration, such as an inactive diluent, or lubricants such as magnesium stearate, or preservatives such as paraben or sorbic acid, or antioxidants such as ascorbic acid, tocopherol or cysteine, a disintegrating agent, binders, thickeners, buffers, sweeteners, flavoring agents or perfuming agents. Tablets and pills may be further treated with suitable coating materials known in the art.

Liquid dosage forms for oral administration may be in the form of pharmaceutically acceptable emulsions, syrups, elixirs, suspensions, and solutions, which may contain an inactive diluent, such as water. Pharmaceutical formulations and medicaments may be prepared as liquid suspensions or solutions using a sterile liquid, such as, but not limited to, an oil, water, an alcohol, and combinations of these. Pharmaceutically suitable surfactants, suspending agents, emulsifying agents, may be added for oral or parenteral administration. As noted above, suspensions may include oils. Such oils include, but are not limited to, peanut oil, sesame oil, cottonseed oil, corn oil and olive oil. Suspension preparations may also contain esters of fatty acids such as ethyl oleate, isopropyl myristate, fatty acid glycerides and acetylated fatty acid glycerides. Suspension formulations may include alcohols, such as, but not limited to, ethanol, isopropyl alcohol, hexadecyl alcohol, glycerol and propylene glycol. Ethers, such as but not limited to, poly(ethyleneglycol), petroleum hydrocarbons such as mineral oil and petrolatum; and water may also be used in suspension formulations.

Injectable dosage forms generally include aqueous suspensions or oil suspensions which may be prepared using a suitable dispersant or wetting agent and a suspending agent. Injectable forms may be in solution phase or in the form of a suspension, which is prepared with a solvent or diluent. Acceptable solvents or vehicles include sterilized water, Ringer's solution, or an isotonic aqueous saline solution. Alternatively, sterile oils may be employed as solvents or suspending agents. Typically, the oil or fatty acid is non-volatile, including natural or synthetic oils, fatty acids, mono-, di- or tri-glycerides.

For injection, the pharmaceutical formulation and/or medicament may be a powder suitable for reconstitution with an appropriate solution as described above. Examples of these include, but are not limited to, freeze dried, rotary dried or spray dried powders, amorphous powders, granules, precipitates, or particulates. For injection, the formulations may optionally contain stabilizers, pH modifiers, surfactants, bioavailability modifiers and combinations of these.

For rectal administration, the pharmaceutical formulations and medicaments may be in the form of a suppository, an ointment, an enema, a tablet or a cream for release of compound in the intestines, sigmoid flexure and/or rectum. Rectal suppositories are prepared by mixing one or more compounds of the instant invention, or pharmaceutically acceptable salts or tautomers of the compound, with acceptable vehicles, for example, cocoa butter or polyethylene glycol, which is present in a solid phase at normal storing temperatures, and present in a liquid phase at those temperatures suitable to release a drug inside the body, such as in the rectum. Oils may also be employed in the preparation of formulations of the soft gelatin type and suppositories. Water, saline, aqueous dextrose and related sugar solutions, and glycerols may be employed in the preparation of suspension formulations which may also contain suspending agents such as pectins, carbomers, methyl cellulose, hydroxypropyl cellulose or carboxymethyl cellulose, as well as buffers and preservatives.

Compounds of the invention may be administered to the lungs by inhalation through the nose or mouth. Suitable pharmaceutical formulations for inhalation include solutions, sprays, dry powders, or aerosols containing any appropriate solvents and optionally other compounds such as, but not limited to, stabilizers, antimicrobial agents, antioxidants, pH modifiers, surfactants, bioavailability modifiers and combinations of these. Formulations for inhalation administration contain as excipients, for example, lactose, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate. Aqueous and nonaqueous aerosols are typically used for delivery of inventive compounds by inhalation.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the compound together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (TWEENS, Pluronic, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions. A nonaqueous suspension (e.g., in a fluorocarbon propellant) can also be used to deliver compounds of the invention.

Aerosols containing compounds for use according to the present invention are conveniently delivered using an inhaler, atomizer, pressurized pack or a nebulizer and a suitable propellant, e.g., without limitation, pressurized dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, nitrogen, air, or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be controlled by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Delivery of aerosols of the present invention using sonic nebulizers is advantageous because nebulizers minimize exposure of the agent to shear, which can result in degradation of the compound.

For nasal administration, the pharmaceutical formulations and medicaments may be a spray, nasal drops or aerosol containing an appropriate solvent(s) and optionally other compounds such as, but not limited to, stabilizers, antimicrobial agents, antioxidants, pH modifiers, surfactants, bioavailability modifiers and combinations of these. For administration in the form of nasal drops, the compounds may be formulated in oily solutions or as a gel. For administration of nasal aerosol, any suitable propellant may be used including compressed air, nitrogen, carbon dioxide, or a hydrocarbon based low boiling solvent. Dosage forms for the topical (including buccal and sublingual) or transdermal administration of compounds of the invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, and patches. The active component may be mixed under sterile conditions with a pharmaceutically-acceptable carrier or excipient, and with any preservatives, or buffers, which may be required. Powders and sprays can be prepared, for example, with excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. The ointments, pastes, creams and gels may also contain excipients such as animal and vegetable fats, oils, waxes, paraffins, starch,

tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the invention to the body. Such dosage forms can be made by dissolving or
5 dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the inventive compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Besides those representative dosage forms described above, pharmaceutically
10 acceptable excipients and carriers are generally known to those skilled in the art and are thus included in the instant invention. Such excipients and carriers are described, for example, in "Remingtons Pharmaceutical Sciences" Mack Pub. Co., New Jersey (1991), which is incorporated herein by reference.

The formulations of the invention may be designed to be short-acting, fast-releasing,
15 long-acting, and sustained-releasing as described below. Thus, the pharmaceutical formulations may also be formulated for controlled release or for slow release. The instant compositions may also comprise, for example, micelles or liposomes, or some other encapsulated form, or may be administered in an extended release form to provide a prolonged storage and/or delivery effect. Therefore, the pharmaceutical formulations and
20 medicaments may be compressed into pellets or cylinders and implanted intramuscularly or subcutaneously as depot injections or as implants such as stents. Such implants may employ known inert materials such as silicones and biodegradable polymers.

Specific dosages may be adjusted depending on conditions of disease, the age, body weight, general health conditions, sex, and diet of the subject, dose intervals, administration
25 routes, excretion rate, and combinations of drugs. Any of the above dosage forms containing effective amounts are well within the bounds of routine experimentation and therefore, well within the scope of the instant invention.

In some embodiments of the present invention, methods and compositions are provided for the treatment and prevention of any number of disorders. Therapeutic antibodies
30 find use in the treatment of, for example, cancer, autoimmune disease, infection (e.g., viral infection), etc.

In some embodiments, the immunoglobulins described herein are administering in combination with another therapeutic agent (e.g., anti-cancer, immunosuppressant, or anti-viral agent). In some embodiments, treatment with the immunoglobulin described herein

precedes or follows the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and immunoglobulin are applied separately to the cell, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the multiple therapies would still be able to exert an
5 advantageously combined effect on the cell. In such instances, it is contemplated that cells are contacted with both modalities within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other, with a delay time of only about 12 hours being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2 to 7) to several weeks (1 to 8) lapse
10 between the respective administrations.

In some embodiments, more than one administration of the immunotherapeutic composition of the present invention or the other agent is utilized. Various combinations may be employed, where the immunoglobulin is "A" and the other agent is "B", as exemplified below: A/B/A, B/A/B, B/B/A, A/A/B, B/A/A, A/B/B, B/B/B/A, B/B/A/B,
15 A/A/B/B, A/B/A/B, A/B/B/A, B/B/A/A, B/A/B/A, B/A/A/B, B/B/B/A, A/A/A/B, B/A/A/A, A/B/A/A, A/A/B/A, A/B/B/B, B/A/B/B, B/B/A/B. Other combinations are contemplated.

In some embodiments of the invention, one or more compounds of the invention and an additional active agent are administered to a subject, more typically a human, in a
20 sequence and within a time interval such that the compound can act together with the other agent to provide an enhanced benefit relative to the benefits obtained if they were administered otherwise. For example, the additional active agents can be co-administered by co-formulation, administered at the same time or administered sequentially in any order at different points in time; however, if not administered at the same time, they should be
25 administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. In some embodiments, the compound and the additional active agents exert their effects at times which overlap. Each additional active agent can be administered separately, in any appropriate form and by any suitable route. In other embodiments, the compound is administered before, concurrently or after administration of the additional active agents.
30 In various examples, the compound and the additional active agents are administered less than about 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about

9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In other examples, the compound and the additional active agents are administered concurrently. In yet other examples, the compound and the additional active agents are administered
5 concurrently by co-formulation.

In other examples, the compound and the additional active agents are administered at about 2 to 4 days apart, at about 4 to 6 days apart, at about 1 week apart, at about 1 to 2 weeks apart, or more than 2 weeks apart.

In certain examples, the inventive compound and optionally the additional active
10 agents are cyclically administered to a subject. Cycling therapy involves the administration of a first agent for a period of time, followed by the administration of a second agent and/or third agent for a period of time and repeating this sequential administration. Cycling therapy can provide a variety of benefits, e.g., reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one or more of the therapies, and/or improve
15 the efficacy of the treatment.

In other examples, one or more compound of some embodiments of the present invention and optionally the additional active agent are administered in a cycle of less than about 3 weeks, about once every two weeks, about once every 10 days or about once every week. One cycle can comprise the administration of an inventive compound and optionally
20 the second active agent by infusion over about 90 minutes every cycle, about 1 hour every cycle, about 45 minutes every cycle, about 30 minutes every cycle or about 15 minutes every cycle. Each cycle can comprise at least 1 week of rest, at least 2 weeks of rest, at least 3 weeks of rest. The number of cycles administered is from about 1 to about 12 cycles, more typically from about 2 to about 10 cycles, and more typically from about 2 to about 8 cycles.
25 Courses of treatment can be administered concurrently to a subject, i.e., individual doses of the additional active agents are administered separately yet within a time interval such that the inventive compound can work together with the additional active agents. For example, one component can be administered once per week in combination with the other components that can be administered once every two weeks or once every three weeks. In other words, the
30 dosing regimens are carried out concurrently even if the therapeutics are not administered simultaneously or during the same day.

The additional active agents can act additively or, more typically, synergistically with the inventive compound(s). In one example, one or more inventive compound is administered concurrently with one or more second active agents in the same pharmaceutical

composition. In another example, one or more inventive compound is administered concurrently with one or more second active agents in separate pharmaceutical compositions. In still another example, one or more inventive compound is administered prior to or subsequent to administration of a second active agent. The invention contemplates administration of an inventive compound and a second active agent by the same or different routes of administration, e.g., oral and parenteral. In certain embodiments, when the inventive compound is administered concurrently with a second active agent that potentially produces adverse side effects including, but not limited to, toxicity, the second active agent can advantageously be administered at a dose that falls below the threshold that the adverse side effect is elicited.

Virtually any antigen may be targeted by the IgG variants, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens, which includes both soluble factors such as cytokines and membrane-bound factors, including transmembrane receptors: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a,

- CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146,
- 5 CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3,
- 10 CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII,
- 15 Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3),
- 20 GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GTR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu
- 25 (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA
- 30 receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta7, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin

- alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF-1),
- 5 Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR),
- 10 MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellerian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3, -4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta,
- 15 nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PIGF, PLP, PP14, Proinsulin, Prorolaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN,
- 20 R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-
- 25 cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta R1 (ALK-5), TGF-beta RII, TGF-beta RIIB, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha,
- 30 TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R3Apo-2, DR4), TNFRSF10B (TRAIL R2DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3DcR1, LIT, TRID), TNFRSF10D (TRAIL R4DcR2, TRUND), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA,

LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF R1CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1 BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEGFR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors.

One skilled in the art will appreciate that the aforementioned list of targets refers not only to specific proteins and biomolecules, but the biochemical pathway or pathways that comprise them. For example, reference to CTLA-4 as a target antigen implies that the ligands and receptors that make up the T cell co-stimulatory pathway, including CTLA-4, B7-1, B7-2, CD28, and any other undiscovered ligands or receptors that bind these proteins, are also targets. Thus target as used herein refers not only to a specific biomolecule, but the set of proteins that interact with said target and the members of the biochemical pathway to which said target belongs. One skilled in the art will further appreciate that any of the aforementioned target antigens, the ligands or receptors that bind them, or other members of

their corresponding biochemical pathway, may be operably linked to the Fc variants of the present invention in order to generate an Fc fusion. Thus for example, an Fc fusion that targets EGFR could be constructed by operably linking an Fc variant to EGF, TGF- β , or any other ligand, discovered or undiscovered, that binds EGFR. Accordingly, an Fc variant of the present invention could be operably linked to EGFR in order to generate an Fc fusion that binds EGF, TGF- β , or any other ligand, discovered or undiscovered, that binds EGFR. Thus virtually any polypeptide, whether a ligand, receptor, or some other protein or protein domain, including but not limited to the aforementioned targets and the proteins that compose their corresponding biochemical pathways, may be operably linked to the Fc variants of the present invention to develop an Fc fusion.

The choice of suitable antigen depends on the desired application. For anti-cancer treatment it is desirable to have a target whose expression is restricted to the cancerous cells. Some targets that have proven especially amenable to antibody therapy are those with signaling functions. Other therapeutic antibodies exert their effects by blocking signaling of the receptor by inhibiting the binding between a receptor and its cognate ligand. Another mechanism of action of therapeutic antibodies is to cause receptor down regulation. Other antibodies do not work by signaling through their target antigen. In some cases, antibodies directed against infectious disease agents are used.

In one embodiment, the Fc variants of the present invention are incorporated into an antibody against a cytokine. Alternatively, the Fc variants are fused or conjugated to a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. For example, as described in Penichet et al., 2001, J Immunol Methods 248:91-101, expressly incorporated by reference, cytokines may be fused to antibody to provide an array of desirable properties. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth

factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; C5a; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

Cytokines and soluble targets, such as TNF superfamily members, are preferred targets for use with the variants of the present invention. For example, anti-VEGF, anti-CTLA-4, and anti-TNF antibodies, or fragments thereof, are particularly good antibodies for the use of Fc variants that increase the FcRn binding. Therapeutics against these targets are frequently involved in the treatment of autoimmune diseases and require multiple injections over long time periods. Therefore, longer serum half-lives and less frequent treatments, brought about from the variants of the present invention, are particularly preferred.

A number of antibodies and Fc fusions that are approved for use, in clinical trials, or in development may benefit from the Fc variants of the present invention. These antibodies and Fc fusions are herein referred to as "clinical products and candidates". Thus in a preferred embodiment, the Fc polypeptides of the present invention may find use in a range of clinical products and candidates. For example, a number of antibodies that target CD20 may benefit from the Fc polypeptides of the present invention. For example the Fc polypeptides of the present invention may find use in an antibody that is substantially similar to rituximab (Rituxan®, IDEC/Genentech/Roche) (see for example U.S. Pat. No. 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HuMax-CD20, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in U.S. Pat. No. 5,500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), HumaLYM (Intracel), and PRO70769 (PCT/US2003/040426, entitled "Immunoglobulin Variants and Uses Thereof"). A number of antibodies that target members of the family of epidermal growth factor receptors, including EGFR (ErbB-1), Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), may benefit from the Fc polypeptides of the present invention. For example the Fc polypeptides of the present invention may find use in an antibody that is substantially similar to trastuzumab (Herceptin®, Genentech) (see for example U.S. Pat. No. 5,677,171), a humanized anti-Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, Omnitarg™), currently being developed by

Genentech; an anti-Her2 antibody described in U.S. Pat. No. 4,753,894; cetuximab (Erbix®), Imclone) (U.S. Pat. No. 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (U.S. Pat. No. 6,235,883), currently being developed by Abgenix-Immunex-Amgen; HuMax-EGFr (U.S. Ser. No. 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (U.S. Pat. No. 5,558,864; Murthy et al. 1987, Arch Biochem Biophys. 252(2):549-60; Rodeck et al., 1987, J Cell Biochem. 35(4):315-20; Kettleborough et al., 1991, Protein Eng. 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al., 1993, J. Cell Biophys. 1993, 22(1-3):129-46; Modjtahedi et al., 1993, Br J. Cancer. 1993, 67(2):247-53; Modjtahedi et al, 1996, Br J Cancer, 73(2):228-35; Modjtahedi et al, 2003, Int J Cancer, 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (U.S. Pat. No. 5,891,996; U.S. Pat. No. 6,506,883; Mateo et al, 1997, Immunotechnology, 3(1):71-81); mAb-806 (Ludwig Institute for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, Proc Natl Acad Sci USA. 100(2):639-44); KSB-102 (KS Biomedix); MR1-1 (IVAX, National Cancer Institute) (PCT WO 0162931A2); and SC100 (Scancell) (PCT WO 01/88138). In another preferred embodiment, the Fc polypeptides of the present invention may find use in alemtuzumab (Campath®, Millenium), a humanized monoclonal antibody currently approved for treatment of B-cell chronic lymphocytic leukemia. The Fc polypeptides of the present invention may find use in a variety of antibodies or Fc fusions that are substantially similar to other clinical products and candidates, including but not limited to muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/Johnson & Johnson, ibritumomab tiuxetan (Zevalin®), an anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (Amevive®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (ReoPro®), developed by Centocor/Lilly, basiliximab (Simulect®), developed by Novartis, palivizumab (Synagis®), developed by Med immune, infliximab (Remicade®), an anti-TNFalpha antibody developed by Centocor, adalimumab (Humira®), an anti-TNFalpha antibody developed by Abbott, Humicade™, an anti-TNFalpha antibody developed by Celltech, etanercept (Enbrel®), an anti-TNFalpha Fc fusion developed by Immunex/Amgen, ABX-CBL, an anti-CD147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, 90Y-muHMFG1), an anti-MUC1 In development by Antisoma, Therex (R1550), an anti-MUC1 antibody being developed by

- Antisoma, AngioMab (AS1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS1407) being developed by Antisoma, Antegren® (natalizumab), an anti-alpha-4-beta-1 (VLA-4) and alpha-4-beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an anti-TGF- β 2 antibody being developed by Cambridge Antibody Technology, J695, an anti-IL-12 antibody being developed by Cambridge Antibody Technology and Abbott, CAT-192, an anti-TGF β 1 antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxin1 antibody being developed by Cambridge Antibody Technology, LymphoStat-B™ an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-R1 mAb, an anti-TRAIL-R1 antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc., Avastin™ (bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, Xolair™ (Omalizumab), an anti-IgE antibody being developed by Genentech, Raptiva™ (Efalizumab), an anti-CD11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millenium Pharmaceuticals, HuMax CD4, an anti-CD4 antibody being developed by Genmab, HuMax-IL 15, an anti-IL 15 antibody being developed by Genmab and Amgen, HuMax-Inflam, being developed by Genmab and Medarex, HuMax-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GcoSciences, HuMax-Lymphoma, being developed by Genmab and Amgen, HuMax-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti-CD4 antibody being developed by IDEC Pharmaceuticals, IDEC-114, an anti-CD80 antibody being developed by IDEC Pharmaceuticals, IDEC-152, an anti-CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being developed by Imclone, IMC-1C11, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-Cide™ (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LymphoCide™ (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being

developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex, Osidem™ (IDM-1), and anti-Her2 antibody being developed by

5 Medarex and Immuno-Designed Molecules, HuMax™-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL 15, an anti-IL 15 antibody being developed by Medarex and Genmab, CNTO 148, an anti-TNF α antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MOR101 and MOR102, anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) antibodies

10 being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, Nuvion® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HuZAF™, an anti-gamma interferon antibody being developed by Protein Design Labs, Anti- $\alpha 5\beta 1$ Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-

15 CAM antibody being developed by Xoma, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma, all of the above-cited references in this paragraph are expressly incorporated herein by reference.

The Fc polypeptides of the present invention may be incorporated into the aforementioned clinical candidates and products, or into antibodies and Fc fusions that are

20 substantially similar to them. The Fc polypeptides of the present invention may be incorporated into versions of the aforementioned clinical candidates and products that are humanized, affinity matured, engineered, or modified in some other way.

In one embodiment, the Fc polypeptides of the present invention are used for the treatment of autoimmune, inflammatory, or transplant indications. Target antigens and

25 clinical products and candidates that are relevant for such diseases include but are not limited to anti- $\alpha 4\beta 7$ integrin antibodies such as LDP-02, anti-beta2 integrin antibodies such as LDP-01, anti-complement (C5) antibodies such as 5G1.1, anti-CD2 antibodies such as BTI-322, MEDI-507, anti-CD3 antibodies such as OKT3, SMART anti-CD3, anti-CD4 antibodies such as IDEC-151, MDX-CD4, OKT4A, anti-CD11a antibodies, anti-CD14 antibodies such as

30 IC14, anti-CD18 antibodies, anti-CD23 antibodies such as IDEC 152, anti-CD25 antibodies such as Zenapax, anti-CD40L antibodies such as 5c8, Antova, IDEC-131, anti-CD64 antibodies such as MDX-33, anti-CD80 antibodies such as IDEC-114, anti-CD147 antibodies such as ABX-CBL, anti-E-selectin antibodies such as CDP850, anti-gpIIb/IIIa antibodies such as ReoPro/Abcixima, anti-ICAM-3 antibodies such as ICM3, anti-ICE antibodies such

as VX-740, anti-FcR1 antibodies such as MDX-33, anti-IgE antibodies such as rhuMab-E25, anti-IL-4 antibodies such as SB-240683, anti-IL-5 antibodies such as SB-240563, SCH55700, anti-IL-8 antibodies such as ABX-IL8, anti-interferon gamma antibodies, anti-TNF (TNF, TNFa, TNFa, TNF-alpha) antibodies such as CDP571, CDP870, D2E7, Infliximab, MAK-
 5 195F, and anti-VLA-4 antibodies such as Antegren.

Fc variants of the present invention such as those with increased binding to FcRn may be utilized in TNF inhibitor molecules to provide enhanced properties. Useful TNF inhibitor molecules include any molecule that inhibits the action of TNF-alpha in a mammal. Suitable examples include the Fc fusion Enbrel® (etanercept) and the antibodies Humira®
 10 (adalimumab) and Remicade® (infliximab). Monoclonal antibodies (such as Remicade and Humira) engineered using the Fc variants of the present invention to increase FcFn binding, may translate to better efficacy through an increased half-life.

In some embodiments, antibodies against infectious diseases are used. Antibodies against eukaryotic cells include antibodies targeting yeast cells, including but not limited to
 15 *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Kluyveromyces fragilis* and *K. lactis*, *Pichia guilliermondii* and *P. pastoris*, *Schizosaccharomyces pombe*, *plasmodium falciparum*, and *Yarrowia lipolytica*.

Antibodies against additional fungal cells are also useful, including target antigens associated with *Candida* strains including *Candida glabrata*, *Candida albicans*, *C. krusei*, *C.*
 20 *lusitaniae* and *C. maltosa*, as well as species of *Aspergillus*, *Cryptococcus*, *Histoplasma*, *Coccidioides*, *Blastomyces*, and *Penicillium*, among others

Antibodies directed against target antigens associated with protozoa include, but are not limited to, antibodies associated with *Trypanosoma*, *Leishmania* species including *Leishmania donovani*; *Plasmodium* spp., *Pneumocystis carinii*, *Cryptosporidium parvum*,
 25 *Giardia lamblia*, *Entamoeba histolytica*, and *Cyclospora cayetanensis*.

Antibodies against prokaryotic antigens are also useful, including antibodies against suitable bacteria such as pathogenic and non-pathogenic prokaryotes including but not limited to *Bacillus*, including *Bacillus anthracis*; *Vibrio*, e.g. *V. cholerae*; *Escherichia*, e.g. Enterotoxigenic *E. coli*, *Shigella*, e.g. *S. dysenteriae*; *Salmonella*, e.g. *S. typhi*;
 30 *Mycobacterium* e.g. *M. tuberculosis*, *M. leprae*; *Clostridium*, e.g. *C. botulinum*, *C. tetani*, *C. difficile*, *C. perfringens*; *Corynebacterium*, e.g. *C. diphtheriae*; *Streptococcus*, *S. pyogenes*, *S. pneumoniae*; *Staphylococcus*, e.g. *S. aureus*; *Haemophilus*, e.g. *H. influenzae*; *Neisseria*, e.g. *N. meningitidis*, *N. gonorrhoeae*; *Yersinia*, e.g. *Y. lamblia*, *Y. pestis*, *Pseudomonas*, e.g. *P. aeruginosa*, *P. putida*; *Chlamydia*, e.g. *C. trachomatis*; *Bordetella*, e.g. *B. pertussis*;

Treponema, e.g. *T. palladium*; *B. anthracis*, *Y. pestis*, *Brucella* spp., *F. tularensis*, *B. mallei*, *B. pseudomallei*, *B. mallei*, *B. pseudomallei*, *C. botulinum*, *Salmonella* spp., SEB *V. cholerae* toxin B, *E. coli* O157:H7, *Listeria* spp., *Trichosporon beigeli*, *Rhodotorula* species, *Hansenula anomala*, *Enterobacter* sp., *Klebsiella* sp., *Listeria* sp., *Mycoplasma* sp. and the like.

In some aspects, the antibodies are directed against viral infections; these viruses include, but are not limited to, including orthomyxoviruses, (e.g. influenza virus), paramyxoviruses (e.g. respiratory syncytial virus, mumps virus, measles virus), adenoviruses, rhinoviruses, coronaviruses, reoviruses, togaviruses (e.g. rubella virus), parvoviruses, poxviruses (e.g. variola virus, vaccinia virus), enteroviruses (e.g. poliovirus, coxsackievirus), hepatitis viruses (including A, B and C), herpesviruses (e.g. Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus), rotaviruses, Norwalk viruses, hantavirus, arenavirus, rhabdovirus (e.g. rabies virus), retroviruses (including HIV, HTLV-I and -II), papovaviruses (e.g. papillomavirus), polyomaviruses, and picornaviruses, and the like.

Experimental

Example 1

Fc-engineering for improved FcRn binding

The neonatal Fc receptor (FcRn) regulates the 3-week long IgG half-life of IgG in humans. Engineering of IgG antibodies for improved pH dependent binding to FcRn has resulted in examples of antibodies with extended half-life/altered pharmacokinetics and cellular transport properties.

Materials and Methods

Cell culture. The cell line HEK293E was maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin at 37°C in a humid 5% CO₂, 95% air incubator.

Production of soluble recombinant human FcRn. The construction of a eukaryotic pcDNA3 vector encoding a recombinant truncated form of wild-type human FcRn, which contains the cDNA encoding the three extracellular domains (α 1- α 3) C-terminally fused to a cDNA encoding glutathione *S*-transferase (GST) from *Schistosoma japonicum*, has been

described (Berntzen, G., et al., J Immunol Methods, 2005. **298**(1-2): p. 93-104; Andersen, J.T., et al., FEBS J, 2008. **275**(16): p. 4097-110. The vector also contains a cDNA encoding human β_2 -microglobulin and the Epstein-Barr virus origin of replication sequence. GST-tagged hFcRn was produced by transient transfection of human embryonic kidney 293E (HEK293E) cells using polyethyleneimine Max (Polysciences), and the receptor was purified from harvested supernatant using a GStrap FF column as described (Berntzen et al., supra; Andersen et al., supra).

Monomeric His-tagged human FcRn was produced using a Baculovirus expression vector system (Kim, J.K., et al., *FcRn*. Eur J Immunol, 1999. **29**(9): p. 2819-25). A viral stock encoding His-tagged human FcRn was a kind gift from Dr. Sally Ward (University of Texas, Southwestern Medical Center, Dallas, TX). Briefly, human FcRn was purified using a HisTrap HP column supplied with Ni^{2+} ions (GE Healthcare). The column was preequilibrated with $1 \times$ PBS with 0.05% sodium azide, and the pH of the supernatant was adjusted with $1 \times$ PBS, 0.05% sodium azide (pH 10.9) to pH 7.2 before being applied to the HisTrap HP column with a flow rate of 5 ml/min. The column was washed using 200 ml of $1 \times$ PBS followed by 50 ml of 25 mM imidazole, $1 \times$ PBS (pH 7.3), and human FcRn was eluted with 250 mM imidazole, $1 \times$ PBS (pH 7.4). The collected protein was buffer-exchanged to $1 \times$ PBS using Amicon Ultra-10 filter units (Millipore) followed by isolation of the monomeric fraction. A HiLoad 26/600 Superdex 200 prep grade column (GE Healthcare) was used to isolate the monomeric fraction before the protein was concentrated using Amicon Ultra columns (Millipore) and stored at 4°C .

Construction and production of Fc-engineered IgG1 variants. Vectors encoding humanized 9C12 IgG1 variants were based on the pLNOH2/pLNOK expression system (Norderhaug, L., et al., J Immunol Methods, 1997. **204**(1): p. 77-87). Specifically, heavy (H) and light (L) chain variable (V) genes derived from the hybridoma cell line TC31-9C12.C9 (Developmental Studies Hybridoma Bank, University of Iowa) (Varghese, R., et al., J Virol, 2004. **78**(22): p. 12320-32) were synthesized as a cloning cassette flanked by the restriction sites recognized by the endonucleases BsmI/BsiWI. The gene fragments were then subcloned into pLNOH2-^{NIP}hIgG1-WT-oriP and ^{NIP}pLNOK-oriP, resulting in pLNOH2-^{Hexon}hIgG1-WT-oriP and ^{Hexon}pLNOK-oriP encoding a chimeric human H chain and L chain, respectively. The H chain encoding vector pLNOH2-^{Hexon}hIgG1-WT-oriP was further used for the generation of h9C12 variants by exchanging C_H2 and C_H3 gene fragments with fragments containing the desired mutations. C_H2 fragments were exchanged using the unique restriction sites

recognized by the endonucleases *AgeI* and *SfiI*, while C_H3 fragments were exchanged using *SfiI* and *BamHI* (All from New England Biolabs). Following co-transfection of both H chain and L chain vectors into HEK293E cells using Lipofectamine 2000 (Life Technologies), h9C12 IgG1 variants were purified from collected supernatant using a C_H1 specific
5 CaptureSelect column (Life Technologies). Monomeric fractions were isolated by SEC chromatography using a Superdex 200 column (GE Healthcare). Protein integrity was verified by non-reducing SDS-PAGE (Life Technologies).

ELISA. Recombinant AdV5 hexon (Abd Serotech) (diluted to 1 µg/ml in PBS) was coated in
10 96-well plates (Nunc), and incubated O/N at 4°C. Remaining surface area was blocked using PBS/ 4% skimmed milk (S) (Acumedia), before washing four times with PBS/0.005% Tween 20 (T). Titrated amounts of h9C12 variants diluted in PBS/T/S were added to the wells and incubated for 1 hour at room temperature. Following washing as above, soluble GST-tagged human FcRn diluted in PBS/S/T was added to the wells and incubated for 1 hour at room
15 temperature. After washing as above, an HRP-conjugated anti GST antibody (1:8000) was diluted in added PBS/S/T (Rockland Immunochemicals, U.S.A) followed by incubated for 1 hour at room temperature. After washing as above, bound human FcRn was visualized by addition of tetramethylbenzidine (TMB) solution (CalBiochem). The reaction was terminated by addition of 100 µl 1 M HCl, and absorption at 450 nm was recorded using a
20 SUNRISE plate reader (TECAN). The ELISA was performed using PBS with pH 6.0 or pH 7.4.

SPR. A Biacore 3000 instrument (GE Healthcare) was used for all kinetics measurements. Humanized 9C12 IgG1 variants were immobilized by amine coupling on CM5 sensor chips
25 according to the manufacturer instructions. The coupling was performed by injecting 1-2.5 µg/ml of the IgG1 variants dissolved in 10 mM sodium acetate, pH 4.5 (GE Healthcare). HBS-P buffer (0.01 M HEPES, 0.15 NaCl, 0.005% surfactant P20, pH 7.4) was used as running and dilution buffer. Subsequently, concentration series of soluble monomeric human FcRn were injected. Kinetics analysis was performed using the BIAevaluation Software and
30 the binding data were fitted to a simple first order (1:1) Langmuir bio molecular interaction model.

Results

Described herein are Fc-engineered IgG antibodies with altered binding to human FcRn as following. The triple mutant, **Q311R/N434W/M428E**, shows improved pH dependent binding beyond that of published examples.

5

IgG1-Q311R/N434W/M428E	
IgG1-Q311R/N434W	
IgG1-Q311R	
IgG1-N434W	
IgG3(b)-Q311R/N434W/M428E	
IgG3(b)-Q311R/N434W/M438E/R435H	
IgG1-M252Y/S254T/T256E, also named the YTE mutant/platform (Robbie, G.J., et al., Antimicrob Agents Chemother, 2013. 57(12): p. 6147-53; Dall'Acqua, W.F., P.A. Kiener, and H. Wu, J Biol Chem, 2006. 281(33): p. 23514-24).	Lead candidate: AstraZeneca Included as a reference.

Table 1. SPR-derived kinetics for binding of human IgG1 variants to human FcRn				
pH 6.0				
IgG1 variants	Ka (10 ⁴ /ms) ^a	Kd(10 ⁻² /s) ^a	KD (nM) ^a	Fold change from WT
WT	7.04±0.1	5.4±0.2	784.0	-
M252Y/S254T/T256E	8.66±0.2	0.78±0.0	93.0	8.4
Q311R/N434W/M428E	12.2±0.2	0.50±0.0	38.5	20.3
Q311R/N434W	16.1±0.2	0.36±0.0	22.3	35.1
Q311R	8.31±0.1	5.72±0.2	686.9	1.1
N434W	21.7±0.2	0.32±0.1	14.8	52.9

pH 7.4				
WT	NA	NA	NA	-
M252Y/S254T/T256E	NA	NA	NA	-
Q311R/N434W/M428E	NA	NA	NA	-
Q311R/N434W	NA	NA	NA	-
Q311R	NA	NA	NA	-
N434W	NA	NA	1980.0	-

Example 2

This Example describes additional IgG1 and IgG3 variants with altered binding to human FcRn.

5

Materials and Methods

In vivo studies- Tg32-Alb^{-/-} mice on the C57BL/6J genetic background carries the null alleles of FcRn HC (Fcgrt^{tm1Dcr}) and of albumin (Alb^{em12Mvw}), and expresses the genomic transgene of hFcRn HC (FCGRT) under the control of the native hFcRn promoter. The Tg32-
 10 Alb^{-/-} mice (sex – female, aged 7 - 9 weeks, weight between 17 and 27 g, 5 mice/group) received 5 mg/kg of IgG1 variants by intraperitoneal injection. Blood samples (25 µl) were drawn from the retro-orbital sinus at 1, 3, 5, 7, 10, 12, 16, 19, 23, 30 and 37 days after injection. Following sample collection, the blood was immediately mixed with 1 µl 1% K3-EDTA to prevent coagulation and then centrifuged at 17000 × g for 5 min at 4 °C. Plasma
 15 was isolated, diluted 1:10 in 50% glycerol/PBS solution and then stored at -20 °C until analysis by ELISA. In vivo studies were performed in The Jackson Laboratory (Bar Harbor, ME).

Surface plasmon resonance- A Biacore T200 instrument (GE Healthcare) was used for all
 20 kinetics measurements. h9C12 variants were immobilized by amine coupling on CM5 sensor chips according to the manufacturer instructions. For all experiments, phosphate buffer (67 mM phosphate, 0.15 M NaCl, 0.005% Tween20) at pH 6.0, or HBS-P buffer (0.1 M HEPES, 0.15 NaCl, 0.005% surfactant P20) at pH 7.4 were used as running and regeneration buffers, respectively. Concentration series of recombinant human FcRn (1000.0-15.6) nM were
 25 injected at a flow rate of 50 µl/min at 25°C. Kinetics analysis was performed using the BIAevaluation Software and the binding data were fitted to a simple first order (1:1) Langmuir interaction model.

ELISA- Performed as described in Example 1.

Table 2

IgG1-WT
IgG1-Q311R/N434W/M428E
IgG1-M252S/Q311R/N434W/M428E
IgG1-Q311R/N434P/M428E
IgG1-Q311R/N434W/M428D
IgG1-Q311R/N434W/M428E/H433K
IgG1-L309K/Q311R/N434W/M428E
IgG1-L309R/Q311R/N434W/M428E
IgG1-L309S/Q311R/N434W/M428E
IgG3(b)-Q311R/N434W/M428E/R435H

5 Results

Results are shown in Figures 5-7 and Tables 3-5.

Table 3. ELISA binding of human h9C12 IgG1 and IgG3 variants to human FcRn.	
h9C12 variants:	Fold change from WT (ELISA) pH 6.0
IgG1-WT	-
IgG1-Q311R/N434W/M428E*	6
IgG1-M252S/Q311R/N434W/M428E	6
IgG1-Q311R/N434P/M428E	3
IgG1-Q311R/N434W/M428D	3
IgG1-Q311R/N434W/M428E/H433K	9
IgG1-L309K/Q311R/N434W/M428E	6
IgG1-L309R/Q311R/N434W/M428E	6
IgG1-L309S/Q311R/N434W/M428E	6
IgG3(b)-Q311R/N434W/M428E/R435H	3

Table 4. SPR-derived kinetics for binding of human h9C12 IgG1 and IgG3 variants to human FcRn.						
pH 6.0						
h9C12 variants	ka(10 ⁵ /ms)	kd(10 ⁻² /s)	KD(nM)	Fold change from WT (KD) pH 6.0		
IgG1-WT*	3.172	8.677	273	-		
IgG1-Q311R/N434W/M428E*	0.117	1.106	9.3	29.30		
IgG1-M252S/Q311R/N434W/M428E	NT	NT	NT	NT		
IgG1-Q311R/N434P/M428E	0.167	4.047	24.2	11.28		
IgG1-Q311R/N434W/M428D	0.106	3.121	29.4	9.28		
pH 7.4						
IgG1-WT*	NA	NA	NA	-		
IgG1-Q311R/N434W/M428E*	NA	NA	NA	-		
IgG1-M252S/Q311R/N434W/M428E	NT	NT	NT	-		
IgG1-Q311R/N434P/M428E	NA	NA	NA	-		
IgG1-Q311R/N434W/M428D	NA	NA	NA	-		
IgG1 variant	IgG1 specificity	Route	Dose (mg/kg)	Half-Life (days) (n=5)	+/- SD (days)	Mouse Strain
IgG1-WT	h9C12	IV	5	6.4	0.8	Tg32 hemi

						mice
IgG1-Q311R/N434W/M428E	h9C12	IV	5	7.5	2.0	Tg32 hemi mice
IgG1-WT	NIP	IV	5	8.4	2.5	Tg32 hemi mice
IgG1-Q311R/N434W/M428E	NIP	IV	5	12.3	1.3	Tg32 hemi mice

Table 5. Half-life (days) in human FcRn Tg32 hemizygous mice.

All publications and patents mentioned in the above specification are herein

5 incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed

10 the described modes for carrying out the invention which are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

CLAIMS

1. A composition, comprising:
a therapeutic immunoglobulin with altered binding affinity for FcRn, wherein said
5 immunoglobulin comprises at least one mutation in the Fc region of said immunoglobulin.
2. The composition of claim 1, wherein said immunoglobulin is an IgG1, IgG2, IgG3, or
IgG4 subclass.
- 10 3. The composition of claim 1 or 2, wherein said immunoglobulin has mutations at one
or more of positions selected from the group consisting of 311, 434, 428, 438, and 435.
4. The composition of any one of claims 1 to 3, wherein said mutation is selected from
the group consisting of IgG1-Q311R/N434W/M428E, IgG1-Q311R/N434W, IgG1-Q311R,
15 IgG1-N434W, IgG3(b)-Q311R/N434W/M428E, IgG3(b)-Q311R/N434W/M438E/R435H,
IgG1-M252S/Q311R/N434W/M428E, IgG1-Q311R/N434P/M428E, IgG1-
Q311R/N434W/M428D, IgG1-Q311R/N434W/M428E/H433K, IgG1-
L309K/Q311R/N434W/M428E, IgG1-L309R/Q311R/N434W/M428E, IgG1-
L309S/Q311R/N434W/M428E, and IgG3(b)-Q311R/N434W/M428E/R435H.
20
5. The composition of claim 4, wherein the constant region of said immunoglobulin has
an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9,
10, 11, 12, 13, 14, and 15.
- 25 6. The composition of any of claims 1 to 5, wherein said mutation extends the serum
half-life of the immunoglobulin as compared to an immunoglobulin lacking said mutation.
7. A composition, comprising:
an immunoglobulin comprising at least one mutation in the Fc region of said
30 immunoglobulin, wherein said immunoglobulin has altered binding to FcRn, and wherein
said mutation is selected from the group consisting of IgG1-Q311R/N434W/M428E, IgG1-
Q311R/N434W, IgG1-Q311R, IgG1-N434W, IgG3(b)-Q311R/N434W/M428E, IgG3(b)-
Q311R/N434W/M438E/R435H, IgG1-M252S/Q311R/N434W/M428E, IgG1-
Q311R/N434P/M428E, IgG1-Q311R/N434W/M428D, IgG1-

Q311R/N434W/M428E/H433K, IgG1-L309K/Q311R/N434W/M428E, IgG1-L309R/Q311R/N434W/M428E, IgG1-L309S/Q311R/N434W/M428E, and IgG3(b)-Q311R/N434W/M428E/R435H.

- 5 8. The composition of claim 6, wherein said mutation extends the serum half-life of the immunoglobulin as compared to an immunoglobulin lacking said mutation.
9. The composition of claim 7 or 8, wherein the constant region of said immunoglobulin has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16.
- 10 10. A composition comprising:
 an IgG1 immunoglobulin with altered binding to FcRn, wherein said immunoglobulin has Q311R, N434W, and M428E mutations.
- 15 11. The composition of claim 10, wherein said mutation extends the serum half-life of the immunoglobulin as compared to an immunoglobulin lacking said mutation.
12. The composition of any one of claims 1 to 11, wherein said antibody binds to a target selected from the group consisting of a cancer marker, a cytokine, an infectious disease marker, and a growth factor.
- 20 13. The use of the immunoglobulin of any one of claims 1 to 12 to treat or prevent a disease in a subject in need thereof.
- 25 14. The use of claim 13, wherein said disease is selected from the group consisting of cancer, autoimmune disease, inflammatory disease, transplant rejection, and infectious disease.
- 30 15. The use of claim 13 or 14, wherein said antibody binds to a target selected from the group consisting of a cancer marker, a cytokine, an infectious disease marker, and a growth factor.

16. A method of treating or treating or preventing a disease in a subject, comprising:
providing the immunoglobulin of any one of claims 1 to 12 to a subject in need thereof.
- 5 17. The method of claim 16, wherein said disease is selected from the group consisting of cancer, autoimmune disease, inflammatory disease, transplant rejection, and infectious disease.
- 10 18. The method of claim 16 or 17, wherein said antibody binds to a target selected from the group consisting of a cancer marker, a cytokine, an infectious disease marker, and a growth factor.
19. An Fc fusion of the immunoglobulin of any one of claims 1 to 12.
- 15 20. A fusion protein comprising the immunoglobulin of any one of claims 1 to 12 or the Fc fusion of claim 19 fused to an immunogen.
21. A vaccine composition comprising the fusion protein of claim 20.
- 20 22. The use of the vaccine composition of claim 21 to generate an immune response in a subject.
23. A method of generating an immune response in a subject, comprising:
administering the vaccine composition of claim 21 to a subject under conditions such
25 that said subject generates an immune response to said immunogen.