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(54) Title: HUMAN EQUIVALENT MONOCLONAL ANTIBODIES ENGINEERED FROM NONHUMAN VARIABLE REGIONS

(57) Abstract: The present invention is directed to the creation of human equivalent CDRs and antibodies containing them by a method of producing an antibody which specifically binds to an antigen.

HUMAN EQUIVALENT MONOCLONAL ANTIBODIES ENGINEERED FROM NONHUMAN VARIABLE REGIONS

[001] This application claims the benefit under 35 U.S.C. § 119(e) to 61/046,399, filed April 18, 2008; 61/115,449, filed November 17, 2008; and, 61/120,675, filed December 8, 2008 all of which are expressly incorporated by reference in their entirety.

FIELD OF THE INVENTION

[002] The present invention is directed to the creation of human equivalent CDRs and antibodies containing them.

BACKGROUND OF THE INVENTION

[003] Monoclonal antibodies (mAbs) have become an important class of therapeutics, and there are currently approved mAbs available to treat patients suffering from various types of cancers and autoimmune disorders. The hybridoma approach (Kohler and Milstein, 1975, *Nature* 256:495-497) remains the most prevalent way to generate mAbs with high affinity and specificity to a target of interest. However, mAbs generated in this way are of non-human origin (usually murine) and are highly immunogenic when administered to human patients.

[004] Several methods have been introduced in order to decrease the potential risk of immunogenicity with antibodies isolated from hybridomas, namely chimerization and humanization. Creation of chimeric antibodies, composed of murine variable regions and human constant regions (Morrison, et al., 1984, *Proc Natl Acad Sci U S A* 81:6851-6855), was the first such method. However, since a significant portion of the antibody remains non-human, these mAbs still pose a risk of eliciting an immune response. A logical next step was the humanization, or engineering of the variable regions of these mAbs to contain more human sequence content. It was found that the murine complementarity-determining regions (CDRs), which are the principle components of the antibody that confer antigen specificity, could be "grafted" onto human frameworks (FRs) to create an antibody with higher human sequence content. This process, known as CDR-grafting (Jones, et al., 1986, *Nature* 321:522-525), was the first described method of antibody humanization. Since then, several methods of humanization have been described including resurfacing (Roguska, et al., 1994, *Proc Natl Acad Sci U S A* 91:969-973), specificity-determining residue (SDR) grafting (Kashmiri, et al., 2005, *Methods* 36:25-34), superhumanization (Hwang, et al., 2005, *Methods* 36:35-42), human string content optimization (Lazar, et al., 2007, *Mol Immunol* 44:1986-1998), and framework shuffling (Dall'Acqua, et al., 2005, *Methods* 36:43-60; Damschroder, et al., 2007, *Mol Immunol* 44:3049-3060). The underlying assumption of all these methods is

that the greater global sequence identity of the humanized sequence to a natural human sequence results in a lower risk of immunogenicity. However, due to the perceived risk of losing antigen affinity, none of these methods substantially engineer the CDRs, and as such none of these humanization methods reach the global sequence identity levels of human antibodies as they still contain mostly non-human CDRs.

[005] More recently, "fully-human" mAbs generated from recombinant human antibody libraries (Griffiths, et al., 1994, *Embo J* 13:3245-3260; Knappik, et al., 2000, *J Mol Biol* 296:57-86) or transgenic mice comprising human germline configuration immunoglobulin gene sequences (Lonberg, 2005, *Nat Biotechnol* 23:1117-1125; Green, et al., 1994, *Nat Genet* 7:13-21; Lonberg, et al., 1994, *Nature* 368:856-859) have emerged as alternatives to murine generated and subsequently humanized mAbs. These mAbs have both high affinity as well as high human sequence content. Yet there remain a large number of murine antibodies with well-characterized and desirable properties. Moreover, hybridoma technology remains an accessible, efficient, and effective method for generating high quality mAbs. Thus, there is a need for efficient methods to combine the ease of creating high affinity and specificity non-human mAbs from hybridomas with the high human sequence content and expected low immunogenicity of fully-human mAbs. The current invention addresses this need.

BRIEF SUMMARY OF THE INVENTION

[006] The invention disclosed herein provides a novel method for engineering human equivalent antibody variable regions from non-human variable regions, and in some cases, from variable regions in antibodies produced in transgenic mice and/or humans, if the antibodies are immunogenetic.

[007] In one aspect, the invention provides a method of producing an immunoglobulin which specifically binds to an antigen, the method comprising: a) comparing a parent antibody variable region amino acid sequence comprising less than 85% identity in V- and J-segments to a human germline V- and J-segment against a collection of human germline V- and J-segment amino acid sequences; b) scoring the collection of human germline V- and J-segments based on the number of identities to the parent antibody variable region amino acid sequence; c) selecting the human germline V- and J-segments with the highest score in step (b); d) constructing variant immunoglobulin(s) comprising the parent antibody variable region amino acid sequence in (a) and an amino acid substitution selected from the human germline V- and J-segment in step (c) at a position in which the amino acids in the parent antibody variable region and human germline V- and J-segment differ; e) measuring antigen binding of the variant immunoglobulin(s) in step (d) to obtain affinity constants; f) selecting the variants in step (e) which have an affinity constant that is no less than two-fold of that of the parent antibody variable region immunoglobulin; h) combining the variants in step (f) to create an antibody variable region sequence comprising greater than 85% identity in V- and J-segments

to a human germline V- and J-segment.

[008] In some aspects, the identity of the heavy chain is at least about 90%, or at least about 95%. In additional aspects, the identity of the light chain is at least about 90% or at least about 95%.

[009] In additional aspects, the plurality of the at least four amino with an identity to the parent antibody acids are outside the heavy chain CDR3.

[010] In some aspects, the plurality of the at least four amino acids with an identity to the parent antibody are within CDR1 or CDR2 of the heavy chain or the light chain.

[011] In an additional aspect, one or more of the variant antibodies generated using these methods retains at least all the affinity for the antigen of the parent antibody Fv domain. In some aspects, the variant antibodies have no less than about a two fold decrease in affinity binding, and in some embodiments, the variant antibodies have no less than about a three fold decrease.

[012] In some aspects, the method is performed on an antibody comprising a parent antibody Fv domain, wherein the parent antibody Fv domain comprises residues 1-94 and 100-113 of a heavy chain Fv domain and residues 1-107 of a light chain Fv domain, wherein the numbering is according to the system of Kabat et al.. In this aspect, the parent antibody Fv domain comprises complimentarity determining regions (CDRs) derived from non-human germline sequences. After the method is done, the identity of residues1-94 of the heavy chain of the variant antibody Fv domain to a human V-region germline and residues 100-113 of the heavy chain of the variant antibody Fv domain to a human J-region germline is at least 85%. In addition, the identity of residues 1-95 of the light chain of the variant antibody Fv domain to a human V-region germline and residues 96-107 of the light chain of the variant antibody Fv domain to a human J-region germline is at least 85% and wherein the variant antibody Fv domain comprises at least four amino acids with an identity to the parent antibody Fv domain but not an identity to the human germline sequence. Some embodiments utilize more than 4 different amino acids, including from about 4 to 15, 4 to 10, 5 to 10 and 5 to 8, with all combinations of ranges possible.

[013] It is an object of the present invention to provide protein variants of a parent protein that are engineered using the methods described herein. In a preferred embodiment, the parent protein is an immunoglobulin.

[014] It is an object of the present invention to provide experimental methods for screening and testing the protein variants of the present invention.

[015] The present invention provides isolated nucleic acids encoding the protein variants described herein. The present invention provides vectors comprising the nucleic acids, optionally, operably linked to control sequences. The present invention provides host cells containing the vectors, and methods for producing and optionally recovering the protein variants.

[016] The present invention provides compositions comprising the protein variants described herein, and a physiologically or pharmaceutically acceptable carrier or diluent.

[017] The present invention provides novel antibodies and Fc fusions that comprise the protein variants disclosed herein. The novel antibodies and Fc fusions may find use in a therapeutic product.

[018] The present invention provides therapeutic treatment and diagnostic uses for the protein variants disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[019] The following drawings further illustrate aspects of the invention, and are not meant to constrain the present invention to any particular application or theory of operation.

[020] Figure 1. Diagram illustrating the process of the invention in which murine antibody variable region sequences are engineered to be similar to sequences of human equivalent antibody variable regions.

[021] Figure 2. Comparison of the V-segment (V_H and V_L) and J-segment (V_L) of an initial framework optimized anti-CD25 variable region sequence to the five closest matching human germline V- and J-segments. Sequences are numbered according to Kabat et. al., and CDR regions are outlined. Amino acids in the human germline V-regions that differ from the framework optimized sequence are highlighted. Positions that are in CDRs or CDR proximal and/or proximal to the V_H/V_L interface are indicated. For V_L, amino acids in common among all five J-regions are shown and those positions that differ are listed as X's.

[022] Figure 3. Sequences of CDR and V_H/V_L interface positions, antigen binding data, and humanness scores for anti-CD25 variants. Anti-CD25 H0L0 is the chimeric form of the anti-TAC antibody and anti-CD25 H1L1 is the framework optimized variable region antibody. Positions in framework optimized anti-CD25 H1L1 which differ from the closest identity human germline sequences were changed to the corresponding germline amino acid and binding to CD25 was measured with Biacore. Shaded residues indicate differences between each variant and anti-CD25 H1L1. The number of total human 9-mers and identities to the closest human germline for each V_H and V_L pair is also shown.

[023] Figure 4. Diagram illustrating CDA technology used to combine favorable single variants into V_H and V_L combination variants with high humanness scores and high library diversity while minimizing library size. Grey circles represent all possible library members and red stars indicate the members selected for the anti-CD25 V_H combination variant library.

[024] Figure 5. Sequences of CDR and V_H/V_L interface positions, antigen binding data, and humanness scores for anti-CD25 combination variants. Anti-CD25 H0L0 is the chimeric form of the anti-TAC antibody and anti-CD25 H1L1 is the framework optimized variable region antibody. Binding data and humanness scores from the anti-CD25 single variants were used to design a library of combination variants that would maximize humanness scores and maintain antigen affinity. Binding to CD25 was measured with Biacore, and shaded residues

indicate differences between each variant and anti-CD25 H1L1. The number of total human 9-mers and identities to the closest human germline V and J regions for each V_H and V_L pair is also shown. Fold change in the dissociation constant (K_d) for human equivalent anti-CD25 combination variants compared to the chimeric antibody anti-CD25 H0L0 is listed.

[025] Figure 6. Biacore binding data for anti-CD25 variants binding to CD25. The top panel shows binding data for 25 nM CD25 binding to anti-CD25 H0L0, anti-CD25 H1.12L1, anti-CD25 H1L1.20, anti-CD25 H1.12L1.20, and daclizumab immobilized on Protein A on a CM5 chip. The bottom panel shows a plot of affinity vs. humanness for anti-CD25 H0L0 (murine Fv), anti-CD25 H1L1 (framework optimized), engineered human equivalent anti-CD25 V_H/V_L pairs, and human equivalent anti-CD25 H1.12_L1.20.

[026] Figure 7. Amino acid sequence alignments of heavy chain and light chain variable regions for anti-TAC H0L0 (anti-CD25 H0L0 - murine Fv), anti-CD25 H1L1 (framework-optimized), human germlines IGHV1-2*02 (VH) and IGKV3-11*01 (VL), and engineered human equivalent anti-CD25 H1.12L1.20. Amino acid differences between the following sequence pairs are highlighted: framework-optimized and murine Fv, human germline and framework-optimized, and engineered human equivalent anti-CD25 H1.12L1.20 and human germline.

[027] Figure 8. Human equivalent anti-CD25 variants show high activity in a receptor blocking assay. Variants tested were chimeric (anti-CD25 H0L0), framework optimized (anti-CD25 H1L1), anti-CD25 H1.12L1.20, anti-CD25 H1.23L1.43, daclizumab, and a non-CD25 binding antibody as an isotype control. From EC50 values, relative potency of the mAbs was anti-CD25 H1.12L1.20, anti-CD25 H1.23L1.43 > chimeric anti-CD25 H0L0, framework optimized anti-CD25 H1L1 > daclizumab.

[028] Figure 9. Comparison of the V-region (V_H and V_L) and J-region (V_L) of an initial framework optimized anti-VEGF variable region sequence to the five closest matching human germline V-regions. Sequences are numbered according to Kabat et. al., and CDR regions are outlined. Amino acids in the human germline V-regions that differ from the framework optimized sequence are highlighted. Positions that are in CDRs or CDR proximal and/or proximal to the V_H/V_L interface are indicated. For V_L, amino acids in common among all five J-regions are shown and those positions that differ are listed as X's.

[029] Figure 10. Biacore binding data for anti-VEGF Fab variants binding to VEGF. The top panel shows binding data for 100 nM of anti-VEGF H0L0 (murine Fv), anti-VEGF H1.33L1.51, anti-VEGF H1.33L1.55, and bevacizumab binding to immobilized VEGF on a CM5 chip. The bottom panel shows a bar graph of the dissociation constants of these variants.

[030] Figure 11. Sequences of CDR and interface positions, binding data, and humanness scores of anti-VEGF variant Fabs. Binding data and humanness scores from anti-VEGF single variants were used to design a library of combination variants that would maximize humanness scores and maintain antigen affinity. Binding to VEGF was measured with Biacore, and shaded residues indicate differences between each variant and anti-VEGF H1L1. The number of total human 9-mers and identities to the closest human germline V and

J regions for each V_H and V_L pair is also shown. Fold change in the dissociation constant (K_d) for human equivalent anti-VEGF combination variants compared to the chimeric antibody anti-VEGF H0L0 is listed.

[031] Figure 12. Sequence properties and final affinity results for engineered mAbs. Number of CDR mutations, percent of CDR changed, final CDR humanness, and fold affinity change relative to H0L0 are listed for the three engineered mAbs anti-CD25 H1.12L1.20, anti-VEGF H1.33L1.55, and anti-TNF H1.103L1.33. VH-CDR3 is not included in the total percent CDR humanness calculations. Number of mutations are relative to the H0L0 mAbs with murine Fv.

[032] Figure 13. Plot comparing humanness scores (% identity to the closest human germline V- and J-segments and % human 9-mers) for the approved and marketed murine, chimeric, and humanized monoclonal antibodies as well as several fully-human monoclonal antibodies in clinical development to fully-human anti-CD25 H1.12L1.20, anti-VEGF H1.33L1.55, and anti-TNF H1.103L1.33. The fully-human antibodies engineered in this invention are comparable to the fully-human antibodies from transgenic mice and human phage display technologies.

[033] Figure 14. Listing of humanness scores for the approved and marketed murine, chimeric, and humanized monoclonal antibodies as well as several fully-human monoclonal antibodies in clinical development. The fully-human antibodies anti-CD25 H1.12L1.20, anti-VEGF H1.33L1.55, and anti-TNF H1.103L1.33 engineered in this invention are shown to be comparable to the fully-human antibodies from transgenic mice and human phage display technologies.

[034] Figure 15. Heavy chain and light chain variable region sequences for antibodies listed in Figure 14 with murine and humanized variable regions that may benefit from the fully-human engineering method.

[035] Figure 16. Sequences of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[036] Definitions

[037] In order that the invention may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[038] By "affinity" as used herein is meant the propensity of one chemical species to separate or dissociate reversibly from another chemical species. In the present invention, the two chemical species most typically are represented by a protein and its ligand, more specifically an antibody and its target antigen. Affinity herein is measured by the equilibrium constant of dissociation (K_d or Kd) that defines the binding between the two chemical species. The K_d defines how tightly the species bind one another. The smaller the dissociation constant, the more tightly bound the ligand is, or the higher the affinity between ligand and protein. For example, an antigen with a nanomolar (nM) dissociation constant binds more

tightly to a particular antibody than a ligand with a micromolar (μM) dissociation constant. By "greater affinity" or "improved affinity" or "enhanced affinity" or "better affinity" than a parent polypeptide, as used herein is meant that a protein variant binds to its ligand with a significantly higher equilibrium constant of association (K_A or Ka) or lower equilibrium constant of dissociation (K_d or Kd) than the parent protein when the amounts of variant and parent polypeptide in the binding assay are essentially the same. For example, in the context of antibodies, a variant antibody may have greater affinity to the antigen that its parent antibody, for example when the CDRs are humanized, as described herein. Alternatively, and Fc polypeptide may have greater affinity to an Fc receptor, for example, when the Fc variant has greater affinity to one or more Fcγ receptors or the FcRn receptor. IN general, the binding affinity is determined, for example, by the binding methods disclosed herein, including but not limited to Biacore™ assays, by one skilled in the art. Accordingly, by "reduced affinity" as compared to a parent protein as used herein is meant that aprotein variant binds its ligand with significantly lower K_a or higher K_d than the parent protein. Again, in the context of antibodies, this can be either to the target antigen, or to a receptor such as an Fc receptor. Greater or reduced affinity can also be defined relative to an absolute level of affinity. For example, greater or enhanced affinity may mean having a K_d lower than about 10 nM, for example between about 1 nM – about 10 nM, between about 0.1 – about 10 nM, or less than about 0.1 nM.

[039] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids or any non-natural analogues that may be present at a specific, defined position.

[040] By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. The preferred amino acid modification herein is a substitution. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a given position in a protein sequence with another amino acid. For example, the substitution Y50W refers to a variant of a parent polypeptide, in which the tyrosine at position 50 is replaced with tryptophan. By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid at a particular position in a parent polypeptide sequence, usually denoted herein and in the incorporated documents by a "^" after the residue where the insertion occurs. By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence, usually denoted herein and in the incorporated documents by a "#" after the residue to be deleted.

[041] By "antibody" herein is meant a protein consisting of one or more polypeptides substantially encoded by all or part of the recognized immunoglobulin genes. The recognized immunoglobulin genes, for example in humans, include the kappa (κ), lambda (λ), and heavy chain genetic loci, which together comprise the myriad variable region genes, and the

constant region genes mu (υ), delta (δ), gamma (γ), sigma (σ), and alpha (α) which encode the IgM, IgD, IgG (IgG1, IgG2, IgG3, and IgG4), IgE, and IgA (IgA1 and IgA2) isotypes respectively. Antibody herein is meant to include full length antibodies and antibody fragments, and may refer to a natural antibody from any organism, an engineered antibody, or an antibody generated recombinantly for experimental, therapeutic, or other purposes. Antibodies include, include, but not limited full length antibodies, antibody fragments, single chain antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

[042] In one embodiment, the antibody is an antibody fragment. Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment, which consists of a single variable domain, (v) isolated CDR regions, (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site, (viii) bispecific single chain Fv dimers, and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion. The antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains. Examples of antibody formats and architectures are described in Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136, and Carter 2006, Nature Reviews Immunology 6:343-357 and references cited therein, all expressly incorporated by reference.

[043] In one embodiment, an antibody disclosed herein may be a multispecific antibody, and notably a bispecific antibody, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens. Diabodies can be manufactured in a variety of ways known in the art, e.g., prepared chemically or from hybrid hybridomas. In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. In some cases, the scFv can be joined to the Fc region, and may include some or all of the hinge region. For a description of multispecific antibodies see Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136 and references cited therein, all expressly incorporated by reference.

[044] By "constant region" of an antibody as defined herein is meant the region of the antibody that is encoded by one of the light or heavy chain immunoglobulin constant region genes. By "constant light chain" or "light chain constant region" as used herein is meant the region of an antibody encoded by the kappa (Cκ) or lambda (Cλ) light chains. The constant light chain typically comprises a single domain, and as defined herein refers to positions 108-

214 of Cκ or Cλ, wherein numbering is according to the EU index. By "constant heavy chain" or "heavy chain constant region" as used herein is meant the region of an antibody encoded by the mu, delta, gamma, alpha, or epsilon genes to define the antibody's isotype as IgM, IgD, IgG, IgA, or IgE, respectively. For full length IgG antibodies, the constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-terminus of the CH3 domain, thus comprising positions 118-447, wherein numbering is according to the EU index.

[045] By "corresponding" or "equivalent" residues as meant herein are residues that represent similar or homologous sequence and/or structural environments between a first and second protein, or between a first protein and set of multiple proteins. In order to establish homology, the amino acid sequence of a first protein is directly compared to the sequence of a second protein. After aligning the sequences, using one or more of the homology alignment programs known in the art (for example using conserved residues as between species), allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of the first protein are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Corresponding residues may also be defined by determining structural homology between a first and second protein that is at the level of tertiary structure for proteins whose structures have been determined. In this case, equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the proteins (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm of each other after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins.

[046] By "CDR" as used herein is meant a Complementarity Determining Region of an antibody variable domain. Systematic identification of residues included in the CDRs have been developed by Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda) and alternately by Chothia (Chothia & Lesk, 1987, J. Mol. Biol. 196: 901-917; Chothia et al., 1989, Nature 342: 877-883; Al-Lazikani et al., 1997, J. Mol. Biol. 273: 927-948). For the purposes of the present invention, CDRs are defined as a slightly smaller set of residues than the CDRs defined by Chothia. VL CDRs are herein defined to include residues at positions 27-32 (CDR1), 50-56 (CDR2), and 91-97 (CDR3), wherein the numbering is according to Chothia. Because the VL CDRs as defined by Chothia and Kabat are identical, the numbering of these VL CDR positions is also according to Kabat. VH CDRs are herein defined to include residues at positions 27-33 (CDR1), 52-56 (CDR2), and 95-102 (CDR3), wherein the numbering is according to Chothia. These VH CDR positions correspond to Kabat positions 27-35 (CDR1), 52-56 (CDR2), and 95-102 (CDR3).

[047] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein, or any other antibody embodiments as outlined herein.

[048] By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

[049] By "framework" as used herein is meant the region of an antibody variable domain exclusive of those regions defined as CDRs. Each antibody variable domain framework can be further subdivided into the contiguous regions separated by the CDRs (FR1, FR2, FR3 and FR4).

[050] By "germline" as used herein is meant the set of sequences that compose the natural genetic repertoire of antibodies, and its associated alleles.

[051] By "host" as used herein is meant a family, genus, species or subspecies, group of individuals or even a single individual. A host group of individuals can be selected for based upon a variety of criteria, such as MHC allele composition, etc. In a preferred embodiment, a host is canine, murine, primate, or human. In the most preferred embodiment, a host is human. In the context of protein production, a "host cell" is the cell in which the protein is produced, and includes a wide variety of possible hosts, as outlined below, including, but not limited to, mammalian cells, yeast cells, fungal cells, bacterial cells (including E. coli), etc.

[052] By "host string" or "host sequence" as used herein is meant a string or sequence that either encodes any part of a naturally occurring host protein (in the case of a nucleic acid sequence) or is any part of a naturally occurring host protein amino acid sequence. In general, as generally outlined in US Publication No. 20080167449, hereby incorporated by reference in its entirety, and particularly for the definitions and methods, a host string is a contiguous sequence of some number of amino acids identical to a naturally occurring protein. In the context of the present invention, the "host string" frequently refers to a partial contiguous sequence of a germline sequence.

[053] By "humanized" antibody as used herein is meant an antibody comprising a human framework region and one or more CDR's from a non-human (usually mouse or rat) antibody. The non-human antibody providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor". One says that the donor antibody has been "humanized", by the process of "humanization".

[054] By "human equivalent CDR" or "human-like CDR" herein is meant at least one CDR, generally a non-human CDR, that has at least one amino acid substitution that brings the identity of the CDR region closer to at least one CDR of a naturally occurring germline sequence.

[055] By "identity" as used herein is meant the number of residues in a first sequence that are identical to the residues in a second sequence after alignment of the sequences to

achieve the maximum identity.

[056] By "Fc" or "Fc region", as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, as illustrated in Figure 1, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, as described below. By "Fc polypeptide" as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides include antibodies, Fc fusions, isolated Fcs, and Fc fragments.

[057] By "Fc fusion" as used herein is meant a protein wherein one or more polypeptides or small molecules is operably linked to an Fc region or a derivative thereof. Fc fusion is herein meant to be synonymous with the terms "immunoadhesin", "Ig fusion", "Ig chimera", and "receptor globulin" (sometimes with dashes) as used in the prior art (Chamow *et al.*, 1996, *Trends Biotechnol* 14:52-60; Ashkenazi *et al.*, 1997, *Curr Opin Immunol* 9:195-200. incorporated by reference). An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner, which in general can be any protein or small molecule. The role of the non-Fc part of an Fc fusion, i.e. the fusion partner, may be to mediate target binding, and thus it is functionally analogous to the variable regions of an antibody.

[058] By "Fc gamma receptor" or "FcγR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and are substantially encoded by the FcγR genes. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[059] By "Fc ligand" or "effector ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc / Fc ligand complex. Binding of an Fc ligand to Fc preferably elicits or more effector

functions. Fc ligands include but are not limited to Fc receptors, FcγRs, FcαRs, FcεRs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, *staphylococcal* protein A, *streptococcal* protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis *et al.*, 2002, *Immunological Reviews* 190:123-136, incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc.

[060] By "IgG" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3. By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments, and individual immunoglobulin domains. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin that exists as a distinct structural entity as ascertained by one skilled in the art of protein structure. Ig domains typically have a characteristic β-sandwich folding topology. The known Ig domains in the IgG class of antibodies are V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

[061] By "full length antibody" as used herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions. For example, in most mammals, including humans and mice, the full length antibody of the IgG isotype is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain comprising immunoglobulin domains VL and CL, and each heavy chain comprising immunoglobulin domains VH, Cγ1, Cγ2, and Cγ3. In some mammals, for example in camels and llamas, IgG antibodies may consist of only two heavy chains, each heavy chain comprising a variable domain attached to the Fc region.

[062] By " humanlike antibody" or "high human antibody" as used herein is meant an antibody whose variable heavy and light chains have sequences that are greater than about 85% identical to at least one sequence in a human germline immunoglobulin gene sequence, or sequences that have greater than 52% of their 9-mers that are a perfect match with at least one 9-mer in a human germline immunoglobulin gene sequence.

[063] By "immune epitope" or "epitope" herein is meant a linear sequence of amino acids that is located in a protein of interest. Epitopes may be analyzed for their potential for immunogenicity. Epitopes may be any length, preferably 9-mers.

[064] By "immunogenicity" herein is meant the ability of a protein to elicit an immune response, including but not limited to production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, and anaphylaxis.

[065] By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more proteins

substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments, and individual immunoglobulin domains. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin that exists as a distinct structural entity as ascertained by one skilled in the art of protein structure. Ig domains typically have a characteristic β-sandwich folding topology. The known Ig domains in the IgG class of antibodies are V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

[066] By "IgG" or "IgG immunoglobulin" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises the subclasses or isotypes IgG1, IgG2, IgG3, and IgG4. By "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE. Included in the definition of "IgG" are IgG fusions, where the IgG fusion contains sequences from two or more IgG molecules. For example, IgG1/2 fusions find use in a number of applications and are described in US Publication Nos. US Publication Nos. 2004/0132101, 2005/0054832, 2006/0024298, 2006/0121032, 2006/0235208, 2007/01481702007/0275460, , herein incorporated by reference in their entirety. Also included in the definition of IgG molecules are other variant IgGs, such as IgG1 variants that include amino acid substitutions in the Fc region, as described in US Publication Nos. 2004/0132101, 2005/0054832, 2006/0024298, 2006/0121032, 2006/0235208, 2007/01481702007/0275460, PCT US04/077250, herein incorporated by reference in their entirety.

[067] By "natural sequence" or "natural protein" as used herein is meant a protein that has been determined to exist absent any experimental modifications. Also included are sequences that can be predicted to exist in nature based on experimentally determined sequences. An example of such a predicted sequence is an antibody that can be predicted to exist based on the established patterns of germline recombination. In this case the large size of the predicted antibody repertoire makes the actual experimental determination of all mature recombined antibodies not practical.

[068] By "parent" or "parent protein" as used herein is meant a protein that is subsequently modified to generate a variant. The parent protein may be a naturally occurring protein, or a variant or engineered version of a naturally occurring protein. Parent protein may refer to the protein itself, compositions that comprise the parent protein, or the amino acid sequence that encodes it. Accordingly, by "parent antibody" as used herein is meant an antibody that is subsequently modified to generate a variant antibody. Accordingly, by "parent sequence" as used herein is meant the sequence that encodes the parent protein or parent antibody. Accordingly, by "parent CDR" as used herein is meant a CDR that is modified to generate a variant, e.g. a humanized CDR, and by "parent antibody" as used herein is meant an antibody that is modified to generate a variant antibody, in some cases within at least one CDR region.

[069] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example Kabat, Chothia, and/or the EU index as in Kabat.

[070] By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures.

[071] By "reduced immunogenicity" herein is meant a decreased ability to activate the immune system, when compared to the parent protein. For example, a protein variant can be said to have "reduced immunogenicity" if it elicits neutralizing or non-neutralizing antibodies in lower titer or in fewer patients than the parent protein. A protein variant also can be said to have "reduced immunogenicity" if it shows decreased binding to one or more MHC alleles or if it induces T cell activation in a decreased fraction of patients relative to the parent protein.

[072] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, serine 31 (also referred to as Ser31, also referred to as S31) is a residue in the WT anti-TAC VH region.

[073] By "scoring function" herein is meant any equation or method for evaluating the fitness of one or more amino acid modifications in a protein. The scoring function may involve a physical or chemical energy term, or may involve knowledge-, statistical-, sequence-based energy terms, and the like.

[074] By "string" as used herein is meant a contiguous sequence that encodes any part of a protein. Strings may comprise any 2 or more linear residues, with the number of contiguous residues being defined by the "window" or "window size". Window sizes of 2 - 20 are preferred, with 7 - 13 more preferred, with 9 most preferred.

[075] By "target antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. A target antigen may be a protein, carbohydrate, lipid, or other chemical compound. An antibody is said to be "specific" for a given target antigen based on having affinity for the target antigen.

[076] By "target cell" as used herein is meant a cell that expresses a target antigen.

[077] By "variable region" as used herein is meant the region of an antibody that comprises one or more Ig domains substantially encoded by any of the VL (including Vκ and Vλ) and/or V_H genes that make up the light chain (including kappa and lambda) and heavy chain immunoglobulin genetic loci respectively. A light or heavy chain variable region (VL and VH) consists of a "framework" or "FR" region interrupted by three hypervariable regions referred to as "complementarity determining regions" or "CDRs". The extent of the framework region and CDRs have been precisely defined, for example as in Kabat (see "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1983)), and as in Chothia. The framework regions of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the

CDRs, which are primarily responsible for binding to an antigen.

[078] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from a parent protein by virtue of at least one amino acid modification, including substitutions, insertions and/or deletions. In some cases, variant proteins contain a plurality of amino acid modifications; for example, as described herein, a variant antibody may contain one or more humanized CDRs, and/or a variant that adds or removes a glycosylation or conjugation site (including toxins and polymers such as PEG (including PEG derivatives)), and/or variants that confer altered binding to one or more Fc receptors, including, but not limited to, FcyR1, FcyRIIa, FcyRIIb, FcyRIIIa, FcRn, etc. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Accordingly, by "immunoglobulin variant" as used herein is meant an immunoglobulin that differs from a parent immunoglobulin by virtue of at least one amino acid modification. Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification. By "CDR variant" herein is meant a CDR that differs from a parent CDR as described herein.

[079] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature and includes allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified. For example, wild type germline sequences are known and are used as the basis for the humanization of the CDRs as described herein.

[080] Overview

[081] The present invention is directed to the "humanization" of CDR sequences. As is known in the art, there are many therapeutic antibodies that utilize non-human CDRs, particularly murine and particularly mouse CDRs, that are used in conjunction with human framework regions. Alternatively, even "human" antibodies such as produced in transgenic mice may differ from human germline CDR sequences.

[082] The present invention is directed to methods utilizing a starting set of parent CDRs (although as will be appreciated by those in the art, it is also possible to do a single CDR or any combination of CDRs, sequentially or simultaneously) that are non-optimized for "human-ness", and then creating amino acid substitutions based on comparisons with one or more human germline sequences. That is, by making amino acid substitutions in one or more of CDRs that correspond to amino acids in a human germline sequence, the CDR is "humanized" and becomes more "humanlike". In general, this is done separately with the variable light chain (e.g. the 3 light chain CDRs) and the variable heavy chain (e.g. the 3 heavy chain CDRs); that is, the light chain is substituted to be more similar to one germline sequence and the heavy chain is independently substituted to be more similar to another germline sequence, which is most frequently a different germline sequence (although it can be to the same germline sequence). This is referred to herein as "globalized CDR humanization". In addition, this can be done locally, e.g. CDR-by-CDR, with any particular

CDR being substituted to be more similar to a first germline sequence, a second to a second, etc. This is called "localized CDR humanization", as described herein.

[083] As is more fully described below, the substitutions may be done one at a time or as multiple variants. Generally a library of variants is created, and then the variants are tested for binding to the target antigen, and variants that retain binding can be combined as well.

[084] As more fully described below, the invention generally relates to variable regions comprising humanized CDRs that are at least about 80-85-90-95% identical to a corresponding human germline CDR sequence, with at least about 85% identity finding particular use, and/or that retain at least about 10-30% of the affinity of the parent CDRs. As noted herein, this identity number is either a global sequence identity, e.g. the three light chain CDRs and the framework region of the light chain, compared to a parent light chain germline sequence and/or the heavy chain CDRs and the framework region of the heavy chain, compared to a parent heavy chain germline sequence.

[085] In addition, these humanized CDRs (whether there is one humanized CDR or more) can be combined with naturally occurring framework regions (including Fc regions), or with variant regions, including variants that confer stability or serum half-life (e.g. FcRn variants) and/or alterations in FcγR binding, as is generally described in US Publication Nos. 2004/0132101, 2005/0054832, 2006/0024298, 2006/0121032, 2006/0235208, 2007/01481702007/0275460, PCT US04/077250, incorporated by reference. In addition, polypeptides comprising the humanized CDRs, such as antibodies, can also be used in combination with engineered glycoform technologies, conjugation technologies (e.g. toxin conjugation), etc.

[086] Antibodies

[087] Antibodies are immunological proteins that bind a specific antigen. In most mammals, including humans and mice, antibodies are constructed from paired heavy and light polypeptide chains. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin is used for such proteins.

[088] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. IgA has several subclasses, including but not

limited to IgA1 and IgA2. Thus, "isotype" as used herein is meant any of the classes and subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE.

[089] Each of the light and heavy chains are made up of two distinct regions, referred to as the variable and constant regions. 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 constant domain 3 respectively (also referred to as VH-Cγ1-Cγ2-Cγ3, 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. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the variable region.

[090] 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 amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. 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. Sequence and structural features of antibody variable regions are disclosed, for example, in Morea et al., 1997, Biophys Chem 68:9-16; Morea et al., 2000, Methods 20:267-279, hereby entirely incorporated by reference, and the conserved features of antibodies are disclosed, for example, in Maynard et al., 2000, Annu Rev Biomed Eng 2:339-376, hereby entirely incorporated by reference.

[091] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in embodiments described herein are the heavy chain domains, including, the constant heavy (CH) domains and the hinge region. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[092] The antibodies disclosed herein may be substantially encoded by immunoglobulin genes belonging to any of the antibody classes. In certain embodiments, the antibodies disclosed herein comprise sequences belonging to the IgG class of antibodies, including IgG1, IgG2, IgG3, or IgG4. In alternate embodiments, antibodies disclosed herein comprise sequences belonging to the IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG, or IgM classes of antibodies.

[093] The parent antibodies of the invention, that is the antibodies from which the antibodies of the invention were derived, may be substantially encoded by genes from any organism, e.g., mammals (including, but not limited to humans, rodents (including but not limited to mice and rats), lagomorpha (including but not limited to rabbits and hares), camelidae (including but not limited to camels, llamas, and dromedaries), and non-human primates, including but not limited to Prosimians, Platyrrhini (New World monkeys), Cercopithecoidea (Old World monkeys), and Hominoidea including the Gibbons and Lesser and Great Apes. In a certain embodiments, the parent antibodies may be substantially human. The parent antibody need not be naturally occurring. For example, the parent antibody may be an engineered antibody, including but not limited to nonhuman and chimeric antibodies. The parent antibody may be an engineered variant of an antibody that is substantially encoded by one or more natural antibody genes. In one embodiment, the parent antibody has been affinity matured, as is known in the art, or engineered in some other way, such as to alter FcR binding, for example. In some embodiments, the parent antibody is a humanized antibody, containing non-human (e.g. murine) CDRs with the remainder of the molecule comprising human sequences. In some embodiments, the parent antibody has had its framework regions optimized, with non-human CDRs.

[094] The antibodies of the present invention may comprise sequences belonging to the IgG (including IgG1, IgG2, IgG3, IgG4 and fusions of any combination), IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG, IgM (and fusions of any combination) classes of antibodies, with the IgG class being preferred. The less immunogenic antibodies of the present invention may be full length antibodies, or antibody fragments. Constant regions need not be present, but if they are, they will likely be substantially identical to human

immunoglobulin constant regions.

[095] As is well known in the art, antibody polymorphisms exist in the human population. Gm polymorphism is determined by the IGHG1, IGHG2 and IGHG3 genes which have alleles encoding allotypic antigenic determinants referred to as G1m, G2m, and G3m allotypes for markers of the human IgG1, IgG2 and IgG3 molecules (no Gm allotypes have been found on the gamma 4 chain). Markers may be classified into `allotypes` and `isoallotypes`. These are distinguished on different serological bases dependent upon the strong sequence homologies between isotypes. Allotypes are antigenic determinants specified by allelic forms of the Ig genes. Allotypes represent slight differences in the amino acid sequences of heavy or light chains of different individuals. Even a single amino acid difference can give rise to an allotypic determinant, although in many cases there are several amino acid substitutions that have occurred. Allotypes are sequence differences between alleles of a subclass whereby the antisera recognize only the allelic differences. An isoallotype is an allele in one isotype which produces an epitope which is shared with a non-polymorphic homologous region of one or more other isotypes and because of this the antisera will react with both the relevant allotypes and the relevant homologous isotypes (Clark, 1997, IgG effector mechanisms, Chem Immunol. 65:88-110; Gorman & Clark, 1990, Semin Immunol 2(6):457-66, both hereby entirely incorporated by reference).

[096] Allelic forms of human antibodies have been well-characterized (WHO Review of the notation for the allotypic and related markers of human immunoglobulins. J Immunogen 1976, 3: 357-362; WHO Review of the notation for the allotypic and related markers of human immunoglobulins. 1976, Eur. J. Immunol. 6, 599-601; Loghem E van, 1986, Allotypic markers, Monogr Allergy 19: 40-51, all hereby entirely incorporated by reference). Additionally, other polymorphisms have been characterized (Kim et al., 2001, J. Mol. Evol. 54:1-9, hereby entirely incorporated by reference). At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, et al., The human IgG subclasses: molecular analysis of structure, function and regulation. Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. et al., 1979, Hum. Genet.: 50, 199-211, both hereby entirely incorporated by reference). Allotypes that are inherited in fixed combinations are called Gm haplotypes. The antibodies disclosed herein may be substantially encoded by any allotype, isoallotype, or haplotype of any immunoglobulin gene.

[097] The variable region of an antibody, as is well known in the art, can compose sequences from a variety of species. In some embodiments, the antibody variable region can be from a nonhuman source, including but not limited to mice, rats, rabbits, camels, llamas, and monkeys. In some embodiments, the scaffold components can be a mixture from different species. As such, an antibody disclosed herein may be a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric

antibodies" traditionally comprise variable region(s) from a mouse or other nonhuman species and the constant region(s) from a human.

[098] "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (U.S. Pat. No. 5,693,762, incorporated entirely by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein). Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in U.S. Ser. No. 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, that is, to increase the affinity of the variable region for its target antigen. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in U.S. Ser. No. 09/810,502; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 10/153,159 and related applications, all incorporated entirely by reference. In certain variations, the immunogenicity of the antibody is reduced using a method described in USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 3, 2004, incorporated entirely by reference.

[099] The engineered antibodies of the invention are human equivalent antibodies. Historically, fully human antibodies have been obtained using transgenic mice (Bruggemann et al., 1997, Curr Opin Biotechnol 8:455-458) or human antibody libraries coupled with selection methods (Griffiths et al., 1998, Curr Opin Biotechnol 9:102-108). However, the

present invention describes a novel method for engineering human equivalent antibodies from nonhuman parent antibodies. For the purposes of the present invention, "a humanlike antibody" or "human equivalent antibody" is defined as: an antibody whose variable heavy and light chains have sequences that are greater than about 85% identical to at least one sequence in a human germline immunoglobulin gene sequence; an antibody whose individual CDRs have greater than about 50-60-70-80-90% identity to at least one CDR of a human germline sequence (with greater than about 80% finding use in a number of applications); or sequences that have greater than 52% of their 9-mers that are a perfect match with at least one 9-mer in a human germline immunoglobulin gene sequence.

[0100] Engineering of Human Equivalent Antibodies

[0101] Current engineering methods for reducing the immunogenicity of antibodies (e.g. humanization) with non-human variable regions do not succeed in creating antibodies with global sequence identity levels comparable to human antibodies. The principal reason for this is that none of the current methods substantially engineer the CDRs due to the perceived risk of losing antigen affinity. Methods that have ventured into the CDRs, such as SDR-grafting (Kashmiri, et al., 2005, *Methods* 36:25-34; Gonzales, et al., 2004, *Mol Immunol* 41:863-872), have either failed to change the CDRs significantly enough to yield human equivalent levels of global sequence identity or have resulted in variants having a significant decrease in affinity. The present invention is based on the discovery that with precise engineering of the antibody variable region, including the CDRs, it is possible to engineer an antibody with a non-human variable region to have humanness or "human equivalent" levels comparable to fully-human antibodies, and importantly, to maintain antigen affinity to within 3-fold of the of the parent antibody. The method (outlined in Figure 1) consists of five main steps: (1) the optional generation of a framework-optimized template sequence; (2) identification of the closest matching human germline sequence for the (optionally) framework-optimized V_H and V_L; (3) generation and screening of a variant library consisting of all possible single mutations designed to increase the local and/or global sequence identity of the framework-optimized sequence to the closest human germline sequence while maintaining antigen affinity; (4) generation and screening of variants consisting of combinations of neutral or affinity enhancing single mutations for V_H and V_L; and (5) expression and screening of the highest affinity V_H and V_L chains paired together to generate the final human equivalent mAb. In other embodiments, the framework sequences need not be optimized. In other embodiments, either with or without framework optimization, the variant library can contain less than every possible single mutation and/or double or higher numbers of mutations as well.

[0102] When framework optimization is done, the method proceeds as follows. After selection of the non-human parent Fv, the first step in the process is to engineer the framework regions for high human sequence content. This framework-optimized mAb is engineered using a method such as that described in USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof",

filed on December 6, 2004, incorporated herein by reference in it's entirety, and in particular for the methods of framework optimization. Other methods of reducing potential immunogenicity using antibody engineering, such as CDR-grafting (Jones, et al., 1986, *Nature* 321:522-525), resurfacing (Roguska, et al., 1994, *Proc Natl Acad Sci U S A* 91:969-973; Roguska, et al., 1996, *Protein Eng* 9:895-904), SDR-grafting (Kashmiri, et al., 2005, *Methods* 36:25-34; Gonzales, et al., 2004, *Mol Immunol* 41:863-872), superhumanization (Hwang, et al., 2005, *Methods* 36:35-42), and framework shuffling (Dall'Acqua, et al., 2005, *Methods* 36:43-60; Damschroder, et al., 2007, *Mol Immunol* 44:3049-3060) may be used, provided the final framework-optimized mAb has high human sequence content in the framework regions and the antibody maintains antigen affinity equivalent to the original mAb with non-human Fv.

[0103] Following the optional creation of the framework-optimized mAb, the next step in the engineering process is to analyze the sequence of this mAb (or, in the case where no framework optimization occurs, against the starting parent sequence) against the human germline repertoire. The set of human sequences used is an aligned set of human germline immunoglobulin sequences. The human germline repertoire for immunoglobulin heavy chain variable regions and immunoglobulin light chain kappa variable regions has been reported (Matsuda et al., 1998, *J Exp Med* 188: 2151–2162; Zachau, 2000, Biol Chem 381:951–954; Pallares et al., 1999, *Exp Clin Immunogenet* 16(1): 36-60; Barbie & Lefranc, 1998, *Exp Clin Immunogenet* 15(3): 171-83). For many of the genes in the human immunoglobulin germline, several different alleles have been identified. Although the polymorphisms detected in many of the alleles do not change the amino acid sequence of the gene, in a great number of cases the sequence is changed. In choosing a set of sequences to use in the method described herein, different sets of sequences may be chosen. In general, the set may be a single sequence with the best starting identity to the parent sequence, a number of sequences, etc.

[0104] The framework-optimized heavy and light chains are aligned with the human germline V- and J-segments and the germlines ranked based on the number of mutations away from the framework-optimized sequence, the conservativeness of each mutation, and/or the proximity of each mutation to CDRs. That is, in some embodiments, the germline is chosen based on the absolute smallest number of mutations between the germline and the parent molecule, or based on the smalles number of mutations within the set of CDRs or within single CDRs. Similarly, the germline with the highest identity may have non-conservative mutations (based on a BLOSUM matrix, for example), with germlines with lower identity having higher conservativeness, with the latter being a good choice in some cases. Similarly, the distance of the mutations to the CDRs may be important: for example, if one germline has a framework with 30 differences, 20 of them located at a distance from each CDR and 10 close, and another germline has 30 differences but 20 are close and 10 are farther, it may be desirable in some situations to pick the former.

[0105] By using these criteria to select the germline to engineer the parent antibody towards,

the probability of achieving a human equivalent mAb with a minimal loss in antigen affinity is increased. Variants with single mutations representing each of the differences in sequence between the framework-optimized mAb and the closest germline V- and J-segments are constructed and screened using standard techniques. For instance, if the framework-optimized mAb had a serine at position 31 in the heavy chain, but the closest germline had a tyrosine at position 31, then a variant of the framework-optimized mAb with tyrosine at position 31 would be constructed and screened for antigen binding. This procedure is performed for all such differences in the two sequences and the data tabulated. Most mutations will be in the CDRs, but a few may be in framework regions that are known to be proximal to the CDRs or located in the VH/VL interface.

[0106] In the next step, single mutations that resulted in comparable affinity to the framework-optimized Fv are explored in combination. Combinations of heavy chain variants are paired with the framework-optimized light chain and combinations of light chain variants are paired with the framework-optimized heavy chain. Because the additivity of single variants is difficult to predict, it is important to try several possible combinations of variants that have different levels of diversity and human sequence content. However, depending on the number of single variants, the number of possible combination variants can be large. Thus, an approach to limit the number of combination variants by balancing human sequence content and diversity of the library can be useful. A computational approach to designing such diverse libraries, such as Combination Design Automation or CDA™ technology is one such method that can be used. Expression and screening of the combination variant library will result in several variants with either human equivalent heavy or light chains with antigen affinity comparable to the framework-optimized mAb. In vitro and/or in vivo assays are used to evaluate the efficacy and potency of the engineered human equivalent antibodies.

[0107] *Evaluation of humanness*

[0108] "Humanness" or "humanlike" or "human equivalent" evaluations can be done in a variety of ways. In one embodiment, global identity scores are used. Global identity is the number of exact sequence matches between the engineered sequence and any one of the human germline V_H, V_K, J_H, and J_K segments (the D segment for the heavy chain is not included). An additional score can be based just on the CDR identities, rather than the entire germline. A further possible score is the number of total "human 9-mers", which is an exact count of 9-mer stretches in the engineered sequence that perfectly match any one of the corresponding stretches of nine amino acids in our set of functional human germline sequences. Finally, the variant antibody can be evaluated on the basis of actual immunogenicity in a host organism as compared to the parent antibody.

[0109] As shown in figure 11 and figure 12, antibodies with murine variable regions (murine and chimeric antibodies) typically have global identities less than 71% and less than 13% human 9-mers. Human equivalent antibodies typically have global identities between about 78% and about 85% (with from about 60, 65, 70, 75, 80, 85, 90 and 95% possible, with all

possible combination of ranges) and human 9-mers of between about 32% and about 52% (with from about 30, 35, 40, 45, 50, 55, 60, 65 and 70% possible, with all possible combination of ranges). Fully-human antibodies isolated from human antibody libraries or from transgenic mice comprising human germline immunoglobulin gene sequences typically have global identities greater than 85% and human 9-mers greater than 52%.

[0110] Target Antigens

[0111] Virtually any binding partner or antigen may be targeted by the antibodies of the present invention. A number biotherapeutic proteins and antibodies that are approved for use, in clinical trials, or in development may thus benefit from the methods of the present invention.

[0112] Other Antibody Modifications

[0113] The antibodies of the invention may be modified in some way to make them more effective, particularly more effective as therapeutics. A variety of modifications for improving the properties of antibodies are described USSN 10/672,280; USSN 10/822,231; USSN 11/124,620; USSN 11/396,495; USSN 11/538,406; USSN 12/020,443; USSN 12/156,183; USSN 11/274,065; USSN 11/436,266; USSN 11/932,151; USSN 12/341,769; Hinton et al., 2004, J. Biol. Chem. 279(8): 6213-6216, Hinton et al. 2006 Journal of Immunology 176:346-356, USSN 11/102,621; USSN 10/966,673; Shields et al, Journal of Biological Chemistry, 2001, 276(9):6591-6604; US 11/429793; Dall Acqua et al. Journal of Immunology, 2002, 169:5171-5180; US 7,083,784; PCT/US2004/037929; Umaña et al., 1999, Nat Biotechnol 17:176-180; Davies et al., 2001, Biotechnol Bioeng 74:288-294; Shields et al., 2002, J Biol Chem 277:26733-26740; Shinkawa et al., 2003, J Biol Chem 278:3466-3473; Yamane-Ohnuki et al., 2004, Biotechnol Bioeng 87 (5), 614-622; Li et al., 2006, Nature Biotechnology 24(2):210-215; Nechansky et al., 2007, Mol Immunjol 44(7):1826-8; Cox et al., 2006, Nat Biotechnol 24(12):1591-7; and Kaneko et al., 2006, Science 313:670-673, all expressly incorporated by reference.

[0114] Modifications may include amino acid modifications, glycoform modifications, and chemical modifications. Modifications may improve the antibody's effector function properties, pharmacokinetic properties, solution properties, and/or biological activity. The antibodies of the invention may be conjugated or operably linked to another therapeutic compound. The therapeutic compound may be a cytotoxic agent, a chemotherapeutic agent, a toxin, a radioisotope, a cytokine, or other therapeutically active agent. The antibodies of the invention may be conjugated to a protein or molecule for utilization in tumor pretargeting or prodrug therapy. Other modifications of the antibodies are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, for example e.g., polyethylene glycol (PEG).

[0115] Production of Antibodies

[0116] Also disclosed herein are methods for producing and experimentally testing

antibodies. The disclosed methods are not meant to constrain embodiments to any particular application or theory of operation. Rather, the provided methods are meant to illustrate generally that one or more antibodies may be produced and experimentally tested to obtain antibodies. General methods for antibody molecular biology, expression, purification, and screening are described in Antibody Engineering, edited by Duebel & Kontermann, Springer-Verlag, Heidelberg, 2001; and Hayhurst & Georgiou, 2001, *Curr Opin Chem Biol* 5:683-689; Maynard & Georgiou, 2000, *Annu Rev Biomed Eng* 2:339-76; Antibodies: A Laboratory Manual by Harlow & Lane, New York: Cold Spring Harbor Laboratory Press, 1988, all incorporated entirely by reference.

[0117] In one embodiment disclosed herein, nucleic acids are created that encode the antibodies, and that may then be cloned into host cells, expressed and assayed, if desired. Thus, nucleic acids, and particularly DNA, may be made that encode each protein sequence. These practices are carried out using well-known procedures. For example, a variety of methods that may find use in generating antibodies disclosed herein are described in Molecular Cloning - A Laboratory Manual, 3rd Ed. (Maniatis, Cold Spring Harbor Laboratory Press, New York, 2001), and Current Protocols in Molecular Biology (John Wiley & Sons), both incorporated entirely by reference. As will be appreciated by those skilled in the art, the generation of exact sequences for a library comprising a large number of sequences is potentially expensive and time consuming. By "library" herein is meant a set of variants in any form, including but not limited to a list of nucleic acid or amino acid sequences, a list of nucleic acid or amino acid substitutions at variable positions, a physical library comprising nucleic acids that encode the library sequences, or a physical library comprising the variant proteins, either in purified or unpurified form. Accordingly, there are a variety of techniques that may be used to efficiently generate libraries disclosed herein. Such methods include but are not limited to gene assembly methods, PCR-based method and methods which use variations of PCR, ligase chain reaction-based methods, pooled oligo methods such as those used in synthetic shuffling, error-prone amplification methods and methods which use oligos with random mutations, classical site-directed mutagenesis methods, cassette mutagenesis, and other amplification and gene synthesis methods. As is known in the art, there are a variety of commercially available kits and methods for gene assembly, mutagenesis, vector subcloning, and the like, and such commercial products find use in for generating nucleic acids that encode antibodies.

[0118] The antibodies disclosed herein may be produced by culturing a host cell transformed with nucleic acid, e.g., an expression vector, containing nucleic acid encoding the antibodies, under the appropriate conditions to induce or cause expression of the protein. The conditions appropriate for expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. A wide variety of appropriate host cells may be used, including but not limited to mammalian cells, bacteria, insect cells, and yeast. For example, a variety of cell lines that may find use in generating antibodies disclosed herein are described in the ATCC® cell line catalog, available

from the American Type Culture Collection.

[0119] In one embodiment, the antibodies are expressed in mammalian expression systems, including systems in which the expression constructs are introduced into the mammalian cells using virus such as retrovirus or adenovirus. Any mammalian cells may be used, e.g., human, mouse, rat, hamster, and primate cells. Suitable cells also include known research cells, including but not limited to Jurkat T cells, NIH3T3, CHO, BHK, COS, HEK293, PER C.6, HeLa, Sp2/0, NS0 cells and variants thereof. In an alternateembodiment, library proteins are expressed in bacterial cells. Bacterial expression systems are well known in the art, and include *Escherichia coli* (*E. coli*), *Bacillus subtilis*, *Streptococcus cremoris*, and *Streptococcus lividans*. In alternate embodiments, antibodies are produced in insect cells (e.g. Sf21/Sf9, Trichoplusia ni Bti-Tn5b1-4) or yeast cells (e.g. *S. cerevisiae*, *Pichia*, etc). In an alternate embodiment, antibodies are expressed *in vitro* using cell free translation systems. *In vitro* translation systems derived from both prokaryotic (e.g. *E. coli*) and eukaryotic (e.g. wheat germ, rabbit reticulocytes) cells are available and may be chosen based on the expression levels and functional properties of the protein of interest. For example, as appreciated by those skilled in the art, *in vitro* translation is required for some display technologies, for example ribosome display. In addition, the antibodies may be produced by chemical synthesis methods. Also transgenic expression systems both animal (e.g. cow, sheep or goat milk, embryonated hen's eggs, whole insect larvae, etc.) and plant (e.g. corn, tobacco, duckweed, etc.)

[0120] The nucleic acids that encode the antibodies disclosed herein may be incorporated into an expression vector in order to express the protein. A variety of expression vectors may be utilized for protein expression. Expression vectors may comprise self-replicating extra-chromosomal vectors or vectors which integrate into a host genome. Expression vectors are constructed to be compatible with the host cell type. Thus expression vectors which find use in generating antibodies disclosed herein include but are not limited to those which enable protein expression in mammalian cells, bacteria, insect cells, yeast, and in *in vitro* systems. As is known in the art, a variety of expression vectors are available, commercially or otherwise, that may find use for expressing antibodies disclosed herein.

[0121] Expression vectors typically comprise a protein operably linked with control or regulatory sequences, selectable markers, any fusion partners, and/or additional elements. By "operably linked" herein is meant that the nucleic acid is placed into a functional relationship with another nucleic acid sequence. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the antibody, and are typically appropriate to the host cell used to express the protein. In general, the transcriptional and translational regulatory sequences may include promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. As is also known in the art, expression vectors typically contain a selection gene or marker to allow the

selection of transformed host cells containing the expression vector. Selection genes are well known in the art and will vary with the host cell used.

[0122] Antibodies may be operably linked to a fusion partner to enable targeting of the expressed protein, purification, screening, display, and the like. Fusion partners may be linked to the antibody sequence via a linker sequences. The linker sequence will generally comprise a small number of amino acids, typically less than ten, although longer linkers may also be used. Typically, linker sequences are selected to be flexible and resistant to degradation. As will be appreciated by those skilled in the art, any of a wide variety of sequences may be used as linkers. For example, a common linker sequence comprises the amino acid sequence GGGGS. A fusion partner may be a targeting or signal sequence that directs antibody and any associated fusion partners to a desired cellular location or to the extracellular media. As is known in the art, certain signaling sequences may target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. A fusion partner may also be a sequence that encodes a peptide or protein that enables purification and/or screening. Such fusion partners include but are not limited to polyhistidine tags (His-tags) (for example H_6 and H_{10} or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (e.g. Ni^{+2} affinity columns)), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like). As will be appreciated by those skilled in the art, such tags may be useful for purification, for screening, or both. For example, an antibody may be purified using a His-tag by immobilizing it to a Ni^{+2} affinity column, and then after purification the same His-tag may be used to immobilize the antibody to a Ni^{+2} coated plate to perform an ELISA or other binding assay (as described below). A fusion partner may enable the use of a selection method to screen antibodies (see below). Fusion partners that enable a variety of selection methods are well-known in the art. For example, by fusing the members of an antibody library to the gene III protein, phage display can be employed (Kay *et al.*, Phage display of peptides and proteins: a laboratory manual, Academic Press, San Diego, CA, 1996; Lowman *et al.*, 1991, *Biochemistry* 30:10832-10838; Smith, 1985, *Science* 228:1315-1317, incorporated entirely by reference). Fusion partners may enable antibodies to be labeled. Alternatively, a fusion partner may bind to a specific sequence on the expression vector, enabling the fusion partner and associated antibody to be linked covalently or noncovalently with the nucleic acid that encodes them. The methods of introducing exogenous nucleic acid into host cells are well known in the art, and will vary with the host cell used. Techniques include but are not limited to dextran-mediated transfection, calcium phosphate precipitation, calcium chloride treatment, polybrene mediated transfection, protoplast fusion, electroporation, viral or phage infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In the case of mammalian cells, transfection may be either transient or stable.

[0123] In one embodiment, antibodies are purified or isolated after expression. Proteins may

be isolated or purified in a variety of ways known to those skilled in the art. Standard purification methods include chromatographic techniques, including ion exchange, hydrophobic interaction, affinity, sizing or gel filtration, and reversed-phase, carried out at atmospheric pressure or at high pressure using systems such as FPLC and HPLC. Purification methods also include electrophoretic, immunological, precipitation, dialysis, and chromatofocusing techniques. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. As is well known in the art, a variety of natural proteins bind Fc and antibodies, and these proteins can find use for purification of antibodies disclosed herein. For example, the bacterial proteins A and G bind to the Fc region. Likewise, the bacterial protein L binds to the Fab region of some antibodies, as of course does the antibody's target antigen. Purification can often be enabled by a particular fusion partner. For example, antibodies may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-tag is employed, or immobilized anti-flag antibody if a flag-tag is used. For general guidance in suitable purification techniques, see, e.g. incorporated entirely by reference Protein Purification: Principles and Practice, 3rd Ed., Scopes, Springer-Verlag, NY, 1994, incorporated entirely by reference. The degree of purification necessary will vary depending on the screen or use of the antibodies. In some instances no purification is necessary. For example in one embodiment, if the antibodies are secreted, screening may take place directly from the media. As is well known in the art, some methods of selection do not involve purification of proteins. Thus, for example, if a library of antibodies is made into a phage display library, protein purification may not be performed.

[0124] In Vitro Experimentation

[0125] Antibodies may be screened using a variety of methods, including but not limited to those that use *in vitro* assays, *in vivo* and cell-based assays, and selection technologies. Automation and high-throughput screening technologies may be utilized in the screening procedures. Screening may employ the use of a fusion partner or label. The use of fusion partners has been discussed above. By "labeled" herein is meant that the antibodies disclosed herein have one or more elements, isotopes, or chemical compounds attached to enable the detection in a screen. In general, labels fall into three classes: a) immune labels, which may be an epitope incorporated as a fusion partner that is recognized by an antibody, b) isotopic labels, which may be radioactive or heavy isotopes, and c) small molecule labels, which may include fluorescent and colorimetric dyes, or molecules such as biotin that enable other labeling methods. Labels may be incorporated into the compound at any position and may be incorporated *in vitro* or *in vivo* during protein expression.

[0126] In one embodiment, the functional and/or biophysical properties of antibodies are screened in an *in vitro* assay. *In vitro* assays may allow a broad dynamic range for screening properties of interest. Properties of antibodies that may be screened include but are not limited to antigen binding (e.g. affinity for the target antigen), stability, solubility, and affinity for Fc ligands, for example FcγRs. Multiple properties may be screened simultaneously or

individually. Proteins may be purified or unpurified, depending on the requirements of the assay. In one embodiment, the screen is a qualitative or quantitative binding assay for binding of antibodies to a protein or nonprotein molecule that is known or thought to bind the antibody. In one embodiment, the screen is a binding assay for measuring binding to the target antigen. In an alternate embodiment, the screen is an assay for binding of antibodies to an Fc ligand, including but are not limited to the family of FcγRs, the neonatal receptor FcRn, the complement protein C1q, and the bacterial proteins A and G. Said Fc ligands may be from any organism. In one embodiment, Fc ligands are from humans, mice, rats, rabbits, and/or monkeys. Binding assays can be carried out using a variety of methods known in the art, including but not limited to FRET (Fluorescence Resonance Energy Transfer) and BRET (Bioluminescence Resonance Energy Transfer) -based assays, AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay), Scintillation Proximity Assay, ELISA (Enzyme-Linked Immunosorbent Assay), SPR (Surface Plasmon Resonance, also known as BIACORE®), isothermal titration calorimetry, differential scanning calorimetry, gel electrophoresis, and chromatography including gel filtration. These and other methods may take advantage of some fusion partner or label of the antibody. Assays may employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels.

[0127] The biophysical properties of antibodies, for example stability and solubility, may be screened using a variety of methods known in the art. Protein stability may be determined by measuring the thermodynamic equilibrium between folded and unfolded states. For example, antibodies disclosed herein may be unfolded using chemical denaturant, heat, or pH, and this transition may be monitored using methods including but not limited to circular dichroism spectroscopy, fluorescence spectroscopy, absorbance spectroscopy, NMR spectroscopy, calorimetry, and proteolysis. As will be appreciated by those skilled in the art, the kinetic parameters of the folding and unfolding transitions may also be monitored using these and other techniques. The solubility and overall structural integrity of an antibody may be quantitatively or qualitatively determined using a wide range of methods that are known in the art. Methods which may find use for characterizing the biophysical properties of antibodies disclosed herein include gel electrophoresis, isoelectric focusing, capillary electrophoresis, chromatography such as size exclusion chromatography, ion-exchange chromatography, and reversed-phase high performance liquid chromatography, peptide mapping, oligosaccharide mapping, mass spectrometry, ultraviolet absorbance spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, differential scanning calorimetry, analytical ultra-centrifugation, dynamic light scattering, proteolysis, and cross-linking, turbidity measurement, filter retardation assays, immunological assays, fluorescent dye binding assays, protein-staining assays, microscopy, and detection of aggregates via ELISA or other binding assay. Structural analysis employing X-ray crystallographic techniques and NMR spectroscopy may also find use. In one embodiment, stability and/or solubility may be measured by determining the amount of protein solution after

some defined period of time. In this assay, the protein may or may not be exposed to some extreme condition, for example elevated temperature, low pH, or the presence of denaturant. Because function typically requires a stable, soluble, and/or well-folded/structured protein, the aforementioned functional and binding assays also provide ways to perform such a measurement. For example, a solution comprising an antibody could be assayed for its ability to bind target antigen, then exposed to elevated temperature for one or more defined periods of time, then assayed for antigen binding again. Because unfolded and aggregated protein is not expected to be capable of binding antigen, the amount of activity remaining provides a measure of the antibody's stability and solubility.

[0128] In one embodiment, the library is screened using one or more cell-based or *in vitro* assays. For such assays, antibodies, purified or unpurified, are typically added exogenously such that cells are exposed to individual variants or groups of variants belonging to a library. These assays are typically, but not always, based on the biology of the ability of the antibody to bind to the target antigen and mediate some biochemical event, for example effector functions like cellular lysis, phagocytosis, ligand/receptor binding inhibition, inhibition of growth and/or proliferation, apoptosisand the like. Such assays often involve monitoring the response of cells to antibody, for example cell survival, cell death, cellular phagocytosis, cell lysis, change in cellular morphology, or transcriptional activation such as cellular expression of a natural gene or reporter gene. For example, such assays may measure the ability of antibodies to elicit ADCC, ADCP, or CDC. For some assays additional cells or components, that is in addition to the target cells, may need to be added, for example serum complement, or effector cells such as peripheral blood monocytes (PBMCs), NK cells, macrophages, and the like. Such additional cells may be from any organism, e.g., humans, mice, rat, rabbit, and monkey. Crosslinked or monomeric antibodies may cause apoptosis of certain cell lines expressing the antibody's target antigen, or they may mediate attack on target cells by immune cells which have been added to the assay. Methods for monitoring cell death or viability are known in the art, and include the use of dyes, fluorophores, immunochemical, cytochemical, and radioactive reagents. For example, caspase assays or annexin-flourconjugates may enable apoptosis to be measured, and uptake or release of radioactive substrates (e.g. Chromium-51 release assays) or the metabolic reduction of fluorescent dyes such as alamar blue may enable cell growth, proliferation or activation to be monitored. In one embodiment, the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA) is used. Alternatively, dead or damaged target cells may be monitored by measuring the release of one or more natural intracellular proteins, for example lactate dehydrogenase. Transcriptional activation may also serve as a method for assaying function in cell-based assays. In this case, response may be monitored by assaying for natural genes or proteins which may be upregulated or down-regulated, for example the release of certain interleukins may be measured, or alternatively readout may be via a luciferase or GFP-reporter construct. Cell-based assays may also involve the measure of morphological changes of cells as a response to the presence of an antibody. Cell types for such assays may be prokaryotic or eukaryotic,

and a variety of cell lines that are known in the art may be employed. Alternatively, cell-based screens are performed using cells that have been transformed or transfected with nucleic acids encoding the antibodies.

[0129] *In vitro* assays include but are not limited to binding assays, ADCC, CDC, cytotoxicity, proliferation, peroxide/ozone release, chemotaxis of effector cells, inhibition of such assays by reduced effector function antibodies; ranges of activities such as >100x improvement or >100x reduction, blends of receptor activation and the assay outcomes that are expected from such receptor profiles.

[0130] In Vivo Experimentation

[0131] The biological properties of the antibodies disclosed herein may be characterized in cell, tissue, and whole organism experiments. As is known in the art, drugs are often tested in animals, including but not limited to mice, rats, rabbits, dogs, cats, pigs, and monkeys, in order to measure a drug's efficacy for treatment against a disease or disease model, or to measure a drug's pharmacokinetics, toxicity, and other properties. Said animals may be referred to as disease models. In some embodiments, antibodies disclosed herein may be assessed for efficacy in clinically relevant animal models of various human diseases. In many cases, relevant models include various transgenic animals for specific antigens and receptors.

[0132] Other organisms, e.g., mammals, may also be used for testing. For example, because of their genetic similarity to humans, monkeys can be suitable therapeutic models, and thus may be used to test the efficacy, toxicity, pharmacokinetics, or other property of the antibodies disclosed herein. In one embodiment, the testing of antibodies may include study of efficacy in primates (e.g. cynomolgus monkey model) to facilitate the evaluation of depletion of specific target cells harboring the target antigen. Additional primate models include but are not limited to use of the rhesus monkey to assess Fc polypeptides in therapeutic studies of autoimmune, transplantation and cancer. Toxicity studies are performed to determine antibody related-effects that cannot be evaluated in standard pharmacology profiles, or occur only after repeated administration of the agent. The pharmacokinetics (PK) of the antibodies disclosed herein may be studied in a variety of animal systems, with the most relevant being non-human primates such as the cynomolgus and rhesus monkeys.

[0133] Tests of the antibodies disclosed herein in humans are ultimately required for approval as drugs, and thus of course these experiments are contemplated. Thus the antibodies disclosed herein may be tested in humans to determine their therapeutic efficacy, toxicity, pharmacokinetics, and/or other clinical properties.

[0134] Therapeutic Application

[0135] The antibodies of the invention may find use in a wide range of protein products. In one embodiment the antibody is a therapeutic, a diagnostic, or a research reagent, preferably

a therapeutic. Alternatively, the antibody of the invention may be used for agricultural or industrial uses. In a preferred embodiment, the protein is a therapeutic that is used to treat a disease. By "disease" herein is meant a disorder that may be ameliorated by the administration of a pharmaceutical composition comprising a protein of the present invention. Diseases include but are not limited to autoimmune diseases, immunological diseases, infectious diseases, inflammatory diseases, neurological diseases, and oncological and neoplastic diseases including cancer. In one embodiment, an antibody of the invention is the only therapeutically active agent administered to a patient. Alternatively, the antibody of the invention is administered in combination with one or more other therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, or other therapeutic agents. The antibodies of the invention may be combined with other therapeutic regimens. For example, in one embodiment, the patient to be treated with the protein may also receive radiation therapy and/or undergo surgery.

[0136] A "patient" for the purposes disclosed herein includes both humans and other animals, e.g., other mammals. Thus the immunoglobulins disclosed herein have both human therapy and veterinary applications. The term "treatment" or "treating" as disclosed herein is meant to include therapeutic treatment, as well as prophylactic, or suppressive measures for a disease or disorder. Thus, for example, successful administration of an immunoglobulin prior to onset of the disease results in treatment of the disease. As another example, successful administration of an optimized immunoglobulin after clinical manifestation of the disease to combat the symptoms of the disease comprises treatment of the disease. "Treatment" and "treating" also encompasses administration of an optimized immunoglobulin after the appearance of the disease in order to eradicate the disease. Successful administration of an agent after onset and after clinical symptoms have developed, with possible abatement of clinical symptoms and perhaps amelioration of the disease, comprises treatment of the disease. Those "in need of treatment" include mammals already having the disease or disorder, as well as those prone to having the disease or disorder, including those in which the disease or disorder is to be prevented.

[0137] Pharmaceutical compositions are contemplated wherein an antibody of the invention and one or more therapeutically active agents are formulated. The antibodies may find use in a composition that is monoclonal or polyclonal. Formulations are prepared for storage by mixing the protein having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed.,1980), in the form of lyophilized formulations or aqueous solutions. The formulations to be used for *in vivo* administration are preferably sterile. The antibodies disclosed herein may also be formulated as immunoliposomes, or entrapped in microcapsules. The concentration of the protein of the present invention in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the protein is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically

effective dose of the protein of the present invention may be administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.01 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10mg/kg being preferred. Administration of the pharmaceutical composition comprising an antibody of the invention, preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, intraotically, transdermally, topically, intraperitoneally, intramuscularly, intrapulmonary, inhalably, vaginally, parenterally, rectally, or intraocularly. As is known in the art, the pharmaceutical composition may be formulated accordingly depending upon the manner of introduction.

EXAMPLES

[0138] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation.

[0139] For reference to immunoglobulin variable regions, positions are numbered according to the Kabat numbering scheme. For reference to immunoglobulin constant regions, positions are numbered according to the EU index as in Kabat (Kabat et al., 1991, *Sequences of Proteins of Immunological Interest*, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda).

Example 1. Engineering of a human equivalent anti-CD25 monoclonal antibody

[0140] An outline of the process of engineering a human equivalent anti-CD25 mAb from a murine anti-CD25 Fv is shown in Figure 1. The murine anti-CD25 mAb anti-TAC (Uchiyama, et al., 1981, *J Immunol* 126:1393-1397) was chosen as a starting point for engineering of a high affinity human equivalent anti-CD25 mAb. This mAb is the precursor of daclizumab, a humanized and marketed anti-CD25 mAb used for prevention of rejection in organ transplantation. Even though this mAb was humanized by CDR-grafting (Queen, et al., 1989, *Proc Natl Acad Sci U S A* 86:10029-10033), approximately 14% of adults and 34% of pediatric patients receiving this drug develop a low-level immune response (Roche, 2005, *Zenapax prescribing information*), thus engineering a human equivalent mAb from the murine anti-TAC variable region seemed like an excellent test case for our methodology. The murine anti-TAC Fv was engineered into a "framework optimized" anti-CD25 mAb (anti-CD25 H1L1) by reducing the immunogenicity of the variable region using a method described in USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 6, 2004. This method utilizes the homology present in human germline sequences and essentially makes murine to human mutations in order to increase the human string content of the Fv. Positions that are not within

or proximal to the CDRs and V_H/V_L interface are optimized in this step, and the relative humanness of the resulting Fv is comparable to mAbs humanized using CDR-grafting and other humanization techniques. Framework optimized heavy chain H1 and light chain L1 were constructed by gene synthesis, and IgG1 format antibodies were expressed transiently in 293E cells, purified by Protein A chromatography, and evaluated by SDS-PAGE and SEC. Antigen affinity of anti-CD25 H1L1 was compared to that of chimeric anti-CD25 H0L0 by Biacore.

[0141] For kinetic analysis of anti-CD25 antibodies, Protein A was coupled to an activated CM5 biosensor chip and 10-25 nM of anti-CD25 antibody injected at a flow rate of 5-10 µL/min for 1 min. Binding was measured by injection of two-fold serial dilutions of CD25 (0 nM to 50 nM; R&D systems) in buffer at 25 °C with a flow rate of 25-30 µL/min for 2 min followed by a dissociation phase of 4-5 min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (K_d) was calculated as the ratio of k_{off} / k_{on}. K_d values ranged from 0.3 - 1.1 nM for anti-CD25 H1L1 and 0.09 - 0.3 nM for anti-CD25 H0L0, and data from four separate Biacore runs consistently showed that anti-CD25 H1L1 has ~3.5-fold reduced affinity compared to anti-CD25 H0L0 even though absolute K_d values varied somewhat between the separate runs.

[0142] The framework optimized anti-CD25 heavy and light chains (H1 and L1, respectively) were aligned with the human germline V-and J-segments and the germlines were ranked based on the number of mutations away from the framework optimized sequence, the conservativeness of each mutation, and the proximity of each mutation to CDRs (Figure 2). For V_H, the five highest ranking human germlines were IGHV1-2*02, IGHV1-46*01, IGHV1-3*01, IGHV1-8*01, and IGHV1-18*01, respectively. These germlines had 11, 13, 14, 17, and 18 differences from anti-CD25 H1, respectively. For V_L, the five highest ranking human germlines were IGKV3-11*01, IGKV3D-11*01, IGKV3D-7*01, IGKV3-15*01, and IGKV3-20*01, with 19, 22, 23, 26, and 26 differences from anti-CD25 L1, respectively.

[0143] The highest ranking V_H germline IGHV1-2*02 and V_L germline IGKV3-11*01 were chosen as the basis for further engineering. Single variants were constructed in the anti-CD25 H1L1 background and expressed, purified, and assayed as mentioned above in order to assess the impact of the 11 differences between anti-CD25 H1 and IGHV1-2*02, and the 19 differences between anti-CD25 L1 and IGKV3-11*01. The single variants constructed are shown in Figure 3 along with binding data and humanness scores (number of human 9-mers and number of identities (IDs) to the closest matching human germlines) for each variant. Variants that had K_d values no worse than 1.5-fold weaker from that of anti-CD25 H1L1 were selected to be combined in the next round of engineering. Surprisingly, the majority of variants had increased or retained affinity. For V_H, ten out of eleven variants had neutral or higher affinity, with the only exception being R33Y in CDR1 which showed a decreased affinity of 5.6-fold. Three V_H variants had a more than 2-fold increase in affinity: I69M (2.4-

fold), Y56G (2.6-fold), and Y50W (6.2-fold). In V_L, twelve out of nineteen variants met our cutoff with H34A, T50D, T51A, H89Q, T93N, Y94W, and an insertion of serine at position 31 showing substantially reduced binding. No V_L variants with substantially increased affinity were identified.

[0144] In the combination step, the ten heavy chain variants that met our affinity cutoff were explored in various combinations paired with the L1 light chain, and the twelve light chain variants in various combinations were paired with the H1 heavy chain. Since we were unsure how additive beneficial mutations would be, and since the number of possible combination variants is large (1024 for V_H and 4096 for V_L), we developed a computational approach (Combination Design Automation or CDA™ technology) to the design of the combination variant library whereby humanness and diversity were balanced based on the number of variants desired (Figure 4). We chose to construct a library of 48 V_H variants paired with L1 and 48 V_L variants paired with H1. Figure 4 shows how CDA™ technology was used to design the library of 48 V_H variants (shown as red circles) from all possible 1024 variants (grey circles). As can be seen from Figure 4, the 48 variants with the best balance of humanness, number of substitutions, and mutational diversity were chosen for construction. All 96 total V_H and V_L combination variants were expressed, purified, and assayed for CD25 binding and results for four selected V_H and V_L combinations are shown in Figure 5. These four V_H combination variants all had substantially tighter binding than the starting template anti-CD25 H1L1: anti-CD25 H1.12L1 - 8.0-fold tighter; anti-CD25 H1.14L1 - 2.4-fold tighter; anti-CD25 H1.22L1 - 4.9-fold tighter; and anti-CD25 H1.23L1 - 4.4-fold tighter. The single mutations in V_H combined remarkably well and the variant that contained all 10 single mutations (anti-CD25 H1.12L1) had the highest affinity, demonstrating a high degree of additivity. For the V_L combinations, all four of the shown variants had binding affinities within 2-fold of anti-CD25 H1L1.

[0145] The four final V_H and V_L chains were paired with one another to create a library of sixteen human equivalent V_H/V_L combination variants. Binding results and humanness scores are shown in Figure 5. Compared to anti-CD25 H1L1, binding affinity ranged from 1.8-fold tighter in the case of variant anti-CD25 H1.14L1.20 to 5.9-fold tighter in the case of anti-CD25 H1.12L1.48. Antigen affinity was also comparable to anti-CD25 H0L0, with affinity differences ranging from 1.9-fold weaker to 1.7-fold tighter. Variant anti-CD25 H1.12L1.20 had the highest level of humanness and was ~1.5-fold tighter binding than anti-CD25 H0L0. Remarkably, this variant has twenty-two mutations compared to framework optimized anti-CD25 H1L1, twelve of which are in the Kabat defined CDRs. Biacore binding curves (25 nM CD25) for anti-CD25 H1.12L1.20 and its individual human equivalent chains paired with H1 or L1 are shown in Figure 6, along with anti-CD25 H0L0 and daclizumab. Also shown in Figure 6 is a plot of affinity versus number of identities to the closest human germline for anti-CD25 H0L0 (murine Fv), anti-CD25 H1L1 (framework optimized), daclizumab, the sixteen engineered human equivalent VH/VL pairs, and anti-CD25 H1.12L1.20, demonstrating that we have progressively engineered the anti-CD25 Fv to be more human equivalent while

simultaneously preserving antigen affinity. Figure 7 shows an amino acid sequence alignment for anti-CD25 H0L0, anti-CD25 H1L1, the closest human germlines used for engineering, and anti-CD25 H1.12L1.20, with differences between the various sequence pairs highlighted.

[0146] To demonstrate that our engineered human equivalent anti-CD25 mAbs had potent activity in another assay format, we evaluated two of our high affinity variants in a CD25 receptor blocking assay (Figure 8). CD25 (R&D systems cat. #223-2A/CF) was coupled to a CM5 chip using standard coupling methods. Antibodies (chimeric anti-CD25 H0L0, framework optimized anti-CD25 H1L1, anti-CD25 H1.12L1.20, anti-CD25 H1.23L1.43, daclizumab, and an isotype control mAb) were serially diluted in half-log increments starting from 1000 nM to 0.1 nM and injected to block CD25 on the chip surface at 10 µL/min for 1 min, followed by injection of rh-IL2 (R&D Systems cat. #202-IL-010/CF) at 100 nM at the same speed for 1 min. To account for any drift resulting from the dissociation of the blocking antibody, IL-2 injection was preceded by injection of buffer alone so that the drift could be subtracted. The chip was regenerated after each cycle by injection of glycine buffer @ pH 1.5 for 30 sec at 10 µL/min. IL-2 binding was calculated from final relative RU values at the end of IL-2 injection. Curves were fit using a four parameter model in Prism 4.03. As can be seen from Figure 9, both engineered human equivalent anti-CD25 mAbs showed a high degree of blocking IL-2 binding to CD25. From EC_{50} values, relative potency of the mAbs was anti-CD25 H1.12L1.20, anti-CD25 H1.23L1.43 > chimeric anti-CD25 H0L0, framework optimized anti-CD25 H1L1 > daclizumab.

Example 2. Engineering of a human equivalent anti-VEGF monoclonal antibody

[0147] An outline of the process of engineering a human equivalent anti-VEGF mAb from a murine anti-VEGF Fv is shown in Figure 1. The murine anti-VEGF mAb A4.6.1(Kim, et al., 1992, *Growth Factors* 7:53-64) was chosen as a starting point for engineering of a high affinity human equivalent anti-VEGF mAb. This mAb is the precursor of bevacizumab, a humanized and marketed anti-VEGF mAb used in the treatment of various types of cancer including colorectal, lung, and breast. The murine A4.6.1 Fv (anti-VEGF H0L0) was engineered into a "framework optimized" anti-VEGF mAb (anti-VEGF H1L1) by reducing the immunogenicity of the variable region using a method described in USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 6, 2004. This method utilizes the homology present in human germline sequences and essentially makes murine to human mutations in order to increase the human string content of the Fv. Positions that are not within or proximal to the CDRs and V_H/V_L interface are optimized in this step, and the relative humanness of the resulting Fv is comparable to mAbs humanized using CDR-grafting and other humanization techniques. Framework optimized heavy chain H1 and light chain L1 were constructed by gene synthesis, and Fab format antibodies were expressed transiently in 293E cells, purified by Ni-NTA chromatography, and evaluated by SDS-PAGE and SEC.

[0148] For kinetic analysis of anti-VEGF antibodies, VEGF was coupled to an activated CM5

biosensor chip using standard NHS-EDC chemistry by injecting 200 nM VEGF at a flow rate of 2 µL/min for 10 min. Binding was measured by injection of two-fold serial dilutions of anti-VEGF Fabs (3.13 nM to 200 nM) in buffer at 25 °C with a flow rate of 30 µL/min for 2 min followed by a dissociation phase of 4 min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (K_d) was calculated as the ratio of k_{off} / k_{on}.

[0149] The framework optimized anti-VEGF heavy and light chains (H1 and L1, respectively) were aligned with the human germline V- and J-segments and the germlines were ranked based on the number of mutations away from the framework optimized sequence, the conservativeness of each mutation, and the proximity of each mutation to CDRs (Figure 9). For V_H, the five highest ranking human germlines were IGHV7-4-1*02, IGHV1-2*02, IGHV1-3*01, IGHV1-46*01, and IGHV1-8*01, respectively. Germline V-segment IGHV7-4-1*02 was clearly the closest by being only eight mutations away from anti-VEGF H1, while the other four closest germlines were 30-31 mutations away. For V_L, the five highest ranking human germlines were IGKV1-33*01, IGKV1D-33*01, IGKV1-16*01, IGKV1-39*01, and IGKV1D-39*01, all with fourteen differences from anti-VEGF L1.

[0150] The highest ranking V_H germline IGHV7-4-1*02 and V_L germline IGKV1-33*01 were chosen as the basis for further engineering. Single variants were constructed in the anti-VEGF H1L1 background and expressed, purified, and assayed as mentioned above in order to assess the impact of the eight differences between anti-VEGF H1 and IGHV7-4-1*02, and the fourteen differences between anti-VEGF L1 and IGKV1-33*01. Variants that had K_d values similar to that of anti-VEGF H1L1 were selected to be combined in the next round of engineering.

[0151] In the combination step, the heavy chain variants that met our affinity cutoff were explored in various combinations paired with the L1 light chain, and the light chain variants in various combinations were paired with the H1 heavy chain. The heavy and light chains with the best combination of antigen affinity and humanness were subsequently combined into human equivalent V_H/V_L pairs. All variants were expressed, purified, and assayed for VEGF binding and results for two selected V_H and V_L combinations along with anti-VEGF H0L0 and bevacizumab are shown in Figures 10 and 11. In Figure 10, the top panel shows binding data for 100 nM of anti-VEGF H0L0 (murine Fv), anti-VEGF H1.33L1.51, anti-VEGF H1.33L1.55 and bevacizumab and the bottom panel shows relative VEGF affinity expressed as -Log[K_d] for the same four variants. Figure 11 shows the human germline mutations included in the final two V_H/V_L combination variants as well as k_{on}, k_{off}, K_d, fold change in K_d compared to anti-VEGF H0L0, number of human 9-mers, and number of identities to the closest matching human germline V- and J-segments for the variants. As demonstrated by the data in figures 10 and 11, the engineered human equivalent anti-VEGF mAbs have antigen affinities comparable to the marketed anti-VEGF mAb bevacizumab and within 3-fold of that of the

chimeric antibody. Anti-VEGF H1.33L1.55 has thirteen more mutations compared to the framework optimized anti-VEGF H1L1, with nine of them located in the Kabat defined CDRs.

Example 3. Engineering of a human equivalent anti-TNFα monoclonal antibody

[0152] An outline of the process of engineering a human equivalent anti-TNFα mAb from a murine anti-TNFα Fv is shown in Figure 1. The murine anti-TNF mAb A2 (Knight, et al., 1993, *Mol Immunol* 30:1443-1453) was chosen as a starting point for engineering of a high affinity human equivalent anti-TNFα mAb. This mAb is the precursor of infliximab, a chimeric, marketed anti-TNFα mAb used in the treatment of rheumatoid arthritis. The murine A2 Fv (anti-TNFα H0L0) was engineered into a "framework optimized" anti-TNFα mAb (anti-TNFα H1L1) by reducing the immunogenicity of the variable region using a method described in USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 6, 2004. This method utilizes the homology present in human germline sequences and essentially makes murine to human mutations in order to increase the human string content of the Fv. Positions that are not within or proximal to the CDRs and V_H/V_L interface are optimized in this step, and the relative humanness of the resulting Fv is comparable to mAbs humanized using CDR-grafting and other humanization techniques. Framework optimized heavy chain H1 and light chain L1 were constructed by gene synthesis, and Fab format antibodies were expressed transiently in 293E cells, purified by Ni-NTA chromatography, and evaluated by SDS-PAGE and SEC.

[0153] For kinetic analysis of anti-TNFα antibodies, TNFα was coupled to an activated CM5 biosensor chip using standard NHS-EDC chemistry by injecting 200 nM TNFα at a flow rate of 2 µL/min for 10 min. Binding was measured by injection of two-fold serial dilutions of anti-TNF Fabs (6.25 nM to 50 nM) in buffer at 25 °C with a flow rate of 30 µL/min for 2 min followed by a dissociation phase of 3 min. For subsequent cycles, the chip was regenerated using pH4.0 acetate buffer. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (K_d) was calculated as the ratio of k_{off} / k_{on}. Data shown in Table 1 are the average of two independent runs with ± standard deviation shown.

[0154] The framework optimized anti-TNFα heavy and light chains (H1 and L1, respectively) were aligned with the human germline V- and J-segments and the germlines were ranked based on the number of mutations away from the framework optimized sequence, the conservativeness of each mutation, and the proximity of each mutation to CDRs. For V_H, the three highest ranking human germlines were IGHV3-73*01, IGHV3-72*01, and IGHV3-15*01, respectively. Germline V-segment IGHV3-73*01 was the closest with seventeen mutations away from anti-TNFα H1, while the other two closest germlines were 21-22 mutations away. For V_L, the three highest ranking human germlines were IGKV6-21*01, IGKV6D-21*01, and IGKV6D-41*01, with 11-24 differences from anti-TNFα L1.

[0155] The highest ranking V_H germline IGHV3-73*01 and V_L germline IGKV6-21*01 were

chosen as the basis for further engineering. Single variants were constructed in the anti-TNFα H1L1 background and expressed, purified, and assayed as mentioned above in order to assess the impact of the seventeen differences between anti-TNFα H1 and IGHV3-73*01, and the eleven differences between anti-TNFα L1 and IGKV6-21*01. Variants that had K_d values similar to that of anti-TNFα H1L1 were selected to be combined in the next round of engineering.

[0156] In the combination step, the heavy chain variants that met our affinity cutoff were explored in various combinations paired with the L1 light chain, and the light chain variants in various combinations were paired with the H1 heavy chain. The heavy and light chains with the best combination of antigen affinity and humanness were subsequently combined into human equivalent V_H/V_L pairs. All variants were expressed, purified, and assayed for TNFα binding and results for four selected V_H and V_L combinations along with anti-TNFα H0L0 (A2; infliximab) and framework optimized anti-TNFα H1L1 are shown in Table 1. As demonstrated by the data in Table 1, the engineered human equivalent anti-TNFα mAbs have antigen affinities comparable to the marketed anti-TNF mAb infliximab and within 3-fold of that of the chimeric antibody. Anti-TNFα H1.103L1.33 has thirteen more mutations compared to the framework optimized anti-TNFα H1L1. Additional statistics for engineered mAbs are shown in Figure 12.

Table 1. Binding measurement of anti-TNFα variants.

Variant	k_a (M/s x 10^5)	k_d (1/s x 10^{-4})	K_d (nM)
Infliximab	5.47 ± 1.88	13.10 ± 0.41	2.4 ± 0.1
Anti-TNFα H1L1	6.05 ± 1.72	11.60 ± 0.40	1.9 ± 0.1
Anti-TNFα H1.101L1.33	2.23 ± 0.41	8.74 ± 0.30	3.9 ± 0.7
Anti-TNFα H1.45L1.33	4.89 ± 1.21	8.70 ± 0.30	1.8 ± 0.2
Anti-TNFα H1.67L1.33	2.00 ± 0.42	7.76 ± 0.35	3.8 ± 0.9
Anti-TNFα H1.103L1.33	2.92 ± 0.71	9.20 ± 0.30	3.1 ± 0.2

Example 4. Comparison of engineered human equivalent mAbs to human equivalent mAbs isolated from transgenic mice and human antibody libraries

[0157] To show that human equivalent mAbs engineered from murine variable regions are comparable in humanness to human equivalent mAbs isolated from transgenic mice or human phage display libraries, we obtained variable region sequences for currently marketed mAbs and several human equivalent mAbs in clinical development from the literature and analyzed their level of humanness as defined by the number of identities to the

closest human germline and the number of human 9-mers. A plot of % identity to the closest human germline V and J-segments and % human 9-mers for approved murine, chimeric, and humanized mAbs as well as the two marketed human equivalent mAbs panitumumab and adalimumab is shown in Figure 13. Also shown are five human equivalent mAbs in clinical development as well as the human equivalent mAbs engineered from murine variable regions, anti-CD25 H1.12L1.20, anti-VEGF H1.33L1.55, and anti-TNF H1.103L1.33 (XmAb human equivalent mAbs). As can be seen from the plot, the sequences of human equivalent mAbs are more similar to those of human germlines than humanized and chimeric mAbs, thus human equivalent mAbs are expected to have less overall risk of immunogenicity. Also shown by the plot is that anti-CD25 H1.12L1.20, anti-VEGF H1.33L1.55, and anti-TNFα H1.103L1.33 have levels of sequence humanness similar to human equivalent mAbs isolated from transgenic mice or human antibody libraries. Figure 14 shows all antibodies included in the analysis and lists antigen, type of Fv (murine, humanized, or human equivalent), clinical status, the number and percent of identities to the closest human germline V- and J-segments, the number and percent of human 9-mers, the Fv length, and the V_H and V_L V- and J-segment germlines that had the highest identities and were used in the analysis.

CLAIMS

1. A method of producing an antibody which specifically binds to an antigen, the method comprising:

a) providing a parent antibody variable region amino acid sequence comprising less than 85% identity in the V- and J-segments to any human germline V- and J-segment;

b) comparing said parent antibody variable region amino acid sequence to a collection of human germline V- and J-segment amino acid sequences;

c) scoring said collection based on the identity of each germline sequence to said parent antibody variable region amino acid sequence;

d) picking the germline sequence with the highest identity as the germline parent sequence;

e) constructing a library of variant immunoglobulin proteins each comprising said parent antibody variable region amino acid sequence with at least one amino acid substitution at a position in which the parent sequence differs from said germline parent sequence, wherein the substitution is to the same amino acid found at the position in the germline parent sequence;

f) measuring the antigen binding of each of said library variant immunoglobulin proteins.

2. A method according to claim 1 further comprising selecting the variant immunoglobulins which have an affinity constant that is no less than two fold of the parent antibody variable region amino acid sequence.

3. A method according to claim 1 or 2 further comprising combining the amino acid substitutions of different variant immunoglobulins to form human equivalent antibodies, and measuring the antigen binding of each of said human equivalent antibodies.

4. A method according to claim 3 wherein at least one of said human equivalent antibodies has an antibody variable region amino acid sequence that is greater than 85% identity in its V- and J- segments to a human germline V- and J- segment.

5. A method according to claim 4 wherein the identity of the variable region amino acid sequence of the heavy chain is at least 90%.

6. A method according to claim 4 wherein the identity of the variable region amino acid sequence of the heavy chain is at least 95%.

7. A method according to claim 4 wherein the identity of the variable region amino acid sequence of the light chain is at least 90%.

8. A method according to claim 4 wherein the identity of the variable region amino acid sequence of the light chain is at least 95%.

9. An antibody that binds CD25, said antibody comprising a heavy chain and/or a light chain, said heavy chain having a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-106, a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 107-109 and a CDR3 comprising the amino acid sequence of SEQ ID NOs: 110; and said light chain having a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 118-121, CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 122-123, and a CDR3 comprising the amino acid sequence of SEQ ID NOs: 124.

10. An antibody according to claim 1, wherein said antibody comprises a variable heavy chain sequence selected from the group consisting of SEQ ID NOS: 1-17, and/or a variable light chain sequence selected from the group consisting of SEQ ID NOS: 43-67.

11. An antibody that binds VEGF, said antibody comprising a heavy chain and/or a light chain, said heavy chain having a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 111-113, a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 114-116 and a CDR3 comprising the amino acid sequence of SEQ ID NOs: 117; and said light chain having a CDR1 comprising the amino acid sequence of SEQ ID NOs: 125, CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 126-131, and a CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 132-133.

12. An antibody according to claim 3, wherein said antibody comprises a variable heavy chain sequence selected from the group consisting of SEQ ID NOS: 18-42, and/or a variable light chain sequence selected from the group consisting of SEQ ID NOS: 68-104.

Figure 1.

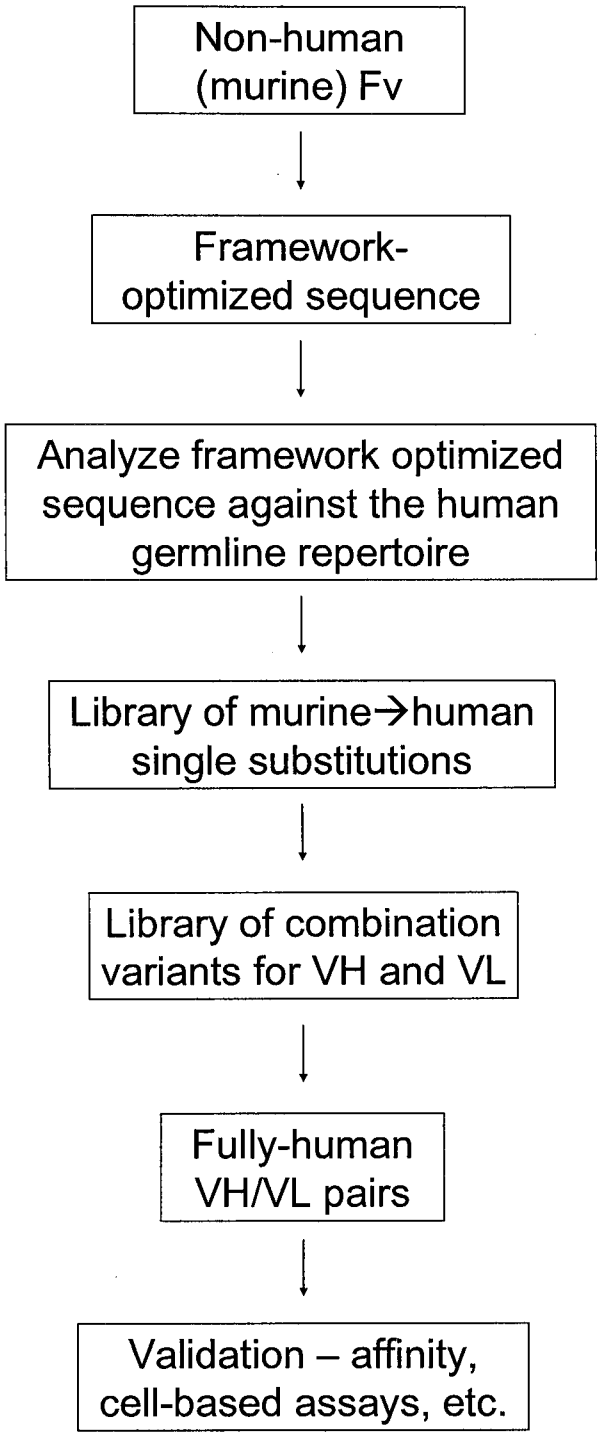

Non-human (murine) Fv
Framework-optimized sequence
Analyze framework optimized sequence against the human germline repertoire
Library of murine→human single substitutions
Library of combination variants for VH and VL
Fully-human VH/VL pairs
Validation – affinity, cell-based assays, etc.

Figure 2.

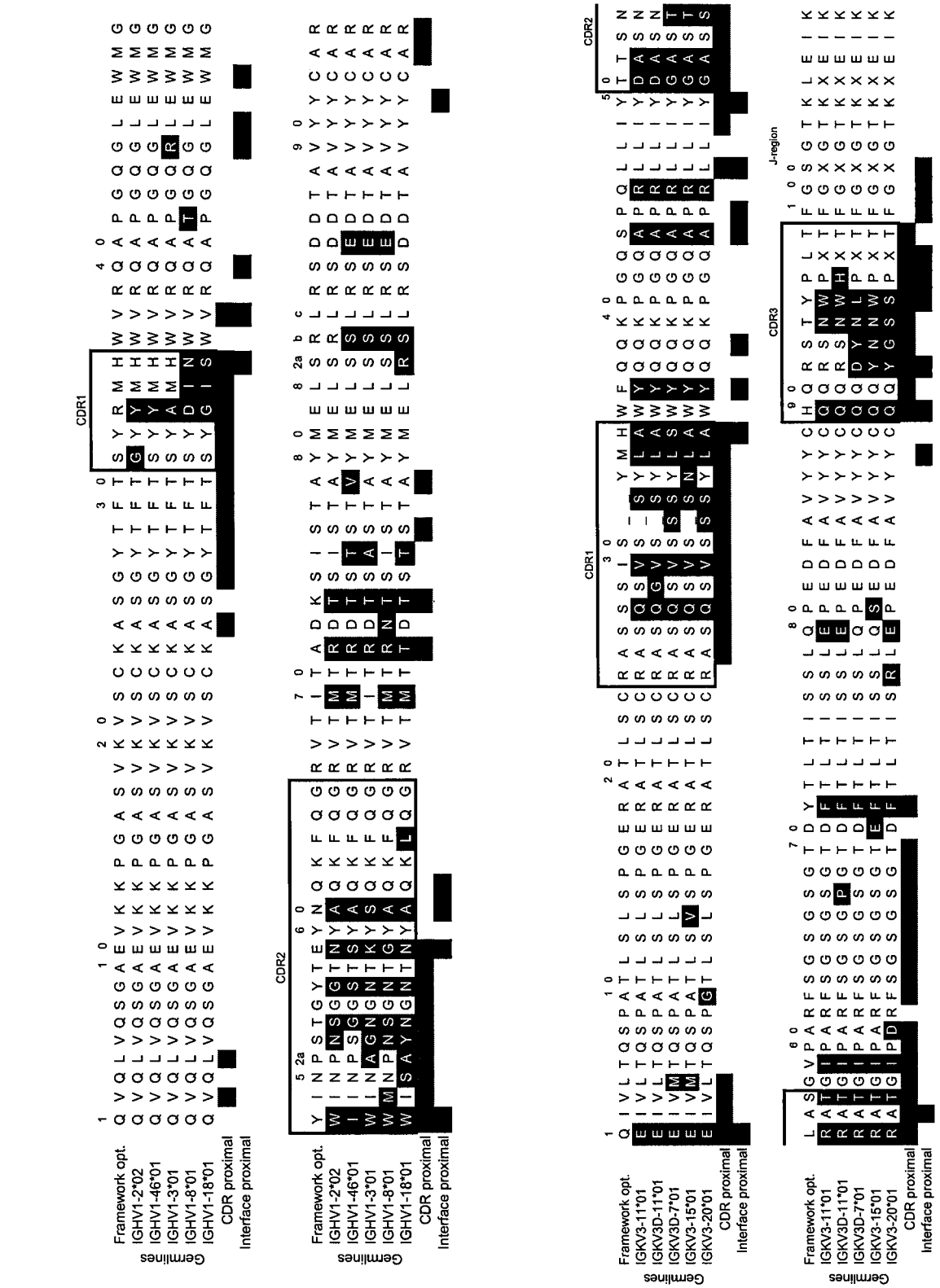

VH
VK
CDR1
CDR2
CDR3
J-region
Framework opt.
IGHV1-2*02
IGHV1-46*01
IGHV1-3*01
IGHV1-8*01
IGHV1-18*01
CDR proximal
Interface proximal
Germlines
IGKV3-11*01
IGKV3D-11*01
IGKV3D-7*01
IGKV3-15*01
IGKV3-20*01

Figure 3.

Sequences of CDR and interface positions, binding data, and humanness scores of anti-CD25 variants

	Heavy chain											Light chain																									
	CDR1		CDR2									CDR1								CDR2							CDR3											
Variant	31	33	50	53	54	56	58	60	69	71	73	1	27	29	31	33	34	36	43	45	50	51	54	56	58	71	79	89	93	94	k_{on} ($10^6 M^{-1}s^{-1}$)	k_{off} ($10^{-4}s^{-1}$)	K_d (nM)	Fold change in K_d vs. H1L1	Human 9-mers	IDs
anti-CD25 H0L0	S	R	Y	S	T	Y	E	N	L	A	K	Q	S	I	-	R	H	F	S	K	T	T	L	S	V	Y	E	H	T	Y	1.6	1.3	0.09		12	155
anti-CD25 H1L1	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.5	5.0	0.32		103	187
daclizumab	S	R	Y	S	T	Y	E	N	I	A	E	D	S	I	-	M	H	Y	A	K	T	T	L	S	V	F	Q	H	T	Y	1.6	2.3	0.15		94	184
IGHV1-2*02 / IGKV3-11*01	G	Y	W	N	S	G	N	A	M	R	T	E	Q	V	S	L	A	Y	A	R	D	A	R	T	I	F	E	Q	N	W						
VH Single variants																																					
anti-CD25 H1.1L1	S	R	Y	S	T	Y	E	N	I	R	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.9	3.1	0.16	2.0	102	188
anti-CD25 H1.2L1	S	R	Y	S	T	Y	N	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.4	4.7	0.35	-1.1	103	188
anti-CD25 H1.3L1	S	R	Y	S	T	Y	E	N	M	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	2.0	2.6	0.13	2.4	101	188
anti-CD25 H1.4L1	S	R	Y	S	T	Y	E	N	I	A	T	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	2.0	6.0	0.30	1.1	106	188
anti-CD25 H1.5L1	S	R	Y	S	T	Y	E	A	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.9	3.5	0.19	1.7	105	188
anti-CD25 H1.6L1	S	Y	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	0.80	14.3	1.79	-5.6	112	188
anti-CD25 H1.7L1	G	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.9	5.8	0.30	1.1	103	188
anti-CD25 H1.8L1	S	R	W	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	2.1	1.1	0.05	6.2	107	188
anti-CD25 H1.9L1	S	R	Y	S	T	G	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	2.2	2.7	0.12	2.6	103	188
anti-CD25 H1.10L1	S	R	Y	N	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.6	3.2	0.20	1.6	103	188
anti-CD25 H1.11L1	S	R	Y	S	S	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.5	4.7	0.32	1.0	103	188
VL Single variants																																					
anti-CD25 H1L1.1	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	Q	T	Y	1.6	16.7	1.06	-3.3	107	188
anti-CD25 H1L1.2	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	V	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.8	4.6	0.25	1.3	103	188
anti-CD25 H1L1.3	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	R	S	V	Y	Q	H	T	Y	1.7	4.8	0.28	1.1	103	188
anti-CD25 H1L1.4	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	L	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.8	5.8	0.33	-1.0	103	188
anti-CD25 H1L1.5	S	R	Y	S	T	Y	E	N	I	A	K	E	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.8	5.2	0.29	1.1	104	188
anti-CD25 H1L1.6	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	R	T	T	L	S	V	Y	Q	H	T	Y	1.8	5.7	0.32	1.0	98	188
anti-CD25 H1L1.7	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	E	H	T	Y	2.0	5.4	0.28	1.2	102	188
anti-CD25 H1L1.8	S	R	Y	S	T	Y	E	N	I	A	K	Q	Q	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.9	6.0	0.32	-1.0	105	188
anti-CD25 H1L1.9	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	A	Q	T	T	L	S	V	Y	Q	H	T	Y	1.9	5.8	0.31	1.0	98	188
anti-CD25 H1L1.10	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	T	V	Y	Q	H	T	Y	1.9	5.2	0.28	1.1	103	188
anti-CD25 H1L1.11	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	D	T	L	S	V	Y	Q	H	T	Y	0.94	153.0	16.20	-50.6	102	188
anti-CD25 H1L1.12	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	A	L	S	V	Y	Q	H	T	Y	1.7	92.7	5.42	-16.9	103	188
anti-CD25 H1L1.13	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	I	Y	Q	H	T	Y	1.7	6.0	0.35	-1.1	105	188
anti-CD25 H1L1.14	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	F	Q	H	T	Y	1.8	4.8	0.27	1.2	103	188
anti-CD25 H1L1.15	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	A	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.1	79.0	7.45	-23.3	103	188
anti-CD25 H1L1.16	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	Y	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.6	4.2	0.26	1.2	103	188
anti-CD25 H1L1.17	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	N	Y	1.7	8.4	0.50	-1.6	103	188
anti-CD25 H1L1.18	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	W	1.5	13.2	0.90	-2.8	103	188
anti-CD25 H1L1.19	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	S	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	0.66	80.5	12.20	-38.1	103	188

Positions in framework-optimized anti-CD25 H1L1 which differ from the closest identity human germline sequence were changed to the corresponding germline amino acid and binding to CD25 was measured with Biacore. Shaded residues indicate differences between each variant and anti-CD25 H1L1. The number of total human 9-mers and identities to the closest human germline for each VH and VL pair is also shown.

Figure 4.

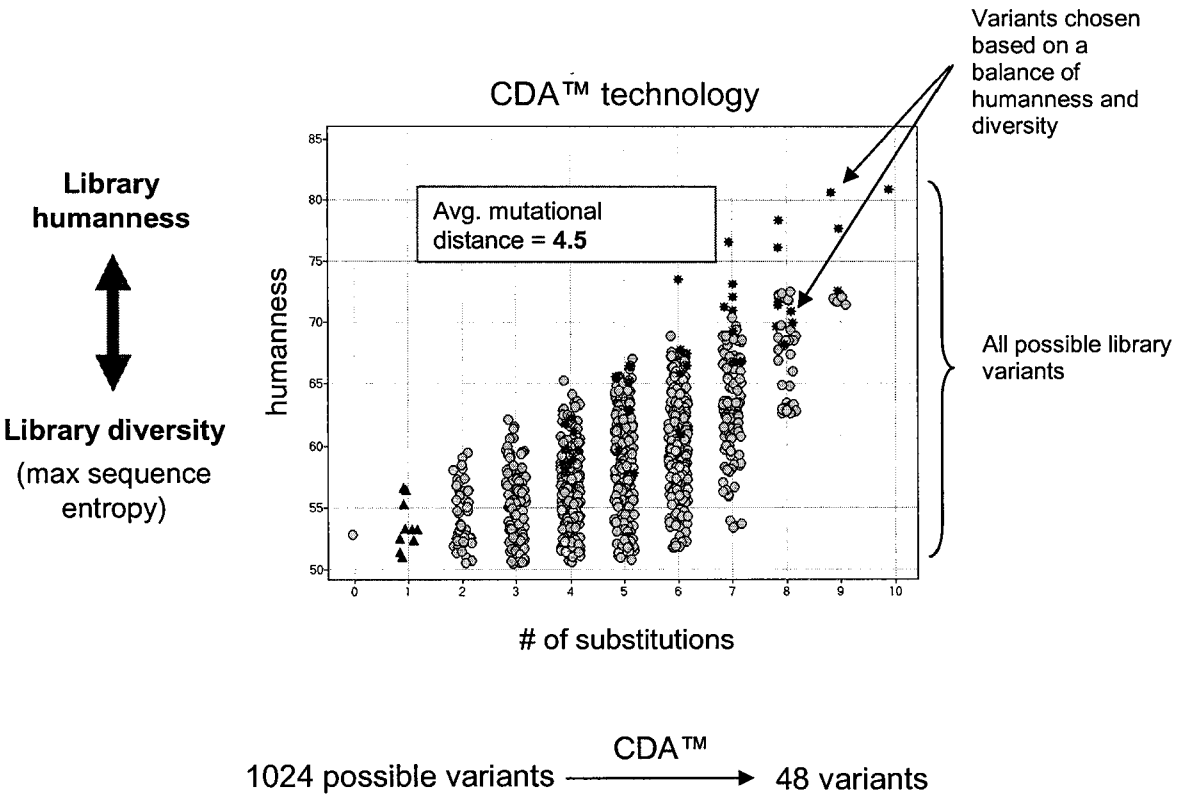

Library humanness
Library diversity
(max sequence entropy)
CDA™ technology
Variants chosen based on a balance of humanness and diversity
Avg. mutational distance = 4.5
All possible library variants
humanness
# of substitutions
1024 possible variants
CDA™
48 variants

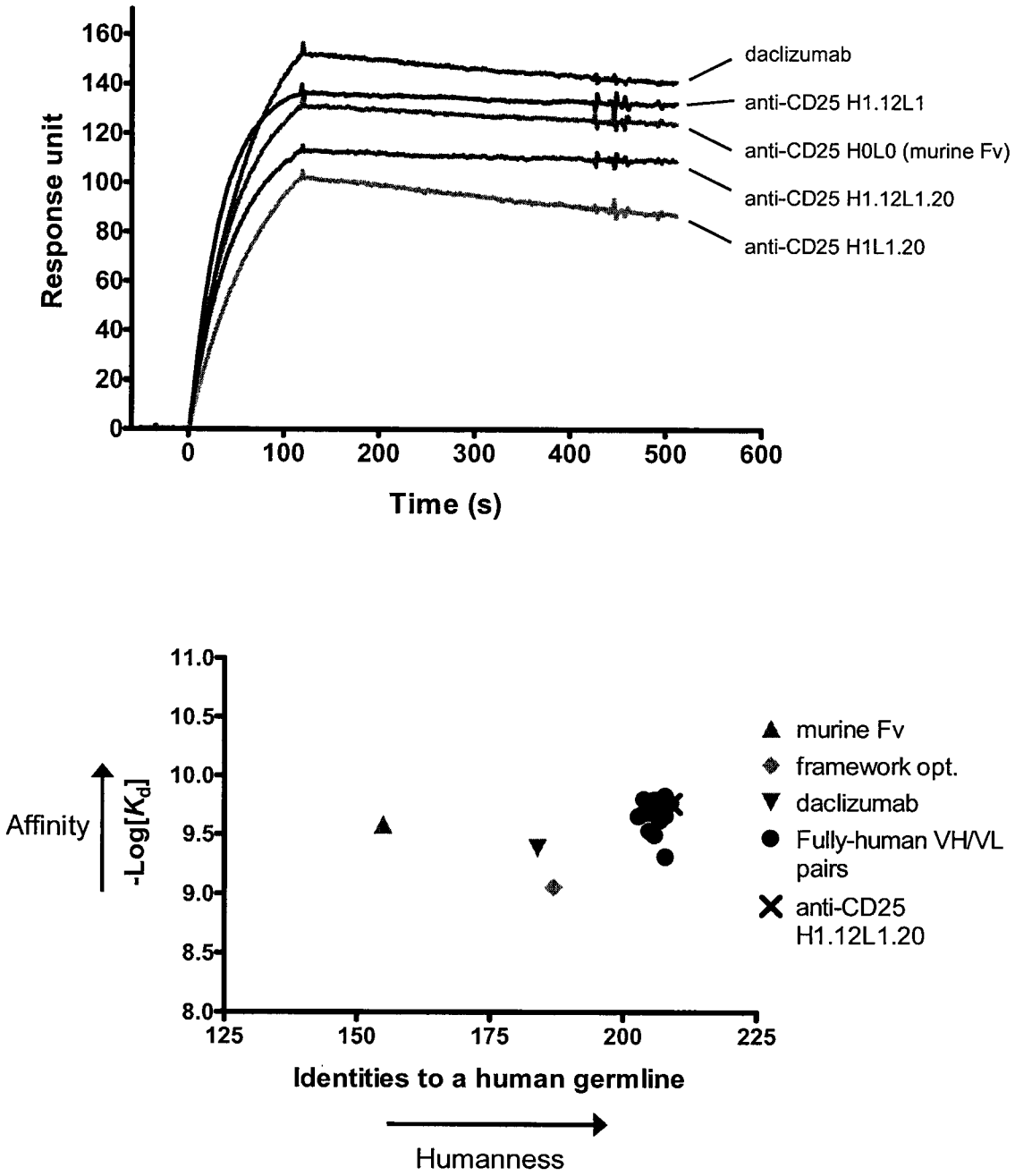
Figure 5.

Sequences of CDR and interface positions, binding data, and humanness scores of anti-CD25 combination variants

Variant	Heavy chain CDR1		Heavy chain CDR2									Light chain CDR1									Light chain CDR2							CDR3			k_{on} ($10^6 M^{-1}s^{-1}$)	k_{off} ($10^{-4} s^{-1}$)	K_d (nM)	Fold change in K_d vs. H0L0	Human 9-mers	IDs
	31	33	50	53	54	56	58	60	69	71	73	1	27	29	31	33	34	36	43	45	50	51	54	56	58	71	79	89	93	94						
anti-CD25 H0L0	S	R	Y	S	T	Y	E	N	L	A	K	Q	S	I	-	R	H	F	S	K	T	T	L	S	V	Y	E	H	T	Y	0.67	1.8	0.26		12	155
anti-CD25 H1L1	S	R	Y	S	T	Y	E	N	I	A	K	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	0.65	5.8	0.88	-3.4	103	187
daclizumab	S	R	Y	S	T	Y	E	N	I	A	E	D	S	I	-	M	H	Y	A	K	T	T	L	S	V	F	Q	H	T	Y	0.57	2.4	0.41		94	184
IGHV1-2*02 / IGKV3-11*01	G	Y	W	N	S	G	N	A	M	R	T	E	Q	V	S	L	A	Y	A	R	D	A	R	T	I	F	E	Q	N	W						
VH Combination variants																																				
anti-CD25 H1.12L1	G	R	W	N	S	G	N	A	M	R	T	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	1.1	1.2	0.11	2.3	131	197
anti-CD25 H1.14L1	G	R	Y	N	S	G	N	A	M	R	T	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	0.77	2.8	0.36	-1.4	122	196
anti-CD25 H1.22L1	S	R	W	N	S	G	N	A	I	R	T	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	0.98	1.7	0.18	1.5	128	195
anti-CD25 H1.23L1	S	R	W	N	S	G	N	A	M	R	T	Q	S	I	-	M	H	F	S	Q	T	T	L	S	V	Y	Q	H	T	Y	0.95	1.9	0.20	1.3	131	196
VL Combination variants																																				
anti-CD25 H1L1.20	S	R	Y	S	T	Y	E	N	I	A	K	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.43	4.8	1.10	-4.2	117	199
anti-CD25 H1L1.43	S	R	Y	S	T	Y	E	N	I	A	K	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	Y	Q	H	T	Y	0.41	5.3	1.30	-5.0	117	197
anti-CD25 H1L1.48	S	R	Y	S	T	Y	E	N	I	A	K	E	Q	V	-	M	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.50	3.1	0.63	-2.4	117	198
anti-CD25 H1L1.56	S	R	Y	S	T	Y	E	N	I	A	K	Q	Q	V	-	L	H	F	S	Q	T	T	R	T	I	F	E	H	T	Y	0.54	7.0	1.30	-5.0	114	195
VH/VL Combination variants																																				
anti-CD25 H1.12L1.20	G	R	W	N	S	G	N	A	M	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.75	1.3	0.17	1.5	145	209
anti-CD25 H1.12L1.43	G	R	W	N	S	G	N	A	M	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	Y	Q	H	T	Y	0.72	1.4	0.19	1.4	145	207
anti-CD25 H1.12L1.48	G	R	W	N	S	G	N	A	M	R	T	E	Q	V	-	M	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.80	1.2	0.15	1.7	145	208
anti-CD25 H1.12L1.56	G	R	W	N	S	G	N	A	M	R	T	Q	Q	V	-	L	H	F	S	Q	T	T	R	T	I	F	E	H	T	Y	0.85	1.8	0.21	1.2	142	205
anti-CD25 H1.14L1.20	G	R	Y	S	S	G	N	A	M	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.58	2.9	0.49	-1.9	136	208
anti-CD25 H1.14L1.43	G	R	Y	N	S	G	N	A	M	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	Y	Q	H	T	Y	0.67	2.2	0.32	-1.3	136	206
anti-CD25 H1.14L1.48	G	R	Y	N	S	G	N	A	M	R	T	E	Q	V	-	M	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.79	1.7	0.21	1.2	136	207
anti-CD25 H1.14L1.56	G	R	Y	N	S	G	N	A	M	R	T	Q	Q	V	-	L	H	F	S	Q	T	T	R	T	I	F	E	H	T	Y	1.0	1.7	0.16	1.6	133	204
anti-CD25 H1.22L1.20	S	R	W	N	S	G	N	A	I	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.72	1.8	0.24	1.1	142	207
anti-CD25 H1.22L1.43	S	R	W	N	S	G	N	A	I	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	Y	Q	H	T	Y	0.64	1.9	0.30	-1.1	142	205
anti-CD25 H1.22L1.48	S	R	W	N	S	G	N	A	I	R	T	E	Q	V	-	M	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.78	1.4	0.17	1.5	142	206
anti-CD25 H1.22L1.56	S	R	W	N	S	G	N	A	I	R	T	Q	Q	V	-	L	H	F	S	Q	T	T	R	T	I	F	E	H	T	Y	0.97	2.2	0.22	1.2	139	203
anti-CD25 H1.23L1.20	S	R	W	N	S	G	N	A	M	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.71	1.6	0.22	1.2	145	208
anti-CD25 H1.23L1.43	S	R	W	N	S	G	N	A	M	R	T	E	Q	V	-	L	H	Y	A	R	T	T	R	T	I	Y	Q	H	T	Y	0.73	1.2	0.16	1.6	145	206
anti-CD25 H1.23L1.48	S	R	W	N	S	G	N	A	M	R	T	E	Q	V	-	M	H	Y	A	R	T	T	R	T	I	F	E	H	T	Y	0.75	1.8	0.23	1.1	145	207
anti-CD25 H1.23L1.56	S	R	W	N	S	G	N	A	M	R	T	Q	Q	V	-	L	H	F	S	Q	T	T	R	T	I	F	E	H	T	Y	0.95	2.0	0.21	1.2	142	204

Binding data and humanness scores from the anti-CD25 single variants were used to design a library of combination variants that would maximize humanness scores and maintain antigen affinity. Binding to CD25 was measured with Biacore, and shaded residues indicate differences between each variant and anti-CD25 H1L1. The number of total human 9-mers and identities to the closest human germline for each VH and VL pair is also shown.

Figure 6.


Response unit
160
140
120
100
80
60
40
20
0
0
100
200
300
400
500
600
Time (s)
daclizumab
anti-CD25 H1.12L1
anti-CD25 H0L0 (murine Fv)
anti-CD25 H1.12L1.20
anti-CD25 H1L1.20
Affinity
-Log[K_d]
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10.5
10.0
9.5
9.0
8.5
8.0
125
150
175
200
225
Identities to a human germline
Humanness
murine Fv
framework opt.
daclizumab
Fully-human VH/VL pairs
anti-CD25 H1.12L1.20

Figure 7.

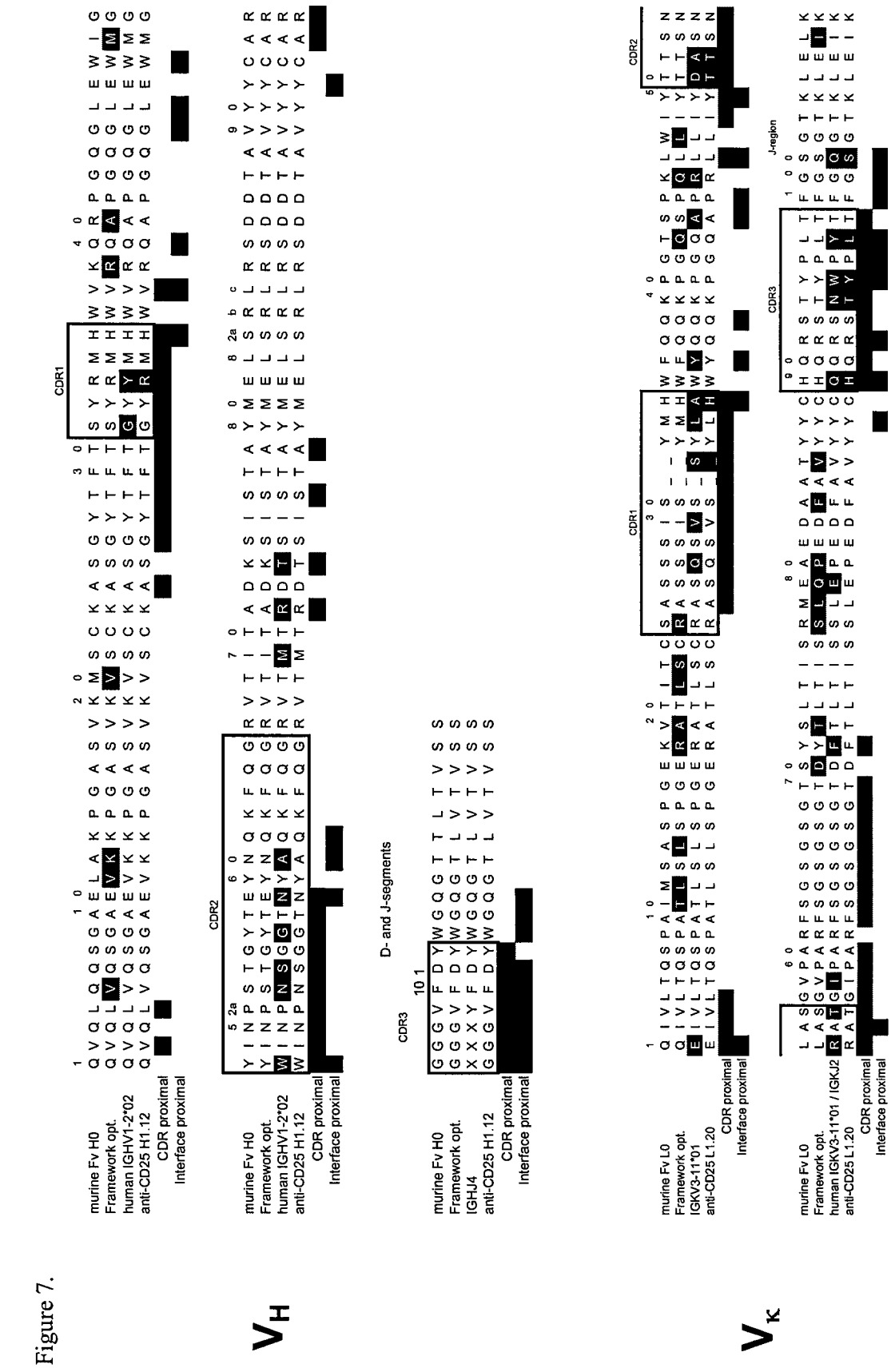

VH
CDR1
CDR2
D- and J-segments
CDR3
murine Fv H0
Framework opt.
human IGHV1-2*02
anti-CD25 H1.12
IGHJ4
CDR proximal
Interface proximal
VK
murine Fv L0
Framework opt.
IGKV3-11*01
human IGKV3-11*01 / IGKJ2
anti-CD25 L1.20
J-region

Figure 8.

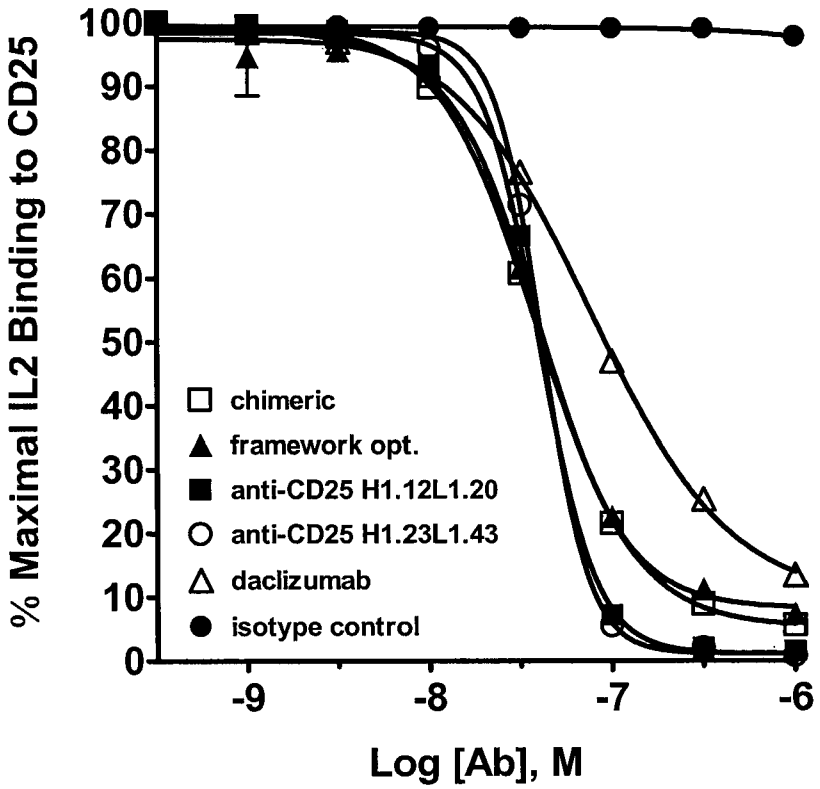
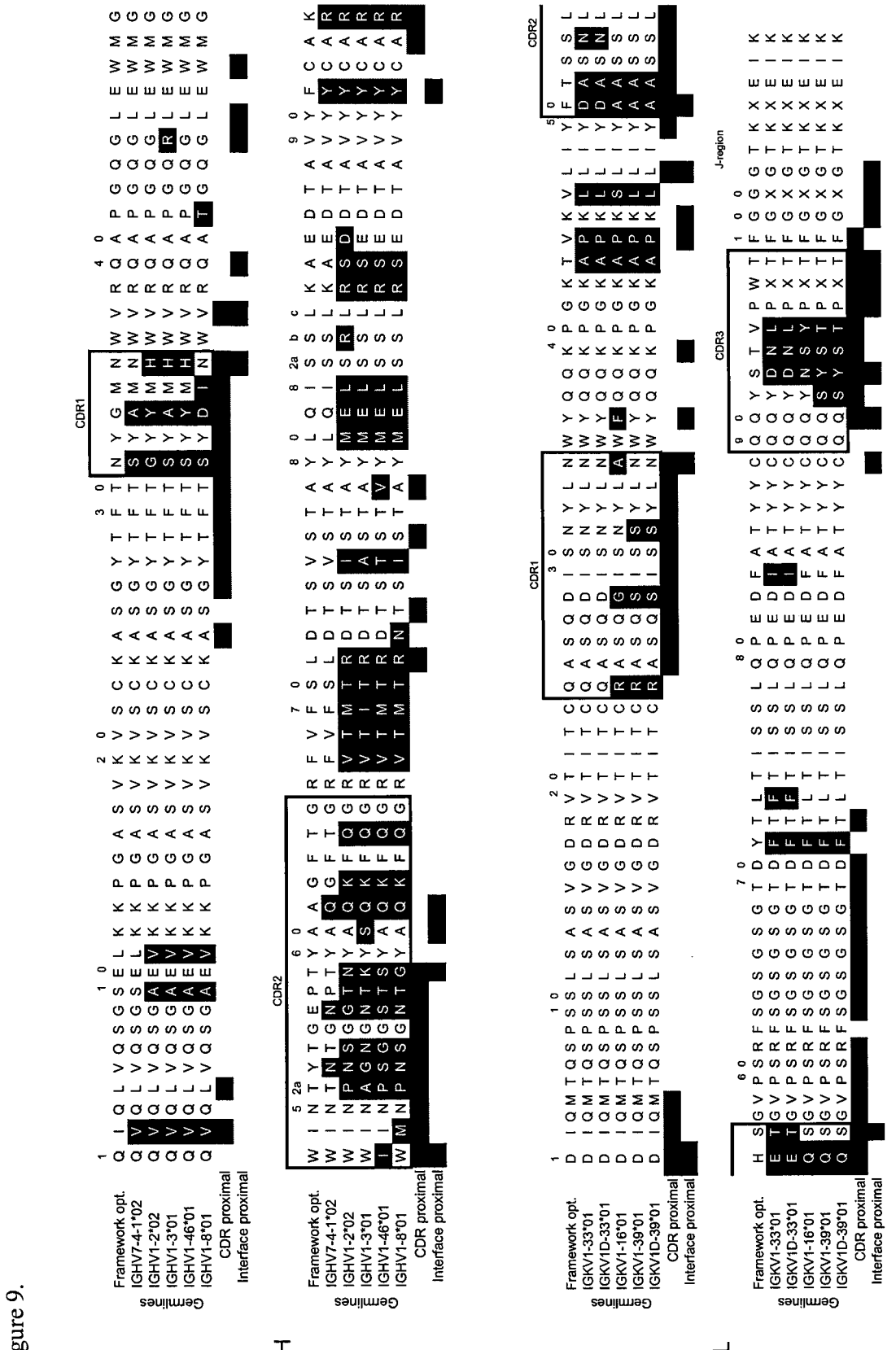

% Maximal IL2 Binding to CD25
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
Log [Ab], M
chimeric
framework opt.
anti-CD25 H1.12L1.20
anti-CD25 H1.23L1.43
daclizumab
isotype control

Figure 9.


VH
VL
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CDR2
CDR3
J-region
Framework opt.
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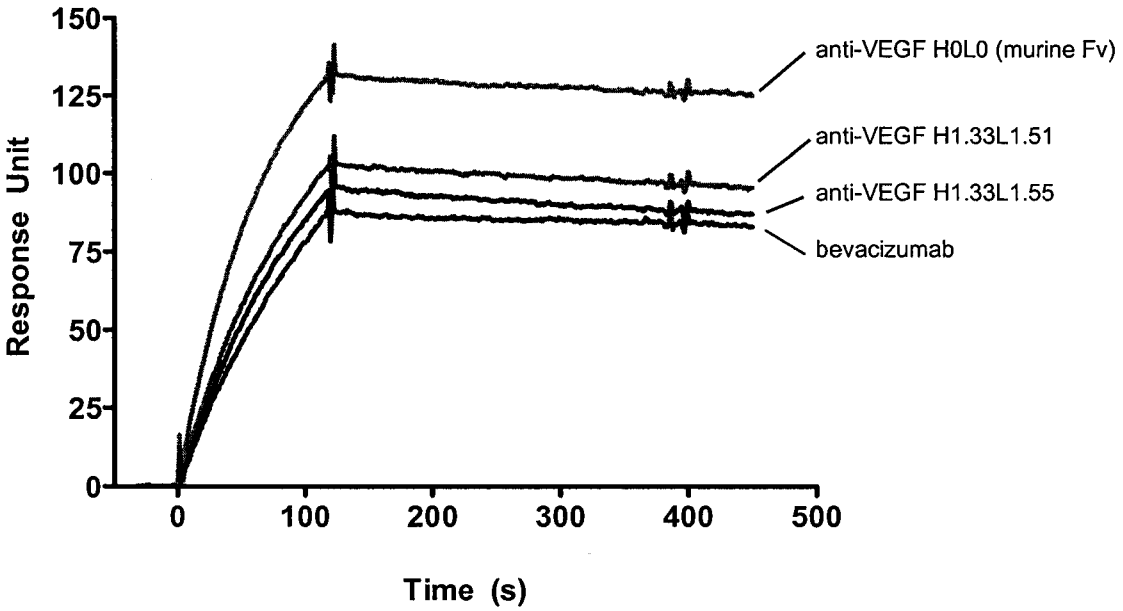
IGHV1-2*02
IGHV1-3*01
IGHV1-46*01
IGHV1-8*01
IGKV1-33*01
IGKV1D-33*01
IGKV1-16*01
IGKV1-39*01
IGKV1D-39*01
CDR proximal
Interface proximal
Germlines

Figure 10.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100
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Response Unit
0
100
200
300
400
500
Time (s)
anti-VEGF H0L0 (murine Fv)
anti-VEGF H1.33L1.51
anti-VEGF H1.33L1.55
bevacizumab

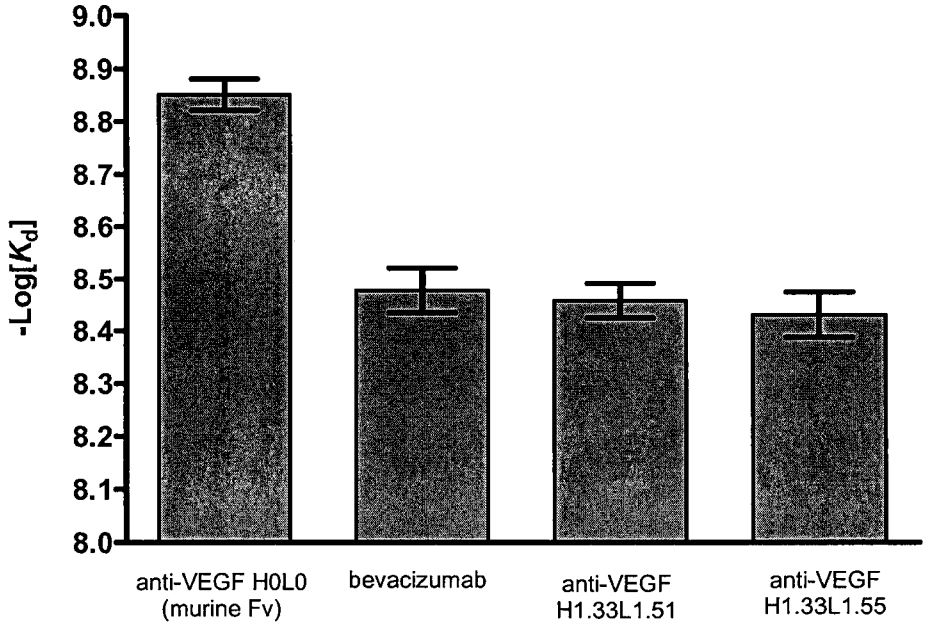
9.0
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
-Log[K_d]
anti-VEGF H0L0 (murine Fv)
bevacizumab
anti-VEGF H1.33L1.51
anti-VEGF H1.33L1.55

Figure 11.

Sequences of CDR and interface positions, binding data, and humanness scores of anti-VEGF variant Fabs

	Heavy chain							Light chain																	
		CDR1	CDR2								CDR2								CDR3			k_{on}	k_{off}		Fold change in K_d vs.	Human 9-	
Variant	2	31	33	53	56	61	91	43	44	46	50	51	53	55	56	71	73	83	92	93	94	($10^4M^{-1}s^{-1}$)	($10^{-4}s^{-1}$)	K_d (nM)	H0L0	mers	IDs
anti-VEGF H0L0 (murine Fv)	I	N	G	Y	E	A	F	T	V	V	F	T	S	H	S	Y	L	I	S	T	V	13.4 ± 0.6	1.7 ± 0.2	1.4 ± 0.2		25	164
anti-VEGF H1L1	I	N	G	Y	E	A	F	T	V	V	F	T	S	H	S	Y	L	F	S	T	V					118	195
bevacizumab	V	N	G	Y	E	A	Y	A	P	V	F	T	S	H	S	F	L	F	S	T	V	6.4 ± 0.3	2.1 ± 0.3	3.4 ± 0.7	- 2.4	103	184
IGHV7-4-1*02 / IGKV1-33*01	V	S	A	N	N	Q	Y	A	P	L	D	A	N	E	T	F	F	I	D	N	L						
VH/VL combination variants																											
anti-VEGF H1.33L1.51	V	N	G	Y	N	Q	Y	A	P	V	F	T	S	H	S	Y	L	F	D	N	L	8.3 ± 0.4	2.9 ± 0.3	3.5 ± 0.5	- 2.5	147	204
anti-VEGF H1.33L1.55	V	N	G	Y	N	Q	Y	A	P	V	F	A	N	E	T	Y	L	F	D	N	L	8.0 ± 0.6	2.8 ± 0.4	3.8 ± 0.7	- 2.7	152	208

Binding data and humanness scores from anti-VEGF single variants were used to design a library of combination variants that would maximize humanness scores and maintain antigen affinity. Binding to VEGF was measured with BIAcore, and shaded residues indicate differences between each variant and anti-VEGF H1L1. The number of total human 9-mers and identities to the closest human germline V and J regions for each VH and VL pair is also shown.

Figure 12.

Sequence properties and final affinity results for engineered mAbs

	VH										
	CDR1			CDR2			CDR3				
Engineered mAb	No. mutations	Percent changed	Final CDR humanness	No. mutations	Percent changed	Final CDR humanness	No. mutations	Percent changed	Final CDR humanness	Total VH mutations	Total percent VH CDR humanness
anti-CD25 H1.12L1.20	1	20.0%	80.0%	8	47.1%	100.0%	0	0.0%	N/A	9	95.5%
anti-VEGF H1.33L1.55	0	0.0%	60.0%	5	29.4%	94.1%	0	0.0%	N/A	5	86.4%
anti-TNF H1.103L1.33	0	0.0%	20.0%	4	21.1%	84.2%	0	0.0%	N/A	4	70.8%

	VL										
	CDR1			CDR2			CDR3				
Engineered mAb	No. mutations	Percent changed	Final CDR humanness	No. mutations	Percent changed	Final CDR humanness	No. mutations	Percent changed	Final CDR humanness	Total VH mutations	Total percent VL CDR humanness
anti-CD25 H1.12L1.20	4	40.0%	81.8%	2	28.6%	83.3%	0	0.0%	55.6%	6	70.4%
anti-VEGF H1.33L1.55	1	10.0%	100.0%	4	57.1%	85.7%	3	33.3%	100.0%	8	96.3%
anti-TNF H1.103L1.33	2	18.2%	90.0%	1	14.3%	85.7%	0	0.0%	55.6%	3	76.9%

Engineered mAb	Total CDR mutations	Total percent CDR humanness	Fold affinity change relative to H0L0
anti-CD25 H1.12L1.20	15	81.6%	+ 1.5 fold
anti-VEGF H1.33L1.55	13	91.8%	- 2.7 fold
anti-TNF H1.103L1.33	7	74.0%	- 1.3 fold

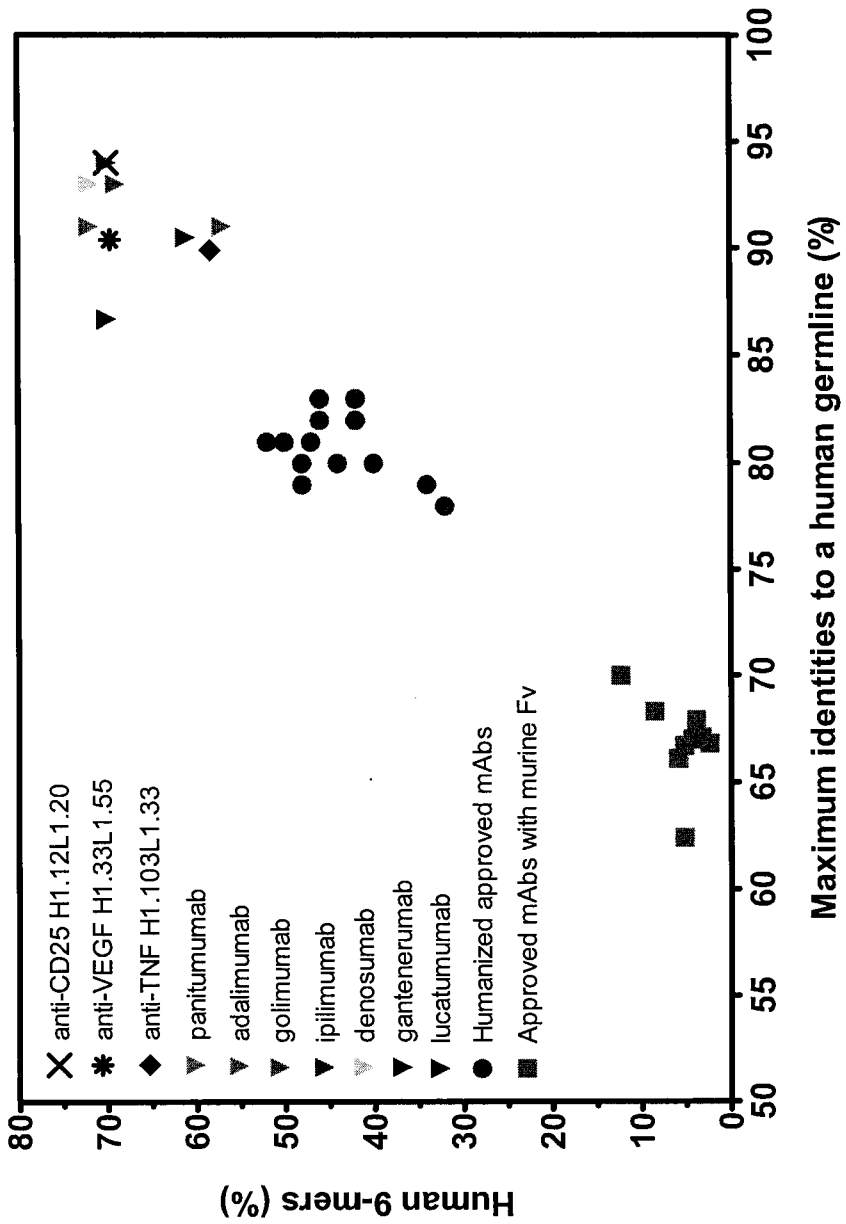
Human 9-mers (%)
Maximum identities to a human germline (%)
anti-CD25 H1.12L1.20
anti-VEGF H1.33L1.55
anti-TNF H1.103L1.33
panitumumab
adalimumab
golimumab
ipilimumab
denosumab
gantenerumab
lucatumumab
Humanized approved mAbs
Approved mAbs with murine Fv
0
10
20
30
40
50
60
70
80
50
55
60
65
70
75
80
85
90
95
100

Figure 13

Figure 14.

Antibody	Antigen	Type of Fv	Status	IDs to closest human germline Fv	Fv length	% ID to closest human germline Fv	Human 9-mers	Human 9-mers (%)	Closest germline (VH)*	Closest germline (VL)*
rituximab	CD20	murine	approved	152	227	67.0%	9	4.3%	1-3 / IGHJ2	1-9 / IGKJ2
cetuximab	EGFR	murine	approved	141	226	62.4%	11	5.2%	2-26 / IGHJ4	6-21 / IGKJ2
infliximab	TNFα	murine	approved	155	227	68.3%	18	8.5%	3-73 / IGHJ4	6-21 / IGKJ2
abciximab	GP IIa/IIIb receptor	murine	approved	151	226	66.8%	5	2.4%	1-3 / IGHJ6	1-8 / IGKJ2
basiliximab	CD25	murine	approved	146	221	66.1%	12	5.9%	1-3 / IGHJ6	3-11 / IGKJ2
ibritumomab	CD20	murine	approved	152	227	67.0%	9	4.3%	1-3 / IGHJ2	1-9 / IGKJ2
tositumomab	CD20	murine	approved	152	228	66.7%	11	5.2%	1-3 / IGHJ2	3-11 / IGKJ2
muromonab	CD3	murine	approved	151	225	67.1%	7	3.3%	1-46 / IGHJ6	3-11 / IGKJ2
satumomab	TAG72	murine	approved	150	221	67.9%	8	3.9%	1-3 / IGHJ1	1-39 / IGKJ2
arcitumomab	CEA	murine	approved	159	227	70.0%	26	12.3%	3-72 / IGHJ4	3-11 / IGKJ2
alemtuzumab	CD52	humanized	approved	180	228	78.9%	72	34.0%	4-30-4 / IGHJ4	1-33 / IGKJ1
bevacizumab	VEGF	humanized	approved	184	230	80.0%	103	48.1%	3-23 / IGHJ2	1-16 / IGKJ1
certolizumab	TNFα	humanized	approved	181	225	80.4%	93	44.5%	3-23 / IGHJ6	1-16 / IGKJ1
daclizumab	CD25	humanized	approved	184	222	82.9%	94	45.6%	1-46 / IGHJ4	1-5 / IGKJ1
eculizumab	C5	humanized	approved	185	229	80.8%	100	46.9%	1-46 / IGHJ2	1-39 / IGKJ1
efalizumab	CD11a	humanized	approved	185	228	81.1%	106	50.0%	3-7 / IGHJ4	1-9 / IGKJ1
gemtuzumab	CD33	humanized	approved	184	227	81.1%	100	47.4%	1-3 / IGHJ4	1-39 / IGKJ1
motavizumab	RSV	humanized[a]	approved	187	226	82.7%	89	42.4%	2-5 / IGHJ6	1-5 / IGKJ4
natalizumab	α4β7 integrin	humanized	approved	184	229	80.3%	85	39.9%	1-3 / IGHJ6	1-33 / IGKJ1
omalizumab	IgE	humanized	approved	188	232	81.0%	112	51.9%	3-66 / IGHJ1	1-39 / IGKJ1
palivizumab	RSV	humanized	approved	186	226	82.3%	89	42.4%	2-5 / IGHJ2	1-5 / IGKJ2
ranibizumab	VEGF	humanized[b]	approved	182	230	79.1%	103	48.1%	3-23 / IGHJ2	1-16 / IGKJ1
tocilizumab	IL-6R	humanized	approved	177	226	78.3%	68	32.4%	2-5 / IGHJ4	1-39 / IGKJ1
trastuzumab	HER2/neu	humanized	approved	186	227	81.9%	96	45.5%	3-66 / IGHJ4	1-39 / IGKJ1
adalimumab	TNFa	fully-human[c]	approved	207	228	90.8%	152	71.7%	3-9 / IGHJ4	1-27 / IGKJ1
panitumumab	EGFR	fully-human[d]	approved	205	226	90.7%	119	56.7%	4-61 / IGHJ3	1-33 / IGKJ4
denosumab	RANKL	fully-human[d]	clinical trials	213	230	92.6%	155	72.4%	3-23 / IGHJ5	3-20 / IGKJ1
gantenerumab	Abeta peptide	fully-human[c]	clinical trials	202	233	86.7%	152	70.0%	3-23 / IGHJ4	3D-7 / IGKJ1
golimumab	TNFα	fully-human[d]	clinical trials	218	233	93.6%	150	69.1%	3-30 / IGHJ6	3-11 / IGKJ3
ipilimumab	CTLA-4	fully-human[d]	clinical trials	213	226	94.2%	147	70.0%	3-30 / IGHJ4	3-20 / IGKJ1
lucatumumab	CD40	fully-human[d]	clinical trials	210	232	90.5%	132	61.1%	3-30 / IGHJ4	2-29 / IGKJ3
anti-CD25 H1.12_L1.20	CD25	XmAb fully-human	pre-clinical	209	222	94.1%	145	70.4%	1-2 / IGHJ4	3-11 / IGKJ2
anti-VEGF H1.33_L1.55	VEGF	XmAb fully-human	pre-clinical	208	230	90.4%	152	71.0%	7-4-1 / IGHJ2	1-33 / IGKJ1
anti-TNF H1.103_L1.33	TNFa	XmAb fully-human	pre-clinical	204	227	89.9%	123	58.3%	3-73 / IGHJ4	6-21 / IGKJ2

*V-segment and J-segment germlines are listed
a: motavizumab is an affinity enhanced version of palivizumab
b: ranibizumab is an affinity enhanced version of bevacizumab
c: phage display technology using human mAb libraries
d: transgenic mouse technology

Figure 15

Rituximab VH

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIY
PGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGG
DWYFNVWGAGTTVTVSA

Rituximab VL

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLA
SGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK

Cetuximab VH

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIW
SGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYYCARALTYYDYEF
AYWGQGTLVTVSA

Cetuximab VL

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESIS
GIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELK

Infliximab VH

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIR
SKSINSATHYAESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGST
YDYWGQGTTLTVSS

Infliximab VL

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASES
MSGIPSRFSGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVK

Abciximab VH

EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYVHWVKQRPEQGLEWIGRIDP
ANGYTKYDPKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCVRPLYDYYAMD
YWGQGTSVTVSS

Abciximab VL

DILMTQSPSSMSVSLGDTVSITCHASQGISSNIGWLQQKPGKSFMGLIYYGTNL
VDGVPSRFSGSGSGADYSLTISSLDSEDFADYYCVQYAQLPYTFGGGTKLEIK

Figure 15 (cont.)

Basiliximab VH

EVQLQQSGTVLARPGASVKMSCKASGYSFTRYWMHWIKQRPGQGLEWIGAIY
PGNSDTSYNQKFEGKAKLTAVTSASTAYMELSSLTHEDSAVYYCSRDYGYYFD
FWGQGTTLTVSS

Basiliximab VL

QIVSTQSPAIMSASPGEKVTMTCSASSSRSYMQWYQQKPGTSPKRWIYDTSKL
ASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQRSSYTFGGGTKLEIK

Ibritumomab VH

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIY
PGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGG
DWYFNVWGAGTTVTVSA

Ibritumomab VL

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLA
SGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK

Tositumomab VH

QAYLQQSGAELVRPGASVKMSCKASGYTFTSYNMHWVKQTPRQGLEWIGAIY
PGNGDTSYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCARVVYYSNS
YWYFDVWGTGTTVTVSG

Tositumomab VL

QIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQQKPGSSPKPWIYAPSNL
ASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGAGTKLELK

Muromonab VH

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYIN
PSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYC
LDYWGQGTTLTVSS

Figure 15 (cont.)

Muromonab VL

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKL
ASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEIN

Satumomab VH

QVQLQQSDAELVKPGASVKISCKASGYTFTDHAIHWAKQKPEQGLEWIGYISP
GNDDIKYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFCKRSYYGHWGQ
GTTLTVSS

Satumomab VL

DIQMTQSPASLSVSVGETVTITCRASENIYSNLAWYQQKQGKSPQLLVYAATNL
ADGVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGTPYTFGGGTRLEIK

Arcitumomab VH

EVKLVESGGGLVQPGGSLRLSCATSGFTFTDYYMNWVRQPPGKALEWLGFIG
NKANGYTTEYSASVKGRFTISRDKSQSILYLQMNTLRAEDSATYYCTRDRGLRF
YFDYWGQGTTLTVSS

Arcitumomab VL

QTVLSQSPAILSASPGEKVTMTCRASSSVTYIHWYQQKPGSSPKSWIYATSNLA
SGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQHWSSKPPTFGGGTKLEIK

Alemtuzumab VH

QVQLQESGPGLVRPSQTLSLTCTVSGFTFTDFYMNWVRQPPGRGLEWIGFIRD
KAKGYTTEYNPSVKGRVTMLVDTSKNQFSLRLSSVTAADTAVYYCAREGHTAA
PFDYWGQGSLVTVSS

Alemtuzumab VL

DIQMTQSPSSLSASVGDRVTITCKASQNIDKYLNWYQQKPGKAPKLLIYNTNNL
QTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCLQHISRPRTFGQGTKVEIK

Figure 15 (cont.)

Bevacizumab VH

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWI
NTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYG
SSHWYFDVWGQGTLVTVSS

Bevacizumab VL

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSL
HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK

Certolizumab VH

EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYGMNWVRQAPGKGLEWMGWI
NTYIGEPIYADSVKGRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARGYRSYAM
DYWGQGTLVTVSS

Certolizumab VL

DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASFL
YSGVPYRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNIYPLTFGQGTKVEIK

Daclizumab VH

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYRMHWVRQAPGQGLEWIGYIN
PSTGYTEYNQKFKDKATITADESTNTAYMELSSLRSEDTAVYYCARGGGVFDY
WGQGTLVTVSS

Daclizumab VL

DIQMTQSPSTLSASVGDRVTITCSASSSISYMHWYQQKPGKAPKLLIYTTSNLAS
GVPARFSGSGSGTEFTLTISSLQPDDFATYYCHQRSTYPLTFGQGTKVEVK

Eculizumab VH

QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQWVRQAPGQGLEWMGEIL
PGSGSTEYTENFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARYFFGSSP
NWYFDVWGQGTLVTVSS

Eculizumab VL

DIQMTQSPSSLSASVGDRVTITCGASENIYGALNWYQRKPGKAPKLLIYGATNL
ADGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQNVLNTPLTFGQGTKVEIK

Figure 15 (cont.)

Efalizumab VH

EVQLVESGGGLVQPGGSLRLSCAASGYSFTGHWMNWVRQAPGKGLEWVGMI
HPSDSETRYNQKFKDRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARGIYFYGT
TYFDYWGQGTLVTVSS

Efalizumab VL

DIQMTQSPSSLSASVGDRVTITCRASKTISKYLAWYQQKPGKAPKLLIYSGSTLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFAFYYCQQHNEYPLTFGQGTKVEIK

Gemtuzumab VH

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIY
PYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDY
WGQGTLVTVSS

Gemtuzumab VL

DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGGAPKLLIYA
ASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGT
KVEIK

Motavizumab VH

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADI
WWDDKKHYNPSLKDRLTISKDTSKNQVVLKVTNMDPADTATYYCARDMIFNFY
FDVWGQGTTVTVSS

Motavizumab VL

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLA
SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCFQGSGYPFTFGGGTKVEIK

Natalizumab VH

QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGQRLEWMGRID
PANGYTKYDPKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCAREGYYGNY
GVYAMDYWGQGTLVTVSS

Figure 15 (cont.)

Natalizumab VL

DIQMTQSPSSLSASVGDRVTITCKTSQDINKYMAWYQQTPGKAPRLLIHYTSAL
QPGIPSRFSGSGSGRDYTFTISSLQPEDIATYYCLQYDNLWTFGQGTKVEIK

Omalizumab VH

EVQLVESGGGLVQPGGSLRLSCAVSGYSITSGYSWNWIRQAPGKGLEWVASIT
YDGSTNYADSVKGRFTISRDDSKNTFYLQMNSLRAEDTAVYYCARGSHYFGH
WHFAVWGQGTLVTVSS

Omalizumab VL

DIQLTQSPSSLSASVGDRVTITCRASQSVDYDGDSYMNWYQQKPGKAPKLLIY
AASYLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSHEDPYTFGQGT
KVEIK

Palivizumab VH

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMSVGWIRQPPGKALEWLADI
WWDDKKDYNPSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCARSMITNWY
FDVWGAGTTVTVSS

Palivizumab VL

DIQMTQSPSTLSASVGDRVTITCKCQLSVGYMHWYQQKPGKAPKLLIYDTSKLA
SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCFQGSGYPFTFGGGTKLEIK

Ranibizumab VH

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWI
NTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYG
TSHWYFDVWGQGTLVTVSS

Ranibizumab VL

DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLH
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK

Figure 15 (cont.)

Tocilizumab VH

QVTLRESGPALVRPTQTLTLTCTFSGFSLSTSGMTVGWIRQPPGEALEWLAHI
WWNDDKYYNPALGKRLAVSKDTSKNQVVLSMNTVGPGDTATYYCARMEDYD
EAMDYWGQGILVTVSS

Tocilizumab VL

DIQMTQSPSSLSASVGDRVTITCRASENIYSNLAWYQQKPGKAPKLLIYAATYLA
DGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQRFWGTPPFGQGTKVEIK

Trastuzumab VH

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYP
TNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFY
AMDYWGQGTLVTVSS

Trastuzumab VL

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFL
YSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

Figure 16

Heavy chains:

>murine anti-CD25 VH (H0) **(SEQ ID NO:1)**
QVQLQQSGAELAKPGASVKMSCKASGYTFTSYRMHWVKQRPGQGLEWIGYINPSTG
YTEYNQKFKDKATLTADKSSSTAYMQLSSLTFEDSAVYYCARGGGVFDYWGQGTTLT
VSS

>framework-optimized anti-CD25 (H1) **(SEQ ID NO:2)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.1 variable region **(SEQ ID NO:3)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTITRDKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.2 variable region **(SEQ ID NO:4)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTNYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.3 variable region **(SEQ ID NO:5)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTMTADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVT
VSS

>anti-CD25 H1.4 variable region **(SEQ ID NO:6)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTITADTSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.5 variable region **(SEQ ID NO:7)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYAQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.6 variable region **(SEQ ID NO:8)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.7 variable region **(SEQ ID NO:9)**
QVQLVQSGAEVKKPGASVKVSCKAGGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

Figure 16 (cont.)

>anti-CD25 H1.8 variable region **(SEQ ID NO:10)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGWINPST
GYTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVT
VSS

>anti-CD25 H1.9 variable region **(SEQ ID NO:11)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSTG
YTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.10 variable region **(SEQ ID NO:12)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPNTG
YTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.11 variable region **(SEQ ID NO:13)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGYINPSSG
YTEYNQKFQGRVTITADKSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVTV
SS

>anti-CD25 H1.12 variable region **(SEQ ID NO:14)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYRMHWVRQAPGQGLEWMGWINPNS
GGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLV
TVSS

>anti-CD25 H1.14 variable region **(SEQ ID NO:15)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYRMHWVRQAPGQGLEWMGYINPNSG
GTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLVT
VSS

>anti-CD25 H1.22 variable region **(SEQ ID NO:16)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGWINPNS
GGTNYAQKFQGRVTITRDTSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLV
TVSS

>anti-CD25 H1.23 variable region **(SEQ ID NO:17)**
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYRMHWVRQAPGQGLEWMGWINPNS
GGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGGVFDYWGQGTLV
TVSS

>murine anti-VEGF VH (H0) **(SEQ ID NO:18)**
EIQLVQSGPELKQPGETVRISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGE
PTYAADFKRRFTFSLETSASTAYLQISNLKNDDTATYFCAKYPHYYGSSHWYFDVWGA
GTTVTVSS

Figure 16 (cont.)

>framework-optimized anti-VEGF (H1) **(SEQ ID NO:19)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.1 variable region **(SEQ ID NO:20)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.2 variable region **(SEQ ID NO:21)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTSYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.3 variable region **(SEQ ID NO:22)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYAMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.4 variable region **(SEQ ID NO:23)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTNTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.5 variable region **(SEQ ID NO:24)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
NPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.6 variable region **(SEQ ID NO:25)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.7 variable region **(SEQ ID NO:26)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.8 variable region **(SEQ ID NO:27)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTGYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

Figure 16 (cont.)

>anti-VEGF H1.9 variable region **(SEQ ID NO:28)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYSMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.10 variable region **(SEQ ID NO:29)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTTYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.11 variable region **(SEQ ID NO:30)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTYYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.12 variable region **(SEQ ID NO:31)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTDYGMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.13 variable region **(SEQ ID NO:32)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTNYDMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.32 variable region **(SEQ ID NO:33)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.33 variable region **(SEQ ID NO:34)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
NPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.34 variable region **(SEQ ID NO:35)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTYTG
NPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.35 variable region **(SEQ ID NO:36)**
QIQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTYTG
NPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

Figure 16 (cont.)

>anti-VEGF H1.36 variable region **(SEQ ID NO:37)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTYTG
NPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.37 variable region **(SEQ ID NO:38)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTYTG
NPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.38 variable region **(SEQ ID NO:39)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTYTG
EPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.39 variable region **(SEQ ID NO:40)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTYYAMNWVRQAPGQGLEWMGWINTYTG
EPTYAAGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.40 variable region **(SEQ ID NO:41)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTYYAMNWVRQAPGQGLEWMGWINTYTG
NPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-VEGF H1.41 variable region **(SEQ ID NO:42)**
QVQLVQSGSELKKPGASVKVSCKASGYTFTYYAMNWVRQAPGQGLEWMGWINTYTG
NPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCAKYPHYYGSSHWYFDVWG
AGTLVTVSS

>anti-TNF H0 variable region (A2; infliximab)
EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSIN
SATHYAESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGT
TLTVSS

>framework-optimized anti-TNF variable region (H1)
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.1 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

Figure 16 (cont.)

>anti-TNF H1.2 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSGHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.3 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNSWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.4 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHAMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.5 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMHWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.6 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGRIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.7 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKAIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.8 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSN
NSATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGT
LVTVSS

>anti-TNF H1.9 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIS
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.10 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
YATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

Figure 16 (cont.)

>anti-TNF H1.11 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATAYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.12 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAASVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.13 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKNIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.14 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSTVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.15 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.16 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKSIN
SATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCTRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.45 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKAIN
YATHYAESVKGRFTISRDDSKSIVYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

>anti-TNF H1.67 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFTFSNHWMNWVRQASGKGLEWVGEIRSKAIS
YATHYAASVKGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGT
LVTVSS

>anti-TNF H1.101 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKAIN
YATHYAESVKGRFTISRDDSKSTAYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQGTL
VTVSS

Figure 16 (cont.)

>anti-TNF H1.103 variable region
EVQLVESGGGLVQPGGSLKLSCAASGFIFSNHWMNWVRQASGKGLEWVGEIRSKAN
NYATHYAASVKGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCSRNYYGSTYDYWGQG
TLVTVSS

Light chains:

>murine anti-CD25 VL (L0) **(SEQ ID NO:43)**
QIVLTQSPAIMSASPGEKVTITCSASSSISYMHWFQQKPGTSPKLWIYTTSNLASGVPAR
FSGSGSGTSYSLTISRMEAEDAATYYCHQRSTYPLTFGSGTKLELK

>framework-optimized anti-CD25 (L1) **(SEQ ID NO:44)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.1 variable region **(SEQ ID NO:45)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCQQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.2 variable region **(SEQ ID NO:46)**
QIVLTQSPATLSLSPGERATLSCRASSSVSYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.3 variable region **(SEQ ID NO:47)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNRASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.4 variable region **(SEQ ID NO:48)**
QIVLTQSPATLSLSPGERATLSCRASSSISYLHWFQQKPGQSPQLLIYTTSNLASGVPAR
FSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.5 variable region **(SEQ ID NO:49)**
EIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.6 variable region **(SEQ ID NO:50)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPRLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.7 variable region **(SEQ ID NO:51)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLEPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.8 variable region **(SEQ ID NO:52)**
QIVLTQSPATLSLSPGERATLSCRASQSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

Figure 16 (cont.)

>anti-CD25 L1.9 variable region **(SEQ ID NO:53)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQAPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.10 variable region **(SEQ ID NO:54)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLATGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.11 variable region **(SEQ ID NO:55)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYDTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.12 variable region **(SEQ ID NO:56)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTASNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.13 variable region **(SEQ ID NO:57)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGIPAR
FSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.14 variable region **(SEQ ID NO:58)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDFTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.15 variable region **(SEQ ID NO:59)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMAWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.16 variable region **(SEQ ID NO:60)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWYQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.17 variable region **(SEQ ID NO:61)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSNYPLTFGSGTKLEIK

>anti-CD25 L1.18 variable region **(SEQ ID NO:62)**
QIVLTQSPATLSLSPGERATLSCRASSSISYMHWFQQKPGQSPQLLIYTTSNLASGVPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTWPLTFGSGTKLEIK

>anti-CD25 L1.19 variable region **(SEQ ID NO:63)**
QIVLTQSPATLSLSPGERATLSCRASSSISSYMHWFQQKPGQSPQLLIYTTSNLASGVP
ARFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

Figure 16 (cont.)

>anti-CD25 L1.20 variable region **(SEQ ID NO:64)**
EIVLTQSPATLSLSPGERATLSCRASQSVSYLHWYQQKPGQAPRLLIYTTSNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.43 variable region **(SEQ ID NO:65)**
EIVLTQSPATLSLSPGERATLSCRASQSVSYLHWYQQKPGQAPRLLIYTTSNRATGIPA
RFSGSGSGTDYTLTISSLQPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.48 variable region **(SEQ ID NO:66)**
EIVLTQSPATLSLSPGERATLSCRASQSVSYMHWYQQKPGQAPRLLIYTTSNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>anti-CD25 L1.56 variable region **(SEQ ID NO:67)**
QIVLTQSPATLSLSPGERATLSCRASQSVSYLHWFQQKPGQSPQLLIYTTSNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCHQRSTYPLTFGSGTKLEIK

>murine anti-VEGF VL (L0) **(SEQ ID NO:68)**
DIQMTQTTSSLSASLGDRVIISCSASQDISNYLNWYQQKPDGTVKVLIYFTSSLHSGVPS
RFSGSGSGTDYSLTISNLEPEDIATYYCQQYSTVPWTFGGGTKLEIK

>framework-optimized anti-VEGF (L1) **(SEQ ID NO:69)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.1 variable region **(SEQ ID NO:70)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.2 variable region **(SEQ ID NO:71)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTPKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.3 variable region **(SEQ ID NO:72)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKLLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.4 variable region **(SEQ ID NO:73)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYDTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.5 variable region **(SEQ ID NO:74)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFASSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.6 variable region **(SEQ ID NO:75)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSNLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

Figure 16 (cont.)

>anti-VEGF L1.7 variable region **(SEQ ID NO:76)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLESGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.8 variable region **(SEQ ID NO:77)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHTGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.9 variable region **(SEQ ID NO:78)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.10 variable region **(SEQ ID NO:79)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTFTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.11 variable region **(SEQ ID NO:80)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDIATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.12 variable region **(SEQ ID NO:81)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYDTVPWTFGGGTKLEIK

>anti-VEGF L1.13 variable region **(SEQ ID NO:82)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSNVPWTFGGGTKLEIK

>anti-VEGF L1.14 variable region **(SEQ ID NO:83)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTLPWTFGGGTKLEIK

>anti-VEGF L1.15 variable region **(SEQ ID NO:84)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLQSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.16 variable region **(SEQ ID NO:85)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYNTVPWTFGGGTKLEIK

>anti-VEGF L1.17 variable region **(SEQ ID NO:86)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYYTVPWTFGGGTKLEIK

>anti-VEGF L1.18 variable region **(SEQ ID NO:87)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKVVKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

Figure 16 (cont.)

>anti-VEGF L1.19 variable region **(SEQ ID NO:88)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLFSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.20 variable region **(SEQ ID NO:89)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKTVKVLIYFTSSLISGVPS
RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.50 variable region **(SEQ ID NO:90)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.51 variable region **(SEQ ID NO:91)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.52 variable region **(SEQ ID NO:92)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFASNLETGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.53 variable region **(SEQ ID NO:93)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.54 variable region **(SEQ ID NO:94)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFASNLETGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.55 variable region **(SEQ ID NO:95)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFASNLETGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.56 variable region **(SEQ ID NO:96)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFASNLETGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.57 variable region **(SEQ ID NO:97)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYDTSSLHSGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.58 variable region **(SEQ ID NO:98)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYDASNLETGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYSTVPWTFGGGTKLEIK

Figure 16 (cont.)

>anti-VEGF L1.59 variable region **(SEQ ID NO:99)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYDASNLETGVP
SRFSGSGSGTDYTLTISSLQPEDFATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.60 variable region **(SEQ ID NO:100)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYDASNLETGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.61 variable region **(SEQ ID NO:101)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFASNLESGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.62 variable region **(SEQ ID NO:102)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFTSNLETGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYSTVPWTFGGGTKLEIK

>anti-VEGF L1.63 variable region **(SEQ ID NO:103)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFASNLESGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPWTFGGGTKLEIK

>anti-VEGF L1.64 variable region **(SEQ ID NO:104)**
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKVLIYFTSNLETGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLPWTFGGGTKLEIK

>murine anti-TNF VL (L0) (A2; infliximab)
DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPS
RFSGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVK

>framework-optimized anti-TNF (L1) variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.1 variable region light chain
EILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.2 variable region light chain
DIVLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.3 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQSVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.4 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFIGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

Figure 16 (cont.)

>anti-TNF L1.5 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSLHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.6 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASQSMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.7 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESFSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.8 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGVP
SRFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.9 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCHQSHSWPFTFGSGTKLEIK

>anti-TNF L1.10 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSSSWPFTFGSGTKLEIK

>anti-TNF L1.11 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSLPFTFGSGTKLEIK

>anti-TNF L1.12 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESASGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.13 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESQSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

>anti-TNF L1.14 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSYSWPFTFGSGTKLEIK

>anti-TNF L1.15 variable region light chain
DILLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESMSGIPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSTPFTFGSGTKLEIK

>anti-TNF L1.30 variable region light chain
EIVLTQSPDFQSVTPKEKVTITCRASQFVGSSIHWYQQKPDQSPKLLIKYASESFSGVPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

Figure 16 (cont.)

>anti-TNF L1.33 variable region light chain
EIVLTQSPDFQSVTPKEKVTITCRASQFIGSSLHWYQQKPDQSPKLLIKYASESFSGVPS
RFSGSGSGTDFTLTINSLEAEDAATYYCQQSHSWPFTFGSGTKLEIK

Heavy chain CDRs:

>anti-CD25 heavy chain CDR1 **(SEQ ID NO:105)**
GYRMH

>anti-CD25 heavy chain CDR1 **(SEQ ID NO:106)**
SYRMH

>anti-CD25 heavy chain CDR2 **(SEQ ID NO:107)**
WINPNSGGTNYAQKFQG

>anti-CD25 heavy chain CDR2 **(SEQ ID NO:108)**
YINPNSGGTNYAQKFQG

>anti-CD25 heavy chain CDR2 **(SEQ ID NO:109)**
YINPSTGYTEYNQKFQG

>anti-CD25 heavy chain CDR3 **(SEQ ID NO:110)**
GGGVFDY

>anti-VEGF heavy chain CDR1 **(SEQ ID NO:111)**
NYGMN

>anti-VEGF heavy chain CDR1 **(SEQ ID NO:112)**
SYAMN

>anti-VEGF heavy chain CDR1 **(SEQ ID NO:113)**
YYAMN

>anti-VEGF heavy chain CDR2 **(SEQ ID NO:114)**
WINTYTGEPTYAAGFTG

>anti-VEGF heavy chain CDR2 **(SEQ ID NO:115)**
WINTNTGNPTYAQGFTG

>anti-VEGF heavy chain CDR2 **(SEQ ID NO:116)**
WINTYTGNPTYAQGFTG

>anti-VEGF heavy chain CDR3 **(SEQ ID NO:117)**
YPHYYGSSHWYFDV

Figure 16 (cont.)

>anti-TNF heavy chain CDR1
NHWMN

>anti-TNF heavy chain CDR2
EIRSKSINSATHYAESVKG

>anti-TNF heavy chain CDR2
EIRSKAINYATHYAESVKG

>anti-TNF heavy chain CDR2
EIRSKAISYATHYAASVKG

>anti-TNF heavy chain CDR2
EIRSKANNYATHYAASVKG

>anti-TNF heavy chain CDR3
NYYGSTYDY

Light chain CDRs:

>anti-CD25 light chain CDR1 **(SEQ ID NO:118)**
RASQSVSYLH

>anti-CD25 light chain CDR1 **(SEQ ID NO:119)**
SASSSISYMH

>anti-CD25 light chain CDR1 **(SEQ ID NO:120)**
RASSSISYMH

>anti-CD25 light chain CDR1 **(SEQ ID NO:121)**
RASQSVSYMH

>anti-CD25 light chain CDR2 **(SEQ ID NO:122)**
TTSNLAS

>anti-CD25 light chain CDR2 **(SEQ ID NO:123)**
TTSNRAT

>anti-CD25 light chain CDR3 **(SEQ ID NO:124)**
HQRSTYPLT

>anti-VEGF light chain CDR1 **(SEQ ID NO:125)**
QASQDISNYLN

Figure 16 (cont.)

>anti-VEGF light chain CDR2 **(SEQ ID NO:126)**
FASNLET

>anti-VEGF light chain CDR2 **(SEQ ID NO:127)**
FTSSLHS

>anti-VEGF light chain CDR2 **(SEQ ID NO:128)**
DTSSLHS

>anti-VEGF light chain CDR2 **(SEQ ID NO:129)**
DASNLET

>anti-VEGF light chain CDR2 **(SEQ ID NO:130)**
FASNLES

>anti-VEGF light chain CDR2 **(SEQ ID NO:131)**
FTSNLET

>anti-VEGF light chain CDR3 **(SEQ ID NO:132)**
QQYSTVPWT

>anti-VEGF light chain CDR3 **(SEQ ID NO:133)**
QQYDNLPWT

>anti-TNF light chain CDR1
RASQFVGSSIH

>anti-TNF light chain CDR1
RASQFVGSSIH

>anti-TNF light chain CDR1
RASQFIGSSLH

>anti-TNF light chain CDR2
YASESMS

>anti-TNF light chain CDR2
YASESFS

>anti-TNF light chain CDR3
QQSHSWPFT
DB2/21083777.1

DB2/21083839.1