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(54) **Title**: ANTIBODIES WITH MODIFIED ISOELECTRIC POINTS AND IMMUNOFILTERING

(57) **Abstract**: The invention relates generally to compositions and methods for altering the isoelectric point of an antibody, and in some cases, resulting in improved plasma pharmacokinetics, e.g. increased serum half-life in vivo.

PATENT APPLICATION

ANTIBODIES WITH MODIFIED ISOELECTRIC POINTS and IMMUNOFILTERING

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ANTIBODIES WITH MODIFIED ISOELECTRIC POINTS

[0001] This application is related to U.S. Provisional Application Serial No. 61/515,745, incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates generally to compositions and methods for altering the isoelectric point of an antibody, and in some cases, resulting in improved plasma pharmacokinetics, e.g. increased serum half-life in vivo.

BACKGROUND OF THE INVENTION

[0003] 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. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin is used for such proteins. Each chain is made up of two distinct regions, referred to as the variable and constant regions. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing feature between these antibody classes is their constant regions, although subtler differences may exist in the V region. IgG antibodies are tetrameric proteins composed of two heavy chains and two light chains. The IgG heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order VH-CH1-CH2-CH3, referring to the heavy chain variable domain, heavy chain constant domain 1, heavy chain constant domain 2, and heavy chain 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.

[0004] Antibodies have serum half-lives in vivo ranging from one to three weeks. This favorable property is due to the preclusion of kidney filtration due to the large size of the full-length molecule, and interaction of the antibody Fc region with the neonatal Fc receptor FcRn. Binding to FcRn recycles endocytosed antibody from the endosome back to the bloodstream (Raghavan et al., 1996, Annu Rev Cell Dev Biol 12:181-220; Ghetie et al., 2000, Annu Rev Immunol 18:739-766, both entirely incorporated by reference).

[0005] Other properties of the antibody may determine its clearance rate (e.g. stability and half-life) in vivo. In addition to antibody binding to the FcRn receptor, other factors that contribute to clearance and half-life are serum aggregation, enzymatic degradation in the serum, inherent immunogenicity of the antibody leading to clearing by the immune system, antigen-mediated uptake, FcR (non-FcRn) mediated uptake and non-serum distribution (e.g. in different tissue compartments).

[0006] Recently it has been suggested that antibodies with variable regions that have lower isoelectric points may also have longer serum half-lives (Igawa et al., 2010 PEDS. 23(5): 385-392; US Publication 2011/0076275 both of which are entirely incorporated by reference). However, the mechanism of this is still poorly understood, and in fact the authors suggest that engineering the variable region is an alternative to engineering the Fc region. Moreover, variable regions differ from antibody to antibody. As such, the methods of the prior art require that each variable region must be altered without significantly affecting the binding affinity.

[0007] Accordingly, the present application defines the impact of charge state on antibody pharmacokinetics, and provides novel engineered variants in the constant regions to improve serum half-life.

BRIEF SUMMARY OF THE INVENTION

Problem to be Solved

[0008] Accordingly, one problem to be solved is to increase serum half life of antibodies by altering the constant domains, thus allowing the same constant regions to be used with different antigen binding sequences, e.g. the variable regions including the CDRs, and minimizing the possibility of immunogenic alterations. Thus providing antibodies with constant region variants with reduced pI and extended half-life provides a more modular approach to improving the pharmacokinetic properties of antibodies, as described herein. In

addition, due to the methodologies outlined herein, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is reduced without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

Summary

[0009] Accordingly, one aspect the invention provides methods for modifying the isoelectric point of an antibody by introducing at least 6 amino acid mutations, including substitutions with non-native amino acids in a constant domain selected from the heavy chain constant domain and light chain constant domain, wherein the substituted amino acids have a pI lower than the native amino acid, such that said isoelectric point of the variant antibody is lowered by at least 0.5 logs. In some cases, only the heavy chain constant domain is altered; in some cases, only the light chain constant domain, and in some cases both the heavy and light constant domains comprise mutated amino acids.

[0010] In another aspect the methods provide for the generation of these variants by amino acid mutations selected from the group consisting of a non-native glutamic acid at position 119; a non-native cysteine at position 131; a non-native arginine, lysine or glutamine at position 133; a non-native glutamic acid at position 137; a non-native serine at position 138; a non-native glutamic acid at position 164; a non native asparagine at position 192; a non native phenylalanine at position 193, a non-native lysine at position 196, a non-native threonine at position 199, a non-native aspartic acid at position 203, a non-native glutamic acid or glutamine at position 205, a non native aspartic acid at position 208, a non-native glutamic acid or glutamine at position 210, a non native threonine at position 214, a non native arginine at position 217 and a non-native cysteine at position 219, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non native threonine at position 339, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine

or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, and a deletion or non-native aspartic acid at position 447, using EU numbering.

[0011] In a further aspect, the invention provides methods for modifying the isoelectric point of an antibody by introducing at least 2 amino acid mutations in the light constant domain, such that said isoelectric point of the variant antibody is lowered by at least 0.5 logs, and wherein said variant antibody comprises substitutions selected from the group consisting of a non-native glutamine or glutamic acid at position 126, a non-native glutamine, glutamic acid or threonine at position 145; a non-native aspartic acid at position 152, a non-native glutamic acid at position 156, a non-native glutamine or glutamic acid at position 169, a non-native glutamic acid at position 199, a non-native glutamic acid at position 202 and a a non-native glutamic acid at position 207 (using EU numbering).

[0012] In additional aspects, the invention provides methods for modifying the isoelectric point of an antibody by introducing: a) at least 6 amino acid mutations in the heavy constant domain, wherein said variant antibody comprises mutations selected from the group consisting of a non-native glutamic acid at position 119; a non-native cysteine at position 131; a non-native arginine, lysine or glutamine at position 133; a non-native glutamic acid at position 137; a non-native serine at position 138; a non-native glutamic acid at position 164; a non native asparagine at position 192; a non native phenylalanine at position 193, a non-native lysine at position 196, a non-native threonine at position 199, a non-native aspartic acid at position 203, a non-native glutamic acid or glutamine at position 205, a non native aspartic acid at position 208, a non-native glutamic acid or glutamine at position 210, a non native threonine at position 214, a non native arginine at position 217 and a non-native cysteine at position 219, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non native threonine at position 339, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, and a deletion or non-native aspartic

acid at position 447; and b) substituting at least 2 non-native amino acids in the light constant domain, wherein said variant antibody comprises substitutions selected from the group consisting of a non-native glutamine or glutamic acid at position 126, a non-native glutamine, glutamic acid or threonine at position 145; a non-native aspartic acid at position 152, a non-native glutamic acid at position 156, a non-native glutamine or glutamic acid at position 169, a non-native glutamic acid at position 199, a non-native glutamic acid at position 202 and a a non-native glutamic acid at position 207 (using EU numbering), such that said isoelectric point of the variant antibody is lowered by at least 0.5 logs.

[0013] In a further aspect, the pI antibodies of the invention, generated using the above methods, has an increased serum half life as compared to an antibody without the mutations.

[0014] In an additional aspect, the invention provides antibodies comprising a variant heavy constant domain polypeptide comprising a variant of SEQ ID NO: 2, comprising at least 6 mutations selected from the group consisting of a non-native glutamic acid at position 119; a non-native cysteine at position 131; a non-native arginine, lysine or glutamine at position 133; a non-native glutamic acid at position 137; a non-native serine at position 138; a non-native glutamic acid at position 164; a non native asparagine at position 192; a non native phenylalanine at position 193, a non-native lysine at position 196, a non-native threonine at position 199, a non-native aspartic acid at position 203, a non-native glutamic acid or glutamine at position 205, a non native aspartic acid at position 208, a non-native glutamic acid or glutamine at position 210, a non native threonine at position 214, a non native arginine at position 217 and a non-native cysteine at position 219, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non native threonine at position 339, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, and a deletion or non-native aspartic acid at position 447.

[0015] In an additional aspect, the invention provides antibodies comprising a variant light constant domain polypeptide comprising of variant of SEQ ID NO:112, wherein said variant

antibody comprises substitutions selected from the group consisting of a non-native glutamine or glutamic acid at position 126, a non-native glutamine, glutamic acid or threonine at position 145; a non-native aspartic acid at position 152, a non-native glutamic acid at position 156, a non-native glutamine or glutamic acid at position 169, a non-native glutamic acid at position 199, a non-native glutamic acid at position 202 and a a non-native glutamic acid at position 207 (using EU numbering).

[0016] In a further aspect, the invention provides nucleic acids encoding the antibodies, including a nucleic acid encoding a variant heavy chain constant domain and/or a nucleic acid encoding a variant light chain constant domain. Host cells containing the nucleic acids and methods of producing the antibodies are also included.

[0017] In an additional aspect, the invention provides antibodies comprising a variant heavy chain constant domain having the formula:

[0018] A-X_{119}-T-K-G-P-S-V-F-P-L-A-P-X_{131}-S-X_{133}-S-T-S-X_{137}-X_{138}-T-A-A-L-G-C-L-V-K-D-Y-F-P-E-P-V-T-V-S-W-N-S-G-A-L-X_{164}-S-G-V-H-T-F-P-A-V-L-Q-S-S-G-L-Y-S-L-S-S-V-V-T-V-P-S-S-X_{192}-X_{193}-G-T-X_{196}-T-Y-X_{199}-C-N-V-X_{203}-H-X_{205}-P-S-X_{208}-T-X_{210}-V-D-K-X_{214}-V-E-X_{217}-K-X_{219}-C-X_{221}-X_{222}-X_{223}-X_{224}-X_{225}-C-P-P-C-P-A-P-X_{233}-X_{234}-X_{235}-X_{236}-G-P-S-V-F-L-F-P-P-K-P-K-D-T-L-M-I-S-R-T-P-E-V-T-C-V-V-V-D-V-S-H-E-D-P-E-V-X_{274}-F-N-W-Y-V-D-G-V-E-V-H-N-A-K-T-K-P-R-E-E-Q-X_{296}-N-S-T-X_{300}-R-V-V-S-V-L-T-V-X_{309}-H-Q-D-W-L-N-G-K-E-Y-X_{320}-C-X_{322}-V-S-N-X_{326}-X_{327}-L-P-A-P-I-E-X_{334}-T-I-S-K-X_{339}-K-G-Q-P-R-E-P-Q-V-Y-T-L-P-P-S-X_{355}-E-E-M-X_{359}-K-N-X_{362}-V-S-L-T-C-L-V-K-G-F-Y-P-S-D-I-A-V-E-W-E-S-X_{384}-G-Q-P-E-X_{389}-N-Y-X_{392}-T-T-P-P-X_{397}-L-D-S-D-G-S-F-F-L-Y-S-K-L-T-V-D-K-S-R-W-X_{418}-X_{419}-G-N-V-F-S-C-S-V-X_{428}-H-E-A-L-H-X_{434}-H-Y-T-Q-K-S-L-S-L-X_{444}-P-G-X_{447},

[0019] wherein X_{119} is selected from the group consisting of S and E;

[0020] wherein X_{131} is selected from the group consisting of S and C;

[0021] wherein X_{133} is selected from the group consisting of K, R, E, and Q;

[0022] wherein X_{137} is selected from the group consisting of G and E;

[0023] wherein X_{138} is selected from the group consisting of G and S;

[0024] wherein X_{164} is selected from the group consisting of T and E;

[0025] wherein X_{192} is selected from the group consisting of S and N;

[0026] wherein X_{193} is selected from the group consisting of L and F;

[0027] wherein X_{196} is selected from the group consisting of Q and K;

[0028] wherein X_{199} is selected from the group consisting of I and T;

[0029] wherein X_{203} is selected from the group consisting of N and D;

[0030] wherein X_{205} is selected from the group consisting of K, E, and Q;

[0031] wherein X_{208} is selected from the group consisting of N and D;

[0032] wherein X_{210} is selected from the group consisting of K, E, and Q;

[0033] wherein X_{214} is selected from the group consisting of K and T;

[0034] wherein X_{217} is selected from the group consisting of P and R;

[0035] wherein X_{219} is selected from the group consisting of S and C;

[0036] wherein X_{220} is selected from the group consisting of C, PLG, and G;

[0037] wherein X_{221} is selected from the group consisting of D and a deletion;

[0038] wherein X_{222} is selected from the group consisting of K, V, and T;

[0039] wherein X_{223} is selected from the group consisting of T and a deletion;

[0040] wherein X_{224} is selected from the group consisting of H and E;

[0041] wherein X_{225} is selected from the group consisting of T and a deletion;

[0042] wherein X_{233} is selected from the group consisting of E and P;

[0043] wherein X_{234} is selected from the group consisting of L and V;

[0044] wherein X_{235} is selected from the group consisting of L, A, and a deletion;

[0045] wherein X_{236} is selected from the group consisting of G, A, and a deletion;

[0046] wherein X_{274} is selected from the group consisting of K, Q, and E;

[0047] wherein X_{296} is selected from the group consisting of Y and F;

[0048] wherein X_{300} is selected from the group consisting of Y and F;

[0049] wherein X_{309} is selected from the group consisting of L and V;

[0050] wherein X_{320} is selected from the group consisting of K and E;

[0051] wherein X_{322} is selected from the group consisting of K and E;

[0052] wherein X_{326} is selected from the group consisting of K and E;

[0053] wherein X_{327} is selected from the group consisting of A and G;

[0054] wherein X_{334} is selected from the group consisting of K and E;

[0055] wherein X_{339} is selected from the group consisting of A and T;

[0056] wherein X_{355} is selected from the group consisting of R, Q, and E;

[0057] wherein is X_{359} is selected from the group consisting of T and E;

[0058] wherein is X_{362} is selected from the group consisting of Q and E;

wherein X_{384} is selected from the group consisting of N and S;

[0059] wherein is X_{389} is selected from the group consisting of N and E;

[0060] wherein X_{392} is selected from the group consisting of K, N, and E;

[0061] wherein X_{397} is selected from the group consisting of V and M;

[0062] wherein X_{418} is selected from the group consisting of Q and E;

[0063] wherein X_{419} is selected from the group consisting of Q and E;

[0064] wherein X_{428} is selected from the group consisting of M and L;

[0065] wherein X_{434} is selected from the group consisting of N and S;

[0066] wherein X_{444} is selected from the group consisting of S and E; and

[0067] wherein X_{447} is selected from the group consisting of K, DEDE, and a deletion;

[0068] wherein said variant heavy chain constant domain comprises at least 6 substitutions as compared to SEQ ID NO: 2 and said variant is not SEQ ID NO: 3.

[0069] In a further aspect the invention provides variant heavy chain constant domain comprises at least 10 or 15 substitutions as compared to SEQ ID NO: 2.

[0070] In an additional aspect, the invention provides antibodies with a variant light chain constant domain having the formula:

[0071] X_{108}-T-V-A-A-P-S-V-F-I-F-P-P-S-D-E-X_{124}-L-X_{126}-S-G-T-A-S-V-V-C-L-L-N-X_{138}-F-Y-P-R-E-A-X_{145}-V-Q-W-K-V-D-X_{152}-A-L-Q-X_{156}-G-N-S-Q-E-S-V-T-E-Q-D-S-X_{169}-D-S-

T-Y-S-L-S-S-T-L-T-L-S-K-A-D-Y-E-K-H-K-V-Y-A-C-E-V-T-H-X_{199}-G-L-X_{202}-S-P-V-T-X_{207}-S-F-N-R-G-E-X_{214},

[0072] wherein X_{108} is selected from the group consisting of R and Q;

[0073] wherein X_{124} is selected from the group consisting of Q and E;

[0074] wherein X_{126} is selected from the group consisting of K, E, and Q;

[0075] wherein X_{138} is selected from the group consisting of N and D;

[0076] wherein X_{145} is selected from the group consisting of K, E, Q, and T;

[0077] wherein X_{152} is selected from the group consisting of N and D;

[0078] wherein X_{156} is selected from the group consisting of S and E;

[0079] wherein X_{169} is selected from the group consisting of K, E, and Q;

[0080] wherein X_{199} is selected from the group consisting of Q and E;

[0081] wherein X_{202} is selected from the group consisting of S and E; and

[0082] wherein X_{207} is selected from the group consisting of K and E; and

[0083] wherein X_{214} is selected from the group consisting of C and CDEDE.

[0084] wherein said variant light chain constant domain comprises at least 2 substitutions as compared to SEQ ID NO: 112.

BRIEF DESCRIPTION OF THE DRAWINGS

[0085] Figure 1. Amino acid sequences of wild-type constant regions used in the invention.

[0086] Figure 2. Engineering of heavy chain CH1 domains. List of CH1 residues for the four IgG isotypes, fraction exposed, and examples of substitutions that can be made to lower pI. Numbering is according to the EU index.

[0087] Figure 3. Engineering of light chain CK domains. List of CK residues, fraction exposed, and substitutions that can be made to lower pI. Numbering is according to the EU index.

[0088] Figure 4. Amino acid sequences of pI engineered constant regions IgG1-CH1-pI(6) and CK-pI(6).

[0089] Figure 5. Amino acid sequences of wild-type anti-VEGF VH and VL variable regions used in the invention.

[0090] Figure 6. Amino acid sequences of the heavy and light chains of pI engineered anti-VEGF antibody XENP9493 IgG1-CH1-pI(6)-CK-pI(6) used in the invention.

[0091] Figure 7. Structure of an antibody Fab domain showing the locations of pI lowering mutations in XENP9493 IgG1-CH1-pI(6)-CK-pI(6).

[0092] Figure 8. Analysis of pI engineered anti-VEGF variants on an Agilent Bioanalyzer showing high purity.

[0093] Figure 9. Analysis of pI engineered anti-VEGF variants on SEC showing high purity.

[0094] Figure 10. Analysis of pI engineered anti-VEGF variants on an IEF gel showing variants have altered pI.

[0095] Figure 11. Binding analysis (Biacore) of bevacizumab and pI engineered anti-VEGF binding to VEGF.

[0096] Figure 12. DSC analysis of CH1 and CK pI engineered anti-VEGF showing high thermostability.

[0097] Figure 13. PK of bevacizumab variants in huFcRn mice. The 9493 variant with pI-engineered CH1 and CK domains extends half-life in vivo.

[0098] Figure 14. PK of a native IgG1 version of bevacizumab in four separate in vivo studies in huFcRn mice. The average IgG1 half-life was 3.2 days.

[0099] Figure 15. PK of a native IgG2 version of bevacizumab in huFcRn mice.

[00100] Figure 16. Correlation between half-life and isoelectric point (pI) of antibody variants with different constant chains.

[00101] Figure 17. Amino acid sequence alignment of the IgG subclasses. Residues with a bounded box illustrate isotypic differences between the IgG's. Residues which contribute to a higher pI (K, R, and H) or lower pI (D and E) are highlighted in bold. Designed substitutions that either lower the pI, or extend an epitope are shown in gray.

[00102] Figure 18. Amino acid sequence of the CK and Cλ light constant chains. Residues which contribute to a higher pI (K, R, and H) or lower pI (D and E) are highlighted in bold. Preferred positions that can be modified to lower the pI are shown in gray.

[00103] Figure 19. Amino acid sequences of pI-engineered variant heavy chains.

[00104] Figure 20. Amino acid sequences of pI-engineered variant light chains.

[00105] Figure 21. PK results of pI-engineered variant bevacizumab antibodies in huFcRn mice.

[00106] Figure 22. PK results of variants that combine pI-engineered modifications with Fc modifications that enhance binding to FcRn.

[00107] Figure 23. Correlation between half-life and isoelectric point (pI) of native bevacizumab antibodies, pI-engineered variant versions with reduced pI, and native and pI-engineered versions that incorporate Fc modifications that improve binding to human FcRn.

[00108] Figure 24. Amino acid sequence alignment of novel isotype IgG-pI-Iso3 with the IgG subclasses. Blue indicates a match between pI-iso3 and residues in the four native IgG's IgG1, IgG2, IgG3, and IgG4. Residues with a bounded box illustrate IgG isotypic differences that have been incorporated into IgG-pI-Iso3 that reduce pI.

[00109] Figure 25. Differences between IgG1 and IgG-pI-Iso3 in the hinge and Fc region.

[00110] Figure 26. Differences between IgG1 and IgG-pI-Iso3 in the CH1 region.

[00111] Figure 27. Amino acid illustration of the CK-pI(4) variant. Red indicates lysine to glutamic acid charge substitutions relative to the native CK light constant chain.

[00112] Figure 28. Amino acid sequences of pI-engineered heavy and light constant chains.

[00113] Figure 29. Analysis of basic residues in the antibody Fc region showing fraction exposed and the calculated energy for substitution to Glu normalized against the energy of the WT residue. Basic residues with a high fraction exposed and a favorable delta E for substitution to Glu are targets for charge swap mutations to lower pI.

[00114] Figure 30. Plot showing the effect of charge swap mutations on antibody pI. As the pI gets lower the change in pI per charge swap decreases.

[00115] Figure 31. PK results of pI-engineered isotypic variant bevacizumab antibodies (IgG-pI-Iso3) and combinations with substitution N434S in huFcRn mice.

[00116] Figure 32. PK results of pI-engineered isotypic variant bevacizumab antibodies and combinations with substitution N434S in huFcRn mice.

[00117] Figure 33. Scatter plot of PK results of pI-engineered isotypic variant bevacizumab antibodies and combinations with substitution N434S in huFcRn mice. Each

point represents a single mouse from the study. It should be noted that the 428L substitution can also be added to each of these pI antibodies.

[00118] Figure 34. Plot showing correlation between pI engineered variant pI and half-life (t1/2).

[00119] Figure 35. Structural alignment of CK and C-lambda domains.

[00120] Figure 36. Literature pIs of the 20 amino acids. It should be noted that the listed pIs are calculated as free amino acids; the actual pI of any side chain in the context of a protein is different, and thus this list is used to show pI trends and not absolute numbers for the purposes of the invention.

[00121] Figure 37. Data table of exemplary pI-engineered variants listing:

XenP#	the internal reference number
Name (HC)	heavy chain sequence designation
SEQ ID NO (HC)	corresponding SEQ ID NO of the heavy chain sequence
Name (LC)	light chain sequence designation
SEQ ID NO (LC)	corresponding SEQ ID NO of the light chain sequence
Calc. pI	calculated pI value for the entire antibody sequence, including heavy and light chain Fv + constant domains, with the Fv of bevacizumab and the constant domains as defined in the table
#KR	number of Lys or Arg residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta KR (vs. WT)	change in the number of Lys or Arg residues relative to IgG1 wild-type sequence of bevacizumab
#DE	number of Asp or Glu residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta DE (vs. WT)	change in the number of Asp or Glu acid residues relative to IgG1 wild-type sequence of bevacizumab
Charge state	derived from the total number of Lys and Arg minus the total number of Asp and Glu residues, assuming a pH of 7
# HC Mutations vs IgG1	number of mutations in the heavy chain constant domain as compared to IgG1
# LC Mutations vs IgG1	number of mutations in the light chain constant domain as compared to IgG1
Total # of Mutations	total number of mutations in the heavy chain and light chain constant domains as compared to IgG1

[00122] Figure 38. Analysis of class II MHC agretopes for DR1 and DR3,4,5 using ImmunoFilter™. IScores of non-human 9-mers for low pI variant IgG1-CH1-pI(6) are shown in dark shading.

[00123] Figure 39. All possible combinations of the 12 substitutions present in the IgG1-CH1-pI(6) and CK-pI(6) chains (6 substitutions in each chain) listing ImmunoFilter™ propensity to bind individual MHC isoforms, number of mutations, and pI.

[00124] Figure 40. Low pI variants with lower non-human 9-mer MHC binding propensity identified by ImmunoFilter™. Variants from each IScore bin with the lowest pI were chosen and constructed for further analysis.

[00125] Figure 41. Analysis of class II MHC agretopes for DR1 and DR3,4,5 using ImmunoFilter™. IScores of non-human 9-mers for low pI and ImmunoFilter™ optimized variant IgG1-CH1-v42 are shown in dark shading.

[00126] Figure 42. Sequence alignment of IgG1-4 showing ImmunoFilter™ optimized heavy chain IgG-pI-CH1-v42, as well as a variant containing extra isotypic substitutions (labeled as "+9-mer optimized Fc").

[00127] Figure 43. Amino acid sequences of ImmunoFilter™ and non-human 9-mer optimized pI-engineered heavy and light constant chains.

[00128] Figure 44. PK results of pI-engineered isotypic variant bevacizumab antibodies and combinations with substitution N434S in huFcRn mice.

[00129] Figure 45. PK results of pI-engineered isotypic variant bevacizumab antibodies and combinations with substitution N434S in huFcRn mice.

[00130] Figure 46. PK results (half-life and AUC) of pI-engineered isotypic variant bevacizumab antibodies and combinations with substitution N434S in huFcRn mice.

[00131] Figure 47. PK results of pI-engineered isotypic variant bevacizumab antibodies and combinations with substitution N434S in cynomolgus monkeys.

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[00132] The present invention is generally directed to compositions and methods relating to decreasing the isoelectric point (pI) of antibodies (to form "pI antibodies") by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or more constant region domains of the antibody. The pI substitutions are chosen such that the pI amino acids have a pI lower than the native amino acid at a particular position in the constant

domain. In various embodiments, the constant domain variants reduce the pI of the antibody, and, as shown herein for the first time, improve serum half-life in vivo. While, as noted above, there is limited data that might suggest that lowering the pI of an antibody by generating variants in the CDR regions of an antibody can lead to increased serum half life. However, the present invention provides a significant benefit to CDR pI engineering, as the constant domains of the present invention can be added in a modular fashion to the variable regions, thus significantly simplifying design of antibodies that have increased serum half lives.

[00133] That is, until the present invention, the fact that decreasing pI of an antibody would lead to increased serum half life was both unpredictable and unexpected.

[00134] In addition, many embodiments of the invention rely on the "importation" of lower pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting protein is lowered, and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significant affect the pI of the variant antibody. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[00135] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g. by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[00136] In addition, by pI engineering both the heavy and light constant domains, significant decreases in pI of the resulting antibody can be seen. As discussed below, lowering the pI by at least 0.5 can increase the half life significantly.

[00137] As will be appreciated by those in the art and described below, a number of factors contribute to the in vivo clearance, and thus the half-life, of antibodies in serum. One factor involves the antigen to which the antibody binds; that is, antibodies with identical

constant regions but different variable regions (e.g. Fv domains), may have different half-lives due to differential ligand binding effects. However, the present invention demonstrates that while the absolute half life of two different antibodies may differ due to these antigen specificity effects, the pI variants (which optionally include FcRn variants as outlined herein), can transfer to different ligands to give the same trends of increasing half-life. That is, in general, the relative "order" of the pI decreases/half life increases will track to antibodies with the same pI variants of antibodies with different Fvs as is discussed herein.

II. Description of the Invention

A. Antibodies

[00138] The present invention relates to the generation of pI variants of antibodies, generally therapeutic antibodies. As is discussed below, the term "antibody" is used generally. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. In general, the term "antibody" includes any polypeptide that includes at least one constant domain, including, but not limited to, CH1, CH2, CH3 and CL.

[00139] 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. The present invention is directed to the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown herein, the present invention covers pI engineering of IgG1/G2 hybrids as is more fully discussed below.

[00140] 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, generally referred to in the art and herein as the "Fv domain" or "Fv region". 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. “Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[00141] Each VH and VL is composed of three hypervariable regions (“complementary determining regions,” “CDRs”) and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00142] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; “L” denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; “H” denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[00143] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g, Kabat *et al.,* supra (1991)).

[00144] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. “Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00145] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which

are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00146] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00147] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning."

[00148] As will be appreciated by those in the art, a wide variant of antigen binding domains, e.g. Fv regions, may find use in the present invention. Virtually any antigen may be targeted by the IgG variants, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens, which includes both soluble factors such as cytokines and membrane-bound factors, including transmembrane receptors: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16,

CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, *Clostridium botulinum* toxin, *Clostridium perfringens* toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-

13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellerian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PlGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta RIIb, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-

2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors.

[00149] In some embodiments, the pI engineering described herein is done to therapeutic antibodies. A number of antibodies that are approved for use, in clinical trials, or in development may benefit from the pI variants of the present invention. These antibodies are herein referred to as “clinical products and candidates”. Thus in a preferred embodiment, the pI engineered constant region(s) of the present invention may find use in a range of

clinical products and candidates. For example, a number of antibodies that target CD20 may benefit from the pI engineering of the present invention. For example the pI variants of the present invention may find use in an antibody that is substantially similar to rituximab (Rituxan®, IDEC/Genentech/Roche) (see for example US 5,736,137), a chimeric anti-CD20 antibody approved to treat Non-Hodgkin's lymphoma; HuMax-CD20, an anti-CD20 currently being developed by Genmab, an anti-CD20 antibody described in US 5,500,362, AME-133 (Applied Molecular Evolution), hA20 (Immunomedics, Inc.), HumaLYM (Intracel), and PRO70769 (PCT/US2003/040426, entitled "Immunoglobulin Variants and Uses Thereof"). A number of antibodies that target members of the family of epidermal growth factor receptors, including EGFR (ErbB-1), Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), may benefit from pI engineered constant region(s) of the invention. For example the pI engineered constant region(s) of the invention may find use in an antibody that is substantially similar to trastuzumab (Herceptin®, Genentech) (see for example US 5,677,171), a humanized anti-Her2/neu antibody approved to treat breast cancer; pertuzumab (rhuMab-2C4, Omnitarg™), currently being developed by Genentech; an anti-Her2 antibody described in US 4,753,894; cetuximab (Erbitux®, Imclone) (US 4,943,533; PCT WO 96/40210), a chimeric anti-EGFR antibody in clinical trials for a variety of cancers; ABX-EGF (US 6,235,883), currently being developed by Abgenix-Immunex-Amgen; HuMax-EGFr (USSN 10/172,317), currently being developed by Genmab; 425, EMD55900, EMD62000, and EMD72000 (Merck KGaA) (US 5,558,864; Murthy et al. 1987, Arch Biochem Biophys. 252(2):549-60; Rodeck et al., 1987, J Cell Biochem. 35(4):315-20; Kettleborough et al., 1991, Protein Eng. 4(7):773-83); ICR62 (Institute of Cancer Research) (PCT WO 95/20045; Modjtahedi et al., 1993, J. Cell Biophys. 1993, 22(1-3):129-46; Modjtahedi et al., 1993, Br J Cancer. 1993, 67(2):247-53; Modjtahedi et al, 1996, Br J Cancer, 73(2):228-35; Modjtahedi et al, 2003, Int J Cancer, 105(2):273-80); TheraCIM hR3 (YM Biosciences, Canada and Centro de Immunologia Molecular, Cuba (US 5,891,996; US 6, 506,883; Mateo et al, 1997, Immunotechnology, 3(1):71-81); mAb-806 (Ludwig Institue for Cancer Research, Memorial Sloan-Kettering) (Jungbluth et al. 2003, Proc Natl Acad Sci U S A. 100(2):639-44); KSB-102 (KS Biomedix); MR1-1 (IVAX, National Cancer Institute) (PCT WO 0162931A2); and SC100 (Scancell) (PCT WO 01/88138). In another preferred embodiment, the pI engineered constant region(s) of the present invention may find use in alemtuzumab (Campath®, Millenium), a humanized monoclonal antibody currently approved for treatment of B-cell chronic lymphocytic leukemia. The pI engineered constant region(s) of the present invention may find use in a variety of antibodies that are substantially similar

to other clinical products and candidates, including but not limited to muromonab-CD3 (Orthoclone OKT3®), an anti-CD3 antibody developed by Ortho Biotech/Johnson & Johnson, ibritumomab tiuxetan (Zevalin®), an anti-CD20 antibody developed by IDEC/Schering AG, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 (p67 protein) antibody developed by Celltech/Wyeth, alefacept (Amevive®), an anti-LFA-3 Fc fusion developed by Biogen), abciximab (ReoPro®), developed by Centocor/Lilly, basiliximab (Simulect®), developed by Novartis, palivizumab (Synagis®), developed by MedImmune, infliximab (Remicade®), an anti-TNFalpha antibody developed by Centocor, adalimumab (Humira®), an anti-TNFalpha antibody developed by Abbott, Humicade™, an anti-TNFalpha antibody developed by Celltech, etanercept (Enbrel®), an anti-TNFalpha Fc fusion developed by Immunex/Amgen, ABX-CBL, an anti-CD147 antibody being developed by Abgenix, ABX-IL8, an anti-IL8 antibody being developed by Abgenix, ABX-MA1, an anti-MUC18 antibody being developed by Abgenix, Pemtumomab (R1549, 90Y-muHMFG1), an anti-MUC1 In development by Antisoma, Therex (R1550), an anti-MUC1 antibody being developed by Antisoma, AngioMab (AS1405), being developed by Antisoma, HuBC-1, being developed by Antisoma, Thioplatin (AS1407) being developed by Antisoma, Antegren® (natalizumab), an anti-alpha-4-beta-1 (VLA-4) and alpha-4-beta-7 antibody being developed by Biogen, VLA-1 mAb, an anti-VLA-1 integrin antibody being developed by Biogen, LTBR mAb, an anti-lymphotoxin beta receptor (LTBR) antibody being developed by Biogen, CAT-152, an anti-TGF-β2 antibody being developed by Cambridge Antibody Technology, J695, an anti-IL-12 antibody being developed by Cambridge Antibody Technology and Abbott, CAT-192, an anti-TGFβ1 antibody being developed by Cambridge Antibody Technology and Genzyme, CAT-213, an anti-Eotaxin1 antibody being developed by Cambridge Antibody Technology, LymphoStat-B™ an anti-Blys antibody being developed by Cambridge Antibody Technology and Human Genome Sciences Inc., TRAIL-R1mAb, an anti-TRAIL-R1 antibody being developed by Cambridge Antibody Technology and Human Genome Sciences, Inc., Avastin™ (bevacizumab, rhuMAb-VEGF), an anti-VEGF antibody being developed by Genentech, an anti-HER receptor family antibody being developed by Genentech, Anti-Tissue Factor (ATF), an anti-Tissue Factor antibody being developed by Genentech, Xolair™ (Omalizumab), an anti-IgE antibody being developed by Genentech, Raptiva™ (Efalizumab), an anti-CD11a antibody being developed by Genentech and Xoma, MLN-02 Antibody (formerly LDP-02), being developed by Genentech and Millenium Pharmaceuticals, HuMax CD4, an anti-CD4 antibody being developed by Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Genmab and Amgen, HuMax-Inflam, being

developed by Genmab and Medarex, HuMax-Cancer, an anti-Heparanase I antibody being developed by Genmab and Medarex and Oxford GcoSciences, HuMax-Lymphoma, being developed by Genmab and Amgen, HuMax-TAC, being developed by Genmab, IDEC-131, and anti-CD40L antibody being developed by IDEC Pharmaceuticals, IDEC-151 (Clenoliximab), an anti-CD4 antibody being developed by IDEC Pharmaceuticals, IDEC-114, an anti-CD80 antibody being developed by IDEC Pharmaceuticals, IDEC-152, an anti-CD23 being developed by IDEC Pharmaceuticals, anti-macrophage migration factor (MIF) antibodies being developed by IDEC Pharmaceuticals, BEC2, an anti-idiotypic antibody being developed by Imclone, IMC-1C11, an anti-KDR antibody being developed by Imclone, DC101, an anti-flk-1 antibody being developed by Imclone, anti-VE cadherin antibodies being developed by Imclone, CEA-Cide™ (labetuzumab), an anti-carcinoembryonic antigen (CEA) antibody being developed by Immunomedics, LymphoCide™ (Epratuzumab), an anti-CD22 antibody being developed by Immunomedics, AFP-Cide, being developed by Immunomedics, MyelomaCide, being developed by Immunomedics, LkoCide, being developed by Immunomedics, ProstaCide, being developed by Immunomedics, MDX-010, an anti-CTLA4 antibody being developed by Medarex, MDX-060, an anti-CD30 antibody being developed by Medarex, MDX-070 being developed by Medarex, MDX-018 being developed by Medarex, Osidem™ (IDM-1), and anti-Her2 antibody being developed by Medarex and Immuno-Designed Molecules, HuMax™-CD4, an anti-CD4 antibody being developed by Medarex and Genmab, HuMax-IL15, an anti-IL15 antibody being developed by Medarex and Genmab, CNTO 148, an anti-TNFα antibody being developed by Medarex and Centocor/J&J, CNTO 1275, an anti-cytokine antibody being developed by Centocor/J&J, MOR101 and MOR102, anti-intercellular adhesion molecule-1 (ICAM-1) (CD54) antibodies being developed by MorphoSys, MOR201, an anti-fibroblast growth factor receptor 3 (FGFR-3) antibody being developed by MorphoSys, Nuvion® (visilizumab), an anti-CD3 antibody being developed by Protein Design Labs, HuZAF™, an anti-gamma interferon antibody being developed by Protein Design Labs, Anti-α5β1 Integrin, being developed by Protein Design Labs, anti-IL-12, being developed by Protein Design Labs, ING-1, an anti-Ep-CAM antibody being developed by Xoma, and MLN01, an anti-Beta2 integrin antibody being developed by Xoma, an pI-ADC antibody being developed by Seattle Genetics, all of the above-cited references in this paragraph are expressly incorporated herein by reference.

[00150] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat *et al.* collected numerous primary sequences of the

variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[00151] 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 the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. 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. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[00152] It should be noted that the sequences depicted herein start at the CH1 region, position 118; the variable regions are not included except as noted. For example, the first amino acid of SEQ ID NO: 2, while designated as position“1” in the sequence listing, corresponds to position 118 of the CH1 region, according to EU numbering.

[00153] Another type of Ig domain of the heavy chain is the hinge region. By “hinge” or “hinge region” or “antibody hinge region” or “immunoglobulin hinge region” herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the “lower hinge” generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[00154] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[00155] Another region of interest for additional substitutions, outlined below, is the Fc region. By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, 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, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[00156] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[00157] Alternatively, the antibodies can be a variety of structures, including, but not limited to, antibody fragments, monoclonal antibodies, multispecific (including bispecific and trispecific) 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. In some embodiments, the antibodies of the present invention are not multispecific antibodies and in some embodiments are not bispecific antibodies.

[00158] In one embodiment, the antibody is an antibody fragment, as long as it contains at least one constant domain which can be pI engineered. 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) 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 (Bird *et al.*, 1988, Science 242:423-426, Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883, entirely incorporated by reference), (iv) "diabodies" or "triabodies", multivalent or

multispecific fragments constructed by gene fusion (Tomlinson et. al., 2000, Methods Enzymol. 326:461-479; WO94/13804; Holliger et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:6444-6448, all entirely incorporated by reference).

[00159] Other antibody fragments that can be used include fragments that contain one or more of the CH1, CH2, CH3, hinge and CL domains of the invention that have been pI engineered. For example, Fc fusions are fusions of the Fc region (CH2 and CH3, optionally with the hinge region) fused to another protein, and are included in the definition of "antibody" for purposes of the inventions described herein. A number of Fc fusions are known the art and can be improved by the addition of the pI variants of the invention. In the present case, antibody fusions can be made comprising CH1; CH1, CH2 and CH3; CH2; CH3; CH2 and CH3; CH1 and CH3, any or all of which can be made optionally with the hinge region, utilizing any combination of pI variants described herein. .

B. Chimeric and Humanized Antibodies

[00160] In some embodiments, the antibody can be a mixture from different species, e.g. 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 rat, in some cases) and the constant region(s) from a human. "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, all entirely incorporated by reference. "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 (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely incorporated 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, entirely incorporated by reference. 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, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen *et al.*, 1988, Science, 239:1534-1536; Queen *et al.*, 1989, Proc Natl Acad Sci, USA 86:10029-33; He *et al.*, 1998, J. Immunol. 160: 1029-1035; Carter *et al.*, 1992, Proc Natl Acad Sci USA 89:4285-9, Presta *et al.*, 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor *et al.*, 1998, Protein Eng 11:321-8, all entirely incorporated by reference. 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, entirely incorporated by reference. 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 USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu *et al.,* 1999, J. Mol. Biol. 294:151-162; Baca *et al.*, 1997, J. Biol. Chem. 272(16):10678-10684; Rosok *et al.,* 1996, J. Biol. Chem. 271(37): 22611-22618; Rader *et al.*, 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss *et al.*, 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan *et al.*, 2002, J. Immunol. 169:1119-1125; De Pascalis *et al.,* 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

[00161] In one embodiment, the antibodies of the invention can be multispecific antibodies, and notably bispecific antibodies, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens, or different epitopes on the same antigen. Diabodies can be manufactured in a variety of ways known in the art (Holliger and Winter, 1993, Current Opinion Biotechnol. 4:446-449, entirely incorporated by reference), e.g., prepared chemically or from hybrid hybridomas. In some cases, multispecific (for example bispecific) antibodies are not preferred.

[00162] In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. Hu *et al.,* 1996, Cancer Res. 56:3055-3061, entirely incorporated by reference. In the present instance, the CH3 domain can be pI engineered. In some cases, the scFv can be joined to the Fc region, and may include some or the entire hinge region.

[00163] The antibodies of the present invention are generally isolated or recombinant. “Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated antibody," refers to an antibody which is substantially free of other antibodies having different antigenic specificities.

[00164] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00165] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[00166] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.

C. pI Variants

[00167] The present invention relates to the generation of pI variants of antibodies. “pI” refers to the isoelectric point of a molecule (including both the individual amino acids

and antibodies) and is the pH at which a particular molecule or surface carries no net electrical charge. In addition, the invention herein sometimes refers to changes in the "charge state" of the proteins at pH 7. That is, wild-type heavy constant region of IgG1 has a charge state of +6, while the heavy constant region of IgG2 has a charge state of 0. Variant 9493 (with a SEQ ID NO: 193 heavy chain constant domain and a SEQ ID NO:117 light chain constant domain) has 12 substitutions in both the heavy and light constant regions resulting in a charge state of -30.

[00168] The present invention relates to the generation of pI variants of antibodies to form "pI antibodies". pI variants are made by introducing amino acid mutations into the parent molecule. "Mutations" in this context are usually amino acid substitutions, although as shown herein, deletions and insertions of amino acids can also be done and thus are defined as mutations.

[00169] By "pI variants" or "isoelectric point variants" or "pI substitutions" or grammatical equivalents thereof herein is meant mutating an amino acid to result in a lower pI at that position. In many embodiments, this means making an amino acid substitution with a lower pI than the original (e.g.wild type) amino acid at the particular position. In some embodiments, this can also mean deleting an amino acid with a high pI (if the structure will tolerate it) or inserting amino acids with lower pIs, for example the low pI "tails" discussed below.

[00170] As shown in Figure 36, the different amino acids have different pIs, although this figure shows the pI of amino acids as individual compositions rather than in the context of a protein, although the trend is identical. pI variants in the context of the invention are made to contribute to the decrease of the pI of the protein, in this case at least the heavy constant domain or the light constant domain of an IgG antibody, or both. An antibody engineered to include one or more of the amino acid mutations outlined herein is sometimes also referred to herein as a "pI antibody".

[00171] In general, "pI variants" refer to the mutation of a higher pI amino acid either via substituting with an amino acid with a lower pI, deleting an amino acid, or inserting low pI amino acids, thus lowering the overall pI of the antibody. (As is noted below, additional non-pI variants are often added to structurally compensate for the pI variants, leading to increased stability, etc.). In the selection of constant domain positions for alteration with a lower pI amino acid, the solvent accessibility of the amino acid is taken into account,

although in general it is not the only factor. That is, based on the known structure of IgG molecules, and as shown in Figure 2, each position will either be fully exposed, fully shielded (e.g. in the interior of the molecule), or partially exposed. This evaluation is shown in Figure 2 as a "fraction exposed" of each residue in the CH1 domain and in Cκ light. In some embodiments, candidate positions for substitution with lower pI amino acids are at least 50% exposed, with exposures of over 60, 70, 80+% finding use in the present invention, as well as those residues that are effectively 100% exposed.

[00172] While not shown, the same calculations can be done for the hinge region, CH2 and CH3 of the heavy chain and the CL domain of the light chain, using standard and commercially available programs to calculate the percentage exposure.

[00173] The lowering of the pI can be done in one of several ways, either replacing a higher pI amino acid (e.g. positive charge state, for example) with a neutral pI, replacing a higher pI amino acid with a lower or low pI amino acid, or replacing a neutral pI amino acid with a low pI amino acid. In some cases, when the structure allows it, deletions or insertions of one or more amino acids can also be done, e.g. deleting a high pI amino acid or inserting one or more low pI amino acids. Thus, for example, an arginine (pI 11.15) can be replaced by lysine (pI 9.59, still high but lower), a more neutral amino acid like glycine or serine, or by low pI variants such as aspartic acid or glutamic acid.

[00174] pI variants are defined as variants by comparison to the starting or parent sequence, which frequently is the wild-type IgG constant domain (either heavy or light or both, as outlined herein). That is, the amino acid at a particular position in the wild-type is referred to as the "native" amino acid, and an amino acid substitution (or deletion or insertion) at this position is referred to as a "non-native" amino acid. For example, many embodiments herein use the IgG1 heavy chain constant region as a parent sequence in which pI mutations are made. Thus, in some embodiments, a "non-native" amino acid is as compared to the IgG1 sequence. For example, at position 119, IgG1 has a serine, and thus the non-native amino acid that can be substituted is glutamic acid. Thus, SEQ ID NO: 193 has a non-native glutamic acid at position 119. Similarly, when starting with IgG2 constant domain(s), the native and non-native amino acids are compared to the wild-type IgG2 sequence.

[00175] As will be appreciated by those in the art, it is possible to make fusions or hybrids from the various IgG molecules. Thus, for example, SEQ ID NO: 28 is a hybrid

IgG1/G2 molecule, and SEQ ID NO: 164 is a hybrid IgG2/G1 molecule. In this context, "non-native" or "non-wild type" substitutions means that the amino acid at the position in question is different from the parent wild-type sequence from whence that position came; that is, if the cross-over point is between amino acids 100 and 101, such that the N-terminus is from IgG1 and the C-terminus is from IgG2, a "non-native" amino acid at position 90 will be compared to the IgG1 sequence. The nomenclature designation herein of "hybrid" in the names, for example of "XENP number 10625, refers to the IgG1/G2 parent (SEQ NO:28). IgG hybrids are disclosed in USSN 11/256,060, for example, the disclosure relating to the creation of the hybrids as well as the rest of the specification being incorporated by reference in its entirety.

[00176] Thus, it is possible to use non-wild type IgG domains, e.g. IgG domains that already have variants, as the starting or parent molecule. In these cases, as above, a substitution will be "non-native" as long as it does not revert back to a wild type sequence.

[00177] In general, the pI variants of the invention are chosen to decrease the positive charge of the pI antibody.

Heavy Chain pI Variants

[00178] In some embodiments, the pI variants are made at least in the CH1 region of the heavy chain domain of an IgG antibody. In this embodiment, the mutations can be independently and optionally selected from position 119, 131, 133, 137, 138, 164, 192, 193, 196, 199, 203, 205, 208, 210,214, 217 and 219. All possible combinations of these 17 positions can be made; e.g. a pI antibody may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 CH1 pI substitutions. In addition, as is described herein, any single or combination CH1 variant(s) can be combined, optionally and individually, with any CH2, CH3, hinge and LC variant(s) as well, as is further described below.

[00179] In addition, the substitution of aspartic acid or glutamic acid at positions 121, 124, 129, 132, 134, 126, 152, 155, 157, 159, 101, 161, 162, 165, 176, 177, 178, 190, 191, 194, 195, 197, 212, 216 and 218 can be made, as shown in Figure 2.

[00180] Specific substitutions that find use in lowering the pI of CH1 domains include, but are not limited to, a non-native glutamic acid at position 119; a non-native cysteine at position 131; a non-native arginine, lysine or glutamine at position 133; a non-native glutamic acid at position 137; a non-native serine at position 138; a non-native glutamic acid at position 164; a non native asparagine at position 192; a non native phenylalanine at

position 193, a non-native lysine at position 196, a non-native threonine at position 199, a non-native aspartic acid at position 203, a non-native glutamic acid or glutamine at position 205, a non native aspartic acid at position 208, a non-native glutamic acid or glutamine at position 210, a non native threonine at position 214, a non native arginine at position 217 and a non-native cysteine at position 219. As is discussed herein, these substitutions can be made individually and in any combination, with preferred combinations shown in the SEQ ID listings and described below. In some cases, only pI substitutions are done in the CH1 domain, and in others, these substitution(s) are added to other pI variants in other domains in any combination.

[00181] In some embodiments, mutations are made in the hinge domain, including positions 221, 222, 223, 224, 225, 233, 234, 235 and 236. It should be noted that changes in 233-236 can be made to increase effector function (along with 327A) in the IgG2 backbone. Thus, pI mutations and particularly substitutions can be made in one or more of positions 221-225, with 1, 2, 3, 4 or 5 mutations finding use in the present invention. Again, all possible combinations are contemplated, alone or with other pI variants in other domains.

[00182] Specific substitutions that find use in lowering the pI of hinge domains include, but are not limited to, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235 and a deletion or a non-native alanine at position 236. Again, as above, these mutations can be made individually and in any combination, with preferred combinations shown in the SEQ ID listings and described below. In some cases, only pI substitutions are done in the hinge domain, and in others, these substitution(s) are added to other pI variants in other domains in any combination.

[00183] In some embodiments, mutations can be made in the CH2 region, including positions 274, 296, 300, 309, 320, 322, 326, 327, 334 and 339. Again, all possible combinations of these 10 positions can be made; e.g. a pI antibody may have 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 CH2 pI substitutions.

[00184] Specific substitutions that find use in lowering the pI of CH2 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327,

a non-native glutamic acid at position 334, a non native threonine at position 339, and all possible combinations within CH2 and with other domains.

[00185] In this embodiment, the mutations can be independently and optionally selected from position 355, 359, 362, 384, 389,392, 397, 418, 419, 444 and 447. All possible combinations of these 11 positions can be made; e.g. a pI antibody may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 CH1 pI mutations. In addition, as is described herein, any single or combination CH3 variant(s) can be combined, optionally and individually, with any CH2, CH1, hinge and LC variant(s) as well, as is further described below.

[00186] Specific substitutions that find use in lowering the pI of CH3 domains include, but are not limited to, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, a non native glutamic acid at position 359, a non native glutamic acid at position 362, a non native glutamic acid at position 389, a non native glutamic acid at position 418, a non native glutamic acid at position 444, and a deletion or non-native aspartic acid at position 447.

[00187] Thus, taken together, any possible combination of the following heavy chain constant domain mutations can be made, with each mutation being optionally included or excluded: a non-native glutamic acid at position 119; a non-native cysteine at position 131; a non-native arginine, lysine or glutamine at position 133; a non-native glutamic acid at position 137; a non-native serine at position 138; a non-native glutamic acid at position 164; a non native asparagine at position 192; a non native phenylalanine at position 193, a non-native lysine at position 196, a non-native threonine at position 199, a non-native aspartic acid at position 203, a non-native glutamic acid or glutamine at position 205, a non native aspartic acid at position 208, a non-native glutamic acid or glutamine at position 210, a non native threonine at position 214, a non native arginine at position 217 and a non-native cysteine at position 219, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, and a deletion at position 235, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at

position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non native threonine at position 339, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, a non native glutamic acid at position 359, a non native glutamic acid at position 362, a non native glutamic acid at position 389, a non native glutamic acid at position 418, a non native glutamic acid at position 444, and a deletion or non-native aspartic acid at position 447.

[00188] Taken together, some embodiments utilize variant heavy chain domains with 0 (when the pI engineering is done in the light constant domain only), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 26, 27, 28 and 29 mutations or higher (as compared to IgG1) can be made, as depicted in Figure 37In addition, as discussed herein, other amino acid substitutions can be done, for example in the Fc region, to alter binding to receptors (e.g. FcγRs and/or FcRn).

<u>Light Chain pI Variants</u>

[00189] In some embodiments, the pI variants are made at least in the light chain domain of an IgG antibody. In this embodiment, the mutations can be independently and optionally selected from positions 126, 145, 152, 156, 169, 199, 202 and 207. All possible combinations of these 8 positions can be made; e.g. a pI antibody may have 1, 2, 3, 4, 5, 6, 7 or light constant domain pI mutations. In addition, as is described herein, any single or combination CL domain mutations can be combined with any heavy chain constant domain pI variants.

[00190] Specific mutations that find use in lowering the pI of light chain constant domains include, but are not limited to, a non-native glutamine or glutamic acid at position 126, a non-native glutamine, glutamic acid or threonine at position 145; a non-native aspartic acid at position 152, a non-native glutamic acid at position 156, a non-native glutamine or glutamic acid at position 169, a non-native glutamic acid at position 199, a non-native glutamic acid at position 202 and a a non-native glutamic acid at position 207.

[00191] Taken together, some embodiments utilize variant light chain domains with 0 (when the pI engineering is done in the heavy constant domain only), 1, 2, 3, 4, 5, 6, or 10 mutations (as compared to Cκ) can be made, as depicted in Figure 37.

<u>Heavy and Light Chain pI Variants</u>

[00192] As is shown in Figure 37, a number of pI antibodies have been generated with heavy and light chain pI variants. As outlined herein and specifically meant to be included in the present invention, any pI engineered heavy chain depicted in Figure 37 and in the sequence listing can be combined with either a wild-type constant light domain or a pI engineered light constant domain. Similarly, an pI engineered light chain constant domain can be combined with either a wild-type constant heavy domain or a pI engineered heavy constant domain, even if not specifically present in Figure 37. That is, the column of "HC names" and "LC names" are meant to form a matrix, with all possible combinations possible.

[00193] Thus, taken together, any possible combination of the following heavy chain constant domain mutations and light chain constant domains can be made, with each mutation being optionally included or excluded: a) heavy chain: a non-native glutamic acid at position 119; a non-native cysteine at position 131; a non-native arginine, lysine or glutamine at position 133; a non-native glutamic acid at position 137; a non-native serine at position 138; a non-native glutamic acid at position 164; a non native asparagine at position 192; a non native phenylalanine at position 193, a non-native lysine at position 196, a non-native threonine at position 199, a non-native aspartic acid at position 203, a non-native glutamic acid or glutamine at position 205, a non native aspartic acid at position 208, a non-native glutamic acid or glutamine at position 210, a non native threonine at position 214, a non native arginine at position 217 and a non-native cysteine at position 219, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, and a deletion at position 235, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non native threonine at position 339, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, a non native glutamic acid at position 359, a non native glutamic acid at position 362, a non native glutamic acid at position 389, a non native glutamic acid at position 418, a non native glutamic acid at position 444, and a deletion or

non-native aspartic acid at position 447; and b) light chain: a non-native glutamine or glutamic acid at position 126, a non-native glutamine, glutamic acid or threonine at position 145; a non-native aspartic acid at position 152, a non-native glutamic acid at position 156, a non-native glutamine or glutamic acid at position 169, a non-native glutamic acid at position 199, a non-native glutamic acid at position 202 and a a non-native glutamic acid at position 207.

[00194] Similarly, the number of mutations that can be generated in suitable pairs of heavy and light constant domains are shown in Figure 37 ("total # of mutations" column), ranging from 1 to 40.

III. Other Amino Acid Substitutions

[00195] As will be appreciated by those in the art, the pI antibodies of the invention can contain additional amino acid substitutions in addition to the pI variants.

[00196] In some embodiments, amino acid substitutions are imported from one isotype into the pI antibody despite either a neutrality of charge state or even an increase of charge state, so as to accommodate the pI variants. These are sometimes referred to as "non-pI isotypic variants". For example, the replacement of the native lysine at position 133 of IgG1 with an arginine from IgG2 is such a change, as is the replacement of the native glutamine in IgG1 at position 196 with the IgG2 lysine, the replacement of native IgG1 proline at position 217 with the IgG2 arginine, etc. It should be noted in this instance that as described above, pI variants can be made at position 133 as well, substituting non-native glutamic acid or glutamine at position 133.

[00197] In the hinge region (positions 233-236), changes can be made to increase effector function. That is, IgG2 has lowered effector function, and as a result, amino acid substitutions at these positions from PVA(deletion) can be changed to ELLG, and an additional G327A variant generated as well.

[00198] In the CH3 region, a mutation at position 384 can be made, for example substituting a non-native serine.

[00199] Additional mutations that can be made include adding either N-terminal or C-terminal (depending on the structure of the antibody or fusion protein) "tails" or sequences of one or more low pI amino acids; for example, glutamic acids and aspartic acids can be added to the CH3 C-terminus; generally, from 1 to 5 amino acids are added.

Properties of the pI Antibodies of the Invention

[00200] The pI antibodies of the present invention display decreased pIs. In general, decreases of at least 0.5 log (e.g. corresponding to half a pH point) are seen, with decreases of at least about 1, 1.5, 2, 2.5 and 3 finding particular use in the invention. The pI can be either calculated or determined experimentally, as is well known in the art. In addition, it appears that pI antibodies with pIs ranging from 5. to 5.5 to 6 exhibit good extended serum half lives. As will be appreciated by those in the art and depicted in Figure 30, pIs lower than this are difficult to achieve, as more and more mutations are required and the physical limits are reached.

[00201] The pI antibodies of the present invention display increased serum half life. As shown in the Figures, surprisingly, every tested pI antibody has exhibited an increase in half life as compared to the starting molecule. While half-life is affected by a number of factors, including the Fv portion, increases of 25, 50, 75, 100, 150, 200 and 250% or more can be obtained using the pI antibodies of the present invention. As shown in Figure 34, pI variants can increase half-life from around 4 days to over 15.

[00202] In addition, some variants herein are generated to increase stability. As noted herein, a number of properties of antibodies affect the clearance rate (e.g. stability for half-life) in vivo. In addition to antibody binding to the FcRn receptor, other factors that contribute to clearance and half-life are serum aggregation, enzymatic degradation in the serum, inherent immunogenicity of the antibody leading to clearing by the immune system, antigen-mediated uptake, FcR (non-FcRn) mediated uptake and non-serum distribution (e.g. in different tissue compartments).

[00203] Accordingly, some additional amino acid substitutions can be made that effect one or more of these properties. As shown in Figure 37, this include, but are not limited to, 222K, 274K, 296Y, 300Y, 339A, 355R, 384N, 392K, 397V, 419Q, 296Y/300Y, 384N/392K/397V, 137G, 138G, 192S, 193L, 199I, 203N, 214K, 137G/138G, 192S/193G, 199I/203N, 214K/222K, 138G/192S/193L and 137G/138G/192S/193L.

IV. Optional and Additional Fc Engineering

FcRn Modifications

[00204] In some embodiments, the pI variants of the present invention can be combined with amino acid substitutions in the FcRn binding domain. Surprisingly, the present invention shows that pI variants can be independently and optionally combined with

Fc variants that result in both higher binding to the FcRn receptor as well as increased half-lives.

[00205] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless other wise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. In some cases, the FcRn variants bind to the human FcRn receptor, or it may be desirable to design variants that bind to rodent or primate receptors in addition, to facilitate clinical trials.

[00206] A variety of such substitutions are known and described in USSN 12/341,769, hereby incorporated by reference in its entirety but especially for the disclosure of variants that increase binding to FcRn and/or increase in vivo half life. In some embodiments, a pI antibody can be engineered to include any of the following substitutions, alone or in any combination: 436I, 436V, 311I, 311V, 428L, 434S, 428L/434S, 259I, 308F, 259I/308F, 259I/308F/428L, 307Q/434S, 434A, 434H, 250Q/428L, M252Y/S254T/T256E, 307Q/434A, 307Q//380A/434A, and 308P/434A. Numbering is EU as in Kabat, and it is understood that the substitution is non-native to the starting molecule. As has been shown previously, these FcRn substitutions work in IgG1, IgG2 and IgG1/G2 hybrid backbones, and are specifically included for IgG3 and IgG4 backbones and derivatives of any IgG isoform as well.

[00207] In some embodiments, it is also possible to do pI engineering on variable regions, either framework or CDRs, as is generally described in US Publication 2011/0076275, expressly incorporated herein by reference.

[00208] In other embodiments, no pI variants are made in the variable region(s) of the antibodies, e.g. no amino acid substitutions are made that purposefully decrease the pI of the amino acid at a position, nor of the total protein. This is to be distinguished from affinity maturation substitutions in the variable region(s) that are made to increase binding affinity of the antibody to its antigen but may result in a lower pI amino acid being added. That is, a pI variant in the variable region(s) is generally significantly "silent" with respect to binding affinity.

<u>Fc engineering</u>

[00209] In addition to substitutions made to increase binding affinity to FcRn and/or increase serum half life, other substitutions can be made in the Fc region, in general for altering binding to FcγR receptors.

[00210] By "Fc gamma receptor", "FcγR" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. 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) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), 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-1 (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00211] There are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495, 11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L and 299T.

V. Other Antibody Modifications

<u>Affinity Maturation</u>

[00212] In some embodiments, one or more amino acid modifications are made in one or more of the CDRs of the antibody. In general, only 1 or 2 or 3amino acids are substituted

in any single CDR, and generally no more than from 4, 5, 6, 7, 8 9 or 10 changes are made within a set of CDRs. However, it should be appreciated that any combination of no substitutions, 1, 2 or 3 substitutions in any CDR can be independently and optionally combined with any other substitution.

[00213] In some cases, amino acid modifications in the CDRs are referred to as "affinity maturation". An "affinity matured" antibody is one having one or more alteration(s) in one or more CDRs which results in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some cases, although rare, it may be desirable to decrease the affinity of an antibody to its antigen, but this is generally not preferred.

[00214] Affinity maturation can be done to increase the binding affinity of the antibody for the antigen by at least about 10% to 50-100-150% or more, or from 1 to 5 fold as compared to the "parent" antibody. Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by known procedures. See, for example, Marks et al., 1992, Biotechnology 10:779-783 that describes affinity maturation by variable heavy chain (VH) and variable light chain (VL) domain shuffling. Random mutagenesis of CDR and/or framework residues is described in: Barbas, et al. 1994, Proc. Nat. Acad. Sci, USA 91:3809-3813; Shier et al., 1995, Gene 169:147-155; Yelton et al., 1995, J. Immunol. 155:1994-2004; Jackson et al., 1995, J. Immunol. 154(7):3310-9; and Hawkins et al, 1992, J. Mol. Biol. 226:889-896, for example.

[00215] Alternatively, amino acid modifications can be made in one or more of the CDRs of the antibodies of the invention that are "silent", e.g. that do not significantly alter the affinity of the antibody for the antigen. These can be made for a number of reasons, including optimizing expression (as can be done for the nucleic acids encoding the antibodies of the invention).

[00216] Thus, included within the definition of the CDRs and antibodies of the invention are variant CDRs and antibodies; that is, the antibodies of the invention can include amino acid modifications in one or more of the CDRs of Ab79 and Ab19. In addition, as outlined below, amino acid modifications can also independently and optionally be made in any region outside the CDRs, including framework and constant regions.

<u>ADC Modifications</u>

[00217] In some embodiments, the pI antibodies of the invention are conjugated with drugs to form antibody-drug conjugates (ADCs). In general, ADCs are used in oncology applications, where the use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents allows for the targeted delivery of the drug moiety to tumors, which can allow higher efficacy, lower toxicity, etc. An overview of this technology is provided in Ducry et al., Bioconjugate Chem., 21:5-13 (2010), Carter et al., Cancer J. 14(3):154 (2008) and Senter, Current Opin. Chem. Biol. 13:235-244 (2009), all of which are hereby incorporated by reference in their entirety

[00218] Thus the invention provides pI antibodies conjugated to drugs. Generally, conjugation is done by covalent attachment to the antibody, as further described below, and generally relies on a linker, often a peptide linkage (which, as described below, may be designed to be sensitive to cleavage by proteases at the target site or not). In addition, as described above, linkage of the linker-drug unit (LU-D) can be done by attachment to cysteines within the antibody. As will be appreciated by those in the art, the number of drug moieties per antibody can change, depending on the conditions of the reaction, and can vary from 1:1 to 10:1 drug:antibody. As will be appreciated by those in the art, the actual number is an average.

[00219] Thus the invention provides pI antibodies conjugated to drugs. As described below, the drug of the ADC can be any number of agents, including but not limited to cytotoxic agents such as chemotherapeutic agents, growth inhibitory agents, toxins (for example, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (that is, a radioconjugate) are provided. In other embodiments, the invention further provides methods of using the ADCs.

[00220] Drugs for use in the present invention include cytotoxic drugs, particularly those which are used for cancer therapy. Such drugs include, in general, DNA damaging agents, anti-metabolites, natural products and their analogs. Exemplary classes of cytotoxic agents include the enzyme inhibitors such as dihydrofolate reductase inhibitors, and thymidylate synthase inhibitors, DNA intercalators, DNA cleavers, topoisomerase inhibitors, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, diynenes, the podophyllotoxins, dolastatins, maytansinoids, differentiation inducers, and taxols.

[00221] Members of these classes include, for example, methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, melphalan, leurosine, leurosideine, actinomycin, daunorubicin, doxorubicin, mitomycin C, mitomycin A, caminomycin, aminopterin, tallysomycin, podophyllotoxin and podophyllotoxin derivatives such as etoposide or etoposide phosphate, vinblastine, vincristine, vindesine, taxanes including taxol, taxotere retinoic acid, butyric acid, N8-acetyl spermidine, camptothecin, calicheamicin, esperamicin, ene-diynes, duocarmycin A, duocarmycin SA, calicheamicin, camptothecin, maytansinoids (including DM1), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), and maytansinoids (DM4) and their analogues.

[00222] Toxins may be used as antibody-toxin conjugates and include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) J. Nat. Cancer Inst. 92(19):1573-1581; Mandler et al (2000) Bioorganic & Med. Chem. Letters 10:1025-1028; Mandler et al (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342). Toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[00223] Conjugates of an pI antibody and one or more small molecule toxins, such as a maytansinoids, dolastatins, auristatins, a trichothecene, calicheamicin, and CC1065, and the derivatives of these toxins that have toxin activity, are contemplated.

[00224] Maytansinoids

[00225] Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. As described below, drugs may be modified by the incorporation of a functionally active group such as a thiol or amine group for conjugation to the antibody.

[00226] Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared by demethylation using Streptomyces or Actinomyces or dechlorination using LAH); and C-20-demethoxy, C-20-

acyloxy (--OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared by acylation using acyl chlorides) and those having modifications at other positions

[00227] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared by the reaction of maytansinol with H2S or P2S5); C-14-alkoxymethyl(demethoxy/CH2OR) (U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH2OH or CH2OAc) (U.S. Pat. No. 4,450,254) (prepared from Nocardia); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared by the conversion of maytansinol by Streptomyces); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (isolated from Trewia nudlflora); C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

[00228] Of particular use are DM1 (disclosed in US Patent No. 5,208,020, incorporated by reference) and DM4 (disclosed in US Patent No. 7,276,497, incorporated by reference). See also a number of additional maytansinoid derivatives and methods in 5,416,064, WO/01/24763, 7,303,749, 7,601,354, USSN 12/631,508, WO02/098883, 6,441,163, 7,368,565, WO02/16368 and WO04/1033272, all of which are expressly incorporated by reference in their entirety.

[00229] ADCs containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described ADCs comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay.

[00230] Chari et al., Cancer Research 52:127-131 (1992) describe ADCs in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansonoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3x105 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar

to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

[00231] Auristatins and Dolastatins

[00232] In some embodiments, the ADC comprises an pI antibody conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (U.S. Pat. Nos. 5,635,483; 5,780,588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al (1998) Antimicrob. Agents Chemother. 42:2961-2965). The dolastatin or auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172).

[00233] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented Mar. 28, 2004 and described in United States Patent Publication No. 2005/0238648, the disclosure of which is expressly incorporated by reference in its entirety.

[00234] An exemplary auristatin embodiment is MMAE (shown in Figure 10 wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate; see US Patent No. 6,884,869 expressly incorporated by reference in its entirety).

[00235] Another exemplary auristatin embodiment is MMAF, shown in Figure 10 wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate (US 2005/0238649, 5,767,237 and 6,124,431, expressly incorporated by reference in their entirety):

[00236] Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):

[00237] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schroder and K. Lubke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in

the field of peptide chemistry. The auristatin/dolastatin drug moieties may be prepared according to the methods of: U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit et al (1998) Anti-Cancer Drug Design 13:243-277; Pettit, G. R., et al. Synthesis, 1996, 719-725; Pettit et al (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863; and Doronina (2003) Nat Biotechnol 21(7):778-784.

[00238] Calicheamicin

[00239] In other embodiments, the ADC comprises an antibody of the invention conjugated to one or more calicheamicin molecules. For example, Mylotarg is the first commercial ADC drug and utilizes calicheamicin γ1 as the payload (see US Patent No. 4,970,198, incorporated by reference in its entirety). Additional calicheamicin derivatives are described in US Patent Nos. 5,264,586, 5,384,412, 5,550,246, 5,739,116, 5,773,001, 5,767,285 and 5,877,296, all expressly incorporated by reference. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ1I, α2I, α2I, N-acetyl- γ1I, PSAG and θI1 (Hinman et al., Cancer Research 53:3336-3342 (1993), Lode et al., Cancer Research 58:2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

[00240] Duocarmycins

CC-1065 (see 4,169,888, incorporated by reference) and duocarmycins are members of a family of antitumor antibiotics utilized in ADCs. These antibiotics appear to work through sequence-selectively alkylating DNA at the N3 of adenine in the minor groove, which initiates a cascade of events that result in apoptosis.

[00241] Important members of the duocarmycins include duocarmycin A (US Patent No. 4,923,990, incorporated by reference) and duocarmycin SA (U.S. Pat. No. 5,101,038, incorporated by reference), and a large number of analogues as described in US Patent Nos. 7,517,903, 7,691,962, 5,101,038; 5,641,780; 5,187,186; 5,070,092; 5,070,092; 5,641,780;

5,101,038; 5,084,468, 5,475,092, 5,585,499, 5,846,545, WO2007/089149, WO2009/017394A1, 5,703,080, 6,989,452, 7,087,600, 7,129,261, 7,498,302, and 7,507,420, all of which are expressly incorporated by reference.

VI. Other Cytotoxic Agents

[00242] Other antitumor agents that can be conjugated to the antibodies of the invention include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Pat. Nos. 5,053,394, 5,770,710, as well as esperamicins (U.S. Pat. No. 5,877,296).

[00243] Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published Oct. 28, 1993.

[00244] The present invention further contemplates an ADC formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[00245] For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu.

[00246] The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as Tc99m or I123, Re186, Re188 and In111 can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate Iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[00247] For compositions comprising a plurality of antibodies, the drug loading is represented by p, the average number of drug molecules per Antibody. Drug loading may

range from 1 to 20 drugs (D) per Antibody. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Antibody-Drug-Conjugates in terms of p may also be determined.

[00248] In some instances, separation, purification, and characterization of homogeneous Antibody-Drug-conjugates where p is a certain value from Antibody-Drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. In exemplary embodiments, p is 2, 3, 4, 5, 6, 7, or 8 or a fraction thereof.

[00249] The generation of Antibody-drug conjugate compounds can be accomplished by any technique known to the skilled artisan. Briefly, the Antibody-drug conjugate compounds can include an pI antibody as the Antibody unit, a drug, and optionally a linker that joins the drug and the binding agent.

[00250] A number of different reactions are available for covalent attachment of drugs and/or linkers to binding agents. This is can be accomplished by reaction of the amino acid residues of the binding agent, for example, antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic and aspartic acid, the sulfhydryl groups of cysteine and the various moieties of the aromatic amino acids. A commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of an antibody molecule.

[00251] Also available for attachment of drugs to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a Schiff base with amino groups of the binding agent. Isothiocyanates can also be used as coupling agents for covalently attaching drugs to binding agents. Other techniques are known to the skilled artisan and within the scope of the present invention.

[00252] In some embodiments, an intermediate, which is the precursor of the linker, is reacted with the drug under appropriate conditions. In other embodiments, reactive groups are used on the drug and/or the intermediate. The product of the reaction between the drug and

the intermediate, or the derivatized drug, is subsequently reacted with an pI antibody of the invention under appropriate conditions.

[00253] It will be understood that chemical modifications may also be made to the desired compound in order to make reactions of that compound more convenient for purposes of preparing conjugates of the invention. For example a functional group e.g. amine, hydroxyl, or sulfhydryl, may be appended to the drug at a position which has minimal or an acceptable effect on the activity or other properties of the drug

VII. Linker Units

[00254] Typically, the antibody-drug conjugate compounds comprise a Linker unit between the drug unit and the antibody unit. In some embodiments, the linker is cleavable under intracellular or extracellular conditions, such that cleavage of the linker releases the drug unit from the antibody in the appropriate environment. For example, solid tumors that secrete certain proteases may serve as the target of the cleavable linker; in other embodiments, it is the intracellular proteases that are utilized. In yet other embodiments, the linker unit is not cleavable and the drug is released, for example, by antibody degradation in lysosomes.

[00255] In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long or more.

[00256] Cleaving agents can include,without limitation, cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, e.g., Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). Peptidyl linkers that are cleavable by enzymes that are present in CD38-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a Phe-Leu or a Gly-Phe-Leu-Gly linker (SEQ ID NO: X)). Other examples of such linkers are described, e.g., in U.S. Pat. No. 6,214,345, incorporated herein by reference in its entirety and for all purposes.

[00257] In some embodiments, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker or a Phe-Lys linker (see, e.g., U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker).

[00258] In other embodiments, the cleavable linker is pH-sensitive, that is, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) may be used. (See, e.g., U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Pat. No. 5,622,929).

[00259] In yet other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT. (See, e.g., Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Pat. No. 4,880,935.)

[00260] In other embodiments, the linker is a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

[00261] In yet other embodiments, the linker unit is not cleavable and the drug is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety and for all purposes).

[00262] In many embodiments, the linker is self-immolative. As used herein, the term "self-immolative Spacer" refers to a bifunctional chemical moiety that is capable of

covalently linking together two spaced chemical moieties into a stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved. See for example, WO 2007059404A2, WO06110476A2, WO05112919A2, WO2010/062171, WO09/017394, WO07/089149, WO 07/018431, WO04/043493 and WO02/083180, which are directed to drug-cleavable substrate conjugates where the drug and cleavable substrate are optionally linked through a self-immolative linker and which are all expressly incorporated by reference.

[00263] Often the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, 15%, 10%, 5%, 3%, or no more than about 1% of the linkers, in a sample of antibody-drug conjugate compound, are cleaved when the antibody-drug conjugate compound presents in an extracellular environment (for example, in plasma).

[00264] Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating with plasma the antibody-drug conjugate compound for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free drug present in the plasma.

[00265] In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the therapeutic agent (that is, in the milieu of the linker-therapeutic agent moiety of the antibody-drug conjugate compound as described herein). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the auristatin compound and the pI antibodies of the invention.

[00266] A variety of exemplary linkers that can be used with the present compositions and methods are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317 (each of which is incorporated by reference herein in its entirety and for all purposes).

VIII. Drug Loading

[00267] Drug loading is represented by p and is the average number of Drug moieties per antibody in a molecule. Drug loading (“p”) may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more moieties (D) per antibody, although frequently the average number is a fraction or a decimal. Generally, drug loading of from 1 to 4 is frequently useful,

and from 1 to 2 is also useful. ADCs of the invention include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy and, ELISA assay.

[00268] The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as electrophoresis.

[00269] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in the exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. p>5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the drug loading for an ADC of the invention ranges from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8; from about 3.2 to about 3.7; from about 3.2 to about 3.6; from about 3.3 to about 3.8; or from about 3.3 to about 3.7. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5. See US 2005-0238649 A1 (herein incorporated by reference in its entirety).

[00270] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[00271] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, e.g., by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number and/or position of linker-drug attachements (such as thioMab or thioFab prepared as disclosed herein and in WO2006/034488 (herein incorporated by reference in its entirety)).

[00272] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography.

[00273] In some embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Methods of Determining Cytotoxic Effect of ADCs

[00274] Methods of determining whether a Drug or Antibody-Drug conjugate exerts a cytostatic and/or cytotoxic effect on a cell are known. Generally, the cytotoxic or cytostatic activity of an Antibody Drug conjugate can be measured by: exposing mammalian cells expressing a target protein of the Antibody Drug conjugate in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based in vitro assays can be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the Antibody Drug conjugate.

[00275] For determining whether an Antibody Drug conjugate exerts a cytostatic effect, a thymidine incorporation assay may be used. For example, cancer cells expressing a target antigen at a density of 5,000 cells/well of a 96-well plated can be cultured for a 72-hour period and exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of the 72-hour period. The incorporation of ^{3}H-thymidine into cells of the culture is measured in the presence and absence of the Antibody Drug conjugate.

[00276] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) can be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on cancer cells indicates that an Antibody Drug conjugate is useful in the treatment of cancers.

[00277] Cell viability can be measured by determining in a cell the uptake of a dye such as neutral red, trypan blue, or ALAMAR™ blue (see, e.g., Page *et al*., 1993, Intl. J. Oncology 3:473-476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytoxicity (Skehan et al., 1990, J. Natl. Cancer Inst. 82:1107-12).

[00278] Alternatively, a tetrazolium salt, such as MTT, is used in a quantitative colorimetric assay for mammalian cell survival and proliferation by detecting living, but not dead, cells (see, e.g., Mosmann, 1983, J. Immunol. Methods 65:55-63).

[00279] Apoptosis can be quantitated by measuring, for example, DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica, 1999, no. 2, pp. 34-37 (Roche Molecular Biochemicals).

[00280] Apoptosis can also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al. eds., 1992, pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage.

[00281] The presence of apoptotic cells can be measured in both the attached and "floating" compartments of the cultures. For example, both compartments can be collected by

removing the supernatant, trypsinizing the attached cells, combining the preparations following a centrifugation wash step (e.g., 10 minutes at 2000 rpm), and detecting apoptosis (e.g., by measuring DNA fragmentation). (See, e.g., Piazza et al., 1995, Cancer Research 55:3110-16).

[00282] *In vivo*, the effect of a therapeutic composition of the pI antibody of the invention can be evaluated in a suitable animal model. For example, xenogenic cancer models can be used, wherein cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Klein *et al.*, 1997, Nature Medicine 3: 402-408). Efficacy can be measured using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

[00283] The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

[00284] Glycosylation

[00285] Another type of covalent modification is alterations in glycosylation. In another embodiment, the antibodies disclosed herein can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to the antibody, wherein said carbohydrate composition differs chemically from that of a parent antibody. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. A preferred form of engineered glycoform is afucosylation, which has been shown to be correlated to an increase in ADCC function, presumably through tighter binding to the FcγRIIIa receptor. In this context, "afucosylation" means that the majority of the antibody produced in the host cells is substantially devoid of fucose, e.g. 90-95-98% of the generated antibodies do not have appreciable fucose as a component of the carbohydrate moiety of the antibody (generally attached at N297 in the Fc region). Defined functionally,

afucosylated antibodies generally exhibit at least a 50% or higher affinity to the FcγRIIIa receptor.

[00286] Engineered glycoforms may be generated by a variety of methods known in the art (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; US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1, all entirely incorporated by reference; (Potelligent® technology [Biowa, Inc., Princeton, NJ]; GlycoMAb® glycosylation engineering technology [Glycart Biotechnology AG, Zürich, Switzerland]). Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells, by regulating enzymes involved in the glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4- N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed. For example, the "sugar engineered antibody" or "SEA technology" of Seattle Genetics functions by adding modified saccharides that inhibit fucosylation during production; see for example 20090317869, hereby incorporated by reference in its entirety. Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an antibody can include an engineered glycoform.

[00287] Alternatively, engineered glycoform may refer to the IgG variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[00288] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose,

or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[00289] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[00290] Another means of increasing the number of carbohydrate moieties on the antibody is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306, both entirely incorporated by reference.

[00291] Removal of carbohydrate moieties present on the starting antibody (e.g. post-translationally) may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin *et al.*, 1987, Arch. Biochem. Biophys. 259:52 and by Edge *et al.*, 1981, Anal. Biochem. 118:131, both entirely incorporated by reference. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, 1987, Meth. Enzymol. 138:350, entirely incorporated by reference. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.*, 1982, J. Biol. Chem. 257:3105, entirely incorporated by reference. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[00292] Another type of covalent modification of the antibody comprises linking the antibody to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in, for example, 2005-2006 PEG Catalog from Nektar Therapeutics (available at the Nektar website) US Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337, all entirely incorporated by reference. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037A1, entirely incorporated by reference.

Nucleic Acids and Host Cells

[00293] Included within the invention are the nucleic acids encoding the pI antibodies of the invention. In the case where both a heavy and light chain constant domains are included in the pI antibody, generally these are made using nucleic acids encoding each, that are combined into standard host cells (e.g. CHO cells, etc.) to produce the tetrameric structure of the antibody. If only one pI engineered constant domain is being made, only a single nucleic acid will be used.

IX. Antibody Compositions for In Vivo Administration

[00294] The use of the pI antibodies of the invention in therapy will depend on the antigen binding component; e.g. in the case of full length standard therapeutic antibodies, on the antigen to which the antibody's Fv binds. That is, as will be appreciated by those in the art, the treatment of specific diseases can be done with the additional benefit of increased half life of the molecule. This can result in a variety of benefits, including, but not limited to, less frequent dosing (which can lead to better patient compliance), lower dosing, and lower production costs.

[00295] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody 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. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or

benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00296] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to provide antibodies with other specifcities. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or small molecule antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00297] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[00298] The formulations to be used for in vivo administration should be sterile, or nearly so. This is readily accomplished by filtration through sterile filtration membranes.

[00299] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as

the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[00300] When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

X. Administrative modalities

[00301] The antibodies and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

XI. Treatment modalities

[00302] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[00303] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI)

scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[00304] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[00305] Thus for B cell tumors, the subject may experience a decrease in the so-called B symptoms, i.e., night sweats, fever, weight loss, and/or urticaria. For pre-malignant conditions, therapy with an pI therapeutic agent may block and/or prolong the time before development of a related malignant condition, for example, development of multiple myeloma in subjects suffering from monoclonal gammopathy of undetermined significance (MGUS).

[00306] An improvement in the disease may be characterized as a complete response. By "complete response" is intended an absence of clinically detectable disease with normalization of any previously abnormal radiographic studies, bone marrow, and cerebrospinal fluid (CSF) or abnormal monoclonal protein in the case of myeloma.

[00307] Such a response may persist for at least 4 to 8 weeks, or sometimes 6 to 8 weeks, following treatment according to the methods of the invention. Alternatively, an improvement in the disease may be categorized as being a partial response. By "partial response" is intended at least about a 50% decrease in all measurable tumor burden (i.e., the number of malignant cells present in the subject, or the measured bulk of tumor masses or the quantity of abnormal monoclonal protein) in the absence of new lesions, which may persist for 4 to 8 weeks, or 6 to 8 weeks.

[00308] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[00309] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[00310] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[00311] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00312] In another embodiment, the reduced pI variants of the invention can be utilized for intraocular/intravitreal administration of antibody against a variety of targets, including but not limited to VEGF, Ang-2, and the compliment C3 and C5 protein (or their cleavage products C3a and C5a). Due to the near-neutral pH of the eye, coupled with the high initial concentrations of injected therapeutic antibodies, there is a general risk of low solubility when the pI of the antibody approaches that of the pH in the ocular environment. The low pI variants of the invention can therefore be applied to reduce the pI of administered antibodies well below neutral , thus promoting high solubility upon administration.

[00313] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00314] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00315] The efficient dosages and the dosage regimens for the pI antibodies used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[00316] An exemplary, non-limiting range for a therapeutically effective amount of an pI antibody used in the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, or about 3 mg/kg. In another embodiment, he antibody is administered in a dose of 1 mg/kg or more, such as a dose of from 1 to 20 mg/kg, e.g. a dose of from 5 to 20 mg/kg, e.g. a dose of 8 mg/kg.

[00317] A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician or a veterinarian could start doses of the medicament employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[00318] In one embodiment, the pI antibody is administered by infusion in a weekly dosage of from 10 to 500 mg/kg such as of from 200 to 400 mg/kg Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours.

[00319] In one embodiment, the pI antibody is administered by slow continuous infusion over a long period, such as more than 24 hours, if required to reduce side effects including toxicity.

[00320] In one embodiment the pI antibody is administered in a weekly dosage of from 250 mg to 2000 mg, such as for example 300 mg, 500 mg, 700 mg, 1000 mg, 1500 mg or 2000 mg, for up to 8 times, such as from 4 to 6 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months. The dosage may be determined or adjusted by measuring the amount of compound of the present invention in the blood upon administration by for instance taking out a biological sample and using anti-idiotypic antibodies which target the antigen binding region of the pI antibody.

[00321] In a further embodiment, the pI antibody is administered once weekly for 2 to 12 weeks, such as for 3 to 10 weeks, such as for 4 to 8 weeks.

[00322] In one embodiment, the pI antibody is administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

[00323] In one embodiment, the pI antibody is administered by a regimen including one infusion of an pI antibody followed by an infusion of an pI antibody conjugated to a radioisotope. The regimen may be repeated, e.g., 7 to 9 days later.

[00324] As non-limiting examples, treatment according to the present invention may be provided as a daily dosage of an antibody in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

[00325] In some embodiments the pI antibody molecule thereof is used in combination with one or more additional therapeutic agents, e.g. a chemotherapeutic agent. Non-limiting examples of DNA damaging chemotherapeutic agents include topoisomerase I inhibitors (*e.g.*, irinotecan, topotecan, camptothecin and analogs or metabolites thereof, and doxorubicin); topoisomerase II inhibitors (*e.g.*, etoposide, teniposide, and daunorubicin); alkylating agents (*e.g.*, melphalan, chlorambucil, busulfan, thiotepa, ifosfamide, carmustine, lomustine, semustine, streptozocin, decarbazine, methotrexate, mitomycin C, and cyclophosphamide); DNA intercalators (*e.g.*, cisplatin, oxaliplatin, and carboplatin); DNA intercalators and free radical generators such as bleomycin; and nucleoside mimetics (*e.g.*, 5-fluorouracil, capecitibine, gemcitabine, fludarabine, cytarabine, mercaptopurine, thioguanine, pentostatin, and hydroxyurea).

[00326] Chemotherapeutic agents that disrupt cell replication include: paclitaxel, docetaxel, and related analogs; vincristine, vinblastin, and related analogs; thalidomide, lenalidomide, and related analogs (*e.g.*, CC-5013 and CC-4047); protein tyrosine kinase inhibitors (*e.g.*, imatinib mesylate and gefitinib); proteasome inhibitors (*e.g.*, bortezomib); NF-κB inhibitors, including inhibitors of IκB kinase; antibodies which bind to proteins overexpressed in cancers and thereby downregulate cell replication (e.g., trastuzumab, rituximab, cetuximab, and bevacizumab); and other inhibitors of proteins or enzymes known

to be upregulated, over-expressed or activated in cancers, the inhibition of which downregulates cell replication.

[00327] In some embodiments, the antibodies of the invention can be used prior to, concurrent with, or after treatment with Velcade® (bortezomib).

EXAMPLES

[00328] 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. For all constant region positions discussed in the present invention, numbering is 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, entirely incorporated by reference). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

[00329] EXAMPLE 1. Design of non-native charge substitutions to reduce pI

[00330] Antibody constant chains were modified with lower pI by engineering substitutions in the constant domains. Reduced pI can be engineered by making substitutions of basic amino acids (K or R) to acidic amino acids (D or E), which result in the largest decrease in pI. Mutations of basic amino acids to neutral amino acids and neutral amino acids to acidic amino acids will also result in a decrease in pI. A list of amino acid pK values can be found in Table 1 of Bjellqvist et al., 1994, Electrophoresis 15:529-539.

[00331] We chose to explore substitutions in the antibody CH1 (Cγ1) and CL (Ckappa or CK) regions (sequences are shown in Figure 1) because, unlike the Fc region, they do not interact with native ligands that impact the antibody's pharmacological properties. In deciding which positions to mutate, the surrounding environment and number of contacts the WT amino acid makes with its neighbors was taken into account such as to minimize the impact of a substitution or set of substitutions on structure and/or function. The solvent accessibility or fraction exposed of each CH1 and CK position was calculated using relevant

crystal structures of antibody Fab domains. The results are shown in Figures 2 and 3 for the Cγ1 and CK respectively. Design was guided further by examining the CH1 and CL domains for positions that are isotypic between the immunoglobulin isotypes (IgG1, IgG2, IgG3, and IgG4). Because such variations occur naturally, such position are expected to be amenable to substitution. Based on this analysis, a number of substitutions were identified that reduce pI but are predicted to have minimal impact on the biophysical properties of the domains.

[00332] EXAMPLE 2. Anti-VEGF antibodies with engineered CH1 and CK regions having lower pI

[00333] Amino acid modifications were engineered in the CH1 and CK domains of an IgG1 antibody to lower the pI of the antibody. Based on the above analysis, chosen substitutions for the heavy chain CH1 were 119E, 133E, 164E, 205E, 208D, and 210E, and substitutions for the light chain Cκ substitutions were 126E, 145E, 152D, 156E, 169E, and 202E. These variant constant chains are referred to as IgG1-CH1-pI(6) and CK-pI(6) respectively, and their amino acid sequences are provided in Figure 4.

[00334] CH1 and CK variants were engineered in the context of an antibody targeting vascular endothelial factor (VEGF). The heavy and light chain variable regions (VH and VL) are those of a humanized version of the antibody A4.6.1, also referred to as bevacizumab (Avastin®), which is approved for the treatment of a variety of cancers. These variable region sequences are provided in Figure 5. The anti-VEGF antibody variant containing the low pI substitutions is referred to as XENP9493 Bevacizumab-IgG1-CH1-pI(6)-CK-pI(6), and the amino acid sequences of the heavy and light chains of this antibody are provided in Figure 6. A structural model of the Fab domain showing the 6 substitutions of CH1-pI(6) and the 6 substitutions of CK-pI(6) is shown in Figure 7. The calculated pI of WT anti-VEGF (bevacizumab) is 8.14. The calculated pI of the engineered anti-VEGF CH1 variant is 6.33 and that of the anti-VEGF CK variant is 6.22. When the heavy chain and light chain pI engineered anti-VEGF variants are co-transfected, the full-length anti-VEGF mAb has a calculated pI of 5.51.

[00335] Genes encoding the heavy and light chains of the anti-VEGF antibodies were constructed in the mammalian expression vector pTT5. The human IgG1 constant chain gene was obtained from IMAGE clones and subcloned into the pTT5 vector. VH and VL genes encoding the anti-VEGF antibodies were synthesized commercially (Blue Heron Biotechnologies, Bothell WA), and subcloned into the vectors encoding the appropriate CL

and IgG1 constant chains. Amino acid modifications were constructed using site-directed mutagenesis using the QuikChange® site-directed mutagenesis methods (Stratagene, La Jolla CA). All DNA was sequenced to confirm the fidelity of the sequences.

[00336] Plasmids containing heavy chain gene (VH-Cγ1-Cγ2-Cγ3) were co-transfected with plasmid containing light chain gene (VL-Cκ) into 293E cells using llipofectamine (Invitrogen, Carlsbad CA) and grown in FreeStyle 293 media (Invitrogen, Carlsbad CA). After 5 days of growth, the antibodies were purified from the culture supernatant by protein A affinity using the MabSelect resin (GE Healthcare). Antibody concentrations were determined by bicinchoninic acid (BCA) assay (Pierce).

[00337] The pI engineered anti-VEGF mAbs were characterized by SDS PAGE on an Agilent Bioanalyzer (Figure 8), by size exclusion chromatography (SEC) (Figure 9), isoelectric focusing (IEF) gel electrophoresis (Figure 10), binding to antigen VEGF by Biacore (Figure 11), and differential scanning calorimetry (DSC) (Figure 12). All mAbs showed high purity on SDS-PAGE and SEC. IEF gels indicated that each variant had the designed isoelectric point. VEGF binding analysis on Biacore showed that pI engineered anti-VEGF bound to VEGF with similar affinity as bevacizumab, indicating that the designed substitutions did not perturb the function of the mAb. DSC showed that the anti-VEGF variant with both CH1 and CL engineered substitutions had high thermostability with a Tm of 71.9 °C.

[00338] Pharmacokinetic experiments were performed in B6 mice that are homozygous knock-outs for murine FcRn and heterozygous knock-ins of human FcRn ($mFcRn^{-/-}$, $hFcRn^{+}$) (Petkova et al., 2006, Int Immunol 18(12):1759-69, entirely incorporated by reference), herein referred to as hFcRn or $hFcRn^{+}$ mice. Samples tested included the parent IgG1/2 constant region, the pI-engineered variant with a pI of 5.51, referred to as IgG1_CH-CL_pI_eng, and an Fc variant version of IgG1/2 containing the substitution N434S, which improves affinity to human FcRn.

[00339] A single, intravenous tail vein injection of anti-VEGF antibody (2 mg/kg) was given to groups of 4-7 female mice randomized by body weight (20-30g range). Blood (~50ul) was drawn from the orbital plexus at each time point, processed to serum, and stored at -80°C until analysis. Antibody concentrations were determined using an ELISA assay. Serum concentration of antibody was measured using a recombinant VEGF (VEGF-165 , PeproTech, Rocky Hill, NJ) as capture reagent, and detection was carried out with

biotinylated anti-human kappa antibody and europium-labeled streptavidin. The time resolved fluorescence signal was collected. PK parameters were determined for individual mice with a non-compartmental model using WinNonLin (Pharsight Inc, Mountain View CA). Nominal times and dose were used with uniform weighing of points.

[00340] Results are shown in Figure 13. Fitted half-life (t1/2) values, which represents the beta phase that characterizes elimination of antibody from serum, are shown in Table 1. The pI-engineered variant, containing substitutions in CH1 and CL that reduce the pI, extended half-life to 7.4 days, an improvement of approximately 2.6-fold relative to IgG1/2. The pI-engineered variant had a comparable half-life to the Fc variant version N434S. Combinations of antibody variants are contemplated that reduce pI and improve affinity for FcRn for extending the half-lives of antibodies and Fc fusions.

Table 1. PK results of pI-engineered variant

Group	Variant	n	Individual mice t1/2 (days)				Average t1/2 (days)	St. Dev. (days)
			n1	n2	n3	n4		
7349	IgG1/2_WT	4	2.9	2.5	3.2	2.8	*2.9*	*0.3*
7350	IgG1/2_N434S	4	6.3	7.7	7.3	6.5	*7.0*	*0.7*
9493	IgG1_CH-CL_pI_eng	3	7.4	8.4	6.4		*7.4*	*1.0*

[00341] EXAMPLE 3. PK analysis of IgG constant regions

[00342] PK studies of IgG1 and IgG2 isotype versions of bevacizumab were carried out in the huFcRn mice as described above. The IgG1 results from four separate PK studies are shown in Figure 14. The half-lives from the four studies were 3.0, 3.9, 2.8, and 2.9 days, resulting in an average half-life of 3.2 days. The PK results from the IgG2 study are shown in Figure 15. The half-life of IgG2 was 5.9 days.

[00343] The PK results from the the IgG1 and IgG2 were analyzed with the results from the IgG1/2 and pI-engineered versions of bevacizumab. Table 2 shows the half-lives of the antibodies along with their calculated pI. These data are plotted in Figure 16.

Table 2. PK results of antibodies with identical Fv (bevacizumab) but constant regions with different pI's

XENP	IgG	pI	Average t 1/2 (days)
4547	IgG1	8.1	3.2
7349	IgG1/2	8.1	2.9
6384	IgG2	7.3	5.9
9493	IgG1_CH-CL_pI_eng	5.6	7.4

	[aka IgG1-pI(12)]		

[00344] A correlation was observed between half-life and the pI of the antibodies. These data further suggest that engineering of antibody constant chains, including heavy and light chain constant regions, for reduced isoelectric point is potentially a novel generalizable approach to extending the serum half-lives of antibodies and Fc fusions.

[00345] EXAMPLE 4. Engineering approaches to constant region pI engineering

[00346] Reduction in the pI of a protein or antibody can be carried out using a variety of approaches. At the most basic level, residues with high pKa's (lysine, arginine, and to some extent histidine) are replaced with neutral or negative residues, and/or neutral residues are replaced with low pKa residues (aspartic acid and glutamic acid). The particular replacements may depend on a variety of factors, including location in the structure, role in function, and immunogenicity.

[00347] Because immunogenicity is a concern, efforts can be made to minimize the risk that a substitution that lowers the pI will elicit immunogenicity. One way to minimize risk is to minimize the mutational load of the variants, i.e. to reduce the pI with the fewest number of mutations. Charge swapping mutations, where a K, R, or H is replaced with a D or E, have the greatest impact on reducing pI, and so these substitutions are preferred. Another approach to minimizing the risk of immunogenicity while reducing pI is to utilize substitutions from homologous human proteins. Thus for antibody constant chains, the isotypic differences between the IgG subclasses (IgG1, IgG2, IgG3, and IgG4) provide low-risk substitutions. Because immune recognition occurs at a local sequence level, i.e. MHC II and T-cell receptors recognize epitopes typically 9 residues in length, pI-altering substitutions may be accompanied by isotypic substitutions proximal in sequence. In this way, epitopes can be extended to match a natural isotype. Such substitutions would thus make up epitopes that are present in other human IgG isotypes, and thus would be expected to be tolerized.

[00348] Figure 17 shows an amino acid sequence alignment of the IgG subclasses. Residues with a bounded box illustrate isotypic differences between the IgG's. Residues which contribute to a higher pI (K, R, and H) or lower pI (D and E) are highlighted in bold. Designed substitutions that either lower the pI, or extend an epitope to match a natural isotype are shown in gray.

[00349] Figure 18 shows the amino acid sequence of the CK and Cλ light constant chains. Homology between Cκ and Cλ is not as high as between the IgG subclasses. Nonetheless the alignment may be used to guide substitutions. Residues which contribute to a higher pI (K, R, and H) or lower pI (D and E) are highlighted in bold. Gray indicates lysine, arginines, and histidines that may be substituted, preferably with aspartic or glutatmic acids, to lower the isoelectric point.

[00350] Another approach to engineering lower pI into proteins and antibodies is to fuse negatively charged residues to the N- or C-termini. Thus for example, peptides consisting principally of aspartic acids and glutamic acid may be fused to the N-terminus or C-terminus to the antibody heavy chain, light chain or both. Because the N-termini are structurally close to the antigen binding site, the C-termini are preferred.

[00351] Based on the described engineering approaches, a number of variants were designed to reduce the isoelectric point of both the antibody heavy chain and light chain. The heavy chain variants comprise various combinations of isotypic substitutions, as well as C-terminal negatively charged peptides. Relative to a native IgG1, the variants comprise one or more isotypic substitutions from the group consisting of G137E, G138S, S192N, L193F, I199T, N203D, K214T, K222T,substitution of 221-225 DKTHT to VE, H268Q, K274Q, R355Q, N384S, K392N, V397M, Q419E, and a deletion of K447 (referred to as K447#), wherein numbering is according to the EU index. The light chain variants comprise various combinations of non-isotypic substitutions and C-terminal negatively charged peptides. Cκ variants comprise one or more substitutions from the group consisting of K126E, K145E, N152D, S156E, K169E, and S202E, wherein numbering is according to the EU index.

[00352] Sequences of the variant heavy chains are provided in Figure 19, and sequences of the variant light chains are provided in Figure 20. Table 3 lists the variants constructed, along with the calculated pI's of the heavy constant chain, light constant chain, as well as the pI of the full length monoclonal antibody (mAb) containing the variable region (Fv) of the anti-VEGF antibody Bevacizumab.

Table 3. pI-engineered antibody constant chain variants

Heavy Chain		Light Chain		Fv			mAb[b]
Identity	**pI**	**Identity**	**pI**	**Identity[a]**	**VH pI**	**VL pI**	**pI**
IgG1-WT	8.46	Ck-WT	6.1	Bev	6.99	6.75	8.10
IgG1-WT	8.46	Ck-pI(3)	4.6	Bev	6.99	6.75	6.58
IgG1-WT	8.46	Ck-pI(6)	4.4	Bev	6.99	6.75	6.21

IgG1-WT	8.46	Ck-pI(6-DEDE)	4.3	Bev	6.99	6.75	5.85
IgG2-WT	7.66	Ck-WT	6.1	Bev	6.99	6.75	7.31
IgG2-WT	7.66	Ck-pI(3)	4.6	Bev	6.99	6.75	6.16
IgG2-WT	7.66	Ck-pI(6)	4.4	Bev	6.99	6.75	5.88
IgG2-WT	7.66	Ck-pI(6-DEDE)	4.3	Bev	6.99	6.75	5.58
pI-iso1	5.93	Ck-WT	6.1	Bev	6.99	6.75	6.16
pI-iso1(NF)	5.93	Ck-WT	6.1	Bev	6.99	6.75	6.16
pI-iso1(NF-VE)	5.85	Ck-WT	6.1	Bev	6.99	6.75	6.11
pI-iso1(NF-VE)	5.85	Ck-pI(3)	4.6	Bev	6.99	6.75	5.58
pI-iso1(NF-VE)	5.85	Ck-pI(6)	4.4	Bev	6.99	6.75	5.38
pI-iso1(NF-VE)	5.85	Ck-pI(6-DEDE)	4.3	Bev	6.99	6.75	5.18
pI-iso1(NF-VE-DEDE)	5.36	Ck-WT	6.1	Bev	6.99	6.75	5.74
pI-iso1(NF-VE-DEDE)	5.36	Ck-pI(3)	4.6	Bev	6.99	6.75	5.32
pI-iso1(NF-VE-DEDE)	5.36	Ck-pI(6)	4.4	Bev	6.99	6.75	5.18
pI-iso1(NF-VE-DEDE)	5.36	Ck-pI(6-DEDE)	4.3	Bev	6.99	6.75	5.03

[a] Bev = the variable region of the anti-VEGF antibody Bevacizumab

[b] mAb pI = the pI of the full length monoclonal antibody containing the Fv of Bevacizumab

[00353] EXAMPLE 5. Determination of charge-dependency of pI engineering and potential combination with Fc variants that enhance binding to FcRn

[00354] A series of new pI-engineered variants were generated to test two aspects of the relationship between low pI and extended half-life. First, the parameter of charge was investigated by making a controlled set of variants based on the 9493 IgG1-pI(12) variant. These variants, 10017, 10018, and 10019, are described in Table 4, along with their pI and the differences in positively and negatively charged residues relative to bevacizumab IgG1 WT.

Table 4. Engineered constructs exploring charge and Fc variants

XENP	HC Identity	HC Substitutions	LC Substitutions	pI	Charge State	# KR	# DE
4547	IgG1-WT			8.1	(+6)	0	0
9493	IgG1-pI(12)	CH1-pI(6)	Ck-pI(6)	5.6	(-30)	(-12)	(+24)
9992	IgG1-pI(12)	CH1-pI(6) + N434S	Ck-pI(6)	5.6	(-30)	(-12)	(+24)
9993	IgG1-pI(12)	CH1-pI(6) + M428L/N434S	Ck-pI(6)	5.6	(-30)	(-12)	(+24)

10017	IgG1-pI(6)-Neutral-to-DE	S119E T164E N208D	N152D S156E S202E	6.6	(-6)	0	(+12)
10018	IgG1-pI(6)-KR-to-Neutral	K133Q K205Q K210Q	K126Q K145Q K169Q	6.6	(-6)	(-12)	0
10019	IgG1-pI(6)-KR-to-DE	K133E K205E K210E	K126E K145E K169E	5.9	(-18)	(-12)	(+12)

CH1-pI(6) = S119E K133E T164E K205E N208D K210E

Ck-pI(6) = K126E K145E N152D S156E K169E S202E

pI calculated with Fv = Bevacizumab

[00355] The experimental rationale here is as follows. If all the mechanism for improved half-life is based on removal of positive charge, 10018 and 10019 should be as good as 9493 while 10017 would not be extended. If the mechanism is based on an increase in negative charge, 10018 will not be extended, while 10017 and 10019 will have equivalent half-life that is extended relative to IgG1 but shorter than 9493. If overall pI (or charge state) is the basis, the result will be 9493 > 10019 > 10017 = 10018.

[00356] In addition to the charge-controlled variant set, the 9493 IgG1-pI(12) variant was combined with substitutions that improve binding to FcRn at pH 6.0 in order to test whether the two mechanisms of half-life improvement, charge state and FcRn, are compatible. These variants, 9992 IgG1-pI(12)-N434S and 9993 IgG1-pI(12)-M428L/N434S, are listed in Table 4.

[00357] Antibody variants were constructed with the variable region of bevacizumab using molecular biology techniques as described above. Antibodies were expressed, purified, and characterized as described above. PK studies of the variant and control antibodies were carried out in the huFcRn mice as described above. The group mean averages of the serum concentrations are plotted in Figures 21 and 22, along with the half-lives obtained from the fits of the data.

[00358] The results indicate that both reducing positive charge and increasing negative charge contribute to improved half-life. In addition, the results indicate that engineered lower pI and increased binding to FcRn can be used in combination to obtain even greater enhancements in half-life. A plot of the half-life vs. pI relationship is provided in Figure 23 for variant and native IgG's of identical Fv (bevacizumab) that have been tested in the huFcRn mice. The graph illustrates again the inverse relationship between half-life and pI, as well as the combinability of variants engineered for lower pI and Fc variants that improve binding to FcRn.

[00359] EXAMPLE 6. New pI-engineered constructs

[00360] As described above, efforts can be made to minimize the risk that substitutions that lower pI will elicit immunogenicity by utilizing the isotypic differences between the IgG subclasses (IgG1, IgG2, IgG3, and IgG4). A new set of novel isotypes was designed based on this principal. Again, because immune recognition occurs at a local sequence level, i.e. MHC II and T-cell receptors recognize epitopes typically 9 residues in length, pI-altering substitutions were accompanied by isotypic substitutions proximal in sequence. In this way, epitopes were extended to match a natural isotype. Such substitutions would thus make up epitopes that are present in other human IgG isotypes, and thus would be expected to be tolerized.

[00361] The designed low-pI isotypes, referred to as IgG-pI-Iso2, IgG-pI-Iso2-SL, IgG-pI-Iso2-charges-only, IgG-pI-Iso3, IgG-pI-Iso3-SL, and IgG-pI-Iso3-charges-only are described in Table 5, along with their pI and effector function properties. Figure 24 provides a sequence alignment of IgG-pI-Iso3 with the native IgG isotypes, and depicts residue identities and residues that reduce pI relative to one or more of the native IgG isotypes. Figures 25 and 26 illustrate the structural differences between IgG1 and IgG-pI-Iso3. IgG-pI-Iso2, IgG-pI-Iso2-SL, and IgG-pI-Iso2-charges-only were designed to have low (weak) effector function, as determined by IgG2-like residues in the hinge (233P, 234V, 235A) and CH2 domain (327G). IgG-pI-Iso3, IgG-pI-Iso3-SL, and IgG-pI-Iso3-charges-only were designed to have high (strong) effector function, as determined by IgG1-like residues in the hinge (233E, 234L, 235L, 236G) and CH2 domain (327A). Isotypic low pI variants with the "SL" designation indicate that these variants differ from IgG-pI-Iso2 and IgG-pI-Iso3 by having 192S and 193L. Serine and leucine at these positions were found to be more compatible than 192N/193F due to differences in neighboring residues that are present in IgG1 and IgG2. Low pI isotype variants designated as "charges only" contain charge affecting isotypic substitutions, but do not contain the neighboring non-charge altering substitutions. The novel isotypes can be combined with a native light chain constant region (Ckappa or Clambda), or a variant version engineered with substitutions to further reduce the pI. An example of a pI-engineered light constant chain is a new variant referred to as CK-pI(4), described schematically in Figure 27. In addition, the novel isotypes can be engineered with Fc variants that improve affinity to FcRn, thereby further enabling extended half-life. Such Fc variants may include, for example 434S or 428L/434S as described in Table 5, or other Fc variants as described herein. Amino acid sequences of IgG-pI-Iso2, IgG-pI-Iso2-SL,

IgG-pI-Iso2-charges-only, IgG-pI-Iso3, IgG-pI-Iso3-SL, IgG-pI-Iso3-charges-only and CK-pI(4) are provided in Figure 28.

Table 5. Novel IgG isotypes with low pI

XENP	Heavy	Light	Fc variant	pI	Effector Function
10178	IgG-pI-Iso2	WT		6.3	Low
10470	IgG-pI-Iso2-SL	WT		6.3	Low
10180	IgG-pI-Iso2	WT	434S	6.3	Low
10471	IgG-pI-Iso2-SL	WT	434S	6.3	Low
10182	IgG-pI-Iso2	CK-pI(4)		5.6	Low
10184	IgG-pI-Iso2	CK-pI(4)	434S	5.6	Low
10427	IgG-pI-Iso2-charges-only	WT		6.3	Low
10473	IgG-pI-Iso2-charges-only	WT	434S	6.3	Low
10179	IgG-pI-Iso3	WT		6.2	High
10286	IgG-pI-Iso3-SL	WT		6.2	High
10181	IgG-pI-Iso3	WT	434S	6.2	High
10466	IgG-pI-Iso3-SL	WT	434S	6.2	High
10467	IgG-pI-Iso3-SL	WT	428L/434S	6.2	High
10183	IgG-pI-Iso3	CK-pI(4)		5.5	High
10185	IgG-pI-Iso3	CK-pI(4)	434S	5.5	High
10525	IgG-pI-Iso3-SL	CK-pI(4)	434S	5.5	High
10426	IgG-pI-Iso3-charges-only	WT		6.2	High
10472	IgG-pI-Iso3-charges-only	WT	434S	6.2	High

SL = 192S/193L

CK-pI(4) = K126E/K145E/K169E/K207E

pI calculated with Fv = Bevacizumab

[00362] The novel engineered isotypes can be combined with other Fc variants to generate antibodies or Fc fusions with extended half-life and other improved properties. For example, IgG-pI-Iso2-SL and/or IgG-pI-Iso3-SL may incorporate variants 239D, 332E, 267E, and/or 328F that modulate binding to FcγRs to provide enhanced effector function or immunomodulatory properties. The novel isotypes may be combined with other Fc variants that improve binding to FcRn, including for example 428L, 428L/434S, T250Q/M428L, M252Y/S254T/T256E, and N434A/T307Q, thereby potentially further extending in vivo half-life. Exemplary heavy chains are described in Table 6. Such variants may be expressed with a light chain that has a native constant light chain (CK or Cλ), or one that also incorporates constant light chain modifications that reduce pI, including for example any of the engineered constant light chains described herein, including for example CK-pI(4).

Table 6. Engineered combinations of pI isotype variants with other variants.

Heavy	Fc
IgG-pI-Iso3-SL	332E
IgG-pI-Iso3-SL	239D/332E
IgG-pI-Iso3-SL	332E/434S
IgG-pI-Iso3-SL	239D/332E/434S
IgG-pI-Iso2-SL	267E/328F
IgG-pI-Iso2-SL	434S/267E/328F
IgG-pI-Iso3-SL	267E/328F
IgG-pI-Iso3-SL	434S/267E/328F
IgG-pI-Iso2-SL	428L/434S
IgG-pI-Iso3-SL	428L/434S
IgG-pI-Iso2-SL	428L
IgG-pI-Iso3-SL	428L
IgG-pI-Iso2-SL	250Q/428L
IgG-pI-Iso3-SL	250Q/428L
IgG-pI-Iso2-SL	252Y/254T/256E
IgG-pI-Iso3-SL	252Y/254T/256E
IgG-pI-Iso2-SL	434A/307Q
IgG-pI-Iso3-SL	434A/307Q

[00363] In order to reduce pI even further, additional variant heavy constant chains with reduced pI were designed to minimize mutational load by introducing charge swapping mutations, i.e. where K and R were replaced with D or E, as described above. To aid in the design of these variants, fraction exposed as well as the energy change upon substitution to Glu were calculated for each K and R residue in the Fc region (Figure 29). These new variants are referred to as pI(7) and pI(11). pI(7) incorporated amino acid modifications K133E, K205E, K210E, K274E, R355E, K392E, and a deletion of the Lys at 447, and pI(11) incorporated amino acid modifications K133E, K205E, K210E, K274E, K320E, K322E, K326E, K334E, R355E, K392E, and a deletion of the Lys at 447 These modifications were introduced into heavy constant chains to result in antibodies with strong effector function, IgG1-pI(7) and IgG1-pI(11), and weak effector function IgG1/2-pI(7) and IgG1/2-pI(11). As can be seen in Figure 30, as mAb pI gets lower, it requires a greater number of charge swap substitutions to decrease pI further. These pI-engineered variants are described in Table 7, and amino acid sequences are provided in Figure 28.

Table 7. Engineered charge swaps

XENP	Heavy	Fc variant	Light	pI
10107	IgG1-pI(7)		CK-pI(4)	5.3
10108	IgG1-pI(11)		CK-pI(4)	5.0
10109	IgG1/2-pI(7)		CK-pI(4)	5.4
10110	IgG1/2-pI(11)		CK-pI(4)	5.0
10476	IgG1/2-pI(7)	434S	CK-pI(4)	5.4

IgG1-pI(7) = K133E/K205E/K210E/K274E/R355E/K392E/K447#

IgG1-pI(11) = K133E/K205E/K210E/K274E/K320E/K322E/K326E/K334E/R355E/K392E/K447#

IgG1/2-pI(7) = K133E/K205E/K210E/Q274E/R355E/K392E/K447#

IgG1/2-pI(11) = K133E/K205E/K210E/Q274E/K320E/K322E/K326E/K334E/R355E/K392E/K447#

CK-pI(4) = K126E/K145E/K169E/K207E

pI calculated with Fv = Bevacizumab

[00364] Antibody variants were constructed with the variable region of bevacizumab using molecular biology techniques as described above. Antibodies were expressed, purified, and characterized as described above. PK studies of the variant and control antibodies were carried out in the huFcRn mice as described above. The group mean averages of the serum concentrations are plotted in Figure 31 and Figure 32, along with the half-lives obtained from the fits of the data. Half-lives for individual mice are plotted in Figure 33. The data clearly demonstrate the additivity of low pI from isotypic pI variants as well as enhanced FcRn binding from the N434S substitution as shown by a plot of half-life vs. pI as shown in Figure 34.

[00365] EXAMPLE 7. Isotypic light chain constant region variants

[00366] Homology between CK and Cλ is not as high as between the IgG subclasses (as shown in Figure 18), however the sequence and structural homology that exists may still be used to guide substitutions to create an isotypic low-pI light chain constant region. In Figure 18, positions with residues contributing to a higher pI (K, R, and H) or lower pI (D and E) are highlighted in bold. Gray indicates lysine, arginines, and histidines that may be substituted, preferably with aspartic or glutatmic acids, to lower the isoelectric point. A structural alignment of CK and Cλ was constructed (Figure 35) and used along with the sequence alignment as a guide to make several CK/Cλ isotypic variants. These pI-engineered variants are described in Table 8, and amino acid sequences are provided in Figure 28.

TABLE 8. Engineered low-pI variants containing isotypic light chain constant regions

XENP	Heavy	Light	Fc variant	pI	Effector Function
10324	IgG-pI-Iso3	CK-Iso(3)		5.9	High
10325	IgG-pI-Iso3	CK-Iso(4)		5.8	High
10326	IgG-pI-Iso3	CK-Iso(5)		5.8	High
10327	IgG-pI-Iso3	CK-Iso(6)		5.7	High
10511	IgG-pI-Iso3-SL	CK-Iso(3)		5.9	High
10512	IgG-pI-Iso3-SL	CK-Iso(4)		5.8	High
10513	IgG-pI-Iso3-SL	CK-Iso(5)		5.8	High
10517	IgG-pI-Iso3-SL	CK-Iso(3)	434S	5.9	High
10518	IgG-pI-Iso3-SL	CK-Iso(4)	434S	5.8	High
10519	IgG-pI-Iso3-SL	CK-Iso(5)	434S	5.8	High
10520	IgG-pI-Iso3-SL	CK-Iso(3)	428L/434S	5.9	High
10521	IgG-pI-Iso3-SL	CK-Iso(4)	428L/434S	5.8	High
10522	IgG-pI-Iso3-SL	CK-Iso(5)	428L/434S	5.8	High
10526	IgG-pI-Iso3	CK-Iso(5)	434S	5.8	High
10527	IgG-pI-Iso2-SL	CK-Iso(5)	434S	5.8	Low

[00367] Antibody variants were constructed with the variable region of bevacizumab using molecular biology techniques as described above. Antibodies were expressed, purified, and characterized as described above. PK studies of the variant and control antibodies were carried out in the huFcRn mice as described above. The group mean averages of the serum concentrations as well as the half-lives obtained from fits of the data for one of these variants (XENP10519 – IgG-pI-Iso3-SL-434S-CK-Iso(5)) are plotted in Figure 32 and the half-lives for individual mice in Figure 33. This variant is also included in the correlation plot shown in Figure 34. The benefit of lower pI due to the CK-Iso(5) light chain is clearly shown.

[00368] EXAMPLE 8. Anti-VEGF antibodies with engineered CH1 and CK regions having lower pI and low expected immunogenicity

[00369] Clinical immunogenicity of naturally occurring and engineered therapeutic proteins is of increasing concern. It will frequently reduce the therapeutic efficacy of a protein drug and at worst it can trigger a new autoimmunity in the patient. ImmunoFilter™ technology focuses on an early event in the recognition of antigens by the immune system: class II MHC recognition of protein-derived peptides generated by intracellular processing mechanisms. Class II MHC types relevant for protein immunogenicity include DR, DP, and DQ. A challenge in this approach is the considerable polymorphism in each class: for example, any patient can possess two different copies of more than 400 known DR isoforms. The ImmunoFilter™ software allows one to rapidly scan protein sequences for the presence of class II MHC agretopes. Each nine-mer peptide within a candidate protein sequence is evaluated and scored for propensity to bind individual MHC isoforms. A summary score that incorporates interaction probability and allele frequencies is also determined in order to assess the potential population impact of a detected agretope.

[00370] Using ImmunoFilter™, efforts can be made to minimize the risk that a substitution that lowers the pI will elicit immunogenicity. Heavy chain IgG1-CH1-pI(6) and light chain CK-pI(6) contain non-isotypic substitutions which may elicit immunogenicity. We used ImmunoFilter™ to scan the sequence of XENP9493 (IgG1-CH1-pI(6)-CK-pI(6)) for the presence of class II MHC agretopes. The analysis indicated several non-human 9-mers with high IScores (potentially high propensity to bind MHC class II) (Figure 38). In silico, we constructed all possible combinations of the 12 substitutions present in the IgG1-CH1-pI(6) and CK-pI(6) chains (6 substitutions in each chain), and used ImmunoFilter™ to rank the resulting combinations for propensity to bind individual MHC isoforms (Figure 39). Various combinations of substitutions were identified that decreased non-human 9-mer MHC binding propensity and still had low pI. Variants from each IScore bin with the lowest pI were chosen and constructed with the variable region of bevacizumab using molecular biology techniques as described above. Antibodies were expressed, purified, and characterized as described above. The variants chosen for further analysis are shown in Figure 40. These variants have substantially reduced non-human 9-mer IScores, as can be seen for variant IgG1-pI-CH1-v42 in when compared to those IScores of IgG1-CH1-pI(6) shown in Figure 39.

[00371] In addition, we wished to combine these new variants containing ImmunoFilter™ optimized CH1/CK regions with low immunogenicity and low pI isotypic constant regions. For this approach, we utilized the CH1-Fc isotype alignment and added in only those isotypic substitutions that preserved the natural 9-mer epitopes containing that substitution. A sequence alignment showing ImmunoFilter optimized heavy chain IgG-pI-CH1-v42, as well as this variant containing extra isotypic substitutions (labeled as "+9-mer optimized Fc") and IgGs is shown in Figure 42. This would result in a variant with lower pI and potentially low immunogenicity. These variants can be evaluated in PK models in huFcRn mice as discussed above. The variant IgG-pI-CH1-v42 with a 9-mer optimized Fc is called IgG-pI-CH1-v42-SLFFV-Iso-434S and when combined with the ImmunoFilter optimized light chain is IgG-pI-CH1-v42-SLFFV-Iso-434S-CK-v23.

[00372] Variants containing ImmunoFilter optimized CH1/CK regions were combined with various isotypic Fc regions expected to have both high and low effector function. Variants containing the ImmunoFilter optimized CH1/CK were also combined with Fc regions containing unnatural charge substitutions (designated as "Fc-charges") at positions with high solvent accessibility and percent exposure in order to further decrease pI.

TABLE 9. Engineered low-pI variants containing isotypic and charged constant regions

XENP	Heavy	Light	Fc variant	pI	Effector Function
10621	IgG1-pI-IF16-ISO	CK-v23		5.6	High
10622	IgG1-pI-IF10-ISO	CK-v12		5.9	High

10623	IgG2-pI-IF10-ISO-N434S	CK-v12	434S	5.7	Low
10624	IgG2-pI-IF16-ISO-N434S	CK-v23	434S	5.4	Low
10625	Hybrid-pI-IF16-ISO-N434S	CK-v23	434S	5.6	High
10626	Hybrid-pI-IF10-ISO-N434S	CK-v12	434S	5.9	High
10628	Hybrid-2-1-2-pI-IF16-ISO-N434S	CK-v23	434S	5.3	High
10629	Hybrid-2-1-2-pI-IF10-ISO-N434S	CK-v12	434S	5.5	High
10648	IgG1-IF10-CH1-Fc-charges	CK-v12		5.9	High
10649	IgG1-IF16-CH1-Fc-charges	CK-v23		5.4	High
10650	IgG1-IF10-ISO-CH1-Fc-charges	CK-v12		5.8	High
10651	IgG1-IF16-ISO-CH1-Fc-charges	CK-v23		5.3	High

[00373] Pharmacokinetic studies of these variants were carried out as previously described using huFcRn transgenic mice. Resulting PK parameters are shown in Figures 44-46. These results show significant improvement in half-life of engineered low-pI variants compared to wild-type IgG1. The variant IgG1-pI-IF16/Ck-v23 was compared against wild-type bevacizumab in a cynomolgus monkey PK study. Four animals were given a single 4 mg/kg dose of each test article via 1 hr IV infusion. Blood samples were taken at various time points up to day 90. Figure 47 shows the resulting PK parameters, including half-life and AUC data. As can be seen from the data, the engineered pI variant with low expected immunogenicity has an ~23% increase in half-life (~3 days longer).

[00374] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims. All references cited herein are incorporated in their entirety.

CLAIMS

1. A method for modifying the isoelectric point of an antibody comprising modifying at least six amino acid residues in a constant domain selected from the heavy and light chain constant domains wherein the modified amino acids have a pI lower than the native amino acid, such that said isoelectric point of the variant antibody is lowered by at least 0.5 logs.

2. A method according to claim 1 wherein said constant domain is the heavy chain constant domain.

3. A method according to claim 1 wherein said constant domain is the light chain constant domain.

4. A method according to claim 1 wherein both said heavy and light constant domains comprise said modified amino acids.

5. An antibody comprising a variant heavy chain constant domain having the formula:

A-X119-T-K-G-P-S-V-F-P-L-A-P-X131-S-X133-S-T-S-X137-X138-T-A-A-L-G-C-L-V-K-D-Y-F-P-E-P-V-T-V-S-W-N-S-G-A-L-X164-S-G-V-H-T-F-P-A-V-L-Q-S-S-G-L-Y-S-L-S-S-V-V-T-V-P-S-S-X192-X193-G-T-X196-T-Y-X199-C-N-V-X203-H-X205-P-S-X208-T-X210-V-D-K-X214-V-E-X217-K-X219-C-X221-X222-X223-X224-X225-C-P-P-C-P-A-P-X233-X234-X235-X236-G-P-S-V-F-L-F-P-P-K-P-K-D-T-L-M-I-S-R-T-P-E-V-T-C-V-V-V-D-V-S-H-E-D-P-E-V-X274-F-N-W-Y-V-D-G-V-E-V-H-N-A-K-T-K-P-R-E-E-Q-X296-N-S-T-X300-R-V-V-S-V-L-T-V-X309-H-Q-D-W-L-N-G-K-E-Y-X320-C-X322-V-S-N-X326-X327-L-P-A-P-I-E-X334-T-I-S-K-X339-K-G-Q-P-R-E-P-Q-V-Y-T-L-P-P-S-X355-E-E-M-X359-K-N-X362-V-S-L-T-C-L-V-K-G-F-Y-P-S-D-I-A-V-E-W-E-S-X384-G-Q-P-E-X389-N-Y-X392-T-T-P-P-X397-L-D-S-D-G-S-F-F-L-Y-S-K-L-T-V-D-K-S-R-W-X418-X419-G-N-V-F-S-C-S-V-X428-H-E-A-L-H-X434-H-Y-T-Q-K-S-L-S-L-X444-P-G-X447,

wherein X119 is selected from the group consisting of S and E;
wherein X131 is selected from the group consisting of S and C;
wherein X133 is selected from the group consisting of K, R, E, and Q;
wherein X137 is selected from the group consisting of G and E;
wherein X138 is selected from the group consisting of G and S;
wherein X164 is selected from the group consisting of T and E;

wherein X192 is selected from the group consisting of S and N;
wherein X193 is selected from the group consisting of L and F;
wherein X196 is selected from the group consisting of Q and K;
wherein X199 is selected from the group consisting of I and T;
wherein X203 is selected from the group consisting of N and D;
wherein X205 is selected from the group consisting of K, E, and Q;
wherein X208 is selected from the group consisting of N and D;
wherein X210 is selected from the group consisting of K, E, and Q;
wherein X214 is selected from the group consisting of K and T;
wherein X217 is selected from the group consisting of P and R;
wherein X219 is selected from the group consisting of S and C;
wherein X220 is selected from the group consisting of C, PLG, and G;
wherein X221 is selected from the group consisting of D and a deletion;
wherein X222 is selected from the group consisting of K, V, and T;
wherein X223 is selected from the group consisting of T and a deletion;
wherein X224 is selected from the group consisting of H and E;
wherein X225 is selected from the group consisting of T and a deletion;
wherein X233 is selected from the group consisting of E and P;
wherein X234 is selected from the group consisting of L and V;
wherein X235 is selected from the group consisting of L, A, and a deletion;
wherein X236 is selected from the group consisting of G, A, and a deletion;
wherein X274 is selected from the group consisting of K, Q, and E;
wherein X296 is selected from the group consisting of Y and F;
wherein X300 is selected from the group consisting of Y and F;
wherein X309 is selected from the group consisting of L and V;
wherein X320 is selected from the group consisting of K and E;
wherein X322 is selected from the group consisting of K and E;
wherein X326 is selected from the group consisting of K and E;
wherein X327 is selected from the group consisting of A and G;
wherein X334 is selected from the group consisting of K and E;
wherein X339 is selected from the group consisting of A and T;
wherein X355 is selected from the group consisting of R, Q, and E;
wherein is X359 is selected from the group consisting of T and E;
wherein is X362 is selected from the group consisting of Q and E;

wherein X384 is selected from the group consisting of N and S;

wherein is X389 is selected from the group consisting of N and E;

wherein X392 is selected from the group consisting of K, N, and E;

wherein X397 is selected from the group consisting of V and M;

wherein X418 is selected from the group consisting of Q and E;

wherein X419 is selected from the group consisting of Q and E;

wherein X428 is selected from the group consisting of M and L;

wherein X434 is selected from the group consisting of N and S;

wherein X444 is selected from the group consisting of S and E; and

wherein X447 is selected from the group consisting of K, DEDE, and a deletion;

wherein said variant has at least 6 amino acid changes as compared to SEQ ID NO:2 and is not SEQ ID NO:3.

6. An antibody comprising a variant heavy chain constant domain as compared to SEQ ID NO:2, said variant domain comprises an aspartic acid at position 208, a glutamine at position 274, a glutamine at position 355, a serine at position 384, an asparagine at position 392, a methionine at position 397 and a glutamic acid at position 419.

7. An antibody according to claim 6, wherein said variant heavy chain constant domain is selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76 and SEQ ID NO:77.

8. An antibody according to claim 5 comprising a variant light chain constant domain having the sequence of SEQ ID NO:40 and a variant heavy chain constant domain selected from the group consisting of SEQ ID NO:70, SEQ ID NO:73; SEQ ID NO:74 and SEQ ID NO:76.

9. An antibody according to claim 5 comprising a variant light chain constant domain having the sequence of SEQ ID NO:41 and a variant heavy chain constant domain selected from the group consisting of SEQ ID NO:71, SEQ ID NO:72; SEQ ID NO:75 and SEQ ID NO:77.

10. An antibody according to claim 5 comprising a variant light chain constant domain having the sequence of SEQ ID NO:8 and a variant heavy chain constant domain selected from the group consisting of SEQ ID NO:7, SEQ ID NO:52 and SEQ ID NO:53.

11. A nucleic acid encoding the variable heavy chain domain of claim 5.

12. A host cell comprising the nucleic acid of claim 11 and a nucleic acid encoding a light chain constant domain.

13. A method of producing an antibody comprising culturing the host cell of claim 12 under conditions wherein said antibody are produced.

14. A method of treating a patient in need thereof by administering the antibody of claim 5.

Figure 1

Kappa constant light chain (CK) (SEQ ID NO: 1)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

IgG1 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 2)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

IgG2 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 3)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

IgG3 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 4)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCD
TPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
YPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEAL
HNRFTQKSLSLSPGK

IgG4 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 5)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

IgG1/2 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO: 6)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK

Figure 2

EU	Domain	IgG1	IgG2	IgG3	IgG4	Fraction Exposed	Notes	Subs
118	CH1	A	A	A	A	0.44		
119	CH1	S	S	S	S	0.69		DE
120	CH1	T	T	T	T	0.40		
121	CH1	K	K	K	K	0.37		DE
122	CH1	G	G	G	G	0.27		
123	CH1	P	P	P	P	0.10		
124	CH1	S	S	S	S	0.38		DE
125	CH1	V	V	V	V	0.05		
126	CH1	F	F	F	F	0.07		
127	CH1	P	P	P	P	0.21		
128	CH1	L	L	L	L	0.02		
129	CH1	A	A	A	A	0.22		DE
130	CH1	P	P	P	P	0.05		
131	CH1	S	C	C	C	1.00		DE
132	CH1	S	S	S	S	1.00		DE
133	CH1	K	R	R	R	1.00	Isotypic	DE
134	CH1	S	S	S	S	1.00		DE
135	CH1	T	T	T	T	1.00		DE
136	CH1	S	S	S	S	1.00		DE
137	CH1	G	E	G	E	1.00	Isotypic	DE
138	CH1	G	S	G	S	1.00		DE
139	CH1	T	T	T	T	0.55		
140	CH1	A	A	A	A	0.08		
141	CH1	A	A	A	A	0.02		
142	CH1	L	L	L	L	0.00		
143	CH1	G	G	G	G	0.00		
144	CH1	C	C	C	C	0.00		
145	CH1	L	L	L	L	0.02		
146	CH1	V	V	V	V	0.00		
147	CH1	K	K	K	K	0.06	Interface w/ CL	
148	CH1	D	D	D	D	0.26		
149	CH1	Y	Y	Y	Y	0.00		
150	CH1	F	F	F	F	0.10		
151	CH1	P	P	P	P	0.01		
152	CH1	E	E	E	E	0.27		DE
153	CH1	P	P	P	P	0.47		
154	CH1	V	V	V	V	0.12		
155	CH1	T	T	T	T	0.44		DE
156	CH1	V	V	V	V	0.14		
157	CH1	S	S	S	S	0.32		DE
158	CH1	W	W	W	W	0.01		
159	CH1	N	N	N	N	0.20		DE
160	CH1	S	S	S	S	0.82		DE
161	CH1	G	G	G	G	0.47		DE
162	CH1	A	A	A	A	0.79		DE
163	CH1	L	L	L	L	0.18		
164	CH1	T	T	T	T	0.71		DE
165	CH1	S	S	S	S	0.66		DE
166	CH1	G	G	G	G	0.38		
167	CH1	V	V	V	V	0.24		

Figure 2 cont.

EU	Domain	IgG1	IgG2	IgG3	IgG4	Fraction Exposed	Notes	Subs
168	CH1	H	H	H	H	0.12		
169	CH1	T	T	T	T	0.37		
170	CH1	F	F	F	F	0.02		
171	CH1	P	P	P	P	0.42		
172	CH1	A	A	A	A	0.24		
173	CH1	V	V	V	V	0.23		
174	CH1	L	L	L	L	0.47		
175	CH1	Q	Q	Q	Q	0.13		
176	CH1	S	S	S	S	1.00		DE
177	CH1	S	S	S	S	0.61		DE
178	CH1	G	G	G	G	0.35		DE
179	CH1	L	L	L	L	0.19		
180	CH1	Y	Y	Y	Y	0.19		
181	CH1	S	S	S	S	0.06		
182	CH1	L	L	L	L	0.08		
183	CH1	S	S	S	S	0.02		
184	CH1	S	S	S	S	0.00		
185	CH1	V	V	V	V	0.01		
186	CH1	V	V	V	V	0.00		
187	CH1	T	T	T	T	0.21		
188	CH1	V	V	V	V	0.04		
189	CH1	P	P	P	P	0.54		
190	CH1	S	S	S	S	0.42		DE
191	CH1	S	S	S	S	0.81		DE
192	CH1	S	N	S	S	0.16		
193	CH1	L	F	L	L	0.16		
194	CH1	G	G	G	G	0.91		DE
195	CH1	T	T	T	T	0.82		DE
196	CH1	Q	Q	Q	K	0.44		DE
197	CH1	T	T	T	T	0.39		DE
198	CH1	Y	Y	Y	Y	0.04		
199	CH1	I	T	T	T	0.26		DE
200	CH1	C	C	C	C	0.00		
201	CH1	N	N	N	N	0.15		
202	CH1	V	V	V	V	0.02		
203	CH1	N	D	N	D	0.18	Isotypic	DE
204	CH1	H	H	H	H	0.00		
205	CH1	K	K	K	K	0.62		DE
206	CH1	P	P	P	P	0.30		
207	CH1	S	S	S	S	0.26		
208	CH1	N	N	N	N	0.80		DE
209	CH1	T	T	T	T	0.21		
210	CH1	K	K	K	K	0.73		DE
211	CH1	V	V	V	V	0.28		
212	CH1	D	D	D	D	0.66		DE
213	CH1	K	K	K	K	0.20	Interface w/ CL	
214	CH1	K/R	T	R	R	0.43	Isotypic	DE
215	CH1	V	V	V	V	0.03		
216	CH1	E	E	E	E	0.50		DE
217	CH1	P	R	L	S	0.41		
218	CH1	K	K	K	K	0.86		DE

Figure 2 cont.

EU	Domain	IgG1	IgG2	IgG3	IgG4	Fraction Exposed	Notes	Subs
219	CH1	S	C	T	Y			DE
220	CH1	C	C	P	G			

Figure 3

EU	Ckappa	Fraction Exposed	Notes	Subs
108	R	0.33		DE
109	T	0.68		DE
110	V	0.42		DE
111	A	0.20		
112	A	0.38		DE
113	P	0.09		
114	S	0.46		DE
115	V	0.08		
116	F	0.20		
117	I	0.11		
118	F	0.02		
119	P	0.40		
120	P	0.08		
121	S	0.11		
122	D	0.54		DE
123	E	0.46		DE
124	Q	0.01		
125	L	0.07		
126	K	0.76		DE
127	S	0.65		DE
128	G	0.37		DE
129	T	0.34		DE
130	A	0.00		
131	S	0.02		
132	V	0.00		
133	V	0.00		
134	C	0.00		
135	L	0.00		
136	L	0.00		
137	N	0.04		
138	N	0.31		
139	F	0.00		
140	Y	0.12		
141	P	0.16		
142	R	0.37	Interface w/ VL	
143	E	0.67		DE
144	A	0.25		
145	K	0.48		DE
146	V	0.15		
147	Q	0.17		DE
148	W	0.01		
149	K	0.27		DE
150	V	0.04		
151	D	0.43		DE
152	N	0.72		DE
153	A	0.47		DE
154	L	0.56	exposed hydrophobic	DE
155	Q	0.22		
156	S	0.80		DE

Figure 3 cont.

EU	Ckappa	Fraction Exposed	Notes	Subs
157	G	0.97		DE
158	N	0.35		
159	S	0.36		
160	Q	0.34		
161	E	0.46		
162	S	0.11		
163	V	0.28		
164	T	0.11		
165	E	0.42		
166	Q	0.04		
167	D	0.27		DE
168	S	0.36		DE
169	K	0.79	Interface w/ VL	DE
170	D	0.38		DE
171	S	0.03		
172	T	0.04		
173	Y	0.04		
174	S	0.00		
175	L	0.02		
176	S	0.05		
177	S	0.01		
178	T	0.17		
179	L	0.00		
180	T	0.42		DE
181	L	0.12		
182	S	0.37		DE
183	K	0.33		DE
184	A	0.53		DE
185	D	0.45		DE
186	Y	0.05		
187	E	0.46		DE
188	K	0.65		DE
189	H	0.30		
190	K	0.44		DE
191	V	0.35		DE
192	Y	0.00		
193	A	0.11		DE
194	C	0.00		
195	E	0.24		DE
196	V	0.00		
197	T	0.34		DE
198	H	0.05		
199	Q	0.66		DE
200	G	0.37		DE
201	L	0.16		
202	S	0.98		DE
203	S	0.55		DE
204	P	0.51		
205	V	0.26		
206	T	0.50		DE
207	K	0.36		DE

Figure 3 cont.

EU	Ckappa	Fraction Exposed	Notes	Subs
208	S	0.50		DE
209	F	0.14		
210	N	0.30		DE
211	R	0.26		DE
212	G	0.97		DE
213	E	0.91		DE
214	C			

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Figure 4

IgG1-CH1-pI(6) (SEQ ID NO: 7)

AETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHEPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

CK-pI(6) (SEQ ID NO: 8)

RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDDALQEGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGEC

IgG1-CH1-pI(6)-434S (SEQ ID NO: 52)

AETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHEPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHSHYTQKSLSLSPGK

IgG1-CH1-pI(6)-428L/434S (SEQ ID NO: 53)

AETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHEPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVLHEALHSHYTQKSLSLSPGK

Figure 5

Anti-VEGF VH (SEQ ID NO: 9)

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPT
YAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLV
TVSS

Anti-VEGF VL (SEQ ID NO: 10)

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK

Figure 6

Heavy chain of XENP9493_Bevacizumab-IgG1-CH1-pI(6)-CK-pI(6) (SEQ ID NO: 11)

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPT
YAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLV
TVSSAETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHEPSDTEVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light chain of XENP9493_Bevacizumab-IgG1-CH1-pI(6)-CK-pI(6) (SEQ ID NO: 12)

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LESGTASVVCLLNNFYPREAEVQWKVDDALQEGNSQESVTEQDSEDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLESPVTKSFNRGEC

Figure 7

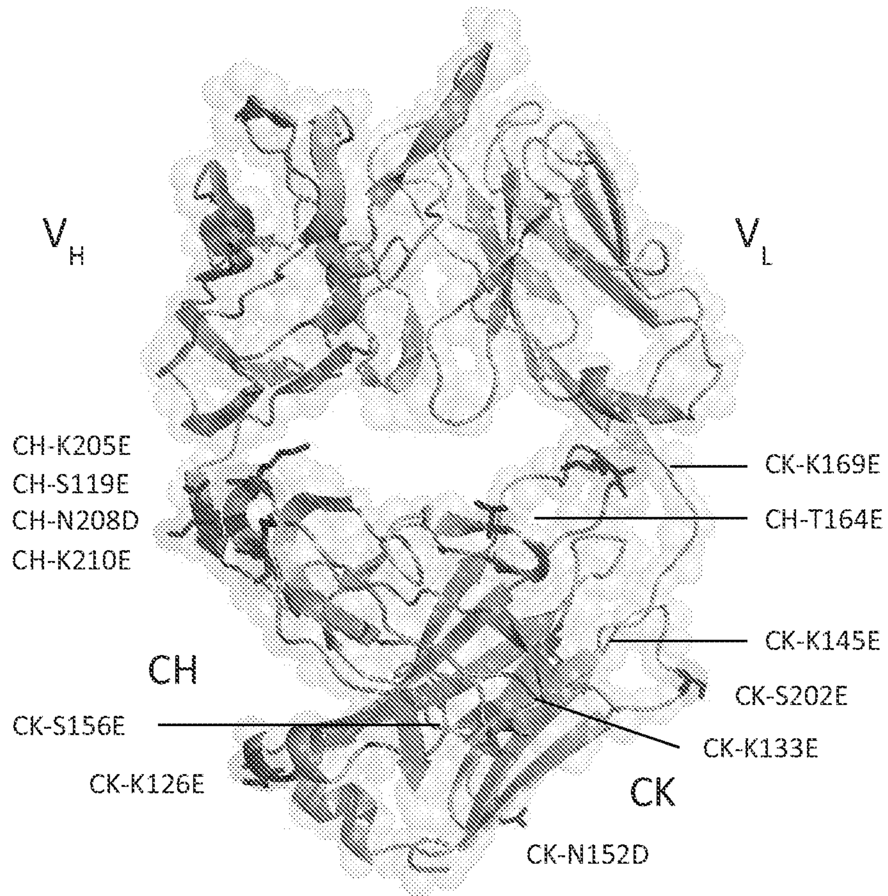
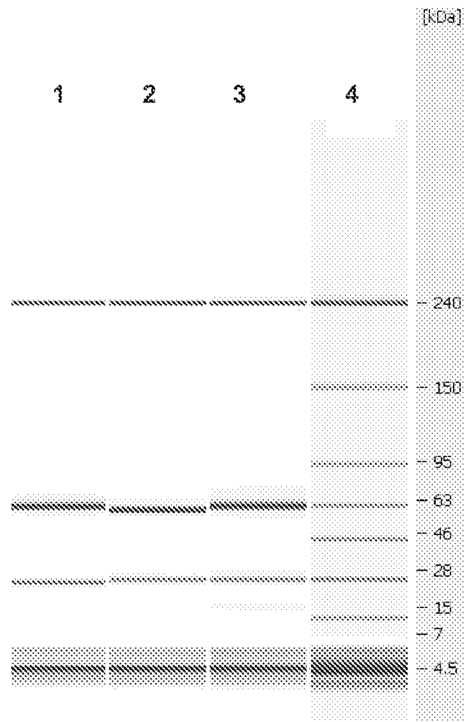


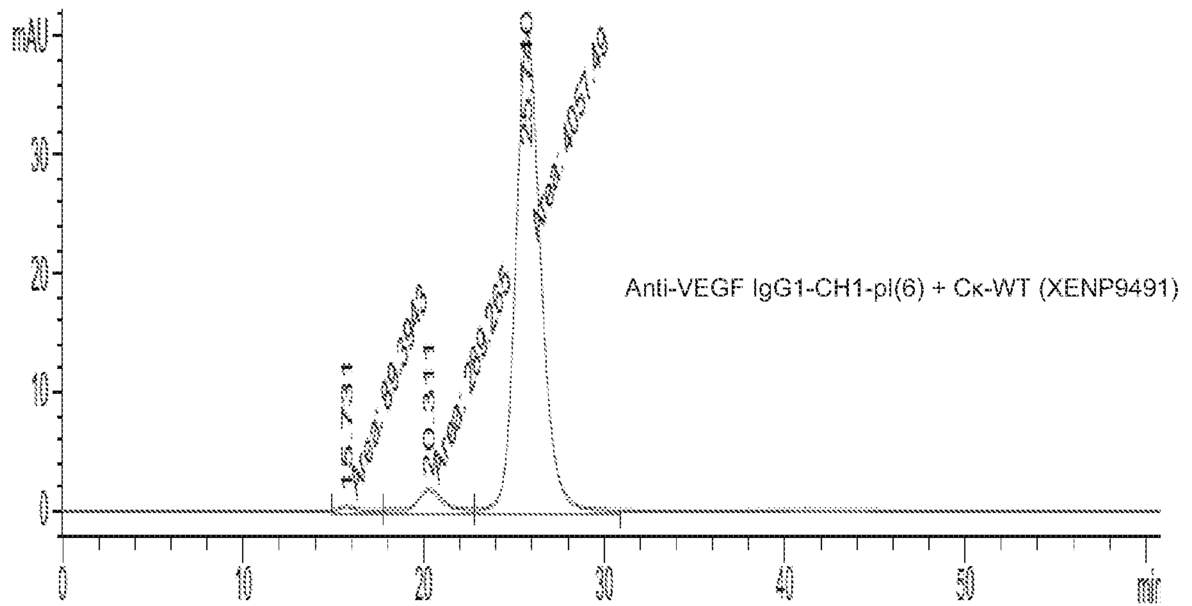
Figure 8



Lanes:

1 – Anti-VEGF IgG1-CH1-pI(6) + Cκ-WT (XENP9491)
2 – Anti-VEGF IgG1-CH1-WT + Ck-pI(6) (XENP9492)
3 – Anti-VEGF IgG1-CH1-pI(6) + Ck-pI(6) (XENP9493)
4 – Ladder

Figure 9



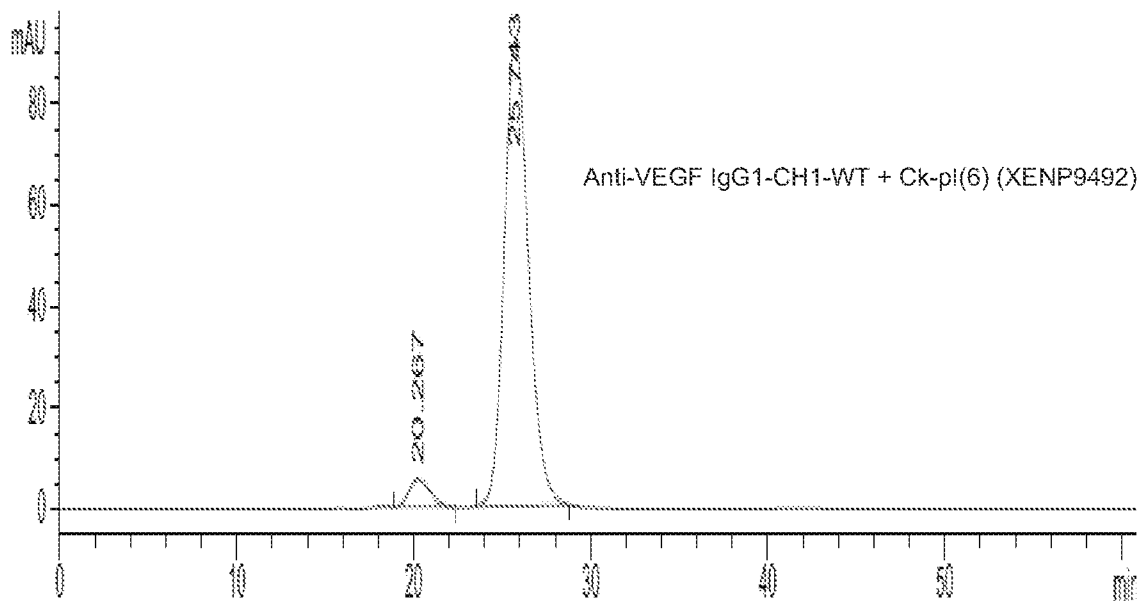


Figure 9 (continued)

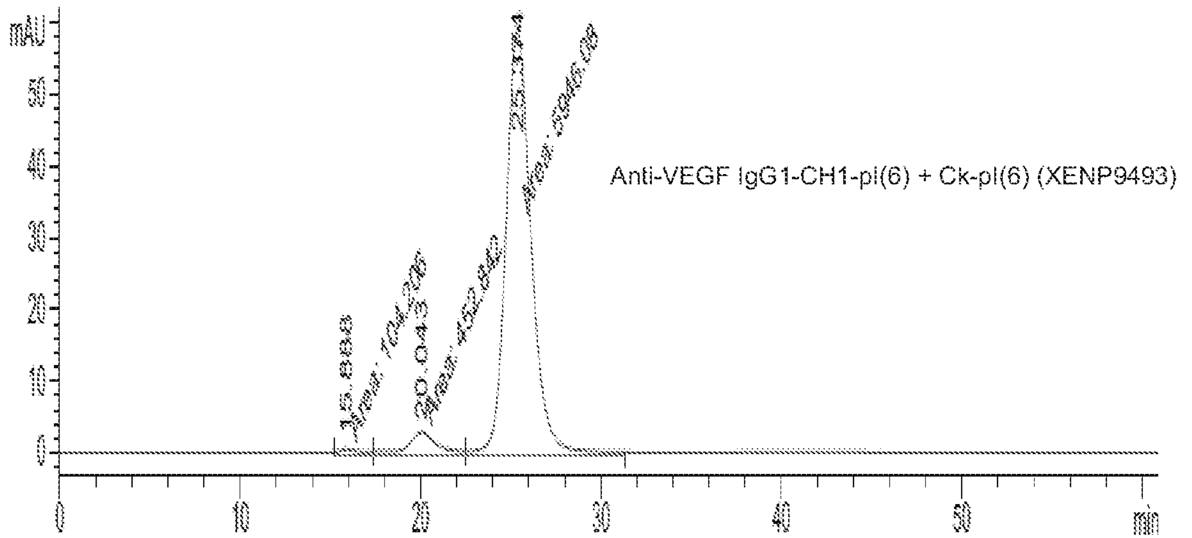
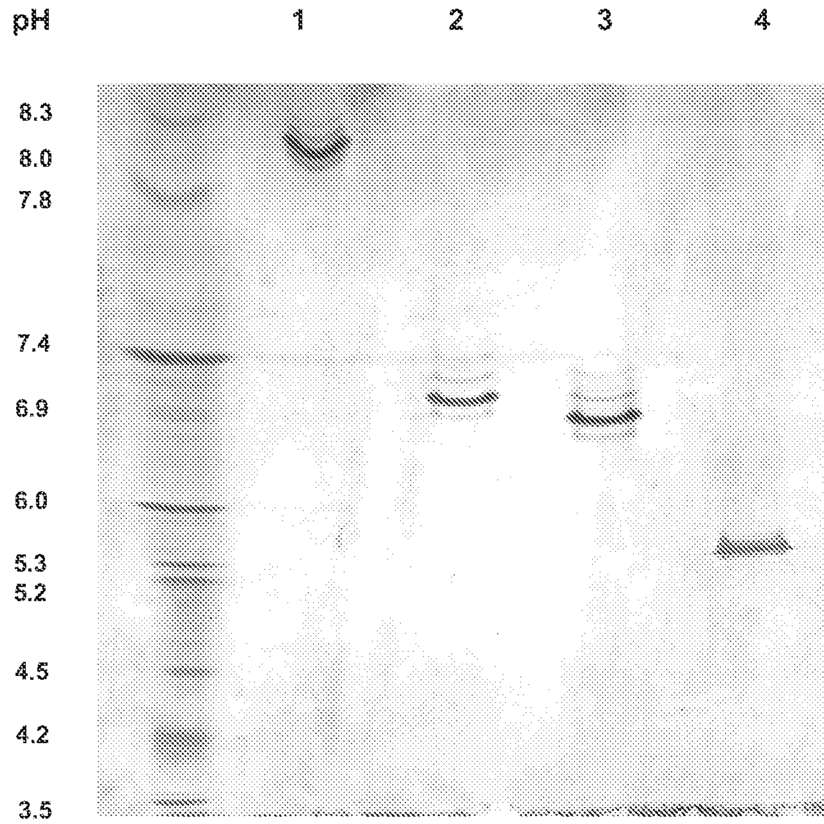


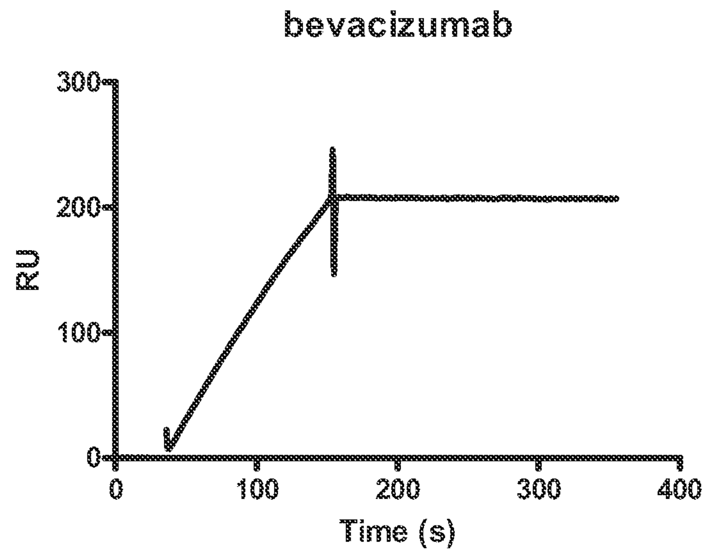
Figure 10



Lanes:

Ladder
1 – bevacizumab
2 – Anti-VEGF IgG1-CH1-pI(6) + Cκ-WT (XENP9491)
3 – Anti-VEGF IgG1-CH1-WT + Ck-pI(6) (XENP9492)
4 – Anti-VEGF IgG1-CH1-pI(6) + Ck-pI(6) (XENP9493)

Figure 11



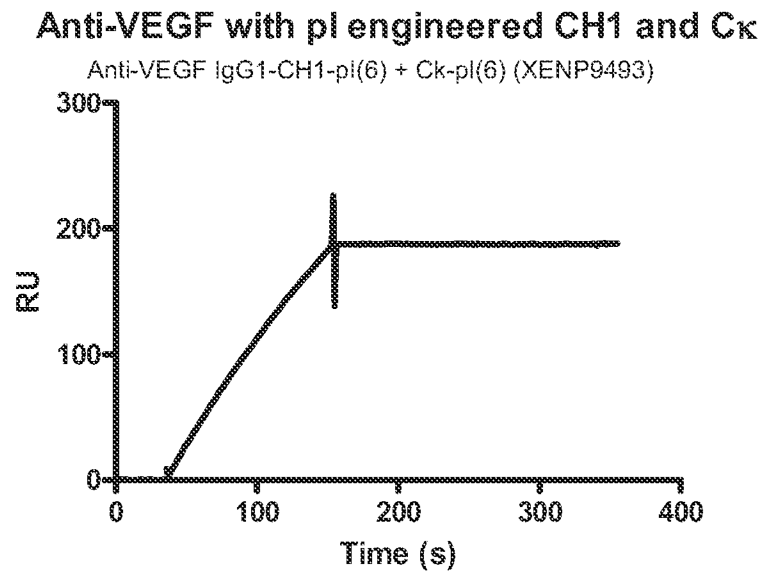


Figure 12

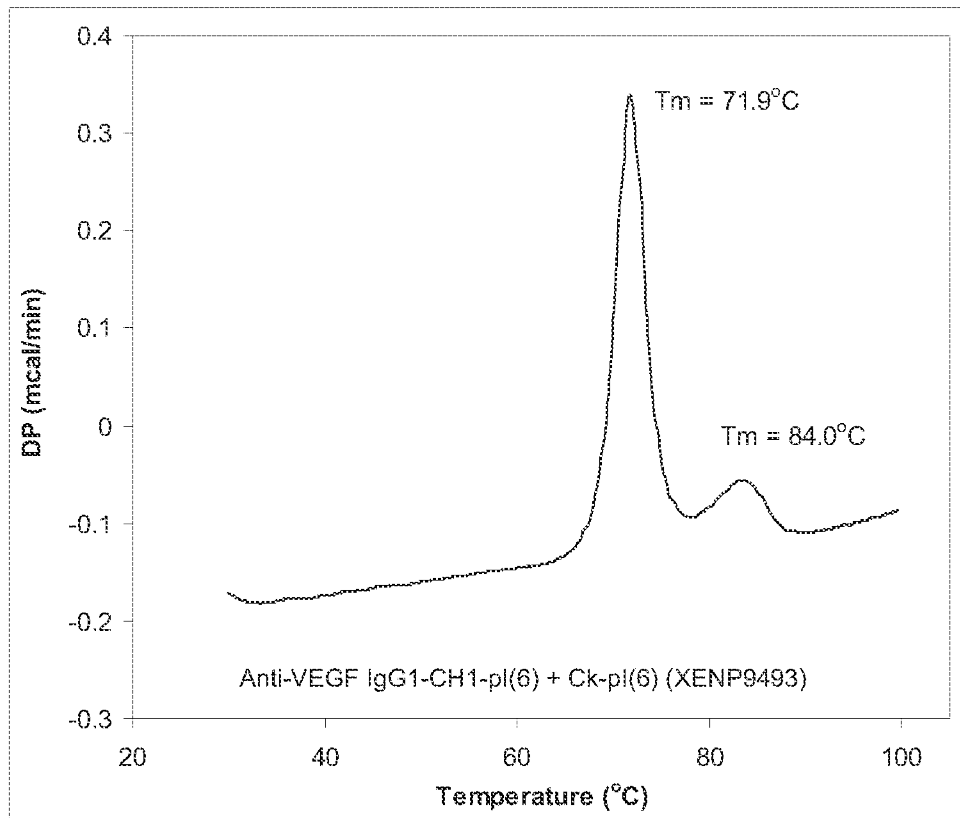


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Figure 13

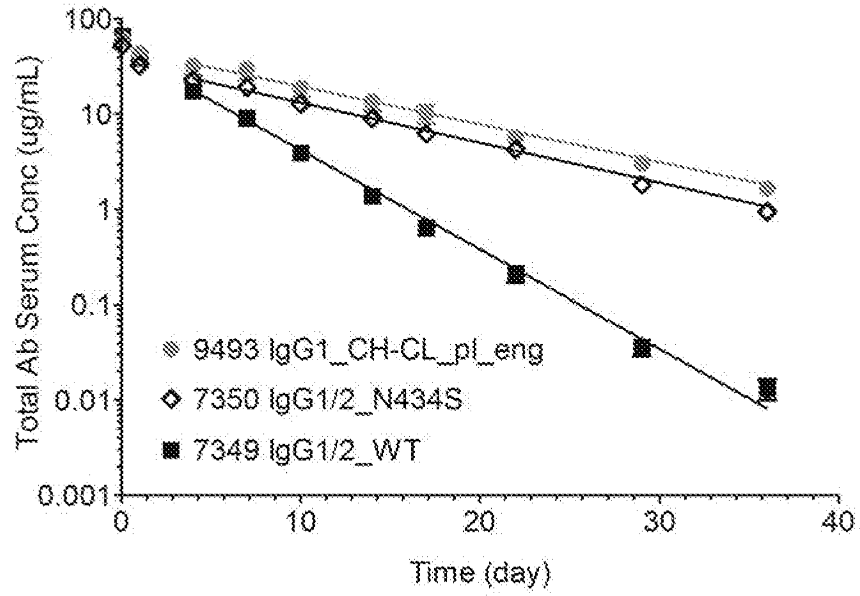
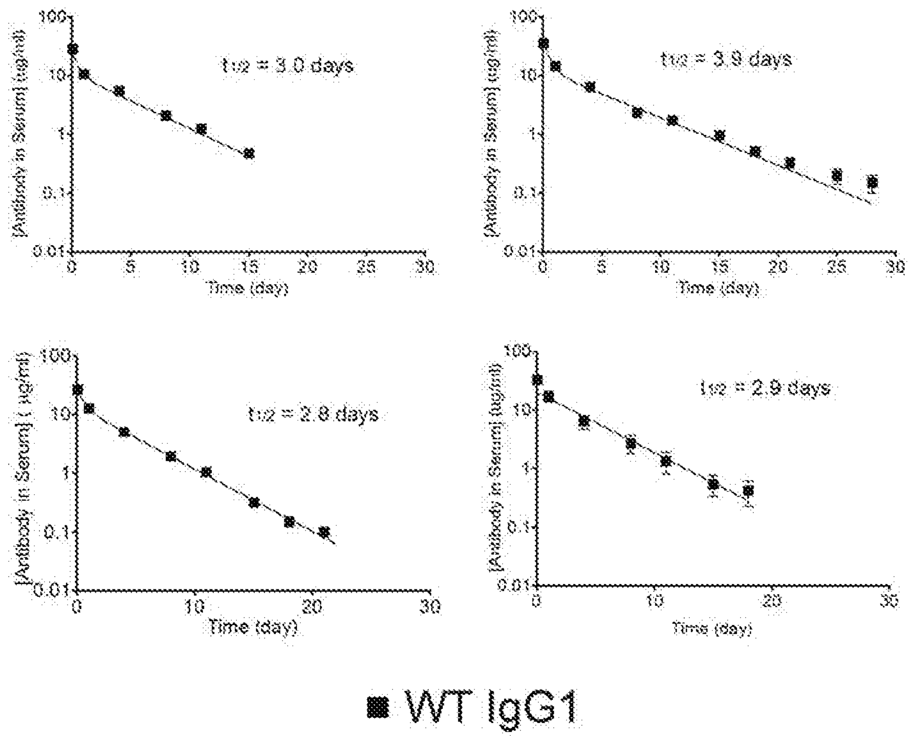


Figure 14



■ WT IgG1

Figure 15

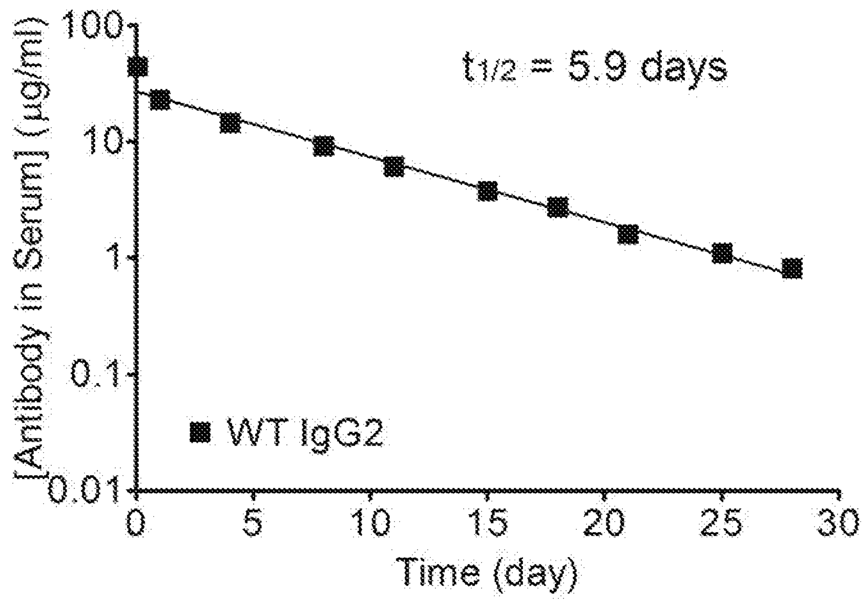


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Figure 16

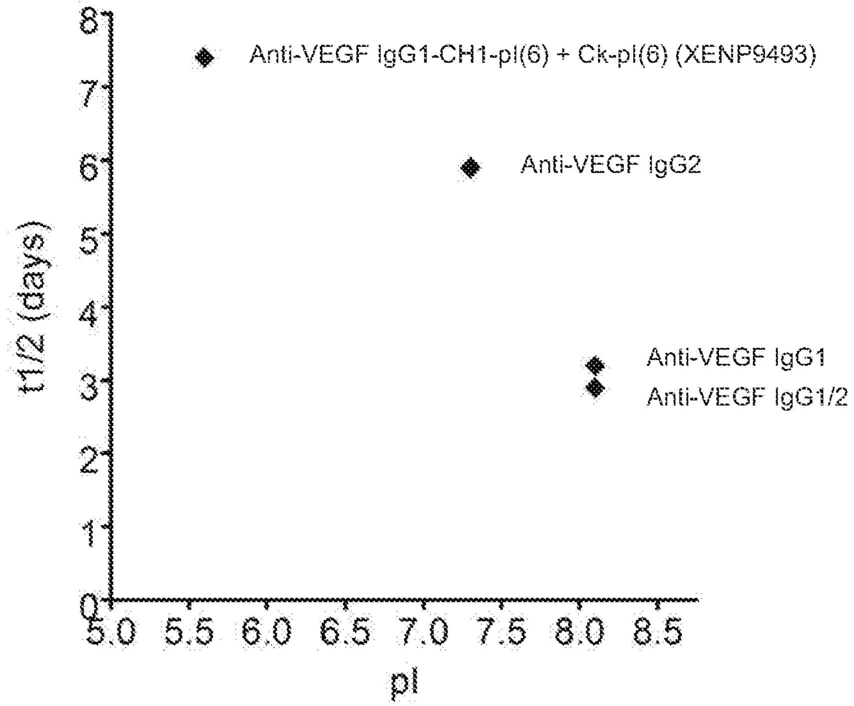

Figure 17

EU Index	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S

EU Index	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159
IgG1	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG2	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG3	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG4	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N

EU Index	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
IgG1	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG2	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG3	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG4	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y

EU Index	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201
IgG1	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N
IgG2	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N
IgG3	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	T	C	N
IgG4	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	K	T	Y	T	C	N

EU Index	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220		
IgG1	V	N	H	K	P	S	N	T	K	V	D	K	K/R	V	E	P	K	S	C		
IgG2	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C		
IgG3	V	N	H	K	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P		
IgG4	V	D	H	K	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G		

EU Index			221	222	223	224	225	226	227	228											
IgG1			D	K	T	H	T	C	P	P											
IgG2				V		E		C	P	P											
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C	D	T	P	P
IgG4						P	P	C	P	S											

EU Index																					
IgG1																					
IgG2																					
IgG3	P	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P
IgG4																					

EU Index														229	230	231	232	233	234	235	236
IgG1														C	P	A	P	E	L	L	G
IgG2														C	P	A	P	P	V	A	
IgG3	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P	A	P	E	L	L	G
IgG4														C	P	A	P	E	F	L	G

Figure 17 (continued)

EU Index	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P

EU Index	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278
IgG1	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y
IgG2	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y
IgG3	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y
IgG4	E	V	T	C	V	V	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y

EU Index	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299
IgG1	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T
IgG2	V	D	G	V/M	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T
IgG3	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T
IgG4	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T

EU Index	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320
IgG1	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K
IgG2	F	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K
IgG3	F	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K
IgG4	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K

EU Index	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	
IgG1	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K	
IgG2	C	K	V	S	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K	
IgG3	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K	
IgG4	C	K	V	S	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K	

EU Index	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D/E	E	L/M	T	K	N
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N

EU Index	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382
IgG1	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG2	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG3	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG4	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E

EU Index	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403
IgG1	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S
IgG2	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	M	L	D	S	D	G	S
IgG3	S	S	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S
IgG4	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S

EU Index	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424
IgG1	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
IgG2	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
IgG3	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	I	F	S
IgG4	F	F	L	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S

EU Index	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445
IgG1	C	S	V	M	H	E	A/G	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG2	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG3	C	S	V	M	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P
IgG4	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L

EU Index	446	447				
IgG1	G	K				
IgG2	G	K				
IgG3	G	K				
IgG4	G	K				
			D	E	D	E

Figure 18

EU Index	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
Cκ	**R**	T	V	A	A	P	S	V	F	I	F	P	P	S	**D**	**E**	Q	L
Cλ	Q	P	K	A	A	P	S	V	T	L	F	P	P	S	S	E	E	L
EU Index	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
Cκ	**K**	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	**R**	**E**
Cλ	Q	A	N	K	A	T	L	V	C	L	I	S	D	F	Y	P	G	A
EU Index	144	145	146	147	148	149	150	151	152	153		154	155	156	157	158	159	160
Cκ	A	**K**	V	Q	W	**K**	V	**D**	N	A		L	Q	S	G	N	S	Q
Cλ	V	T	V	A	W	K	A	D	S	S	P	V	K	A	G			V
EU Index	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178
Cκ	**E**	S	V	T	**E**	Q	**D**	S	**K**	**D**	S	T	Y	S	L	S	S	T
Cλ	E	T	T	T	P	S	K	Q	S	N	N	K	Y	A	A	S	S	Y
EU Index	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196
Cκ	L	T	L	S	**K**	A	**D**	Y	**E**	**K**	**H**	**K**	V	Y	A	C	**E**	V
Cλ	L	S	L	T	P	E	Q	W	K	S	H	R	S	Y	S	C	Q	V
EU Index	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214
Cκ	T	**H**	Q	G	L	S	S	P	V	T	**K**	S	F	N	**R**	G	**E**	C
Cλ	T	H	E	G			S	T	V	E	K	T	V	A	P	T	E	C

Figure 19

IgG1-WT (SEQ ID NO: 2)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

IgG2-WT (SEQ ID NO: 3)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

pI-iso1 (SEQ ID NO: 13)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSPG

pI-iso1(NF) (SEQ ID NO: 14)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSPG

pI-iso1(NF-VE) (SEQ ID NO: 15)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCVECPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSPG

pI-iso1(NF-VE-DEDE) (SEQ ID NO:16)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCVECPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSPGDEDE

Bevacizumab VH (SEQ ID NO: 9)
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPT
YAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLV
TVSS

Figure 19 cont.

IgG1-434S (SEQ ID NO: 50)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHSHYTQKSLSLSPGK

IgG2-434S (SEQ ID NO: 51)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC_V_E_CPPCPAPPV_AGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHSHYTQKSLSLSPGK

IgG1-pI(6)-Neutral-to-DE (SEQ ID NO: 55)
AETKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

IgG1-pI(6)-KR-to-Neutral (SEQ ID NO: 56)
ASTKGPSVFPLAPSSQSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHQPSNTQVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

IgG1-pI(6)-KR-to-DE (SEQ ID NO: 57)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

Figure 20

CK-WT (SEQ ID NO: 1)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

CK-pI(3) (SEQ ID NO: 17)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDNALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

CK-pI(6) (SEQ ID NO: 8)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDDALQEGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGEC

CK-pI(6-DEDE) (SEQ ID NO: 18)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDDALQEGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGECDEDE

Bevacizumab VL (SEQ ID NO: 10)
DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK

CK-pI(3) (SEQ ID NO: 54)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDNALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

CK-N152D S156E S202E (SEQ ID NO: 58)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDDALQEGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGEC

CK-K126Q K145Q K169Q (SEQ ID NO: 59)
RTVAAPSVFIFPPSDEQLQSGTASVVCLLNNFYPREAQVQWKVDNALQSGNSQESVTEQD
SQDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

CK-K126E K145E K169E (SEQ ID NO: 60)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDNALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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Figure 21

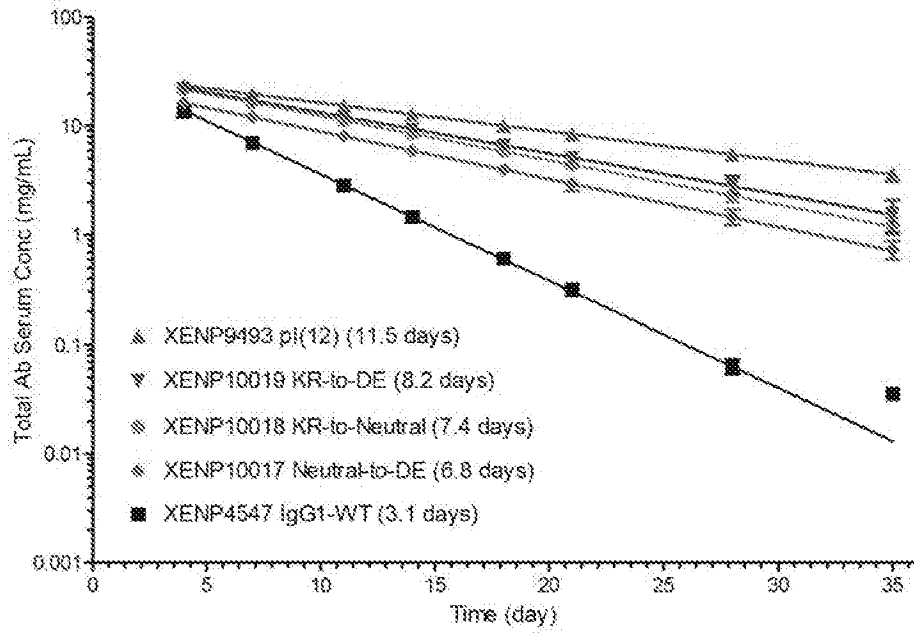

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Figure 22

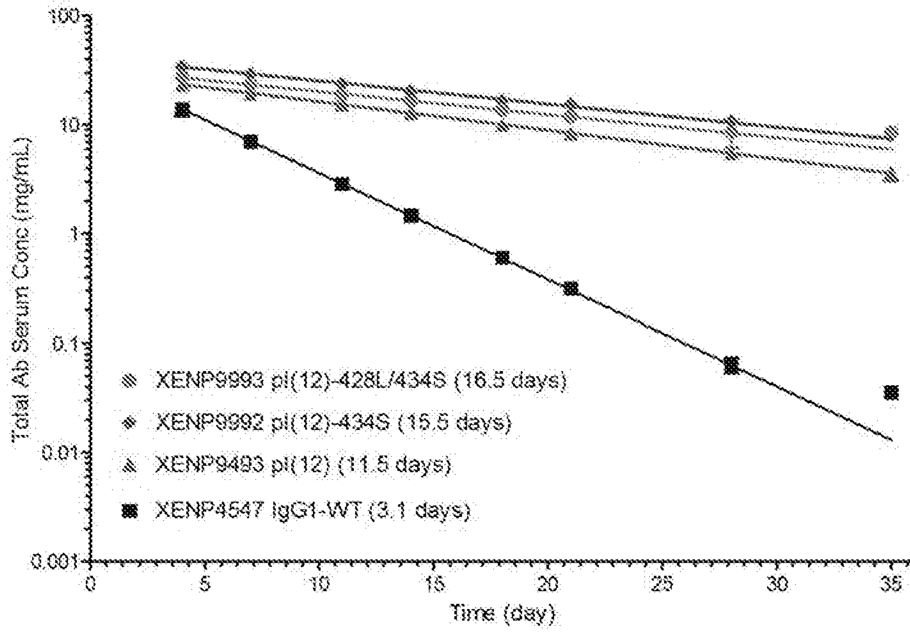

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Figure 23

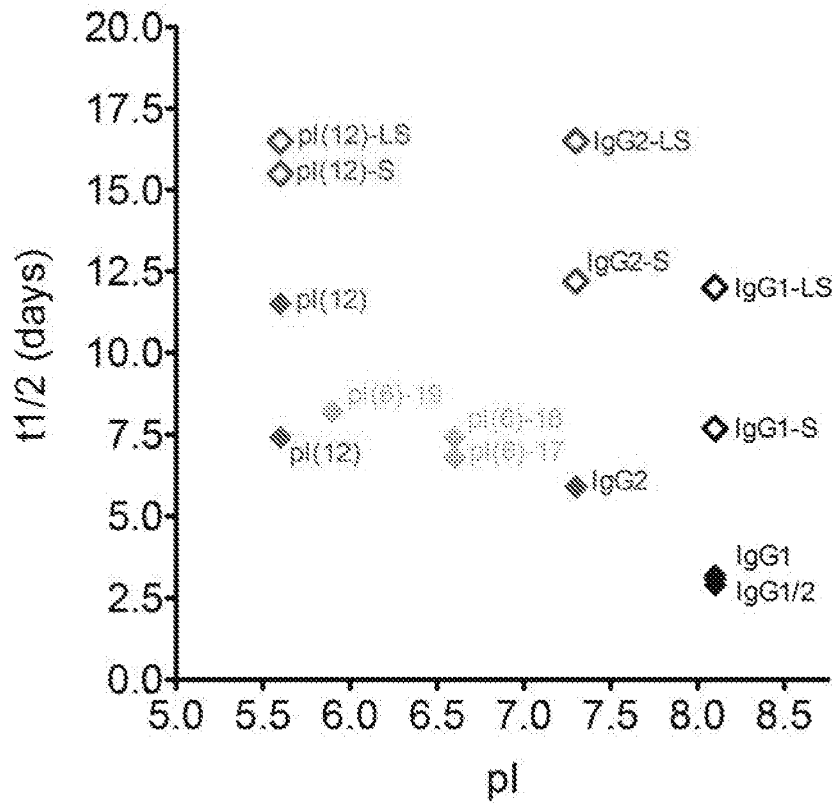


Figure 24

CH1

EU	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139
pI-iso3	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	E	S	T
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	T
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G	T
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	T

EU	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161
pI-iso3	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG1	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG2	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG3	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG4	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G

EU	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183
pI-iso3	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG1	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG2	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG3	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG4	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S

EU	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205
pI-iso3	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N	V	D	H	K
IgG1	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N	V	N	H	K
IgG2	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N	V	D	H	K
IgG3	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	T	C	N	V	N	H	K
IgG4	S	V	V	T	V	P	S	S	S	L	G	T	K	T	Y	T	C	N	V	D	H	K

EU	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
pI-iso3	P	S	N	T	K	V	D	K	T	V	E	P	K	S	C
IgG1	P	S	N	T	K	V	D	K	K/R	V	E	P	K	S	C
IgG2	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C
IgG3	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P
IgG4	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G

Hinge

EU			221	222	223	224	225															
pI-iso3			D	T	T	H	T															
IgG1			D	K	T	H	T															
IgG2				V		E																
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P
IgG4						P	P															

EU																						
pI-iso3																						
IgG1																						
IgG2																						
IgG3	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P	E	P
IgG4																						

EU									226	227	228	229	230	231	232	233	234	235	236			
pI-iso3									C	P	P	C	P	A	P	E	L	L	G			
IgG1									C	P	P	C	P	A	P	E	L	L	G			
IgG2									C	P	P	C	P	A	P	P	V	A				
IgG3	K	S	C	D	T	P	P	P	C	P	R	C	P	A	P	E	L	L	G			
IgG4									C	P	S	C	P	A	P	E	F	L	G			

Figure 24 (continued)

CH2

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258
pI-iso3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E

EU	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280
pI-iso3	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y	V	D
IgG1	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D
IgG2	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y	V	D
IgG3	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y	V	D
IgG4	V	T	C	V	V	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y	V	D

EU	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302
pI-iso3	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T	F	R	V
IgG1	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V
IgG2	G	V/M	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T	F	R	V
IgG3	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	F	R	V
IgG4	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T	Y	R	V

EU	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324
pI-iso3	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG1	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG2	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG3	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG4	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S

EU	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
pI-iso3	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG1	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
IgG2	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG3	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG4	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K

SUBSTITUTE SHEET (RULE 26)

Figure 24 (continued)

CH3

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	**355**	356	357	358	359	360	361	362
pI-iso3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	**Q**	E	E	M	T	K	N	Q
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D/E	E	L/M	T	K	N	Q
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N	Q

EU	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384
pI-iso3	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	S
IgG1	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N
IgG2	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N
IgG3	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	S
IgG4	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N

EU	385	386	387	388	389	390	391	**392**	393	394	395	396	397	398	399	400	401	402	403	404	405	406
pI-iso3	G	Q	P	E	N	N	Y	**N**	T	T	P	P	M	L	D	S	D	G	S	F	F	L
IgG1	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L
IgG2	G	Q	P	E	N	N	Y	K	T	T	P	P	M	L	D	S	D	G	S	F	F	L
IgG3	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S	F	F	L
IgG4	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L

EU	407	408	409	410	411	412	413	414	415	416	417	418	**419**	420	421	422	423	424	425	426	427	428
pI-iso3	Y	S	K	L	T	V	D	K	S	R	W	Q	**E**	G	N	V	F	S	C	S	V	M
IgG1	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M
IgG2	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M
IgG3	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	I	F	S	C	S	V	M
IgG4	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S	C	S	V	M

EU	429	430	431	432	433	434	**435**	436	437	438	439	440	441	442	443	444	445	446	**447**
pI-iso3	H	E	A	L	H	N	**H**	Y	T	Q	K	S	L	S	L	S	P	G	
IgG1	H	E	A/G	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG2	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG3	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P	G	K
IgG4	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L	G	K

Figure 25

```
IgG1      DKTHTCPPCPAPELLG
pI_Iso3   DTTHTCPPCPAPELLG

DTTHT present in IgG3
```

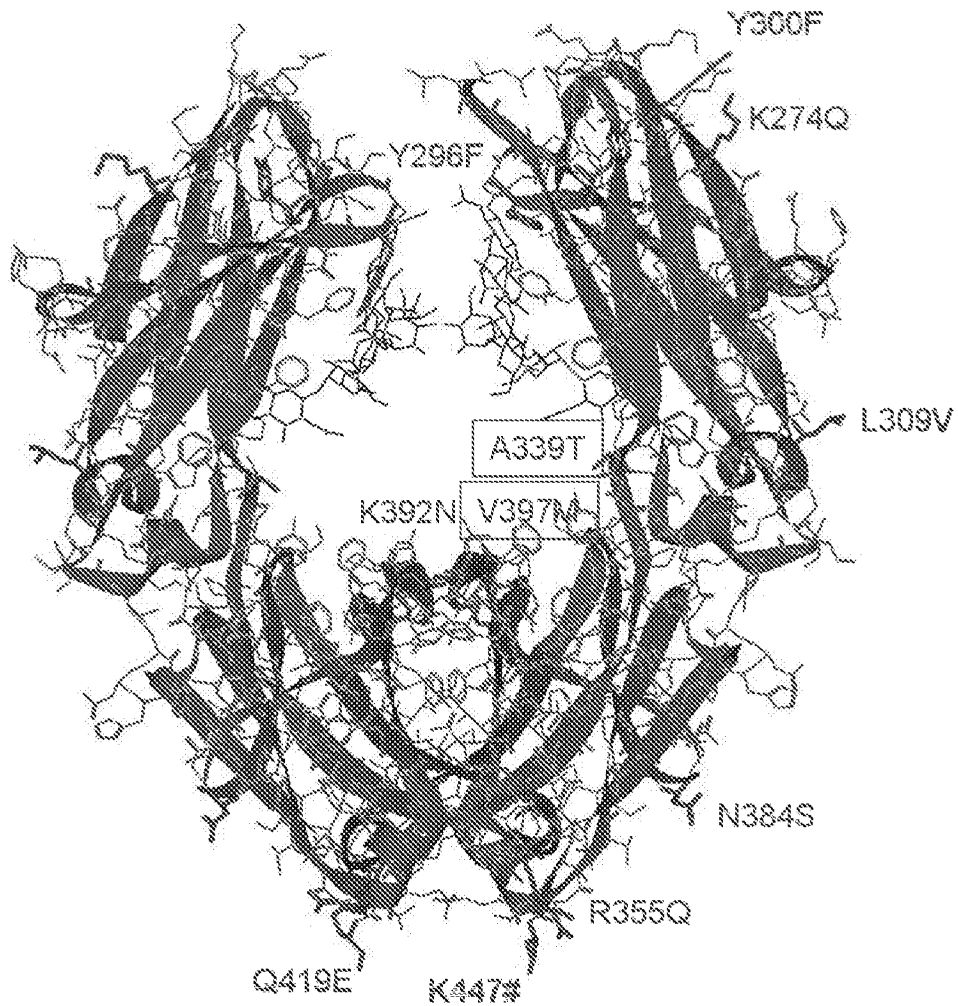
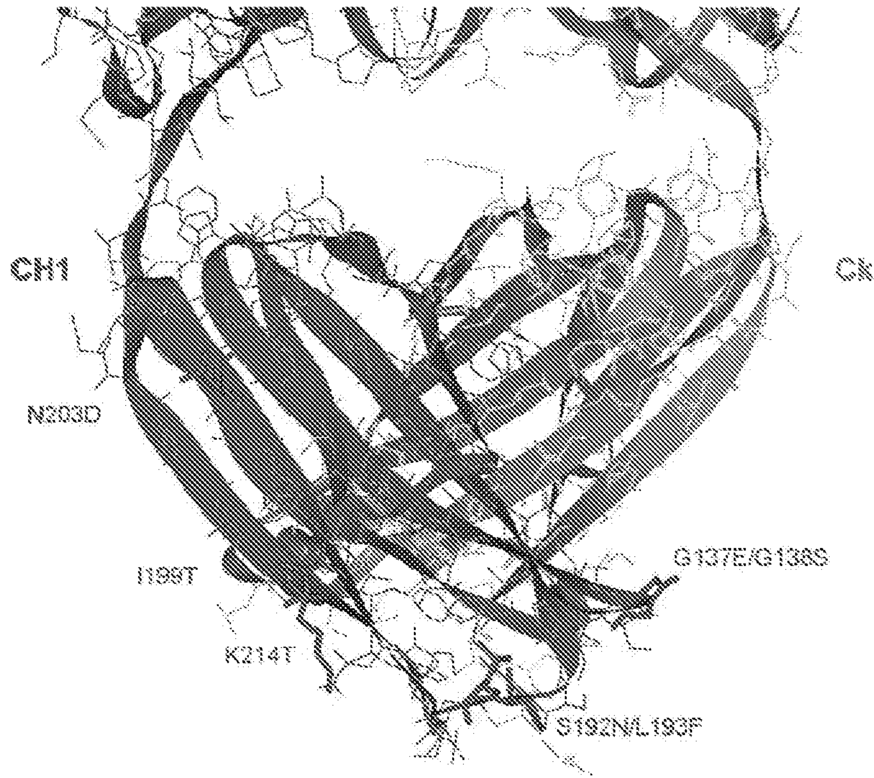


Figure 26



*pI iso3-SL has 192S/193L

+pI-iso3-charges-only contains all pI lowering substitutions (e.g. N203D), but does not contain neighboring isotypic mutations (e.g. I199T) that do not affect charge.

Figure 27

EU	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
C_κ	R	T	V	A	A	P	S	V	F	I	F	P	P	S	D	E	Q	L
EU	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143
C_κ	E	S	G	T	A	S	V	V	C	L	L	N	N	F	Y	P	R	E
EU	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	
C_κ	A	E	V	Q	W	K	V	D	N	A	L	Q	S	G	N	S	Q	
EU	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178
C_κ	E	S	V	T	E	Q	D	S	E	D	S	T	Y	S	L	S	S	T
EU	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196
C_κ	L	T	L	S	K	A	D	Y	E	K	H	K	V	Y	A	C	E	V
EU	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214
C_κ	T	H	Q	G	L	S	S	P	V	T	E	S	F	N	R	G	E	C

Figure 28

IgG-pI-Iso2 (SEQ ID NO: 19)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFS
CSVMHEALHNHYTQKSLSLSPG

IgG-pI-Iso2-434S (SEQ ID NO: 61)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFS
CSVMHEALHSHYTQKSLSLSPG

IgG-pI-Iso2-SL-434S (SEQ ID NO: 65)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTVEPKSCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFS
CSVMHEALHSHYTQKSLSLSPG

IgG-pI-Iso2-SL (SEQ ID NO: 20)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTVEPKSCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFS
CSVMHEALHNHYTQKSLSLSPG

IgG-pI-Iso2-charges-only (SEQ ID NO: 21)
ASTKGPSVFPLAPSSKSTSEGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVDHKPSNTKVDKTVEPKSCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKGLPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYNTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSC
SVMHEALHNHYTQKSLSLSPG

IgG-pI-Iso2-charges-only-434S (SEQ ID NO: 67)
ASTKGPSVFPLAPSSKSTSEGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVDHKPSNTKVDKTVEPKSCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKGLPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYNTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSC
SVMHEALHSHYTQKSLSLSPG

IgG-pI-Iso3 (SEQ ID NO: 22)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKN

Figure 28 cont.

QVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSPG

IgG-pI-Iso3-434S (SEQ ID NO: 62)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSR
WQEGNVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-Iso3-SL-434S (SEQ ID NO: 63)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFN
STFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSR
WQEGNVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-Iso3-SL-428L/434S (SEQ ID NO: 64)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGN
VFSCSVLHEALHSHYTQKSLSLSPG

IgG-pI-Iso3-SL (SEQ ID NO: 23)
ASTKGPSVFPLAPSSKSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSPG

IgG-pI-Iso3-charges-only (SEQ ID NO: 24)
ASTKGPSVFPLAPSSKSTSEGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPPVLDSDGSFFLYSKLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSPG

IgG-pI-Iso3-charges-only-434S (SEQ ID NO: 66)
ASTKGPSVFPLAPSSKSTSEGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVDHKPSNTKVDKTVEPKSCDTTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPPVLDSDGSFFLYSKLTVDKSRWQEGN
VFSCSVMHEALHSHYTQKSLSLSPG

IgG1-pI(7) (SEQ ID NO: 25)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWYVDGVEVHNAKTKPREEQYNSTY

RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSEEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPG

IgG1-pI(7)-434S (SEQ ID NO: 68)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSEEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHSHYTQKSLSLSPG

IgG1-pI(11) (SEQ ID NO: 26)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYECEVSNEALPAPIEETISKAKGQPREPQVYTLPPSEEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPG

IgG1/2-pI(7) (SEQ ID NO: 27)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSEEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPMLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPG

IgG1/2_pI(7)-434S (SEQ ID NO: 69)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSEEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPMLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHSHYTQKSLSLSPG

IgG1/2-pI(11) (SEQ ID NO: 28)
ASTKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHEPSNTEVDKKVEPKSCDKTHTCPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYECEVSNEGLPAPIEETISKTKGQPREPQVYTLPPSEEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPMLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPG

CK-pI(4) (SEQ ID NO: 29)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDNALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTESFNRGEC

CK-Iso(3) (SEQ ID NO: 30)
QTVAAPSVFIFPPSDEQLQSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHEGLSSPVTKSFNRGEC

CK-Iso(4) (SEQ ID NO: 31)
QTVAAPSVFIFPPSDEQLQSGTASVVCLLNNFYPREATVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHEGLSSPVTKSFNRGEC

Figure 28 cont.

CK-Iso(5) (SEQ ID NO: 32)
QTVAAPSVFIFPPSDEELQSGTASVVCLLNNFYPREATVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHEGLSSPVTKSFNRGEC

CK-Iso(6) (SEQ ID NO: 33)
QTVAAPSVFIFPPSDEELQSGTASVVCLLNDFYPREATVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHEGLSSPVTKSFNRGEC

Figure 29

Position	WT	fraction exposed (avg)	Delta E Glu (Avg)
246	K	0.60	-0.65
248	K	0.21	-0.16
255	R	0.37	1.36
274	K	0.57	-0.95
288	K	0.58	-0.81
290	K	0.42	-0.23
292	R	0.51	0.64
301	R	0.29	0.17
317	K	0.28	1.75
320	K	0.26	-0.22
322	K	0.21	-0.43
326	K	0.71	-0.58
334	K	0.35	-0.20
338	K	0.08	1.21
340	K	0.72	-0.57
344	R	0.45	0.28
355	R	0.78	-0.28
360	K	0.32	-1.26
370	K	0.13	-0.45
392	K	0.31	0.08
409	K	0.01	1.19
414	K	0.22	0.19
416	R	0.28	0.07
439	K	0.30	-0.15

Figure 30

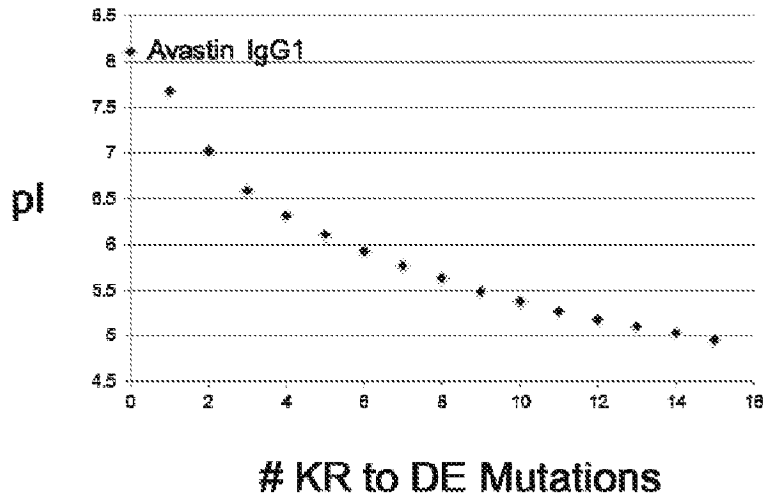


Figure 31

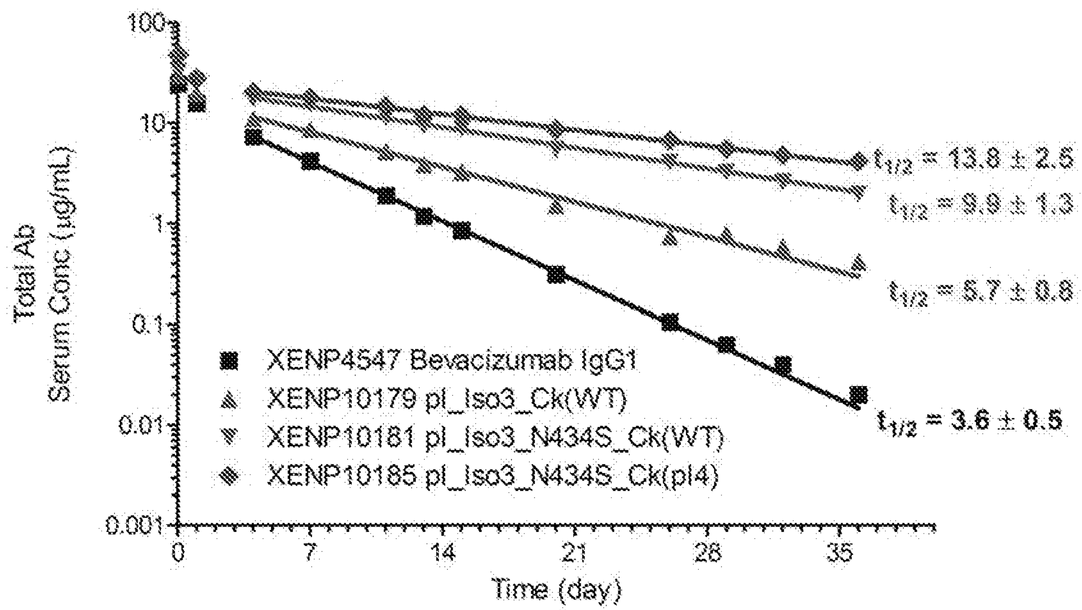
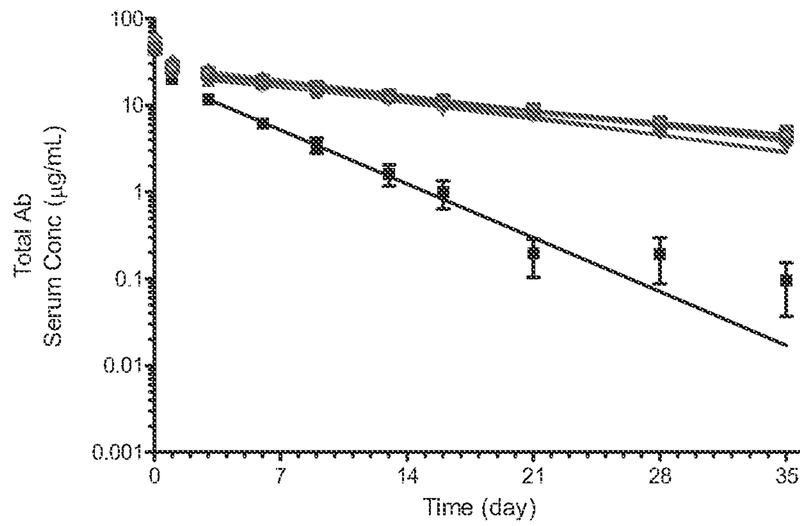


Figure 32



Bevacizumab (IgG1-WT)	**$t_{1/2}$ = 3.7 ± 1.1**
IgG2-434S	$t_{1/2}$ = 14.4 ± 1.3
IgG1-CH1-pI(6)-434S-CK-pI(6)	$t_{1/2}$ = 15.8 ± 2.8
IgG-pI-Iso3-SL-434S-CK-WT	$t_{1/2}$ = 11.5 ± 1.3
IgG-pI-Iso2-SL-434S-CK-WT	$t_{1/2}$ = 10.7 ± 1.7
IgG-pI-Iso3-charges-only-434S-CK-WT	$t_{1/2}$ = 12.0 ± 2.1
IgG-pI-Iso3-SL-434S-CK-Iso(5)	$t_{1/2}$ = 13.2 ± 1.2

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Figure 33

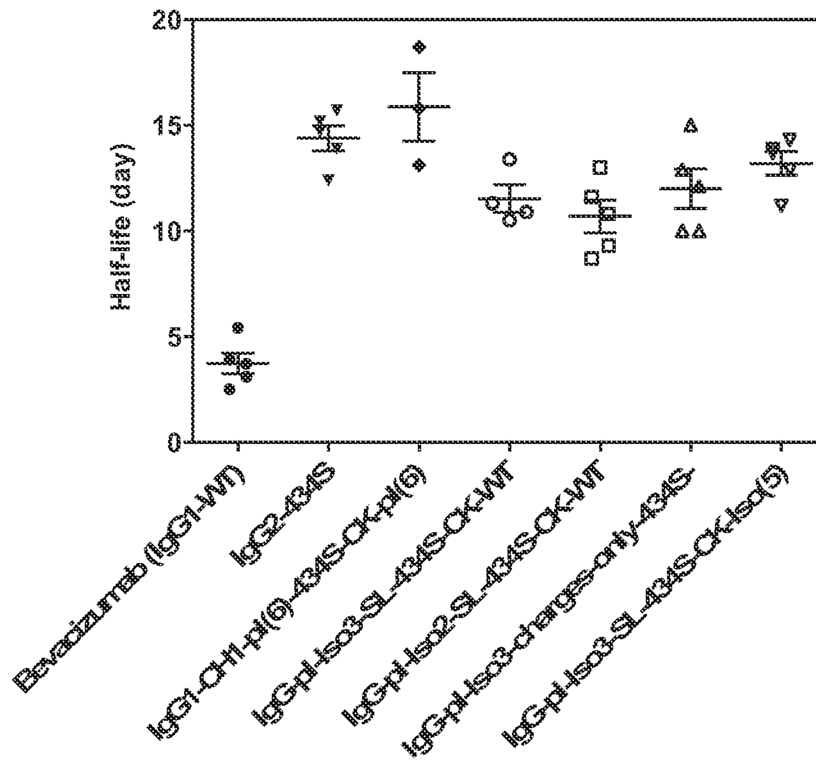
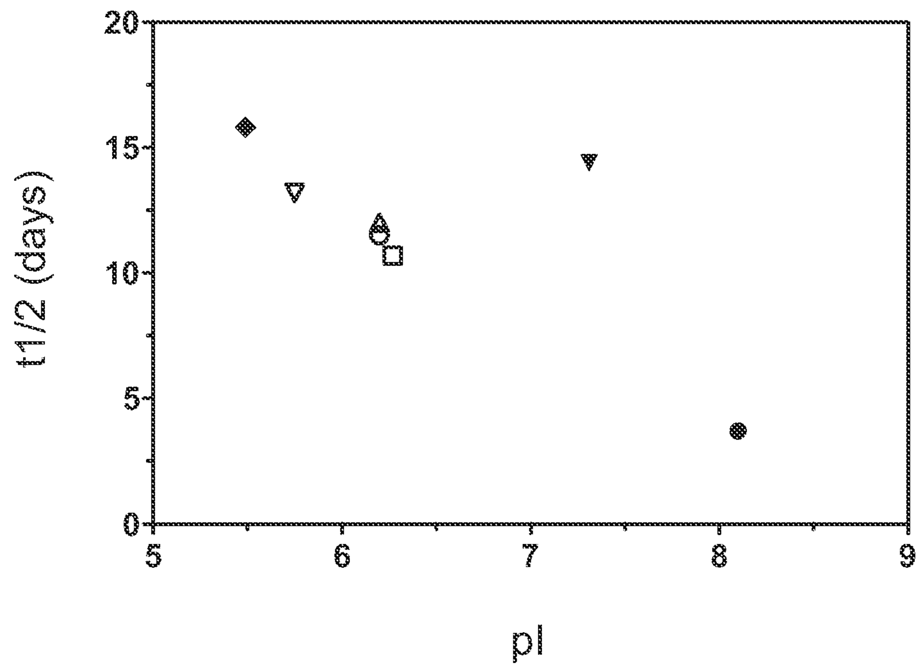
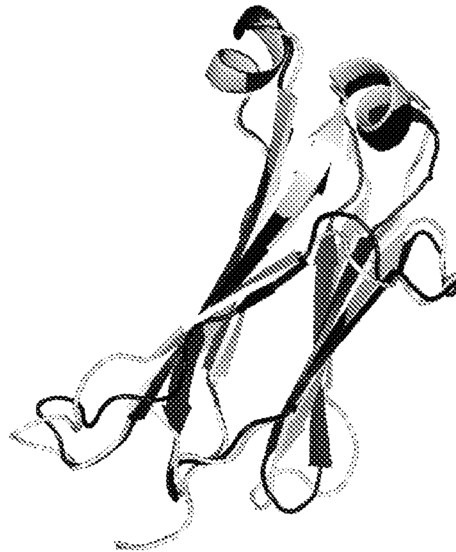


Figure 34



- Bevacizumab (IgG1-WT)
- IgG2-434S
- IgG1-CH1-pI(6)-434S-CK-pI(6)
- IgG-pI-Iso3-SL-434S-CK-WT
- IgG-pI-Iso2-SL-434S-CK-WT
- IgG-pI-Iso3-charges-only-434S-CK-WT
- IgG-pI-Iso3-SL-434S-CK-Iso(5)

Figure 35



Grey = C-kappa
Black = C-lambda

Figure 36

AMINO ACID	pI
Alanine Ala A	6.00
Arginine Arg R	11.15
Asparagine Asn N	5.41
Aspartic acid Asp D	2.77
Cysteine Cys C	5.02
Glutamic acid Glu E	3.22
Glutamine Gln Q	5.65
Glycine Gly G	5.97
Histidine His H	7.47
Isoleucine Ile I	5.94
Leucine Leu L	5.98
Lysine Lys K	9.59
Methionine Met M	5.74
Phenylalanine Phe F	5.48
Proline Pro P	6.30
Serine Ser S	5.68
Threonine Thr T	5.64
Tryptophan Trp W	5.89
Tyrosine Tyr Y	5.66
Valine Val V	5.96

Figure 37

XenP#	Protein_Name	Name (HC)	SEQ ID NO (HC)
XENP004547	Bevacizumab - Avastin - IgG1 WT	IgG1-WT	2
XENP006384	Bevacizumab_Avastin_IgG2_WT	IgG2-WT	3
NA	IgG3 example	IgG3-WT	4
NA	IgG4 example	IgG4-WT	5
XENP007349	Bevacizumab Avastin IgG1/2 WT	IgG1/2-HC	6
XENP005653	Bevacizumab_Avastin_IgG1_N434S	IgG1-434S	50
XENP006389	Bevacizumab_Avastin_IgG2_N434S	IgG2-434S	51
XENP009491	Bevacizumab_IgG1_S119E/K133E/T164E/K205E/N208D/K210E	IgG1-CH1-pI(6)	7
XENP009492	Bevacizumab_IgG1_CL_mutations_K126E/K145E/N152D/S156E/K169E/S202E	IgG1-WT	2
XENP009493	Bevacizumab_IgG1_CH-CL_pI_engineered_combo1	IgG1-CH1-pI(6)	7
XENP009992	Bevacizumab_Avastin_N434S_IgG1_CH1_pI(6)_CK_pI(6)	IgG1-CH1-pI(6)-434S	52
XENP009993	Bevacizumab_Avastin_N434S/M428L_IgG1_CH1_pI(6)_CK_pI(6)	IgG1-CH1-pI(6)-428L/434S	53
XENP010088	Bevacizumab_Avastin_IgG1_CK_pI(3)	IgG1-WT	2
XENP010089	Bevacizumab_Avastin_IgG1_CK_pI(6-DEDE)	IgG1-WT	2
XENP010090	Bevacizumab_Avastin_IgG2_CK_pI(3)	IgG2-WT	3
XENP010091	Bevacizumab_Avastin_IgG2_CK_pI(6)	IgG2-WT	3
XENP010092	Bevacizumab_Avastin_IgG2_CK_pI(6-DEDE)	IgG2-WT	3
XENP010093	Bevacizumab_Avastin_pI-iso1_CK_WT	pI-Iso1	13
XENP010094	Bevacizumab_Avastin_pI-iso1(NF)_CK_WT	pI-Iso1(NF)	14
XENP010095	Bevacizumab_Avastin_pI-iso1(NF-VE)_CK_WT	pI-Iso1(NF-VE)	15
XENP010096	Bevacizumab_Avastin_pI-iso1(NF-VE)_CK_pI(3)	pI-Iso1(NF-VE)	15
XENP010101	Bevacizumab_Avastin_pI-iso1(NF-VE)_CK_pI(6)	pI-Iso1(NF-VE)	15
XENP010102	Bevacizumab_Avastin_pI-iso1(NF-VE)_CK_pI(6-DEDE)	pI-Iso1(NF-VE)	15
XENP010103	Bevacizumab_Avastin_pI-iso1(NF-VE-DEDE)_CK_WT	pI-Iso1(NF-VE-DEDE)	16
XENP010104	Bevacizumab_Avastin_pI-iso1(NF-VE-DEDE)_CK_pI(3)	pI-Iso1(NF-VE-DEDE)	16
XENP010105	Bevacizumab_Avastin_pI-iso1(NF-VE-DEDE)_CK_pI(6)	pI-Iso1(NF-VE-DEDE)	16
XENP010106	Bevacizumab_Avastin_pI-iso1(NF-VE-DEDE)_CK_pI(6-DEDE)	pI-Iso1(NF-VE-DEDE)	16
XENP010107	Bevacizumab_Avastin_IgG1_pI(7)_CK_pI(4)	IgG1-pI(7)	25
XENP010108	Bevacizumab_Avastin_IgG1_pI(11)_CK_pI(4)	IgG1-pI(11)	26
XENP010109	Bevacizumab_Avastin_IgG1/2_pI(7)_CK_pI(4)	IgG1/2-pI(7)	27
XENP010110	Bevacizumab_Avastin_IgG1/2_pI(11)_CK_pI(4)	IgG1/2-pI(11)	28
XENP010017	Bevacizumab_Avastin_IgG1_CH/CL_charge_neutral_to_negative	IgG1-pI(6)-Neutral-to-DE	55
XENP010018	Bevacizumab_Avastin_IgG1_CH/CL_charge_positive_to_neutral	IgG1-pI(6)-KR-to-Neutral	56

Figure 37 (cont.)

XenP#	Name (LC)	SEQID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP004547	Ck-WT	1	8.10	122	0	116	0	6			
XENP006384	Ck-WT	1	7.31	118	-4	118	2	0	27	0	
NA	Ck-WT	1							22		
NA	Ck-WT	1							28		
XENP007349	Ck-WT	1	8.11	120	-2	114	-2	6	11	0	11
XENP005653	Ck-WT	1	8.1	122	0	116	0	6	1	0	1
XENP006389	Ck-WT	1	7.31	118	-4	118	2	0	28	0	28
XENP009491	Ck-WT	1	6.21	116	-6	128	12	-18	6	0	6
XENP009492	Ck-pI(6)	8	6.21	116	-6	128	12	-18	0	6	6
XENP009493	Ck-pI(6)	8	5.49	110	-12	140	24	-30	6	6	12
XENP009992	Ck-pI(6)	8	5.49	110	-12	140	24	-30	7	6	13
XENP009993	Ck-pI(6)	8	5.49	110	-12	140	24	-30	8	6	14
XENP010088	Ck-pI(3)	17	6.58	116	-6	122	6	-6	0	3	3
XENP010089	Ck-pI(6-DEDE)	18	5.85	116	-6	136	20	-20	0	10	10
XENP010090	Ck-pI(3)	54	6.16	112	-10	124	8	-12	27	3	30
XENP010091	Ck-pI(6)	8	5.88	112	-10	130	14	-18	27	6	33
XENP010092	Ck-pI(6-DEDE)	18	5.58	112	-10	138	22	-26	27	10	37
XENP010093	Ck-WT	1	6.20	110	-12	122	6	-12	13	0	13
XENP010094	Ck-WT	1	6.20	110	-12	122	6	-12	15	0	15
XENP010095	Ck-WT	1	6.16	110	-12	122	6	-12	19	0	19
XENP010096	Ck-pI(3)	54	5.63	104	-18	128	12	-24	19	3	22
XENP010101	Ck-pI(6)	8	5.43	104	-18	134	18	-30	19	6	25
XENP010102	Ck-pI(6-DEDE)	18	5.23	104	-18	142	26	-38	19	10	29
XENP010103	Ck-WT	1	5.79	110	-12	130	14	-20	22	0	22
XENP010104	Ck-pI(3)	54	5.37	104	-18	136	20	-32	22	3	25
XENP010105	Ck-pI(6)	8	5.22	104	-18	142	26	-38	22	6	28
XENP010106	Ck-pI(6-DEDE)	18	5.07	104	-18	150	34	-46	22	10	32
XENP010107	Ck-pI(4)	29	5.31	100	-18	136	20	-38	7	4	11
XENP010108	Ck-pI(4)	29	4.98	92	-22	144	28	-50	11	4	15
XENP010109	Ck-pI(4)	29	5.36	100	-30	134	18	-48	17	4	21
XENP010110	Ck-pI(4)	29	5.01	92	-22	142	26	-48	21	4	25
XENP010017	CK-N152D S156E S202	58	6.59	122	0	128	12	-6	3	3	6
XENP010018	CK-K126Q K145Q K169	59	6.58	110	-12	116	0	-6	3	3	6

23416002 1 : All variants

Figure 37 (cont.)

XenP#	Protein_Name	Name (HC)	SEQ ID NO (HC)
XENP010019	Bevacizumab_Avastin_IgG1_pI(3)_CH/CL_charge_positive_to_negative	IgG1-pI(6)-KR-to-DE	57
XENP010178	Bevacizumab_Avastin_pI_iso2_CK_WT	IgG-pI-Iso2	19
XENP010179	Bevacizumab_Avastin_pI_iso3_CK_WT	IgG-pI-Iso3	22
XENP010180	Bevacizumab_Avastin_pI_iso2_N434S_CK_WT	IgG-pI-Iso2-434S	61
XENP010181	Bevacizumab_Avastin_pI_iso3_N434S_CK_WT	IgG-pI-Iso3-434S	62
XENP010182	Bevacizumab_Avastin_pI_iso2_CK_pI(4)	IgG-pI-Iso2	19
XENP010183	Bevacizumab_Avastin_pI_iso3_CK_pI(4)	IgG-pI-Iso3	22
XENP010184	Bevacizumab_Avastin_pI_iso2_N434S_CK_pI(4)	IgG-pI-Iso2-434S	61
XENP010185	Bevacizumab_Avastin_pI_iso3_N434S_CK_pI(4)	IgG-pI-Iso3-434S	62
XENP010265	Bevacizumab_Avastin_pI_iso3_T222K		
XENP010266	Bevacizumab_Avastin_pI_iso3_Q274K		
XENP010267	Bevacizumab_Avastin_pI_iso3_F296Y		
XENP010268	Bevacizumab_Avastin_pI_iso3_F300Y		
XENP010269	Bevacizumab_Avastin_pI_iso3_V309L		
XENP010270	Bevacizumab_Avastin_pI_iso3_T339A		
XENP010271	Bevacizumab_Avastin_pI_iso3_Q355R		
XENP010272	Bevacizumab_Avastin_pI_iso3_S384N		
XENP010273	Bevacizumab_Avastin_pI_iso3_N392K		
XENP010274	Bevacizumab_Avastin_pI_iso3_M397V		
XENP010275	Bevacizumab_Avastin_pI_iso3_E419Q		
XENP010276	Bevacizumab_Avastin_pI_iso3_F296Y/F300Y		
XENP010277	Bevacizumab_Avastin_pI_iso3_S384N/N392K/M397V		
XENP010278	Bevacizumab_Avastin_pI_iso3_E137G		
XENP010279	Bevacizumab_Avastin_pI_iso3_S138G		
XENP010280	Bevacizumab_Avastin_pI_iso3_N192S		
XENP010281	Bevacizumab_Avastin_pI_iso3_F193L		
XENP010282	Bevacizumab_Avastin_pI_iso3_T199I		
XENP010283	Bevacizumab_Avastin_pI_iso3_D203N		
XENP010284	Bevacizumab_Avastin_pI_iso3_T214K		
XENP010285	Bevacizumab_Avastin_pI_iso3_E137G/S138G		
XENP010286	Bevacizumab_Avastin_pI_iso3_N192S/F193L	IgG-pI-Iso3-SL	23
XENP010287	Bevacizumab_Avastin_pI_iso3_T199I/D203N		
XENP010288	Bevacizumab_Avastin_pI_iso3_T214K/T222K		

23416002 1 : All variants

Figure 37 (cont.)

XenP#	Name (LC)	SEQID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010019	CK-K126E K145E K169!	60	5.92	110	-12	128	12	-18	3	3	6
XENP010178	Ck-WT	1	6.27	110	-12	120	4	-10	28	0	28
XENP010179	Ck-WT	1	6.20	110	-12	122	6	-12	19	0	19
XENP010180	Ck-WT	1	6.27	110	-12	120	4	-10	29	0	29
XENP010181	Ck-WT	1	6.20	110	-12	122	6	-12	20	0	20
XENP010182	Ck-pI(4)	29	5.55	102	-20	128	12	-26	28	4	32
XENP010183	Ck-pI(4)	29	5.54	102	-20	130	14	-28	19	4	23
XENP010184	Ck-pI(4)	29	5.55	102	-20	128	12	-26	29	4	33
XENP010185	Ck-pI(4)	29	5.54	102	-20	130	14	-28	20	4	24
XENP010265			6.31	112	-10	122	6	-10	18	0	18
XENP010266			6.31	112	-10	122	6	-10	18	0	18
XENP010267			6.20	110	-12	122	6	-12	18	0	18
XENP010268			6.20	110	-12	122	6	-12	18	0	18
XENP010269			6.20	110	-12	122	6	-12	18	0	18
XENP010270			6.20	110	-12	122	6	-12	18	0	18
XENP010271			6.31	112	-10	122	6	-10	18	0	18
XENP010272			6.20	110	-12	122	6	-12	18	0	18
XENP010273			6.31	112	-10	122	6	-10	18	0	18
XENP010274			6.20	110	-12	122	6	-12	18	0	18
XENP010275			6.31	110	-12	120	4	-10	18	0	18
XENP010276			6.20	110	-12	122	6	-12	17	0	17
XENP010277			6.31	112	-10	122	6	-10	16	0	16
XENP010278			6.31	110	-12	120	4	-10	18	0	18
XENP010279			6.20	110	-12	122	6	-12	18	0	18
XENP010280			6.20	110	-12	122	6	-12	18	0	18
XENP010281			6.20	110	-12	122	6	-12	18	0	18
XENP010282			6.20	110	-12	122	6	-12	18	0	18
XENP010283			6.31	110	-12	120	4	-10	18	0	18
XENP010284			6.31	112	-10	122	6	-10	18	0	18
XENP010285			6.31	110	-12	120	4	-10	17	0	17
XENP010286	Ck-WT	1	6.20	110	-12	122	6	-12	17	0	17
XENP010287			6.31	110	-12	120	4	-10	17	0	17
XENP010288			6.44	114	-8	122	6	-8	17	0	17

23416002 1 : All variants

Figure 37 (cont.)

XenP#	Protein_Name	Name (HC)	SEQ ID NO (HC)
XENP010289	Bevacizumab_Avastin_pI_iso3_S138G/N192S/F193L		
XENP010290	Bevacizumab_Avastin_pI_iso3_E137G/S138G/N192S/F193L		
XENP010324	Bevacizumab_Avastin_H0_pI_iso3_L0_Ckappa_iso(3)	IgG-pI-Iso3	22
XENP010325	Bevacizumab_Avastin_H0_pI_iso3_L0_Ckappa_iso(4)	IgG-pI-Iso3	22
XENP010326	Bevacizumab_Avastin_H0_pI_iso3_L0_Ckappa_iso(5)	IgG-pI-Iso3	22
XENP010327	Bevacizumab_Avastin_H0_pI_iso3_L0_Ckappa_iso(6)	IgG-pI-Iso3	22
XENP010425	Bevacizumab_Avastin_H0L0_IgG2_CH1_IgG1_CH2_CH3		
XENP010426	Bevacizumab_Avastin_H0L0_pI_iso3_charges_only	IgG-pI-Iso3-charges-only	24
XENP010427	Bevacizumab_Avastin_H0L0_pI_iso2_charges_only	IgG-pI-Iso2-charges-only	21
XENP010428	Bevacizumab_Avastin_H0L0_IgG2_CH1_IgG1_Hinge_CH2_CH3		
XENP010466	Bevacizumab_Avastin_H0L0_pI_iso3_N192S/F193L_N434S	IgG-pI-Iso3-SL-434S	63
XENP010467	Bevacizumab_Avastin_H0L0_pI_iso3_N192S/F193L_M428L/N434S	IgG-pI-Iso3-SL-428L/434S	64
XENP010468	Bevacizumab_Avastin_H0L0_pI_iso3_S138G/N192S/F193L_N434S		
XENP010469	Bevacizumab_Avastin_H0L0_pI_iso3_E137G/S138G/N192S/F193L_N434S		
XENP010470	Bevacizumab_Avastin_H0L0_pI_iso2_N192S/F193L	IgG-pI-Iso2-SL	20
XENP010471	Bevacizumab_Avastin_H0L0_pI_iso2_N192S/F193L_N434S	IgG-pI-Iso2-SL-434S	65
XENP010472	Bevacizumab_Avastin_H0L0_pI_iso3_charges_only_N434S	IgG-pI-Iso3-charges-only-434S	66
XENP010473	Bevacizumab_Avastin_H0L0_pI_iso2_charges_only_N434S	IgG-pI-Iso2-charges-only-434S	67
XENP010474	Bevacizumab_Avastin_H0L0_IgG2_CH1_IgG1_CH2_CH3_N434S		
XENP010475	Bevacizumab_Avastin_H0L0_IgG2_CH1_IgG1_Hinge_CH2_CH3_N434S		
XENP010476	Bevacizumab_Avastin_H0L0_IgG1_pI(7)_N434S_CK_pI(4)	IgG1_pI(7)-434S	68
XENP010477	Bevacizumab_Avastin_H0L0_IgG1/2_pI(7)_N434S_CK_pI(4)	IgG1/2_pI(7)-434S	69
XENP010478	Bevacizumab_Avastin_H0L0_pI_iso1/2_charges_only		
XENP010511	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_L0_Ckappa_iso(3)	IgG-pI-Iso3-SL	23
XENP010512	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_L0_Ckappa_iso(4)	IgG-pI-Iso3-SL	23
XENP010513	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_L0_Ckappa_iso(5)	IgG-pI-Iso3-SL	23
XENP010517	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_N434S_L0_Ckappa_iso(3)	IgG-pI-Iso3-SL-434S	63
XENP010518	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_N434S_L0_Ckappa_iso(4)	IgG-pI-Iso3-SL-434S	63
XENP010519	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_N434S_L0_Ckappa_iso(5)	IgG-pI-Iso3-SL-434S	63
XENP010520	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_M428L/N434S_L0_Ckappa_iso(3)	IgG-pI-Iso3-SL-428L/434S	64
XENP010521	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_M428L/N434S_L0_Ckappa_iso(4)	IgG-pI-Iso3-SL-428L/434S	64
XENP010522	Bevacizumab_Avastin_H0_pI_iso3_N192S/F193L_M428L/N434S_L0_Ckappa_iso(5)	IgG-pI-Iso3-SL-428L/434S	64
XENP010525	Bevacizumab_Avastin_H0L0_pI_iso3_N192S/F193L_N434S_CK_pI(4)	IgG-pI-Iso3-SL-434S	63

23416002 1 : All variants

Figure 37 (cont.)

XenP#	Name (LC)	SEQID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010289			6.20	110	-12	122	6	-12	16	0	16
XENP010290			6.31	110	-12	120	4	-10	15	0	15
XENP010324	Ck-Iso(3)	30	5.92	106	-16	124	8	-18	19	3	22
XENP010325	Ck-Iso(4)	31	5.83	104	-18	124	8	-20	19	4	23
XENP010326	Ck-Iso(5)	32	5.75	104	-18	126	10	-22	19	5	24
XENP010327	Ck-Iso(6)	33	5.68	104	-18	128	12	-24	19	6	25
XENP010425			7.30	120	-2	120	4	0	16	0	16
XENP010426	Ck-WT	1	6.20	110	-12	122	6	-12	9	0	9
XENP010427	Ck-WT	1	6.27	110	-12	120	4	-10	18	0	18
XENP010428			7.67	122	0	120	4	2	9	0	9
XENP010466	Ck-WT	1	6.20	110	-12	122	6	-12	18	0	18
XENP010467	Ck-WT	1	6.20	110	-12	122	6	-12	19	0	19
XENP010468			6.20	110	-12	122	6	-12	17	0	17
XENP010469			6.31	110	-12	120	4	-10	16	0	16
XENP010470	Ck-WT	1	6.27	110	-12	120	4	-10	26	0	26
XENP010471	Ck-WT	1	6.27	110	-12	120	4	-10	27	0	27
XENP010472	Ck-WT	1	6.20	110	-12	122	6	-12	10	0	10
XENP010473	Ck-WT	1	6.27	110	-12	120	4	-10	19	0	19
XENP010474			7.30	120	-2	120	4	0	17	0	17
XENP010475			7.67	122	0	120	4	2	10	0	10
XENP010476	Ck-pI(4)	29	5.31	100	-22	136	20	-36	8	4	12
XENP010477	Ck-pI(4)	29	5.36	100	-22	134	18	-34	18	4	22
XENP010478			6.27	110	-12	120	4	-10	23	0	23
XENP010511	Ck-Iso(3)	30	5.92	106	-16	124	8	-18	17	3	20
XENP010512	Ck-Iso(4)	31	5.83	104	-18	124	8	-20	17	4	21
XENP010513	Ck-Iso(5)	32	5.75	104	-18	126	10	-22	17	5	22
XENP010517	Ck-Iso(3)	30	5.92	106	-16	124	8	-18	18	3	21
XENP010518	Ck-Iso(4)	31	5.83	104	-18	124	8	-20	18	4	22
XENP010519	Ck-Iso(5)	32	5.75	104	-18	126	10	-22	18	5	23
XENP010520	Ck-Iso(3)	30	5.92	106	-16	124	8	-18	19	3	22
XENP010521	Ck-Iso(4)	31	5.83	104	-18	124	8	-20	19	4	23
XENP010522	Ck-Iso(5)	32	5.75	104	-18	126	10	-22	19	5	24
XENP010525	Ck-pI(4)	29	5.54	102	-20	130	14	-28	18	4	22

23416002 1 : All variants

Figure 37 (cont.)

XenP#	Protein_Name	Name (HC)	SEQ ID NO (HC)
XENP010526	Bevacizumab_Avastin_H0_pI_iso3_N434S_L0_Ckappa_iso(5)	IgG-pI-Iso3-434S	62
XENP010527	Bevacizumab_Avastin_H0_pI_iso2_N192S/F193L_N434S_L0_Ckappa_iso(5)	IgG-pI-Iso2-SL-434S	65
XENP010589	Bevacizumab_Avastin_IgG1_pI_variant_CH4CK8	IgG-pI-CH1-v4	34
XENP010590	Bevacizumab_Avastin_IgG1_pI_variant_CH25CK28	IgG-pI-CH1-v25	35
XENP010591	Bevacizumab_Avastin_IgG1_pI_variant_CH42CK23	IgG-pI-CH1-v42	36
XENP010592	Bevacizumab_Avastin_IgG1_pI_variant_CH16CK12	IgG-pI-CH1-v16	37
XENP010593	Bevacizumab_Avastin_pI_variant_CH4_SLFFV_9merISO_N434S_CK8	IgG-pI-CH1-v4-SLFFV-Iso-434S	42
XENP010594	Bevacizumab_Avastin_pI_variant_CH25_SLFFV_9merISO_N434S_CK28	IgG-pI-CH1-v25-SLFFV-Iso-434S	43
XENP010595	Bevacizumab_Avastin_pI_variant_CH42_SLFFV_9merISO_N434S_CK23	IgG-pI-CH1-v42-SLFFV-Iso-434S	44
XENP010596	Bevacizumab_Avastin_pI_variant_CH16_SLFFV_9merISO_N434S_CK12	IgG-pI-CH1-v16-SLFFV-Iso-434S	45
XENP010601	Bevacizumab_Avastin_pI_variant_CH4_SL_9merISO_N434S_CK8	IgG-pI-CH1-v4-SL-Iso-434S	46
XENP010602	Bevacizumab_Avastin_pI_variant_CH25_SL_9merISO_N434S_CK28	IgG-pI-CH1-v25-SL-Iso-434S	47
XENP010603	Bevacizumab_Avastin_pI_variant_CH42_SL_9merISO_N434S_CK23	IgG-pI-CH1-v42-SL-Iso-434S	48
XENP010604	Bevacizumab_Avastin_pI_variant_CH16_SL_9merISO_N434S_CK12	IgG-pI-CH1-v16-SL-Iso-434S	49
XENP010621	Bevacizumab_Avastin_pI_IgG1_IF16_ISO_CK23	IgG1-pI-IF16-ISO	70
XENP010622	Bevacizumab_Avastin_pI_IgG1_IF10_ISO_CK12	IgG1-pI-IF10-ISO	71
XENP010623	Bevacizumab_Avastin_pI_IgG2_IF10_ISO_N434S_CK12	IgG2-pI-IF10-ISO-N434S	72
XENP010624	Bevacizumab_Avastin_pI_IgG2_IF16_ISO_N434S_CK23	IgG2-pI-IF16-ISO-N434S	73
XENP010625	Bevacizumab_Avastin_pI_Hybrid_IF16_ISO_N434S_CK23	Hybrid-pI-IF16-ISO-N434S	74
XENP010626	Bevacizumab_Avastin_pI_Hybrid_IF10_ISO_N434S_CK12	Hybrid-pI-IF10-ISO-N434S	75
XENP010628	Bevacizumab_Avastin_pI_Hybrid_2-1-2_IF16_ISO_N434S_CK23	Hybrid-2-1-2-pI-IF16-ISO-N434S	76
XENP010629	Bevacizumab_Avastin_pI_Hybrid_2-1-2_IF10_ISO_N434S_CK12	Hybrid-2-1-2-pI-IF10-ISO-N434S	77
XENP010648	Bevacizumab_Avastin_pI_IgG1_IF10_CH1-Fc_charges_CK12	IgG1-IF10-CH1-Fc-charges	78
XENP010649	Bevacizumab_Avastin_pI_IgG1_IF16_CH1-Fc_charges_CK23	IgG1-IF16-CH1-Fc-charges	79
XENP010650	Bevacizumab_Avastin_pI_IgG1_IF10_ISO_CH1-Fc_charges_CK12	IgG1-IF10-ISO-CH1-Fc-charges	80
XENP010651	Bevacizumab_Avastin_pI_IgG1_IF16_ISO_CH1-Fc_charges_CK23	IgG1-IF16-ISO-CH1-Fc-charges	81

23416002 1 : All variants

Figure 37 (cont.)

XenP#	Name (LC)	SEQID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010526	Ck-Iso(5)	32	5.75	104	-18	126	10	-22	20	5	25
XENP010527	Ck-Iso(5)	32	5.78	104	-18	124	8	-20	27	5	32
XENP010589	CK-v8	38	5.69	112	-10	136	20	-30	5	5	10
XENP010590	CK-v28	39	5.85	114	-8	134	18	-26	4	5	9
XENP010591	CK-v23	40	6.01	114	-8	130	14	-22	3	4	7
XENP010592	CK-v12	41	6.44	116	-6	124	8	-14	2	2	4
XENP010593	CK-v8	38	5.37	104	-18	138	22	-40	17	5	22
XENP010594	CK-v28	39	5.49	106	-16	136	20	-36	16	5	21
XENP010595	CK-v23	40	5.61	106	-16	132	16	-32	15	4	19
XENP010596	CK-v12	41	5.92	108	-14	126	10	-24	14	2	16
XENP010601	CK-v8	38	5.37	104	-18	138	22	-40	14	5	19
XENP010602	CK-v28	39	5.49	106	-16	136	20	-36	13	5	18
XENP010603	CK-v23	40	5.61	106	-16	132	16	-32	12	4	16
XENP010604	CK-v12	41	5.92	108	-14	126	10	-24	11	2	13
XENP010621	CK-v23	40	5.61	106	-16	132	16	-32	10	4	14
XENP010622	CK-v12	41	5.92	108	-14	126	10	-24	9	2	11
XENP010623	CK-v12	41	5.71	106	-16	128	12	-28	35	2	37
XENP010624	CK-v23	40	5.43	104	-18	134	18	-36	36	4	40
XENP010625	CK-v23	40	5.61	106	-16	132	16	-32	15	4	19
XENP010626	CK-v12	41	5.92	108	-14	126	10	-24	14	2	16
XENP010628	CK-v23	40	5.31	102	-20	138	22	-42	24	4	28
XENP010629	CK-v12	41	5.54	104	-18	132	16	-34	23	2	25
XENP010648	CK-v12	41	5.93	112	-10	130	14	-24	6	2	8
XENP010649	CK-v23	40	5.38	108	-14	142	26	-40	10	4	14
XENP010650	CK-v12	41	5.76	108	-14	130	14	-28	10	2	12
XENP010651	CK-v23	40	5.28	106	-16	144	28	-44	14	4	18

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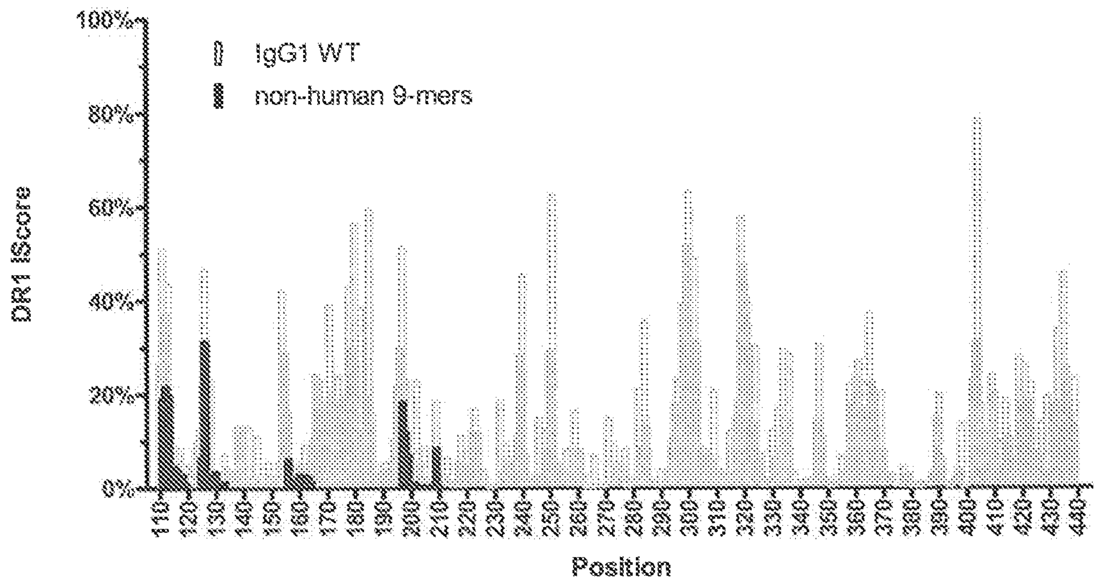
Figure 38



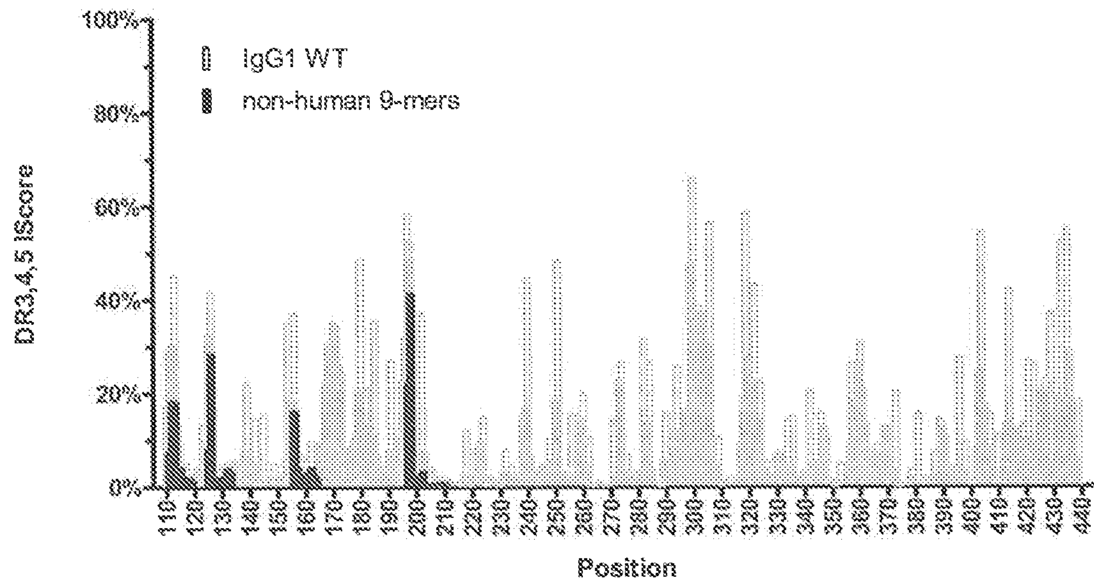


Figure 39

MAX DR1	MAX DR3,4,5	MAX (All DR)	Variant (HC)	119	133	164	205	208	210	# mutations	pI (HC)
NA	NA		IgG1	S	K	T	K	N	K	0	8.38
31.3%	[illegible]	41.3%	IgG1-CH1-pI(6)	E	E	E	E	D	E	6	6.16
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v1	S	E	E	E	D	E	5	6.30
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v2	E	K	E	E	D	E	5	6.46
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v3	E	E	T	E	D	E	5	6.30
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v4	E	E	E	K	D	E	5	6.46
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v5	E	E	E	E	N	E	5	6.30
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v6	E	E	E	E	D	K	5	6.46
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v7	S	K	E	E	D	E	4	6.66
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v8	E	K	T	E	D	E	4	6.66
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v9	E	E	T	K	D	E	4	6.66
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v10	E	E	E	K	N	E	4	6.66
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v11	E	E	E	E	N	K	4	6.66
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v12	S	K	T	E	D	E	3	6.94
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v13	E	K	T	K	D	E	3	7.35
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v14	E	E	T	K	N	E	3	6.94
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v15	E	E	E	K	N	K	3	7.35
8.6%	4.3%	8.6%	IgG1-pI-CH1-v16	S	K	T	K	D	E	2	7.77
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v17	E	K	T	K	N	E	2	7.77
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v18	E	E	T	K	N	K	2	7.77
8.6%	7.4%	8.6%	IgG1-pI-CH1-v19	S	K	T	K	N	E	1	8.05
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v20	E	K	T	K	N	K	1	8.24
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v21	S	E	T	E	D	E	4	6.46
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v22	S	E	E	K	D	E	4	6.66
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v23	S	E	E	E	N	E	4	6.46
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v24	S	E	E	E	D	K	4	6.66
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v25	E	K	E	K	D	E	4	6.94
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v26	E	K	E	E	N	E	4	6.66
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v27	E	K	E	E	D	K	4	6.94
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v28	E	E	T	E	N	E	4	6.46
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v29	E	E	T	E	D	K	4	6.66
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v30	E	E	E	K	D	K	4	6.94

Figure 39 (continued)

MAX DR1	MAX DR3,4,5	MAX (All DR)	Variant (HC)	119	133	164	205	208	210	# mutations	pI (HC)
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v31	S	K	E	E	D	K	3	7.35
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v32	E	K	E	K	D	K	3	7.77
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v33	S	K	T	E	N	E	2	7.35
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v34	S	E	T	K	D	E	3	6.94
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v35	S	E	E	K	N	K	2	7.77
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v36	S	K	E	E	N	K	2	7.77
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v37	S	E	E	K	D	K	3	7.35
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v38	S	K	T	E	D	K	2	7.77
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v39	E	E	T	E	N	K	3	6.94
11.8%	5.7%	11.8%	IgG1-pI-CH1-v40	S	K	T	K	D	K	1	8.24
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v41	S	K	E	E	N	E	3	6.94
8.6%	16.2%	16.2%	IgG1-pI-CH1-v42	S	K	E	K	D	E	3	7.35
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v43	E	K	T	E	D	K	3	7.35
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v44	E	K	E	E	N	K	3	7.35
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v45	E	K	T	E	N	E	3	6.94
21.8%	[illegible]	41.3%	IgG1-pI-CH1-v46	E	K	T	E	N	K	2	7.77
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v47	S	E	T	E	D	K	3	6.94
8.6%	16.2%	16.2%	IgG1-pI-CH1-v48	S	K	E	K	N	E	2	7.77
11.8%	16.2%	16.2%	IgG1-pI-CH1-v49	S	K	E	K	D	K	2	8.04
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v50	E	E	T	K	D	K	3	7.35
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v51	S	E	T	K	D	K	2	7.77
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v52	E	K	T	K	D	K	2	8.04
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v53	E	K	E	K	N	K	2	8.04
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v54	S	E	E	K	N	E	3	6.94
6.2%	16.2%	16.2%	IgG1-pI-CH1-v55	S	K	E	K	N	K	1	8.24
[illegible]	18.3%	21.8%	IgG1-pI-CH1-v56	E	K	E	K	N	E	3	7.35
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v57	S	E	T	K	N	K	1	8.05
18.4%	[illegible]	41.3%	IgG1-pI-CH1-v58	S	K	T	E	N	K	1	8.05
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v59	S	E	T	E	N	K	2	7.35
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v60	S	E	E	E	N	K	3	6.94
[illegible]	28.4%	31.3%	IgG1-pI-CH1-v61	S	E	T	K	N	E	2	7.35
31.3%	[illegible]	41.3%	IgG1-pI-CH1-v62	S	E	T	E	N	E	3	6.66

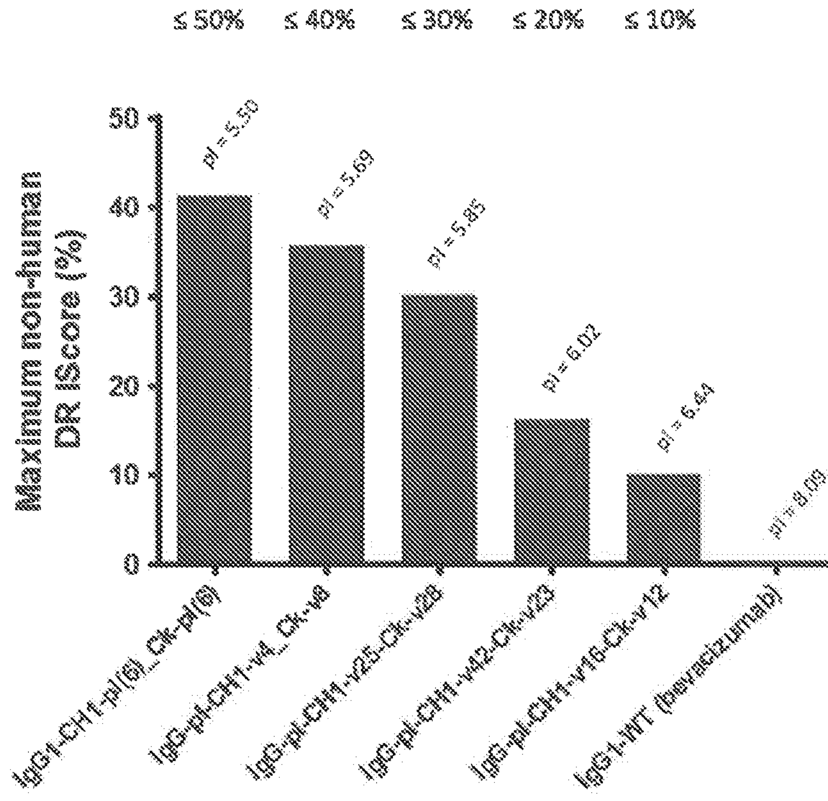
Figure 39 (continued)

MAX DR1	MAX DR3,4,5	MAX (All DR)	Variant (LC)	126	145	152	156	169	202	# mutations	pI (LC)
NA	NA		IgG1-LC	K	K	N	S	K	S	0	6.37
13.6%	[illegible]	27.5%	IgG1-CK1-pI(6)	E	E	D	E	E	E	6	4.70
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v1	E	E	N	E	K	E	4	4.95
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v2	E	K	D	S	K	S	2	5.37
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v3	E	K	N	E	E	S	3	5.06
13.6%	10.0%	13.6%	IgG1-pI-CK1-v4	E	E	N	S	E	S	3	4.92
10.7%	[illegible]	27.5%	IgG1-pI-CK1-v5	K	K	D	E	K	E	3	5.39
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v6	K	E	D	S	K	E	3	5.20
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v7	E	K	N	E	E	E	4	4.95
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v8	E	E	D	S	E	E	5	4.76
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v9	K	E	D	S	E	S	3	5.04
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v10	E	K	N	E	K	S	2	5.38
10.7%	[illegible]	27.5%	IgG1-pI-CK1-v11	K	E	D	E	K	E	4	5.07
2.8%	10.0%	10.0%	IgG1-pI-CK1-v12	K	E	N	S	E	S	2	5.19
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v13	K	E	D	S	E	E	4	4.94
13.6%	12.4%	13.6%	IgG1-pI-CK1-v14	E	E	N	S	K	E	3	5.06
3.8%	[illegible]	27.5%	IgG1-pI-CK1-v15	K	K	D	E	E	S	3	5.20
7.3%	[illegible]	30.1%	IgG1-pI-CK1-v16	K	E	N	E	E	S	3	5.06
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v17	K	K	D	S	K	S	1	5.94
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v18	E	E	D	S	K	S	3	5.04
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v19	E	K	D	S	K	E	3	5.20
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v20	E	E	N	E	K	S	3	5.06
10.7%	[illegible]	27.5%	IgG1-pI-CK1-v21	K	K	D	E	E	E	4	5.07
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v22	E	K	D	E	K	S	3	5.20
13.6%	12.4%	13.6%	IgG1-pI-CK1-v23	E	E	N	S	E	E	4	4.84
2.8%	7.4%	7.4%	IgG1-pI-CK1-v24	K	E	N	S	K	S	1	5.62
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v25	K	K	D	S	E	S	2	5.37
10.7%	[illegible]	30.1%	IgG1-pI-CK1-v26	K	K	N	E	E	E	3	5.21
3.8%	[illegible]	27.5%	IgG1-pI-CK1-v27	K	K	D	E	K	S	2	5.62
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v28	E	E	N	E	E	E	5	4.77
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v29	E	K	D	S	E	S	3	5.04
13.6%	7.4%	13.6%	IgG1-pI-CK1-v30	E	E	N	S	K	S	2	5.19

Figure 39 (continued)

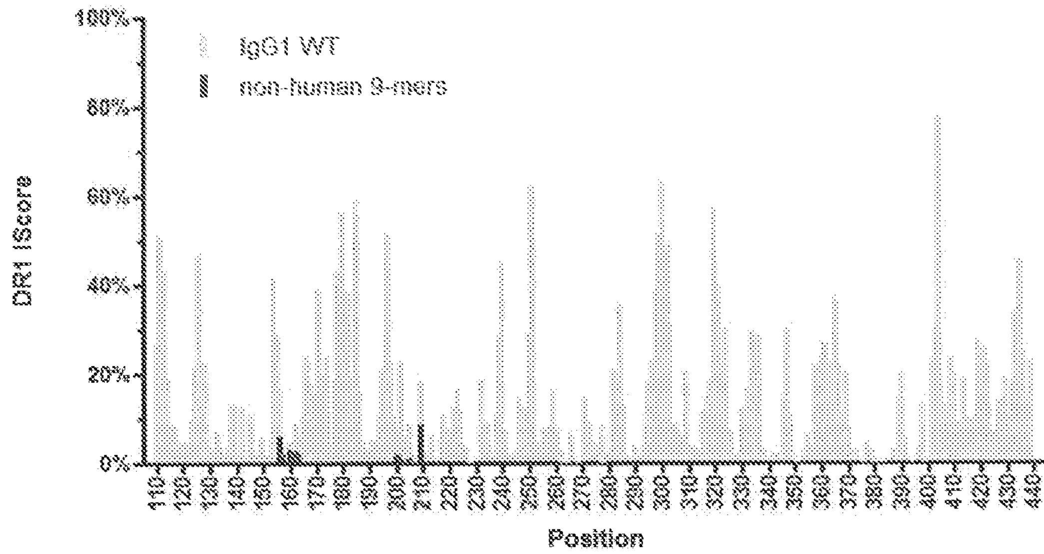
MAX DR1	MAX DR3,4,5	MAX (All DR)	Variant (LC)	126	145	152	156	169	202	# mutations	pI (LC)
7.3%	[illegible]	30.1%	IgG1-pI-CK1-v31	K	K	N	E	K	S	1	5.94
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v32	E	K	N	E	K	E	3	5.21
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v33	K	K	D	S	E	E	3	5.20
7.3%	[illegible]	30.1%	IgG1-pI-CK1-v34	K	E	N	E	K	S	2	5.38
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v35	E	K	D	E	K	E	4	5.07
10.7%	[illegible]	30.1%	IgG1-pI-CK1-v36	K	K	N	E	K	E	2	5.64
10.7%	12.4%	12.4%	IgG1-pI-CK1-v37	K	E	N	S	E	E	3	5.06
3.8%	[illegible]	27.5%	IgG1-pI-CK1-v38	K	E	D	E	E	S	4	4.94
3.8%	[illegible]	27.5%	IgG1-pI-CK1-v39	K	E	D	E	K	S	3	5.20
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v40	E	E	D	S	E	S	4	4.83
[illegible]	12.4%	13.6%	IgG1-pI-CK1-v41	E	K	N	S	E	E	3	5.06
10.7%	[illegible]	30.1%	IgG1-pI-CK1-v42	K	E	N	E	E	E	4	4.95
7.3%	[illegible]	30.1%	IgG1-pI-CK1-v43	K	K	N	E	E	S	2	5.38
10.7%	[illegible]	27.5%	IgG1-pI-CK1-v44	K	E	D	E	E	E	5	4.86
[illegible]	10.0%	13.6%	IgG1-pI-CK1-v45	E	K	N	S	E	S	2	5.19
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v46	E	E	D	E	K	E	5	4.86
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v47	E	K	D	E	E	S	4	4.94
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v48	K	E	D	S	K	S	2	5.37
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v49	E	E	D	S	K	E	4	4.94
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v50	E	K	D	E	E	E	5	4.86
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v51	E	E	D	E	K	S	4	4.94
10.7%	[illegible]	12.4%	IgG1-pI-CK1-v52	K	K	N	S	K	E	1	5.94
10.7%	12.4%	12.4%	IgG1-pI-CK1-v53	K	E	N	S	K	E	2	5.38
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v54	E	K	D	S	E	E	4	4.94
[illegible]	12.4%	13.6%	IgG1-pI-CK1-v55	E	K	N	S	K	E	2	5.38
14.8%	[illegible]	35.6%	IgG1-pI-CK1-v56	K	K	D	S	K	E	2	5.62
10.7%	[illegible]	30.1%	IgG1-pI-CK1-v57	K	E	N	E	K	E	3	5.21
[illegible]	5.5%	13.6%	IgG1-pI-CK1-v58	E	K	N	S	K	S	1	5.62
10.7%	[illegible]	12.4%	IgG1-pI-CK1-v59	K	K	N	S	E	E	2	5.38
13.6%	[illegible]	30.1%	IgG1-pI-CK1-v60	E	E	N	E	E	S	4	4.84
13.6%	[illegible]	27.5%	IgG1-pI-CK1-v61	E	E	D	E	E	S	5	4.76
2.1%	[illegible]	10.0%	IgG1-pI-CK1-v62	K	K	N	S	E	S	1	5.62

Figure 40



The pI shown is that for the full length mAb with bevacizumab Fv

Figure 41



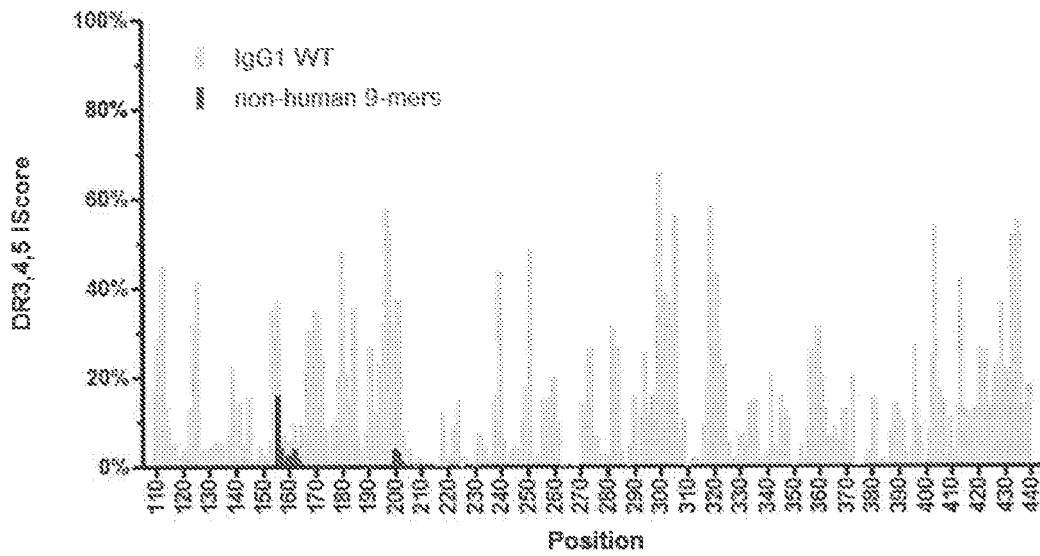


Figure 42

CH1

EU	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139
IgG-pI-CH1-v42	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T
+ 9-mer optimized Fc	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G	T
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	T
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G	T
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S	T

EU	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161
IgG-pI-CH1-v42	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
+ 9-mer optimized Fc	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG1	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG2	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG3	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G
IgG4	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N	S	G

EU	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183
IgG-pI-CH1-v42	A	L	E	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
+ 9-mer optimized Fc	A	L	E	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG1	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG2	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG3	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S
IgG4	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y	S	L	S

EU	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205
IgG-pI-CH1-v42	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N	V	N	H	K
+ 9-mer optimized Fc	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N	V	N	H	K
IgG1	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N	V	N	H	K
IgG2	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N	V	D	H	K
IgG3	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	T	C	N	V	N	H	K
IgG4	S	V	V	T	V	P	S	S	S	L	G	T	K	T	Y	T	C	N	V	D	H	K

EU	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG-pI-CH1-v42	P	S	D	T	E	V	D	K	K	V	E	P	K	S	C
+ 9-mer optimized Fc	P	S	D	T	E	V	D	K	K	V	E	P	K	S	C
IgG1	P	S	N	T	K	V	D	K	K/R	V	E	P	K	S	C
IgG2	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C
IgG3	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P
IgG4	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G

Hinge

EU			221	222	223	224	225															
IgG-pI-CH1-v42			D	K	T	H	T															
+ 9-mer optimized Fc			D	K	T	H	T															
IgG1			D	K	T	H	T															
IgG2				V		E																
IgG3	L	S	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P
IgG4						P	P															

EU																						
IgG-pI-CH1-v42																						
+ 9-mer optimized Fc																						
IgG1																						
IgG2																						
IgG3	C	P	R	C	P	E	P	K	S	C	D	T	P	P	P	C	P	R	C	P	E	P
IgG4																						

EU									226	227	228	229	230	231	232	233	234	235	236
IgG-pI-CH1-v42									C	P	P	C	P	A	P	E	L	L	G
+ 9-mer optimized Fc									C	P	P	C	P	A	P	E	L	L	G
IgG1									C	P	P	C	P	A	P	E	L	L	G
IgG2									C	P	P	C	P	A	P	P	V	A	
IgG3	K	S	C	D	T	P	P	P	C	P	R	C	P	A	P	E	L	L	G
IgG4									C	P	S	C	P	A	P	E	F	L	G

Figure 42 (continued)

CH2

EU	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258
IgG-pI-CH1-v42	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
+ 9-mer optimized Fc	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E

EU	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280
IgG-pI-CH1-v42	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D
+ 9-mer optimized Fc	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y	V	D
IgG1	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y	V	D
IgG2	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y	V	D
IgG3	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y	V	D
IgG4	V	T	C	V	V	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y	V	D

EU	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302
IgG-pI-CH1-v42	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V
+ 9-mer optimized Fc	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T	F	R	V
IgG1	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	Y	R	V
IgG2	G	V/M	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T	F	R	V
IgG3	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T	F	R	V
IgG4	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T	Y	R	V

EU	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324
IgG-pI-CH1-v42	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
+ 9-mer optimized Fc	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG1	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG2	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG3	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S
IgG4	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K	C	K	V	S

EU	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG-pI-CH1-v42	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
+ 9-mer optimized Fc	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG1	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
IgG2	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG3	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG4	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K

Figure 42 (continued)

CH3

EU	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362
IgG-pI-CH1-v42	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q
+ 9-mer optimized Fc	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N	Q
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D/E	E	L/M	T	K	N	Q
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N	Q
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N	Q

EU	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384
IgG-pI-CH1-v42	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N
+ 9-mer optimized Fc	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	S
IgG1	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N
IgG2	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N
IgG3	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	S
IgG4	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E	S	N

EU	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406
IgG-pI-CH1-v42	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L
+ 9-mer optimized Fc	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S	F	F	L
IgG1	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L
IgG2	G	Q	P	E	N	N	Y	K	T	T	P	P	M	L	D	S	D	G	S	F	F	L
IgG3	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S	F	F	L
IgG4	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S	F	F	L

EU	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428
IgG-pI-CH1-v42	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M
+ 9-mer optimized Fc	Y	S	K	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S	C	S	V	M
IgG1	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M
IgG2	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S	C	S	V	M
IgG3	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	I	F	S	C	S	V	M
IgG4	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S	C	S	V	M

EU	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447
IgG-pI-CH1-v42	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
+ 9-mer optimized Fc	H	E	A	L	H	S	H	Y	T	Q	K	S	L	S	L	S	P	G	-
IgG1	H	E	A/G	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG2	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P	G	K
IgG3	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P	G	K
IgG4	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L	G	K

Figure 43

IgG-pI-CH1-v4 (SEQ ID NO: 34)
AETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

IgG-pI-CH1-v25 (SEQ ID NO: 35)
AETKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

IgG-pI-CH1-v42 (SEQ ID NO: 36)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

IgG-pI-CH1-v16 (SEQ ID NO: 37)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

CK-v8 (SEQ ID NO: 38)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDDALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGEC

CK-v28 (SEQ ID NO: 39)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDNALQEGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGEC

CK-v23 (SEQ ID NO: 40)
RTVAAPSVFIFPPSDEQLESGTASVVCLLNNFYPREAEVQWKVDNALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLESPVTKSFNRGEC

CK-v12 (SEQ ID NO: 41)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAEVQWKVDNALQSGNSQESVTEQD
SEDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

IgG-pI-CH1-v4-SLFFV-Iso-434S (SEQ ID NO: 42)
AETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTK

SUBSTITUTE SHEET (RULE 26)

Figure 43 (cont.)

NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEG
NVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v25-SLFFV-Iso-434S (SEQ ID NO: 43)
AETKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEG
NVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v42-SLFFV-Iso-434S (SEQ ID NO: 44)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEG
NVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v16-SLFFV-Iso-434S (SEQ ID NO: 45)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGN
VFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v4-SL-Iso-434S (SEQ ID NO: 46)
AETKGPSVFPLAPSSESTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQE
GNVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v25-SL-Iso-434S (SEQ ID NO: 47)
AETKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQE
GNVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v42-SL-Iso-434S (SEQ ID NO: 48)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQE
GNVFSCSVMHEALHSHYTQKSLSLSPG

IgG-pI-CH1-v16-SL-Iso-434S (SEQ ID NO: 49)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQYNST

Figure 43 (cont.)

YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEG
NVFSCSVMHEALHSHYTQKSLSLSPG

Figure 43 (cont.)

IgG1-pI-IF16-ISO (SEQ ID NO: 70)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSPG

IgG1-pI-IF10-ISO (SEQ ID NO: 71)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSPG

IgG2-pI-IF10-ISO (SEQ ID NO: 72)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHEPSDTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHSHYT
QKSLSLSPG

IgG2-pI-IF16-ISO (SEQ ID NO: 73)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHEPSDTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVD
GVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHSHYT
QKSLSLSPG

Hybrid-pI-IF16-ISO-N434S (SEQ ID NO: 74)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
SHYTQKSLSLSPG

Hybrid-pI-IF10-ISO-N434S (SEQ ID NO: 75)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
SHYTQKSLSLSPG

Hybrid-2-1-2-pI-IF16-ISO-N434S (SEQ ID NO: 76)

DB2/ 23416749.1

ASTKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHEPSDTEVDKTVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
SHYTQKSLSLSPG

Hybrid-2-1-2-pI-IF10-ISO-N434S (SEQ ID NO: 77)
ASTKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHEPSDTEVDKTVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
SHYTQKSLSLSPG

IgG1-IF10-CH1-Fc-charges (SEQ ID NO: 78)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSEEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLEPG

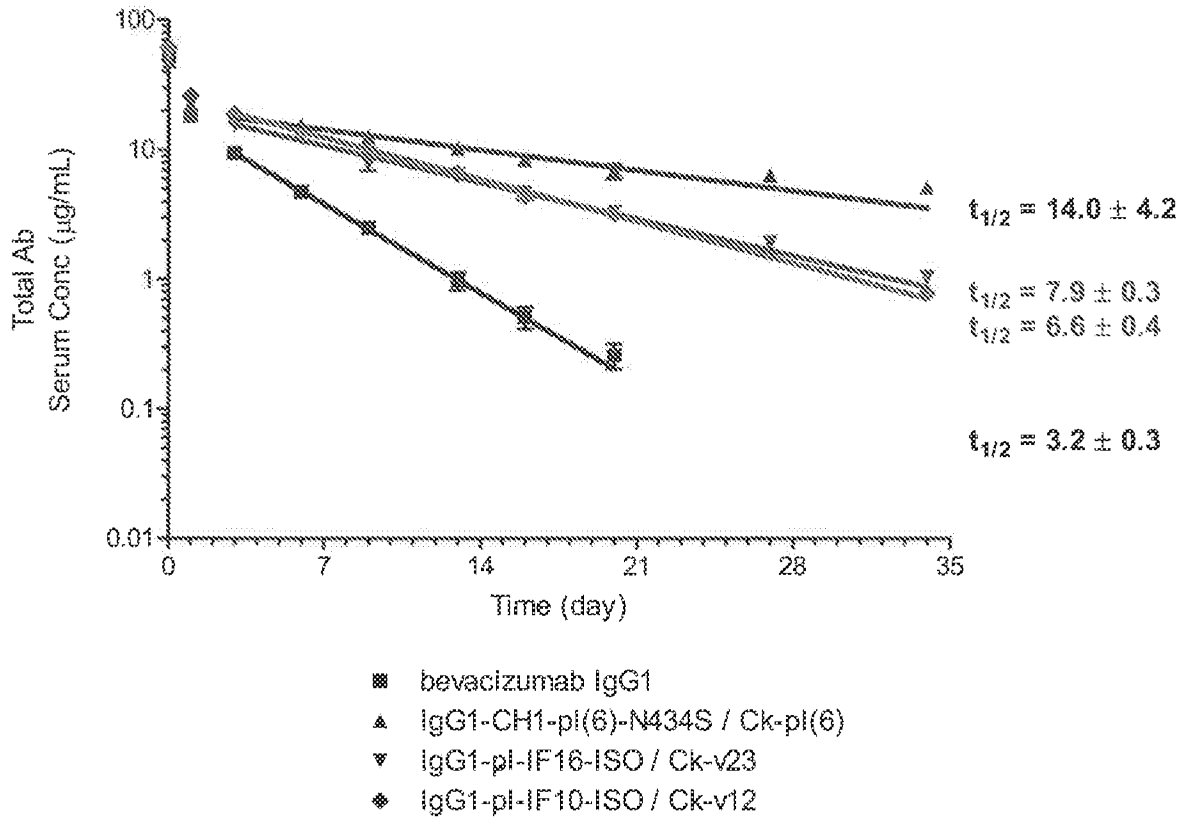
IgG1-IF16-CH1-Fc-charges (SEQ ID NO: 79)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSEEE
MEKNEVSLTCLVKGFYPSDIAVEWESNGQPEENYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLEPG

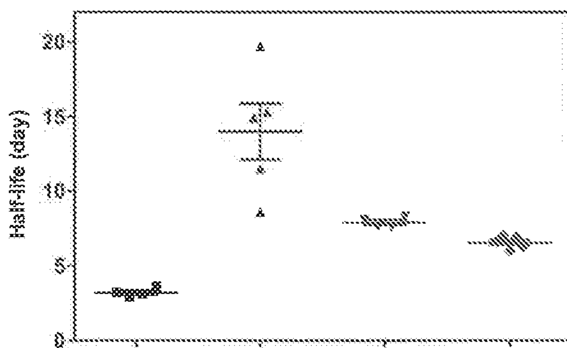
IgG1-IF10-ISO-CH1-Fc-charges (SEQ ID NO: 80)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSEE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLEPG

IgG1-IF16-ISO-CH1-Fc-charges (SEQ ID NO: 81)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALESGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHKPSDTEVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVEFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSEEE
MEKNEVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWEEGNVFSCSVMHEALHN
HYTQKSLSLEPG

DB2/ 23416749.1

Figure 44





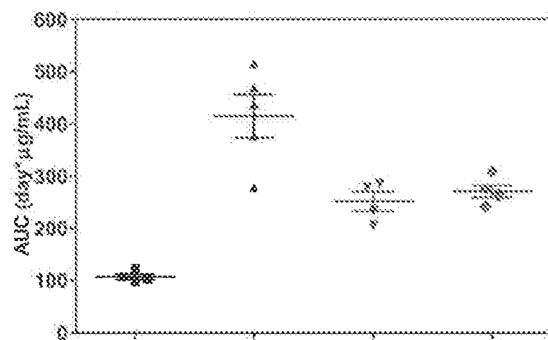


Figure 45

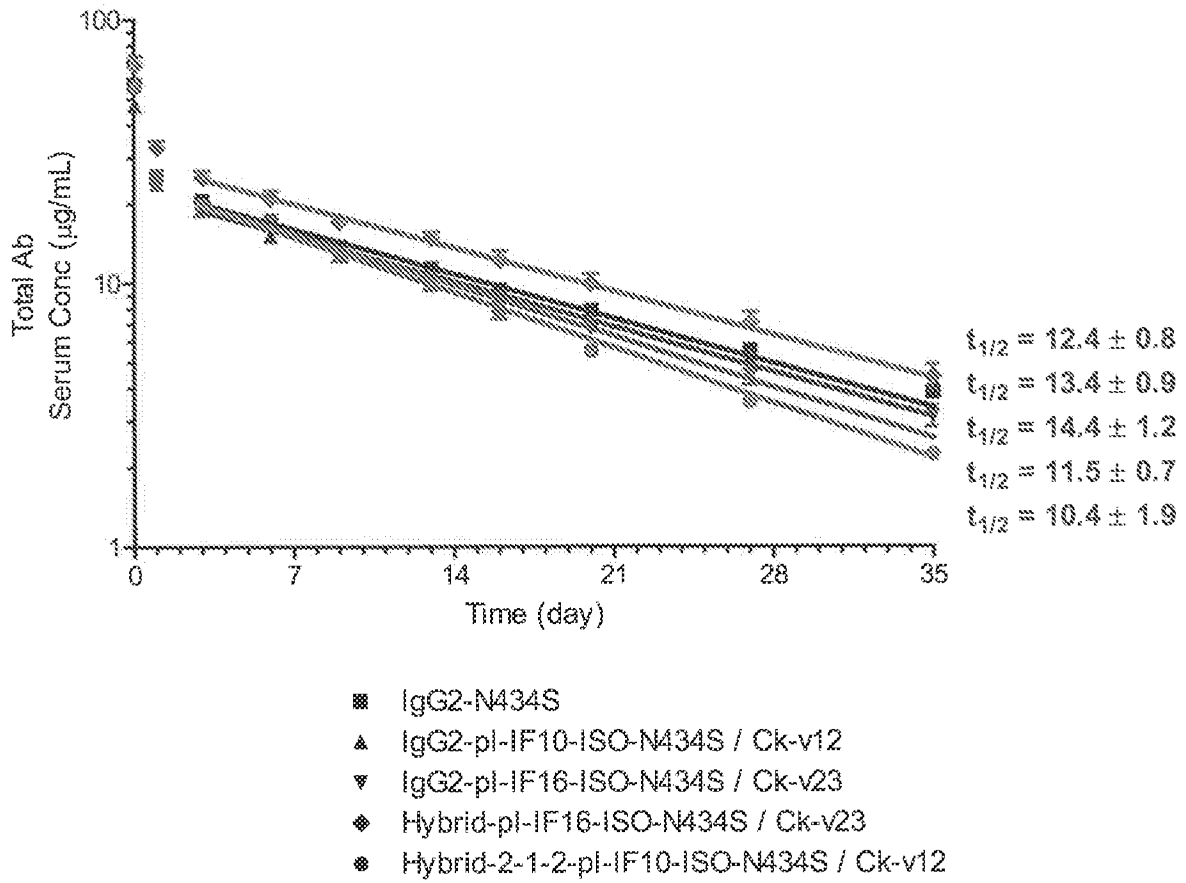


Figure 46

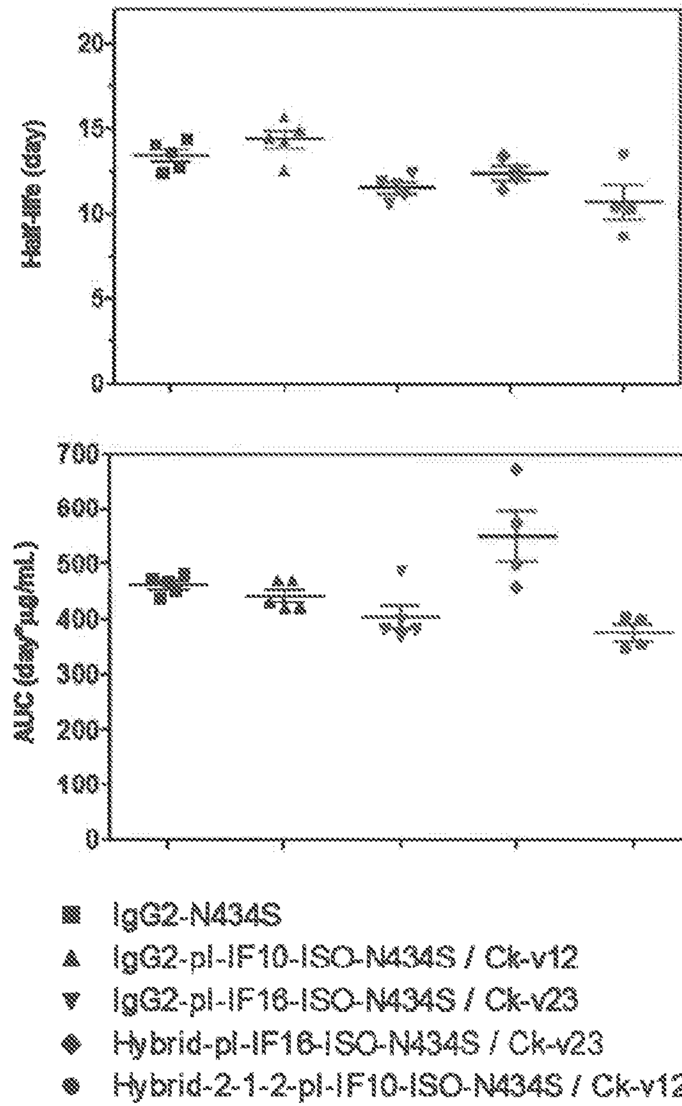
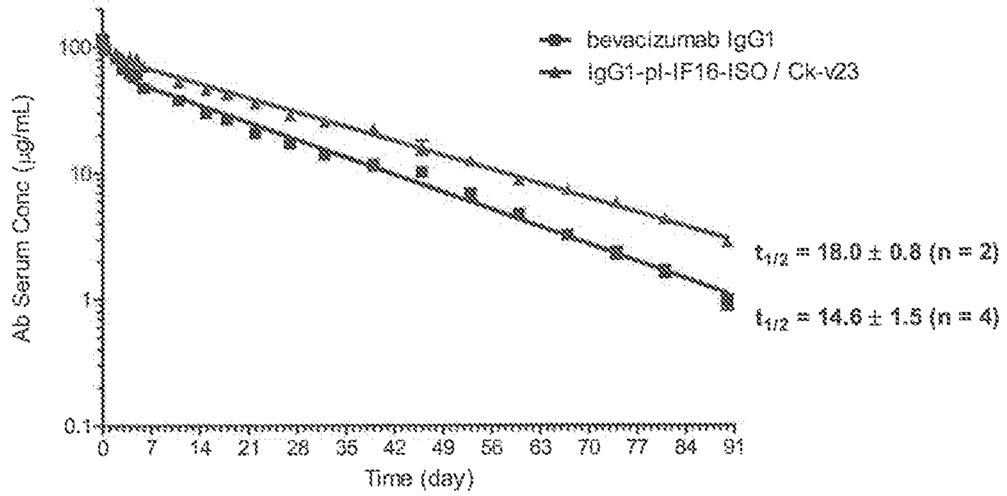
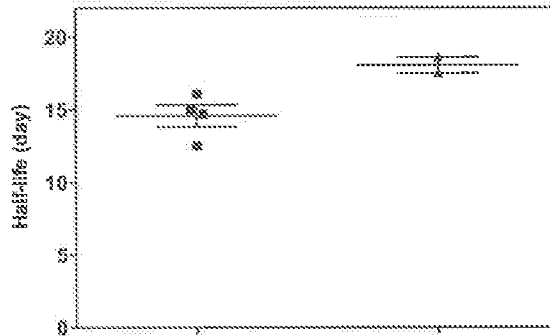
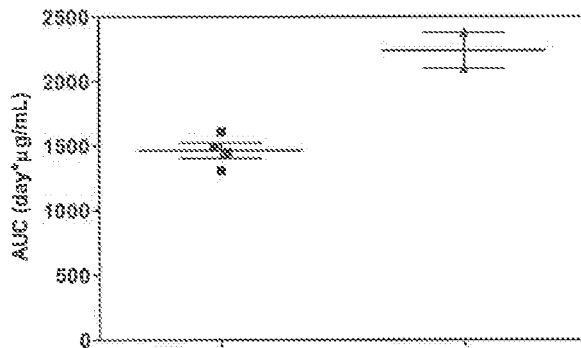


Figure 47







INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/049789

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2012/016227 A2 (XENCOR INC [US]; DAHIYAT BASSIL I [US]; BERNETT MATTHEW J [US]; LAZAR) 2 February 2012 (2012-02-02) the whole document -----	1-14
X,P	WO 2012/058768 A1 (ZYMEWORKS INC [CA]; CABRERA ERIC ESCOBAR [CA]; VON KREUDENSTEIN THOMAS) 10 May 2012 (2012-05-10) page 75 - page 76 -----	1-14
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A	EP 2 006 381 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 24 December 2008 (2008-12-24) the whole document -----	1-14
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2012	18/12/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pérez-Mato, Isabel

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/049789

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [X] on paper

   [X] in electronic form

   b. (time)

   [ ] in the international application as filed

   [ ] together with the international application in electronic form

   [X] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	EP 2 202 245 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 30 June 2010 (2010-06-30) the whole document -----	1-14
A	C. ANDREW BOSWELL ET AL: "Effects of Charge on Antibody Tissue Distribution and Pharmacokinetics", BIOCONJUGATE CHEMISTRY, vol. 21, no. 12, 15 December 2010 (2010-12-15), pages 2153-2163, XP055046782, ISSN: 1043-1802, DOI: 10.1021/bc100261d the whole document in particular figure 3 and page 2158 -----	1-14
A	DE GROOT A S ET AL: "DE-IMMUNIZATION OF THERAPEUTIC PROTEINS BY T-CELL EPITOPE MODIFICATION", DEVELOPMENTS IN BIOLOGICALS, KARGER, BASEL, vol. 122, 1 January 2005 (2005-01-01), pages 171-194, XP008074262, ISSN: 1424-6074 the whole document -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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			EP	2006381	A1	24-12-2008
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			RU	2010116208	A	10-11-2011
			US	2011076275	A1	31-03-2011
			WO	2009041643	A1	02-04-2009