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978-3-52-733317-2 pages 1-44, DOI: 10.1002/9783527675272.ch01, * The whole document, in particular, Tables
1.3 and 1.5 ***

DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to modified antibodies having improved resistance against proteolytic degradation and altered effector functions and/or pharmacokinetic properties. The antibodies are useful in the therapeutic treatment of various disorders, in particular of inflammatory conditions.

BACKGROUND

[0002] Monoclonal antibodies have gained increasing importance as therapeutic reagents in clinical medicine over the last 20 years. For many years, efforts to improve antibodies concentrated on reducing their potential immunogenicity, leading to humanized or even fully human antibodies. Another approach aims to optimize antibodies by improving their effector functions. While direct effects are mediated by the variable antigen binding region of the antibody, indirect effects are mediated by the constant Fc region. Efforts to improve effector functions mainly concentrate on modulating the Fc region. In addition, improving the serum half-life of therapeutic antibodies is desirable, which may reduce the amount of required antibodies, and may increase their convenience for patients by prolonging treatment intervals.

[0003] For therapeutic applications, immunoglobulin G (IgG) has been the preferred class of choice for several reasons; IgGs are easy to purify, are relatively stable on storage, can be administered intravenously, have extended biological half-life in vivo and are able to engage a range of biological effector functions such as activation of complement dependent cytotoxicity (CDC) and recruitment of effector cells through various Fc-receptor interactions (antibody-dependent cellular cytotoxicity; ADCC). Of the five immunoglobulin classes, IgG exhibits the longest biological half-life due to its unique interaction with the IgG recycling receptor, the neonatal Fc receptor (FcRn). One of the known functions of the receptor is to rescue IgG from catalytic degradation. A solved FcRn-Fc cocrystal structure has shown that the interaction with Fc occurs in the IgG hinge-CH₂-CH₃ region. This interaction occurs in a strictly pH-dependent manner at acidic pH of 6.0-6.5 in the endosomes. Bound IgG molecules are recycled back to the cell surface where they are released at physiological pH of 7.4 into the circulation, whereas noncomplexed IgG molecules are destined for lysosomal degradation. This recycling is the mechanism for the extended half-life of IgG; modulation of the FcRn-IgG interaction will therefore allow specific control of the serum half-lives of gamma immunoglobulins and Fc-fusion proteins.

[0004] Depending on the application it may be desirable to increase or reduce the serum residence time of IgG. For therapeutic application a longer half-life is desirable as smaller doses and fewer injections will be required. Several approaches to increase the half-life have

been investigated including the use of polyethylene glycol (PEG), generation of albumin- or Fc-fusion proteins and strengthening the FcRn-IgG interaction. PEGylated pharmaceuticals have been in the clinic since 1990 and PEGylation is an established technology for extension of drug residence in the blood. Since human serum albumin (HSA) is also recycled by FcRn via a pH-dependent interaction, several albumin-fusion proteins to enhance stability and half-life have also been produced. Additionally, antibody fragments fused to albumin or albumin-binding domains have demonstrated prolonged serum residence time in preclinical studies. The generation of Fc-fusion proteins is another strategy that will endow proteins or peptides with properties similar to an intact antibody.

[0005] Modifications of the Fc region that have been investigated are summarized in Saxena (2016) *Frontiers in Immunology*, Vol. 7, Article 580. Various Fc mutations are further described in WO 1998/023289 A1, WO 2000/042072 A2, WO 2010/106180 A2 and WO 2014/108198 A1.

[0006] WO 2012/087746 A1 and Kinder et al. (2013) *The Journal Of Biological Chemistry* Vol. 288, No. 43, pp. 30843-30854 investigated various mutations in the Fc region of an antibody for improving the resistance to proteolytic degradation.

[0007] Biancheri et al. (2015) *Gastroenterology*, 149, pp. 1564-1574 refer to proteolytic degradation of TNF-neutralizing antibodies by certain proteases *in vivo*.

[0008] Rajpal et al. (2014) "Therapeutic Fc-Fusion Proteins", Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp.1-44 disclose several mutations in the Fc region for increasing the half-life of an antibody.

[0009] There is an ongoing need for antibodies having improved effector functions, pharmacokinetics and/or resistance to proteolytic degradation.

SUMMARY OF THE INVENTION

[0010] The inventors of this application found that a combination of specific mutations in the Fc region of an antibody confer favorable properties to the antibody, including improved resistance to proteolytic degradation and increased affinity to FcRn at pH6. Antibodies having the mutations have improved pharmacokinetic properties. In addition, the antibodies exhibit superior effector functions as compared to non-modified antibodies and/or known antibodies such as infliximab (IFX).

[0011] The present invention therefore relates to the subject matter defined in the following items [1] to [88]:

1. [1] An IgG1 antibody comprising a TNFa-binding domain and an FcRn binding site, wherein the amino acid sequence of the antibody comprises
 1. (i) the amino acids 233P, 234V, 235A, and a deletion at amino acid position 236;

2. (ii) the amino acid 434A or the amino acids 252Y, 254T and 256E; and
 3. (iii) the amino acids 239D, 330L and 332E
- in an Fc region of a human IgG; and wherein the antibody comprises (i) a V_L domain having the amino acid sequence as shown in SEQ ID NO:10, and (ii) a V_H domain having the amino acid sequence as shown in SEQ ID NO:9.
2. [2] The antibody of item [1], wherein the antibody is a modified antibody having the substitutions E233P, L234V and L235A and a deletion of G236.
 3. [3] The antibody of item [2], wherein the antibody further has the substitution N434A.
 4. [4] The antibody of item [2], wherein the antibody further has the substitutions M252Y, S254T and T256E.
 5. [5] The antibody of item [1], wherein the antibody is a modified antibody having the substitutions S239D, A330L and I332E.
 6. [6] The antibody of item [1], wherein the amino acid sequence of the antibody comprises the amino acids 233P, 234V, 235A, 239D, 330L, 332E and 434A, and a deletion at amino acid position 236.
 7. [7] The antibody of item [6], wherein the antibody is a modified antibody having the substitutions E233P, L234V, L235A, S239D, A330L, I332E and N434A, and a deletion of G236.
 8. [8] The antibody of item [6] or [7], comprising the amino acid sequence as shown in SEQ ID NO:29.
 9. [9] The antibody of item [1], wherein the amino acid sequence of the antibody comprises the amino acids 233P, 234V, 235A, 239D, 330L, 332E, 252Y, 254T and 256E, and a deletion at amino acid position 236.
 10. [10] The antibody of item [9], wherein the antibody is a modified antibody having the substitutions E233P, L234V, L235A, S239D, A330L, I332E, M252Y, S254T and T256E, and a deletion of G236.
 11. [11] The antibody of item [9] or [10], comprising the amino acid sequence as shown in SEQ ID NO:28.
 12. [12] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 6 that is greater than that of infliximab.
 13. [13] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 6 that is characterized by a dissociation constant K_D of less than 500 nM.
 14. [14] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 6 that is characterized by a dissociation constant K_D of less than 400 nM.
 15. [15] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 6 that is characterized by a dissociation constant K_D of less than 300 nM.
 16. [16] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 6 that is characterized by a dissociation constant K_D of less than 200 nM.
 17. [17] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 6 that is characterized by a dissociation constant K_D in the range from 5 nM to 500 nM, or from 10 nM to 400 nM, or from 25 nM to 300 nM, or from 50 nM to 200 nM, or from 75 nM to 175 nM.
 18. [18] The antibody of any one of the preceding items, wherein said K_D characterizing the

- affinity to human FcRn at pH 6 is determined by surface plasmon resonance (SPR).
19. [19] The antibody of any one of the preceding items, having an affinity to human FcRn at pH 7.4 that is characterized by a dissociation constant K_D of greater than 10 μ M.
 20. [20] The antibody of any one of the preceding items, wherein said K_D characterizing the affinity to human FcRn at pH 7.4 is determined by surface plasmon resonance (SPR).
 21. [21] The antibody of any one of items [1] to [18], wherein its affinity to human FcRn at pH 7.4 is so low that a K_D value cannot be determined by SPR.
 22. [22] The antibody of any one of the preceding items, which binds to human TNF α with a K_D of less than 200 pM.
 23. [23] The antibody of any one of the preceding items, which binds to human TNF α with a K_D of less than 100 pM.
 24. [24] The antibody of any one of the preceding items, which binds to human TNF α with a K_D of less than 50 pM.
 25. [25] The antibody of any one of the preceding items, which binds to human TNF α with a K_D of less than 25 pM.
 26. [26] The antibody of any one of the preceding items, which binds to human TNF α with a K_D of less than 10 pM.
 27. [27] The antibody of any one of the preceding items, which is transported across a polarized cell monolayer from the apical side to the basolateral side.
 28. [28] The antibody of any one of the preceding items, which is transported across a polarized cell monolayer from the apical side to the basolateral side in greater amount than a control antibody comprising a light chain having the amino acid sequence as shown in SEQ ID NO:1 and a heavy chain having the amino acid sequence as shown in SEQ ID NO:2.
 29. [29] The antibody of any one of the preceding items, which is transported across a polarized cell monolayer from the apical side to the basolateral side in greater amount than infliximab.
 30. [30] The antibody of item [28] or [29], wherein said amount refers to the mass of antibody transported across the polarized cell monolayer within four hours.
 31. [31] The antibody of any one of items [27] to [30], wherein the amount of antibody transported across the polarized cell monolayer is greater than two times the amount of a parent immunoglobulin transported across the polarized cell monolayer, wherein said parent immunoglobulin differs from said antibody only in that its Fc region has only wild type amino acids.
 32. [32] The antibody of any one of the preceding items, wherein a greater percentage of the antibody than that of infliximab is transported across a polarized cell monolayer from the apical side to the basolateral side in the presence of a tenfold excess of competing immunoglobulins, wherein the percentage refers to the total mass of immunoglobulins transported across the polarized cell monolayer.
 33. [33] The antibody of item [32], wherein the percentage of the antibody transported across the polarized cell monolayer is greater than two times the percentage of a parent immunoglobulin transported across the polarized cell monolayer, wherein said parent immunoglobulin differs from said antibody only in that its Fc region has only wild type

amino acids.

34. [34] The antibody of any one of items [27] to [33], wherein said polarized cell monolayer is a monolayer of polarized T84 cells.
35. [35] The antibody of any one of the preceding items, binding to CD16a(V) with a K_D of less than 500 nM, or less than 300 nM, or less than 200 nM, or less than 100 nM.
36. [36] The antibody of any one of the preceding items, binding to CD16a(F) with a K_D of less than 10 μ M, or less than 1 μ M.
37. [37] The antibody of any one of the preceding items, binding to CD16b(NA2) with a K_D of less than 10 μ M, or less than 5 μ M, or less than 1 μ M.
38. [38] The antibody of any one of the preceding items, having antibody-dependent cellular cytotoxicity (ADCC).
39. [39] The antibody of any one of the preceding items, capable of inducing CD14⁺CD206⁺ macrophages.
40. [40] The antibody of any one of the preceding items, capable of inducing CD14⁺CD206⁺ macrophages at a level equal to or greater than infliximab.
41. [41] The antibody of any one of the preceding items, capable of suppressing T-cell proliferation.
42. [42] The antibody of any one of the preceding items, capable of suppressing T-cell proliferation at a degree equal to or greater than infliximab.
43. [43] The antibody of any one of the preceding items, which is a non-fucosylated antibody or an antibody with reduced fucosylation.
44. [44] The antibody of any one of the preceding items, comprising a V_H domain having the amino acid sequence as shown in SEQ ID NO:9 and a V_L domain having an amino acid sequence as shown in SEQ ID NO:10.
45. [45] The antibody of any one of the preceding items, comprising a light chain having the amino acid sequence as shown in SEQ ID NO:1 and a heavy chain having the amino acid sequence as shown in SEQ ID NO:11, SEQ ID 12, or SEQ ID NO:13.
46. [46] The antibody of any one of the preceding items, wherein said antibody specifically binds to human TNF α .
47. [47] The antibody of any one of the preceding items, wherein said antibody does not significantly bind to TNF β .
48. [48] The antibody of any one of the preceding items, wherein said antibody
 1. (i) binds to human TNF α with a dissociation constant (K_D) of less than 125 pM;
 2. (ii) is cross-reactive with *Macaca mulatta* TNF α and with *Macaca fascicularis* TNF α ;
 3. (iii) has a greater potency than infliximab, as determined by an L929 assay; and/or
 4. (iv) is capable of binding to human TNF α_{Trimer} in a stoichiometry (antibody : TNF α_{Trimer}) of at least 2.
49. [49] The antibody of any one of the preceding items, which binds to TNF α from *Macaca mulatta* with a K_D of less than 1 nM.
50. [50] The antibody of any one of the preceding items, which binds to TNF α from *Macaca fascicularis* with a K_D of less than 1 nM.

51. [51] The antibody of any one of the preceding items, wherein the potency of the antibody to inhibit TNF α -induced apoptosis relative to that of infliximab (relative potency), determined in an L929 assay, is greater than 3, and wherein said relative potency is the ratio of the IC₅₀ value in ng/mL of infliximab in the L929 assay to the IC₅₀ value in ng/mL of the antibody in the L929 assay.
52. [52] The antibody of any one of the preceding items, wherein the melting temperature of the variable domain of the antibody in scFv format, determined by differential scanning fluorimetry, is at least 65°C.
53. [53] The antibody of any one of the preceding items, wherein the melting temperature of the variable domain of the antibody in scFv format, determined by differential scanning fluorimetry, is at least 68°C.
54. [54] The antibody of any one of the preceding items, wherein the melting temperature, determined by differential scanning fluorimetry, is at least 70°C.
55. [55] The antibody of any one of the preceding items, wherein the antibody is capable of blocking the interaction between human TNF α and TNF receptor I (TNFRI).
56. [56] The antibody of any one of the preceding items, wherein the antibody is capable of blocking the interaction between human TNF α and TNF receptor II (TNFRII).
57. [57] The antibody of any one of the preceding items, which is capable of inhibiting cell proliferation of peripheral blood mononuclear cells in a mixed lymphocyte reaction.
58. [58] The antibody of any one of the preceding items, which is capable of inhibiting LPS-induced secretion of interleukin-1 β from CD14⁺ monocytes.
59. [59] The antibody of item [58], wherein the IC₅₀ value for inhibiting LPS-induced secretion of interleukin-1 β is less than 1 nM.
60. [60] The antibody of item [59], wherein said IC₅₀ value for inhibiting LPS-induced secretion of interleukin-1 β , on a molar basis, is lower than that of adalimumab.
61. [61] The antibody of any one of the preceding items, which is capable of inhibiting LPS-induced secretion of TNF α from CD14⁺ monocytes.
62. [62] The antibody of item [61], wherein the IC₅₀ value for inhibiting LPS-induced secretion of TNF α is less than 1 nM.
63. [63] The antibody of item [62], wherein said IC₅₀ value for inhibiting LPS-induced secretion of TNF α , on a molar basis, is lower than that of adalimumab.
64. [64] The antibody of any one of the preceding items, which is more resistant to proteolytic degradation than a wildtype antibody.
65. [65] The antibody of item [64], wherein said wildtype antibody is infliximab.
66. [66] The antibody of item [64], wherein said wildtype antibody is an immunoglobulin which differs from said antibody only in that its Fc region has only wild type amino acids.
67. [67] The antibody of item [64], wherein said wildtype antibody comprises a light chain having the amino acid sequence as shown in SEQ ID NO:1 and a heavy chain having the amino acid sequence as shown in SEQ ID NO:2.
68. [68] The antibody of any one items [64] to [67], wherein said proteolytic degradation includes degradation by matrix metalloproteinase 3 (MMP-3).
69. [69] The antibody of any one items [64] to [68], wherein said proteolytic degradation includes degradation by immunoglobulin G-degrading enzyme of *S. pyogenes* (IdeS).

70. [70] The antibody of any one items [64] to [69], wherein said proteolytic degradation includes degradation by Endoproteinase Glu-C from *Staphylococcus aureus* strain V8 (GluC).
71. [71] A nucleic acid encoding the antibody of any one of the preceding items.
72. [72] A vector or plasmid comprising the nucleic acid of item [71].
73. [73] A cell comprising the nucleic acid of item [60] or the vector or plasmid of item [72].
74. [74] A method of preparing the antibody of any one of items [1] to [70], comprising culturing the cell of item [71] in a medium under conditions that allow expression of the nucleic acid encoding the antibody, and recovering the antibody from the cells or from the medium.
75. [75] The antibody as defined in any one of items [1] to [70] for use in a method of treating an inflammatory disorder or a TNF α -related disorder.
76. [76] The antibody for use according to item [75], wherein said inflammatory disorder is selected from the list of diseases and disorders listed in Section "*Disorders to be treated*" below.
77. [77] The antibody for use according to item [75], wherein said inflammatory disorder is an inflammatory disorder of the gastrointestinal tract.
78. [78] The antibody for use according to item [77], wherein said inflammatory disorder of the gastrointestinal tract is inflammatory bowel disease.
79. [79] The antibody for use according to item [77] or [78], wherein said inflammatory disorder of the gastrointestinal tract is Crohn's disease.
80. [80] The antibody for use according to item [79], wherein said Crohn's disease is selected from the group consisting of ileal, colonic, ileocolonic, and/or isolated upper Crohn's disease (gastric, duodenal and/or jejunal) and including non-stricturing/non-penetrating, stricturing, penetrating and perianal disease behavior, allowing any combination of localization and disease behavior of any of the above mentioned.
81. [81] The antibody for use according to item [77] or [78], wherein said inflammatory disorder of the gastrointestinal tract is ulcerative colitis.
82. [82] The antibody for use according to item [81], wherein said ulcerative colitis is selected from the group consisting of ulcerative proctitis, sigmoiditis, proctosigmoiditis, left-sided colitis, pan-ulcerative colitis, and pouchitis.
83. [83] The antibody for use according to item [77] or [78], wherein said inflammatory disorder of the gastrointestinal tract is microscopic colitis.
84. [84] The antibody for use according to item [75], wherein said inflammatory disorder is arthritis.
85. [85] The antibody for use according to item [75] or [84], wherein said inflammatory disorder rheumatoid arthritis.
86. [86] The antibody for use according to any one of items [75] to [85], wherein said method comprises orally administering the antibody to a subject.
87. [87] The antibody for use according to any one of items [75] to [85], wherein said method comprises topically applying the antibody.
88. [88] A pharmaceutical composition comprising the antibody of any one of items [1] to [70].

DESCRIPTION OF THE FIGURES

[0012]

Figure 1: Potency of anti-TNF α antibody variants to neutralize human TNFa in the L929 assay. Dose response curves for TNFa antibody variants and the reference infliximab are shown.

Figure 2: Transport of anti-TNF α IgG variants across polarized T84 cells. The amounts of anti-TNF α antibody variants and Infliximab (IFX) from the apical to the basolateral reservoir at 4 hours post adding. Presented as ng/cm². Error bars indicate SD of two to four individual monolayers.

Figure 3: Transport of anti-TNF α IgG variants across polarized T84 cells in the presence of excess amounts of myeloma IgG. The amounts of the anti-TNF α IFX and Ab variants transported from the apical to the basolateral reservoir in the presence of 10-fold excess of human myeloma IgG at 4 hours post adding. Presented as ng/cm². Error bars indicate SD of three to four individual monolayers.

Figure 4: ADCC activity. Induction of ADCC by anti-TNF α antibody variants and Ab-wt.

Figure 5: Induction of CD14⁺CD206⁺ macrophages by each compound relative to the induction of IFX. Summarized data of 4 independent experiments. Bars represent mean, error bars represent SEM.

Figure 6: Suppression of T-cell proliferation by each compound relative to IFX. Summarized data of 3 independent experiments. Bars represent mean, error bars represent SEM.

Figure 7: Resistance to proteolytic degradation by MMP-3.

Figure 8: Resistance to proteolytic degradation by IdeS.

Figure 9: Resistance to proteolytic degradation by GluC.

Figure 10: Schematic presentation of site directed mutagenesis.

DETAILED DESCRIPTION

[0013] The present invention relates to an IgG1 antibody that is capable of binding to TNFa and comprises a modified Fc region. The antibody has improved resistance to proteolytic degradation. The antibody further has a high affinity to human FcRn at pH 6 and low affinity to human FcRn at pH 7.4. The amino acid sequence of the antibody comprises the amino acids

233P, 234V, 235A, and a deletion at amino acid position 236 (EU numbering). The antibody further comprises either the amino acid 434A or the amino acids 252Y, 254T and 256E (EU numbering). Additionally, the antibody comprises the amino acids 239D, 330L and 332E (EU numbering). Moreover, the antibody comprises (i) a V_L domain having the amino acid sequence as shown in SEQ ID NO:10 and (ii) a V_H domain having the amino acid sequence as shown in SEQ ID NO:9.

[0014] Throughout the present specification and claims, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain and residues 1-113 of the heavy chain) (Kabat et al., Sequences of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) expressly incorporated herein by reference). Unless stated otherwise herein, references to residues numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (see e.g., WO 2006/073941).

Antibody

[0015] In the context of the present application, the term "antibody" is used as a synonym for "immunoglobulin" (Ig), which is defined as a protein belonging to the class IgG, IgM, IgE, IgA, or IgD (or any subclass thereof), and includes all conventionally known antibodies and functional fragments thereof. According to the present application, the antibody is an IgG1 antibody. In the context of the present invention, a "functional fragment" of an antibody/immunoglobulin is defined as antigen-binding fragment or other derivative of a parental antibody that essentially maintains one or more of the properties of such parental antibody. An "antigen-binding fragment" or "antigen-binding domain" of an antibody/immunoglobulin is defined as fragment (e.g., a variable region of an IgG) that retains the antigen-binding region. An "antigen-binding region" of an antibody typically is found in one or more hypervariable region(s) of an antibody, i.e., the CDR-1, -2, and/or -3 regions. The antibodies of the present invention may be part of bi- or multifunctional constructs.

[0016] Preferably the antibody is a monoclonal antibody. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. (Harlow and Lane, "Antibodies, A Laboratory Manual" CSH Press 1988,

Cold Spring Harbor N.Y.).

[0017] In other embodiments, including embodiments relating to the *in vivo* use of the anti-TNF α antibodies in humans, chimeric, primatized, humanized, or human antibodies can be used. In a preferred embodiment, the antibody is a human antibody or a humanized antibody, more preferably a monoclonal human antibody or a monoclonal humanized antibody.

TNF α -binding domain

[0018] The antibody of the invention specifically binds to TNF α . As used herein, an antibody "specifically recognizes", or "specifically binds to" human TNF α , when the antibody is able to discriminate between human TNF α and one or more reference molecule(s). Preferably, the IC₅₀ value for binding to each of the reference molecules is at least 1,000 times greater than the IC₅₀ value for binding to TNF α . In its most general form (and when no defined reference is mentioned), "specific binding" is referring to the ability of the antibody to discriminate between human TNF α and an unrelated biomolecule, as determined, for example, in accordance with a specificity assay methods known in the art. Such methods comprise, but are not limited to, Western blots and ELISA tests. For example, a standard ELISA assay can be carried out. Typically, determination of binding specificity is performed by using not a single reference biomolecule, but a set of about three to five unrelated biomolecules, such as milk powder, BSA, transferrin or the like. In one embodiment, specific binding refers to the ability of the antibody to discriminate between human TNF α and human TNF β .

[0019] The antibody of the invention comprises a V_L domain and a V_H domain. The V_L domain comprises a CDR1 region (CDRL1), a CDR2 region (CDRL2), a CDR3 region (CDRL3) and Framework regions. The V_H domain comprises a CDR1 region (CDRH1), a CDR2 region (CDRH2), a CDR3 region (CDRH3) and Framework regions.

[0020] The term "CDR" refers to one of the six hypervariable regions within the variable domains of an antibody that mainly contribute to antigen binding. One of the most commonly used definitions for the six CDRs was provided by Kabat E. A. et al., (1991) Sequences of proteins of immunological interest. NIH Publication 91-3242). As used herein, Kabat's definition of CDRs only apply for CDR1, CDR2 and CDR3 of the light chain variable domain (CDR L1, CDR L2, CDR L3, or L1, L2, L3), as well as for CDR2 and CDR3 of the heavy chain variable domain (CDR H2, CDR H3, or H2, H3). CDR1 of the heavy chain variable domain (CDR H1 or H1), however, as used herein is defined by the following residues (Kabat numbering): It starts with position 26 and ends prior to position 36.

[0021] The antibody of the invention comprises (i) a V_L domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:3, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:4, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:5, and (ii) a V_H domain comprising a CDR1 region having the amino

acid sequence as shown in SEQ ID NO:6, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:7, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:8.

[0022] The antibody of the invention comprises (i) a V_H domain having the amino acid sequence as shown in SEQ ID NO:9, and (ii) a V_L domain having the amino acid sequence as shown in SEQ ID NO:10.

[0023] Further described herein is an antibody comprising (i) a V_L domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:14, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:15, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:16, and (ii) a V_H domain comprising a CDR1 region having the amino acid sequence as shown in SEQ ID NO:17, a CDR2 region having the amino acid sequence as shown in SEQ ID NO:18, and a CDR3 region having the amino acid sequence as shown in SEQ ID NO:19.

[0024] Further described herein is an antibody comprising a V_H domain having the amino acid sequence as shown in SEQ ID NO:20. The antibody of the present disclosure may comprise a V_L domain having the amino acid sequence as shown in SEQ ID NO:21 or SEQ ID NO:22. Furthermore, the antibody of the present disclosure may comprise (i) a V_H domain having the amino acid sequence as shown in SEQ ID NO:21, and (ii) a V_L domain having the amino acid sequence as shown in SEQ ID NO:21 or SEQ ID NO:22.

[0025] The antibody of the invention has a high affinity to human TNF α . The term " K_D ," refers to the dissociation equilibrium constant of a particular antibody-antigen interaction. Typically, the antibody of the invention binds to human TNF α with a dissociation equilibrium constant (K_D) of less than approximately 2×10^{-10} M, preferably less than 1.5×10^{-10} M, preferably less than 1.25×10^{-10} M, more preferably less than 1×10^{-10} M, most preferably less than 7.5×10^{-11} M or even less than 5×10^{-11} M, as determined using surface plasmon resonance (SPR) technology in a BIACORE instrument. In particular, the determination of the K_D is carried out as described in Example 1.

Modifications of the Fc region

[0026] A "modified Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one "amino acid modification" or "mutation" as herein defined. Preferably, the modified Fc region comprises a modified FcRn binding site which has at least one amino acid substitution compared to a native sequence FcRn binding site or to the FcRn binding site of a parent antibody, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a

native sequence FcRn binding site or in the FcRn binding site of the parent antibody. Alternatively, the antibody may have a modification outside the FcRn binding site which affects the affinity to FcRn, e.g. by structural changes. Typically, the affinity to human FcRn at pH 6 is increased due to the modification. It is preferred that the affinity to human FcRn at pH 7.4 is not substantially affected by the modification. The modifications can be generated by methods that are known *per se*, e.g. by site-directed mutagenesis as described in "Antibody Engineering - Methods and Protocols", edited by Patrick Chames, 2nd ed., 2012, Chapter 31 (ISBN 978-1-61779-973-0).

[0027] The amino acid sequence of the antibody of the invention comprises the amino acid proline at position 233, the amino acid valine at position 234, and the amino acid alanine at position 235, and further has a deletion of the amino acid at position 236 (EU numbering). This is referred to as "233P/234V/235A/236del" herein. The native amino acid at position 233 of non-modified human IgG antibodies is glutamic acid (E). The native amino acid at position 234 of non-modified human IgG antibodies is leucine (L). The native amino acid at position 235 of non-modified human IgG antibodies is leucine (L). The native amino acid at position 236 of non-modified human IgG antibodies is glycine (G). Thus, the antibody of the invention can be obtained by introducing the mutations E233P, L234V, L235A and G236del into an antibody. This is referred to as E233P/L234V/L235A/G236del herein. Preferably, the antibody of the invention is obtainable or obtained by substituting proline for glutamic acid at position 233, substituting valine for leucine at position 234, substituting alanine for leucine at position 235, and deleting glycine at position 236.

[0028] The amino acid sequence of the antibody of the invention further comprises (i) the amino acid alanine at position 434, or (ii) the amino acid tyrosine at position 252, the amino acid threonine at position 254 and the amino acid glutamic acid at position 256. This is referred to as 434A and 252Y/254T/256E herein, respectively. The native amino acid at position 434 of non-modified human IgG antibodies is asparagine (N). The native amino acid at position 252 of non-modified human IgG antibodies is methionine (M). The native amino acid at position 254 of non-modified human IgG antibodies is serine (S). The native amino acid at position 256 of non-modified human IgG antibodies is threonine (T). Thus, the antibody of the invention can be obtained by introducing the further mutation(s) N434A or M252Y/S254T/T256E into an antibody.

[0029] That is, the amino acid sequence of the antibody of the invention comprises 233P/234V/235A/236del/434A or 233P/234V/235A/236del/252Y/254T/256E. This amino acid sequence can be obtained by introducing the mutations E233P/L234V/L235A/ G236del/N434A or E233P/L234V/L235A/G236del/M252Y/S254T/T256E into an amino acid sequence of an antibody, e.g. of an antibody the Fc region of which has a non-modified or wild-type amino acid sequence.

[0030] The remaining amino acid sequence of the Fc region may be identical to the native amino acid sequence of a typical human IgG. It is possible, however, that the amino acid sequence of the antibody comprises one or more additional mutations or substitutions to the

native amino acid sequence of the Fc region of a native antibody, as long as the antibody still has TNF α -binding activity, FcRn binding activity at pH 6.0 and one or more effector functions.

[0031] The amino acid sequence of the antibody has the amino acids 239D/330L/332E, preferably obtainable or obtained by introducing the substitutions S239D/A330L/I332E..

[0032] In a preferred embodiment, the amino acid sequence of the antibody of the invention comprises 233P/234V/235A/236del/239D/330L/332E/434A. This antibody can be obtained by introducing the mutations E233P/L234V/L235A/236del/S239D/A330L/I332E/N434A into an amino acid sequence of an antibody, e.g. of an antibody the Fc region of which has a non-modified or wild-type amino acid sequence.

[0033] In another preferred embodiment, the amino acid sequence of the antibody of the invention comprises 233P/234V/235A/236del/239D/330L/332E/252Y/254T/256E. This antibody can be obtained by introducing the mutations E233P/L234V/L235A/236del/S239D/A330L/I332E/ M252Y/S254T/T256E into an amino acid sequence of an antibody, e.g. of an antibody the Fc region of which has a non-modified or wild-type amino acid sequence.

[0034] In a preferred embodiment, the Fc region of the antibody of the invention, including the hinge region, comprises or consists of the amino acid sequence selected from the group consisting of SEQ ID NO:28, and SEQ ID NO:29.

[0035] In one embodiment, the heavy chain of the antibody of the invention has the amino acid sequence as shown in SEQ ID NO:2, wherein the mutations E233P/L234V/L235A/236del/S239D/A330L/I332E/N434A have been introduced. Preferably this antibody further comprises a light chain having the amino acid sequence as shown in SEQ ID NO:1.

[0036] In another embodiment, the heavy chain of the antibody of the invention has the amino acid sequence as shown in SEQ ID NO:2, wherein the mutations E233P/L234V/L235A/236del/S239D/A330L/I332E/M252Y/S254T/T256E have been introduced. Preferably this antibody further comprises a light chain having the amino acid sequence as shown in SEQ ID NO:1.

[0037] In a preferred aspect of the invention, the antibody of the invention is a non-fucosylated antibody or an antibody having reduced fucosylation.

[0038] The term "antibody having reduced fucosylation", as used herein, refers to an antibody in which less than 90% of the N-glycans of the antibody are fucosylated. Methods to determine the percentage of fucosylation are known in the art. In one embodiment, less than 75%, or less than 50%, or less than 25% of the N-glycans of the antibody are fucosylated. Most preferably, less than 10% of the N-glycans of the antibody are fucosylated. In a particular embodiment, the N-glycans of the antibody of the invention do not contain any fucose.

[0039] Preferably, less than 90% of the N-glycans at N297 (EU numbering) of the antibody are

fucosylated. In another embodiment, less than 75%, or less than 50%, or less than 25% of the N-glycans at N297 (EU numbering) of the antibody are fucosylated. Most preferably, less than 10% of the N-glycans at N297 (EU numbering) of the antibody are fucosylated.

[0040] In another embodiment, the N-glycans at N297 of the antibody do not contain any fucose.

[0041] Non-fucosylated antibodies, sometimes also referred to as afucosylated antibodies, can be generated by various methods. For example, the synergistic knockdown of the genes for α 1,6-fucosyltransferase (*FUT8*) and GDP-mannose 4,6-dehydratase (*GMD*) in CHO cells can be used to produce monoclonal antibody variants that are fully afucosylated and ADCC-enhanced (see, e.g., Imai-Nishiya et al. (2007) BMC Biotechnol. 7, 84). A method using zinc-finger nucleases (ZFNs) cleaving the *FUT8* gene in a region encoding the catalytic core of the α 1,6-fucosyltransferase and thus disrupting the corresponding enzymatic function in CHO cells can be used to produce monoclonal antibodies completely lacking core fucose (see, e.g., Malphettes et al. (2010) Biotechnol. Bioeng. 106, 774-783).

[0042] Antibodies having reduced fucosylation can be prepared by addition of a decoy substrate such as 2-deoxy-2-fluoro-2-fucose to the culture medium (see, e.g., Dekker et al. (2016) Sci Rep 6:36964), resulting in a reduced incorporation of fucose in the IgG-Fc glycans.

[0043] In another embodiment, the antibody of the invention has a high sialic acid content. Increase in sialylation can be achieved, e.g. by simultaneous transfection of cytidine monophosphate-sialic acid synthase (CMP-SAS), cytidine monophosphate-sialic acid transporter (CMP-SAT), and α 2,3-sialyltransferases (see, e.g., Son et al. (2011) Glycobiology 21, 1019-1028).

Affinity to FcRn

[0044] The affinity at pH 6 to human FcRn of the antibody of the invention is high. The high affinity binding of the antibody to human FcRn at pH 6 is typically characterized by a K_D value of less than 500 nM. Preferably, the K_D value of the high affinity binding at pH 6 is less than 400 nM, or less than 300 nM, or less than 200 nM. For example, the K_D value characterizing the affinity at pH 6 may be in the range from 5 to 500 nM, or 10 to 400 nM, or 25 to 300 nM, or 50 to 200 nM, or 100 to 175 nM.

[0045] In a preferred embodiment, the affinity of the antibody of the invention to human FcRn at pH 6 is greater than the affinity of infliximab to human FcRn at pH 6.0.

[0046] The affinity of the antibody of the invention to human FcRn is preferably determined by surface plasmon resonance (SPR), for example as described in Example 4 of this application.

[0047] The antibody of the present invention typically has a low affinity to human FcRn at pH 7.4. The low affinity is characterized by a K_D value of greater than 1 μM . Preferably, the low affinity to human FcRn at pH 7.4 is characterized by a K_D value of greater than 2 μM , or greater than 5 μM , or greater than 10 μM .

[0048] In a particular embodiment, the low affinity at pH 7.4 is so low that a K_D value cannot be determined by SPR.

[0049] In a special embodiment, the ratio of (i) a K_D value for binding of the antibody of the invention to human FcRn at pH 7.4 to (ii) a K_D value for binding to human FcRn at pH 6.0, is at least 50. Preferably, this ratio is at least 100, or at least 150, or at least 200.

Functional properties of the antibody

[0050] The antibody of the invention is efficiently transported across a polarized cell monolayer from the apical side to the basolateral side. Typically, the transport across the polarized cell monolayer is in a greater amount than that of infliximab, wherein the amount of antibody in infliximab refers to the mass/cm² of the polarized cell monolayer. The amount of antibody transported across the polarized cell monolayer, relative to the amount of infliximab transported across the polarized cell monolayer, is at least 110%, preferably at least 120%, more preferably at least 130%, or at least 140%, or at least 150% (wherein the amount of transported infliximab is set to 100%).

[0051] Furthermore, the antibody is specifically transported across the polarized cell monolayer from the apical side to the basolateral side in the presence of an excess of competing immunoglobulins. This is referred to as specific transport herein.

[0052] The percentage of the total mass of immunoglobulins transported across the polarized cell monolayer is greater than the percentage of infliximab transported across the polarized cell monolayer from the apical side to the basolateral side in the presence of a 10-fold excess of competing immunoglobulins. The percentage of antibody of the invention transported across the polarized cell monolayer in the presence of a 10-fold excess of unrelated immunoglobulins, relative to the percentage of infliximab transported across the polarized cell monolayer in the presence of a 10-fold excess of unrelated antibodies, is at least 120%, or at least 130%, or at least 140%, or at least 150% (infliximab is set to be 100%).

[0053] Preferably, the polarized cell monolayer is a monolayer of polarized T84 cells. The transport assay mimicking process of transcytosis can be carried out as described in Example 5 of this application.

[0054] The antibody of the invention binds to CD16a(V), CD16a(F) and CD16b(NA2).

[0055] The antibody of the invention typically binds to CD16a(V) with a K_D of less than 1 μM , preferably less than 500 nM, more preferably less than 100 nM.

[0056] The antibody of the invention typically binds to CD16a(F) with a K_D of less than 10 μM , preferably less than 1 μM .

[0057] The antibody of the invention typically binds to CD16b(NA2) with a K_D of less than 10 μM , preferably less than 1 μM .

[0058] The antibody of the invention is further capable of inducing CD14⁺CD206⁺ macrophages. The level of induction is preferably comparable to, equal to, or greater than that of infliximab.

[0059] The antibody of the invention is further capable of suppressing T cell proliferation. The degree of suppression of T cell proliferation is preferably comparable to, equal to, or greater than that of infliximab.

Pharmaceutical Compositions and Treatment

[0060] Treatment of a disease encompasses the treatment of patients already diagnosed as having any form of the disease at any clinical stage or manifestation; the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of the disease; and/or preventing and/or reducing the severity of the disease.

[0061] A "subject" or "patient" to whom an anti-TNF α antibody is administered can be a mammal, such as a non-primate (e.g., cow, pig, horse, cat, dog, rat, etc.) or a primate (e.g., monkey or human). In certain aspects, the human is a pediatric patient. In other aspects, the human is an adult patient.

[0062] Compositions comprising an anti-TNF α antibody and, optionally one or more additional therapeutic agents, such as the second therapeutic agents described below, are described herein. The compositions typically are supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient).

[0063] The anti-TNF α antibodies can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intrathecally, topically or locally, e.g. mucosally. The most suitable route for administration in any given case will depend on the particular antibody, the subject, and the nature and severity of the disease and the physical condition of the subject. In one embodiment, the anti-TNF α antibody is administered intravenously.

[0064] In a particularly preferred embodiment, the antibody of the invention is administered orally. If the administration is via the oral route the antibody is preferably an IgG, most preferably an IgG₁.

[0065] The anti-TNF α antibody may be present in a pharmaceutical composition at a concentration sufficient to permit intravenous administration at 0.5 mg/kg body weight to 20 mg/kg body weight. In some embodiments, the concentration of antibody suitable for use in the compositions and methods described herein includes, but is not limited to, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, 20 mg/kg, or a concentration ranging between any of the foregoing values, e.g., 1 mg/kg to 10 mg/kg, 5 mg/kg to 15 mg/kg, or 10 mg/kg to 18 mg/kg.

[0066] The effective dose of an anti-TNF α antibody can range from about 0.001 to about 750 mg/kg per single (e.g., bolus) administration, multiple administrations or continuous administration, or to achieve a serum concentration of 0.01-5000 μ g/ml per single (e.g., bolus) administration, multiple administrations or continuous administration, or any effective range or value therein depending on the condition being treated, the route of administration and the age, weight and condition of the subject. In case of oral administration, the serum concentration may be very low or even below the detection limit. In certain embodiments, each dose can range from about 0.5 mg to about 50 mg per kilogram of body weight or from about 3 mg to about 30 mg per kilogram body weight. The antibody can be formulated as an aqueous solution.

[0067] In a particularly preferred embodiment, the antibody of the invention is administered orally. If the administration is via the oral route the antibody is preferably an IgG, most preferably an IgG₁. If the antibody is administered orally, the daily dose of antibody is typically in the range of about 0.01 mg/kg to about 100 mg/kg of body weight, or about 0.05 mg/kg to about 50 mg/kg of body weight, or about 0.1 mg/kg to about 25 mg/kg of body weight, or about 0.15 mg/kg to about 10 mg/kg of body weight, or about 0.16 mg/kg to about 5 mg/kg of body weight, or about 0.2 mg/kg to about 2 mg/kg of body weight, or about 0.2 mg/kg to about 1 mg/kg of body weight. Generally, advantageous doses are doses of 1 to 200 mg per day, preferably 5 to 100 or 10 to 50 mg per day.

[0068] Pharmaceutical compositions can be conveniently presented in unit dose forms containing a predetermined amount of an anti-TNF α antibody per dose. Such a unit can contain 0.5 mg to 5 g, for example, but without limitation, 1 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 750 mg, 1000 mg, or any range between any two of the foregoing values, for example 10 mg to 1000 mg, 20 mg to 50 mg, or 30 mg to 300 mg. Pharmaceutically acceptable carriers can take a wide variety of forms depending, e.g., on the condition to be treated or route of administration.

[0069] Determination of the effective dosage, total number of doses, and length of treatment an anti-TNF α antibody thereof is well within the capabilities of those skilled in the art, and can

be determined using a standard dose escalation study.

[0070] Therapeutic formulations of the anti-TNF α antibodies suitable in the methods described herein can be prepared for storage as lyophilized formulations or aqueous solutions by mixing the antibody having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as "carriers"), i.e., buffering agents, stabilizing agents, preservatives, isotonicifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

[0071] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They can present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), citrate-phosphate buffers, succinate buffers (e.g., succinic acid- monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid- disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium gluconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0072] The pharmaceutical composition of the invention may further comprise at least one salt, e.g. sodium chloride. The salt concentration preferably ranges from 100 mM to 200 mM, e.g. about 150 mM.

[0073] Preservatives can be added to retard microbial growth, and can be added in amounts ranging from 0.2%- 1% (w/v). Suitable preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as "stabilizers" can be added to ensure isotonicity of liquid compositions and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the

container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (e.g., peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccharides such as raffinose; and polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10,000 weights per part of weight active protein.

[0074] Non-ionic surfactants or detergents (also known as "wetting agents") can be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.). Non-ionic surfactants can be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, or in a range of about 0.07 mg/ml to about 0.2 mg/ml.

[0075] Additional miscellaneous excipients include bulking agents (e.g., starch), chelating agents (e.g., EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), protease inhibitors and co-solvents.

[0076] The formulation herein can also contain a second therapeutic agent in addition to an anti-TNF α antibody thereof. Examples of suitable second therapeutic agents are provided below.

[0077] The dosing schedule can vary from once a month to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the patient's sensitivity to the anti-TNF α antibody. In specific embodiments, an anti-TNF α antibody thereof is administered daily, twice weekly, three times a week, every other day, every 5 days, once weekly, every 10 days, every two weeks, every three weeks, every four weeks or once a month, or in any range between any two of the foregoing values, for example from every four days to every month, from every 10 days to every two weeks, or from two to three times a week, etc.

[0078] The dosage of an anti-TNF α antibody to be administered will vary according to the particular antibody, the subject, and the nature and severity of the disease, the physical condition of the subject, the therapeutic regimen (e.g., whether a second therapeutic agent is used), and the selected route of administration; the appropriate dosage can be readily

determined by a person skilled in the art.

[0079] It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an anti-TNF α antibody thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the age and condition of the particular subject being treated, and that a physician will ultimately determine appropriate dosages to be used. This dosage can be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in accordance with normal clinical practice.

Disorders to be treated

[0080] The invention relates to a method of treating or preventing a human TNF α -related disease in a subject, comprising administering to the subject the antibody as defined herein. The term "TNF α -related disorder" or "TNF α -related disease" refers to any disorder, the onset, progression or the persistence of the symptoms or disease states of which requires the participation of TNF α . Exemplary TNF α -related disorders include, but are not limited to, chronic and/or autoimmune states of inflammation in general, immune mediated inflammatory disorders in general, inflammatory CNS disease, inflammatory diseases affecting the eye, joint, skin, mucous membranes, central nervous system, gastrointestinal tract, urinary tract or lung, states of uveitis in general, retinitis, HLA-B27+ uveitis, Behçet's disease, dry eye syndrome, glaucoma, Sjögren syndrome, diabetes mellitus (incl. diabetic neuropathy), insulin resistance, states of arthritis in general, rheumatoid arthritis, osteoarthritis, reactive arthritis and Reiter's syndrome, juvenile arthritis, ankylosing spondylitis, multiple sclerosis, Guillain-Barre syndrome, myasthenia gravis, amyotrophic lateral sclerosis, sarcoidosis, glomerulonephritis, chronic kidney disease, cystitis, psoriasis (incl. psoriatic arthritis), hidradenitis suppurativa, panniculitis, pyoderma gangrenosum, SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis), acne, Sweet's syndrome, pemphigus, Crohn's disease (incl. extraintestinal manifestations), ulcerative colitis, asthma bronchiale, hypersensitivity pneumonitis, general allergies, allergic rhinitis, allergic sinusitis, chronic obstructive pulmonary disease (COPD), lung fibrosis, Wegener's granulomatosis, Kawasaki syndrome, Giant cell arteritis, Churg-Strauss vasculitis, polyarteritis nodosa, burns, graft versus host disease, host versus graft reactions, rejection episodes following organ or bone marrow transplantation, systemic and local states of vasculitis in general, systemic and cutaneous lupus erythematoses, polymyositis and dermatomyositis, scleroderma, pre-eclampsia, acute and chronic pancreatitis, viral hepatitis, alcoholic hepatitis, postsurgical inflammation such as after eye surgery (e.g. cataract (eye lens replacement) or glaucoma surgery), joint surgery (incl. arthroscopic surgery), surgery at joint-related structures (e.g. ligaments), oral and/or dental surgery, minimally invasive cardiovascular procedures (e.g. PTCA, atherectomy, stent placement), laparoscopic and/or endoscopic intra-abdominal and gynecological procedures, endoscopic urological procedures (e.g. prostate surgery, ureteroscopy, cystoscopy, interstitial cystitis), or perioperative inflammation (prevention) in general, bullous dermatitis, neutrophilic dermatitis, toxic epidermal necrolysis, pustular dermatitis, cerebral malaria, hemolytic uremic syndrome, allograft

rejection, otitis media, snakebite, erythema nodosum, myelodysplastic syndromes, primary sclerosing cholangitis, seronegative spondylarthropathy, autoimmune hemolytic anemia, orofacial granulomatosis, pyostomatitis vegetans, aphthous stomatitis, geographic tongue, migratory stomatitis, Alzheimer disease, Parkinson's disease, Huntington's disease, Bell's palsy, Creutzfeld-Jakob disease and neuro-degenerative conditions in general.

[0081] Cancer-related osteolysis, cancer-related inflammation, cancer-related pain, cancer-related cachexia, bone metastases, acute and chronic forms of pain, irrespective whether these are caused by central or peripheral effects of TNF α and whether they are classified as inflammatory, nociceptive or neuropathic forms of pain, sciatica, low back pain, carpal tunnel syndrome, complex regional pain syndrome (CRPS), gout, postherpetic neuralgia, fibromyalgia, local pain states, chronic pain syndroms due to metastatic tumor, dysmenorrhea.

[0082] Particular disorders to be treated include states of arthritis in general, rheumatoid arthritis, osteoarthritis, reactive arthritis, juvenile arthritis; psoriasis incl. psoriatic arthritis; inflammatory bowel disease, including Crohn's disease, ulcerative colitis incl. proctitis, sigmoiditis, proctosigmoiditis, left-sided colitis, extensive colitis and pancolitis, undetermined colitis, microscopic colitis incl. collagenous and lymphocytic colitis, colitis in connective tissue disease, diversion colitis, colitis in diverticular disease, eosinophilic colitis and pouchitis.

[0083] Most preferably, the antibody of the invention is used to treat an inflammatory bowel disease, in particular Crohn's disease, ulcerative colitis or microscopic colitis. The Crohn's disease may be ileal, colonic, ileocolonic or isolated upper Crohn's disease (gastric, duodenal and/or jejunal) including non-stricturing/non-penetrating, stricturing, penetrating and perianal disease behavior, allowing any combination of localization and disease behavior of any of the above mentioned. The ulcerative colitis may be ulcerative proctitis, proctosigmoiditis, left-sided colitis, pan-ulcerative colitis and pouchitis.

Combination Therapy and other aspects

[0084] Preferably, the patient being treated with an anti-TNF α antibody thereof is also treated with another conventional medicament. For example, a patient suffering from inflammatory bowel disease, especially if having moderate to severe disease is typically also being treated with mesalazine or derivatives or prodrugs thereof, corticosteroids, e.g. budesonide or prednisolone (oral or i.v.), immunosuppressants, e.g. azathioprine/6-mercaptopurine (6-MP) or methotrexate, cyclosporine or tacrolimus. Other medicaments which can be co-administered to the patient include other anti-TNF α antibodies (e.g. infliximab, adalimumab, etanercept, certolizumab pegol, golimumab), integrin antagonists (e.g. natalizumab, vedolizumab), anti-IL-23 antibodies (e.g. MEDI2070), anti- β 7 antibodies (e.g. etrolizumab), JAK inhibitors in the JAK/STAT pathway (e.g. tofacitinib), and others. Further medicaments which can be co-administered to the patient include immunosuppressants (e.g. azathioprine/6-MP or methotrexate or oral cyclosporine) in order to maintain stable and longer remission. Yet another aspect of the invention is the use of an anti-TNF α antibody as defined hereinabove for

reducing inflammation.

[0085] Yet another aspect of the invention is an anti-TNF α antibody as defined hereinabove for use in reducing inflammation in a patient suffering from an inflammatory condition.

[0086] A further aspect of this invention is a method of treating an inflammatory condition, comprising administering to a patient in need thereof an effective amount of an anti-TNF α antibody as defined hereinabove. The inflammatory condition is preferably one of the conditions described above.

[0087] A further aspect of this invention is a method of preventing an inflammatory condition, comprising administering to a patient in need thereof an effective amount of an anti-TNF α antibody as defined hereinabove. The inflammatory condition is preferably one of the conditions described above.

[0088] Further described herein is a method for improving the transcytosis of an antibody directed against TNF α , comprising introducing the substitutions E233P, L234V and L235A, deleting G236 and introducing the following further substitution(s) (a) or (b):

1. (a) M252Y, S254T and T256E
2. (b) N434A;

and optionally further introducing one or more of the other substitutions described herein; so as to obtain a modified antibody having improved transcytosis. The modified antibody is preferably an antibody as described hereinabove.

[0089] Further described herein is a method for extending the plasma half-life of an antibody directed against TNF α , comprising introducing the substitutions E233P, L234V and L235A, deleting G236 and introducing the following further substitution(s) (a) or (b):

1. (a) M252Y, S254T and T256E
2. (b) N434A;

and optionally further introducing one or more of the other substitutions described herein; so as to obtain a modified antibody having an extended plasma half-life. The modified antibody is preferably an antibody as described hereinabove. The plasma half-life may be increased by at least 10%, or least 20%, or least 30%, or least 40%, or least 50%, relative to the plasma half-life of the non-modified antibody (i.e., the respective parent antibody lacking the recited mutations).

[0090] Further described herein is a method of improving the resistance against proteolytic degradation of an antibody directed against TNF α , comprising introducing the substitutions E233P, L234V and L235A, deleting G236 and introducing the following further substitution(s)

(a) or (b):

(a) M252Y, S254T and T256E

(b) N434A;

and optionally further introducing one or more of the other substitutions described herein; so as to obtain a modified antibody having improved resistance to proteolytic degradation. The modified antibody is preferably an antibody as described hereinabove.

Table 1. Overview of the sequences of the sequence listing.

SEQ ID NO:	Description of the amino acid sequence
1	Light chain of Ab-wt, the parent antibody of the modified antibodies used in the examples
2	Heavy chain of Ab-wt, the parent antibody of the modified antibodies used in the examples
3	CDR L1 of clone 16-22-H05
4	CDR L2 of clone 16-22-H05
5	CDR L3 of clone 16-22-H05
6	CDR H1 of clone 16-22-H05
7	CDR H2 of clone 16-22-H05
8	CDR H3 of clone 16-22-H05
9	V _H of humanized IgG of clone 16-22-H05
10	V _L of humanized IgG of clone 16-22-H05
11	Heavy chain of Ab-YTE-DLE-PVAΔG (based on clone 16-22-H05)
12	Heavy chain of Ab-A-DLE-PVAΔG (based on clone 16-22-H05)
13	Heavy chain of Ab-A-AEA-PVAΔG (based on clone 16-22-H05)
14	CDR L1 of clone 17-22-B03
15	CDR L2 of clone 17-22-B03
16	CDR L3 of clone 17-22-B03
17	CDR H1 of clone 17-22-B03
18	CDR H2 of clone 17-22-B03
19	CDR H3 of clone 17-22-B03
20	V _H of humanized IgG of clone 17-22-B03
21	V _L of humanized IgG of clone 17-22-B03 (sc08)
22	V _L of humanized IgG of clone 17-22-B03 (sc02)
23	Light chain of humanized IgG of clone 17-22-B03 (sc08)
24	Light chain of humanized IgG of clone 17-22-B03 (sc02)
25	Heavy chain of Ab-YTE-DLE-PVAΔG (based on clone 17-22-B03)

SEQ ID NO:	Description of the amino acid sequence
26	Heavy chain of Ab-A-DLE-PVAΔG (based on clone 17-22-B03)
27	Heavy chain of Ab-A-AEA-PVAΔG (based on clone 17-22-B03)
28	Fc region including hinge region of Ab-YTE-DLE-PVAΔG
29	Fc region including hinge region of Ab-A-DLE-PVAΔG
30	Fc region including hinge region of Ab-A-AEA-PVAΔG

Examples

Antibody variants

[0091] Several variants of an anti-TNFα antibody (hereinafter referred to as "parent antibody" or "Ab-wt") were generated by introducing substitutions in the Fc region of the antibody amino acid sequence. The light chain of Ab-wt has the amino acid sequence as shown in SEQ ID NO:1, and the heavy chain of Ab-wt has the amino acid sequence as shown in SEQ ID NO:2. The mutations were introduced by site-directed mutagenesis by established methods. Briefly, mutations were introduced by PCR. The forward primer was designed to contain the intended mutation while the reverse primer was designed so that the 5' ends of the two primers anneal back-to-back (but do not overlap) (Figure 10). PCR was run for 25 cycles (98°C for 10 s, 64°C for 30s, 72°C for 3 min). Before running the PCR product on an agarose gel, the non-mutated PCR template was removed from the pool of PCR products using the restriction enzyme DpnI. Following gel purification of the PCR product the blunt ends were ligated to obtain a circularized plasmid which was transformed into competent E.coli cells. Following overnight incubation several colonies were picked, the plasmid DNA isolated and sequenced to confirm that the mutation had been incorporated..

Table 2: Generated antibody variants of an anti-TNFα antibody (EU numbering)

Designation	Mutations relative to parent antibody
Ab-wt*	None (=parent antibody)
Ab-YTE-DLE-PVAΔG**	M252Y/S254T/T256E-S239D/A330L/I332E-E233P/L234V/L235A/G236del
Ab-A-DLE-PVAΔG**	N434A-S239D/A330L/I332E-E233P/L234V/L235A/G236del
Ab-A-AEA-PVAΔG*	N434A-K326A/I332E/E333A-E233P/L234V/L235A/G236del
* antibody not according to the invention; **antibody according to the invention	

Example 1. Affinity to TNFα

Method:

[0092] Affinity to TNF α was measured by Biacore. A CM5 chip was prepared using standard amine immobilisation Biacore procedures. Upon insertion of a CM5 chip the system was primed and then normalised with BIA-normalising solution (Biacore Preventative Maintenance Kit 2). The chip was added to the system with Phosphate Buffered Saline Tween-20 (PBS-T) running buffer; prior to immobilisation the chip surface was primed with three injections of 50 mM NaOH. Protein A was immobilised on the chip surface. For this, the protein was diluted to 5 μ g/mL into 10 mM acetate buffer at pH 4.5 and injected so to generate a bound response of $\sim$ 1000 RU's in all 4 flow cells. To remove non-covalently bound material from all the chip flow cells, three 15 second 50 mM NaOH washes were performed. On the Protein A chip, antibody was captured in flow cells 2 and 4, with flow cells 1 and 3 used for reference subtraction. The trial antibodies were diluted in PBS-T to 10 nM and 2.5-7.5 μ L injected to obtain 120 RU of captured antibody. The analyte TNF α was prepared at 500 μ g/mL in water as directed by the supplier and further diluted into the running buffer PBS-T. Single cycle kinetics was used to estimate the steady state affinity. For each single cycle analysis cycle a titration of 5 analyte concentrations were injected over the ligand and then the dissociation of the complex was measured. The surface was regenerated using glycine pH 1.7. A double referencing method was employed in which data from the ligand bound capture surface (fc 2 and 4) were subtracted from the references surfaces where no ligand was captured (fc 1 and 3 respectively). Blank injections of buffer were run every 3-4 cycles and then subtracted from analyte injection cycles, to correct for small changes in the ligand capture surface. Repeat injections of analyte at the start and end of each analytical run were used to check for sample degradation, or changes in the instrument performance. All analysis was performed at 25°C and the sample rack was incubated at 10°C during experimental runs. Each experiment was run at least three times. A 1-to-1 binding model was used to fit the resulting kinetic data.

Results:

[0093] All antibodies displayed similar binding kinetics to TNF α indicating that any introduced modification had not led to significant changes in the antigen binding region.

Table 3: Binding kinetics of human IgG1 variants to TNF α as determined by SPR

	$k_a(10^6/\text{Ms})$	$k_d(10^{-5}/\text{s})$	$K_D(\text{pM})$
Ab-wt	8.37 ± 0.11	3.45 ± 0.20	4.13 ± 0.19
Ab-YTE-DLE-PVA Δ G	8.14 ± 0.37	1.28 ± 0.31	1.57 ± 0.31
Ab-A-DLE-PVA Δ G	11.3 ± 3.58	3.04 ± 0.14	2.88 ± 0.87
Ab-A-AEA-PVA Δ G	9.16 ± 3.24	2.19 ± 0.53	2.51 ± 0.75

Example 2. Potency

Method:

[0094] L929 cells were incubated with 0.25 ng/mL of TNF α and 1 μ g/well of actinomycin D in the presence of serial dilutions of anti-TNF α antibody variants. Following incubation for 20 h at 37°C/5% CO₂, the proliferative responses were measured using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and an electron coupling reagent (phenazine ethosulfate, PES). MTS was converted into formazan product by dehydrogenase enzymes present in metabolically active cells. The quantity of formazan product as measured by absorbance at 492 nm was directly proportional to the number of living cells in culture.

Results:

[0095] The results are shown in Figure 1. The introduction of mutations into the Fc region of the anti-TNF α antibody did not affect the potency.

Example 3. Affinity to Fc γ receptors (CD16a, CD16b)

Method:

[0096] Affinity to Fc γ R_s was measured by Biacore. A CM5 chip was prepared using standard amine immobilisation Biacore procedures. Upon insertion of a CM5 chip the system was primed and then normalised with BIA-normalizing solution (Biacore Preventative Maintenance Kit 2). The chip was added to the system with PBS-T running buffer; prior to immobilisation the chip surface was primed with three injections of 50 mM NaOH. Fc γ R_s were immobilised on the chip surface using a His-tag capture system. The anti-His tag chip was prepared according to the Biacore kit instructions, with ~12000 RU's of the antibody deposited on all 4 flow cells. To remove non-covalently bound material from all the chip flow cells, three 30 second 10 mM glycine pH 1.5 washes were performed. The Fc γ receptors were diluted in PBS-T to a range of 0.5-2 μ g/mL, with 2.5-5.0 μ L injected onto the chip generating capture levels between 60 and 200 RU's. Antibodies were diluted into PBS-T prior to analysis. Single cycle kinetics were used to estimate the steady state affinity. For each single cycle analysis cycle a titration of 5 antibody concentrations were injected over the Fc γ R ligand and then the dissociation of the complex was measured. The surface was regenerated using the recommended solution, 10 mM glycine

pH 1.5 for the anti-His capture surface. A double referencing method was employed in which data from the ligand bound capture surface (fc 2 and 4) were subtracted from the references surfaces where no ligand was captured (fc 1 and 3 respectively). Blank injections of buffer were run for every antibody titration cycle and then subtracted from analyte injection cycles, to correct for small changes in the ligand capture surface. All analysis was performed at 25°C and the sample rack was incubated at 10°C during experimental runs. Each experiment was run at least three times.

Results:

[0097] The introduction of the mutations did not affect the affinity to CD16a(V), CD16a(F) and CD16b. However, some antibody variants exhibited increased binding to CD16a. Especially Ab-A-DLE-PVAΔG had an improved binding to the low affinity CD16a receptor and to CD16b.

[0098] Table 4: Affinity to Fcγ receptors CD16a(V), CD16a(F) and CD16b as determined by SPR. The mean and standard deviation affinity calculated from two or more independent experiments is shown.

	Affinity (K _D)		
	CD16a(V) (nM)	CD16a(F) (μM)	CD16b(NA2) (μM)
Ab-wt	184 ± 31.9	nd	> 3.00
Ab-YTE-DLE-PVAΔG	117 ± 13.9	nd	1.03 ± 0.54
Ab-A-DLE-PVAΔG	68.7 ± 14.1	0.11 ± 0.02	0.47 ± 0.10
Ab-A-AEA-PVAΔG	260 ± 9.73	nd	5.18 ± 0.08

Example 4. Affinity to FcRn

Method:

[0099] SPR was performed using a Biacore 3000 instrument with CM5 sensor chips coupled with anti-TNFα IgG1 antibodies (-500 resonance units (RU)) using amine-coupling chemistry as described by the manufacturer. The coupling was performed by injecting 2.0 ug/mL of each protein in 10 mM sodium acetate, pH 4.5, using the amine-coupling kit (GE Healthcare). HBS-P buffer pH 7.4 (10 mM HEPES, 150 mM NaCl, 0.005% surfactant P20) or phosphate buffer pH 6.0 (67 nM phosphate buffer, 150 mM NaCl, 0.005% Tween 20) were used as running and dilution buffer. Binding kinetics were determined by injecting titrated amounts (1000 - 31.2 nM) of monomeric His-tagged human FcRn (hFcRn) over immobilised antibodies at pH 7.4 or pH 6.0. All SPR experiments were conducted at 25°C with a flow rate of 40 ul/min. Binding data

were zero-adjusted, and reference cell value subtracted. The Langmuir 1:1 ligand binding model provided by the BIAevaluation software (version 4.1) was used to determine the binding kinetics.

Results:

[0100] The results showed that the wild-type antibody Ab-wt bound strictly pH dependently to hFcRn. All engineered antibody variants have a higher affinity to FcRn at pH 6.0, but kept their pH dependency and did not bind to the receptor at pH 7.4. All antibody variants showed improved binding to FcRn compared to infliximab which contains a wildtype IgG1 Fc region.

Table 5: Affinity of anti-TNF α antibody variants to FcRn at pH 6.0 and pH 7.4 as determined by SPR

	pH 6.0			pH 7.4
	K _D (nM)	Fold change from wt	Fold change from IFX	K _D (nM)
Ab-wt	1000			NA
Ab-YTE-DLE-PVA Δ G	90.0	11.1	4.7	NA
Ab-A-DLE-PVA Δ G	157	6.4	2.7	NA
IFX	425			NA
NA: not acquired due to weak binding.				

Example 5. Transcytosis

Method:

[0101] Transwell filters (1.12 cm²) with collagen coated polytetrafluoroethylene (PTFE) membranes with 0.4 μ m pore size were incubated O/N in complete growth medium followed by seeding of 1.0 x 10⁶ T84 cells per well. Transepithelial electrical resistance (TEER) was monitored daily using a MILLICELL-ERS-2 volt-ohm meter. The cultures were grown for 4 - 5 days before reaching confluence with a TEER value of ~1000 - 1300 Ω x cm². Prior to experiments the monolayers were starved for 1 h in Hank's Balanced Salt Solution (HBSS). Then, 400 nM of the the antibody variants or IFX alone or together with 4000 nM human myeloma IgG with irrelevant specificity were added to the apical Transwell chamber. Samples were collected from the basolateral reservoir at 0 and 4 h post adding. Antibody concentrations in the basolateral reservoir were determined by ELISA. Briefly, 96-well Maxisorp plates were

coated O/N with either recombinant TNF α or an anti-human Fc specific antibody from goat, both diluted to 1 μ g/ml in PBS. Subsequently, the plates were blocked with PBS containing 4% skimmed milk for 2 h at RT followed by washing 4 times with PBS containing 0.05% Tween 20. Samples collected during the transcytosis experiments were added to the wells and incubated for 2 h at RT before washing as above. Captured antibody variants, IFX or total IgG were detected using an alkaline phosphatase (ALP)-conjugated anti-human Fc specific antibody from goat. Binding was visualized by addition of 100 μ l ALP-substrate and the 405 nm absorption spectrum was recorded. The amount of antibody variants, IFX and total IgG transported were calculated from standard curves of each of the individual antibody variants.

Transcytosis of antibody variants across polarized human epithelial cells

Results:

[0102] The engineered anti-TNF α antibody variants were tested for transcytosis across a cell monolayer and compared to the wt antibody or IFX as another human IgG1 anti-TNF α antibody. The results are depicted in Figure 2. The wt anti-TNF α antibody was transported from the apical to the basolateral reservoir. Of the antibody panel of the engineered variants for improved binding to FcRn, somewhat more of Ab-YTE-DLE-PVA Δ G was shown to be released at the basolateral side compared to Ab-wt. Compared to IFX, another IgG1 antibody with a wt Fc region, Ab-A-DLE-PVA Δ G was transported about 1.8-fold more efficiently.

Transcytosis of antibody variants across polarized human epithelial cells in the presence of competing IgG

Results:

[0103] The total amount of immunoglobulin transported across a polarized T84 cell monolayer from the apical to the basolateral reservoir when the anti-TNF α antibody variants were incubated with a 10-fold excess of human myeloma IgG at 4 hours post adding was comparable for all antibodies. However, an increased affinity to FcRn at pH 6.0 resulted in a significantly higher percentage of specific anti-TNF α transport across the cell monolayer also in the presence of an excess of competing human IgG with irrelevant specificity. The results are depicted in Figure 3.

Example 6. ADCC

Method:

[0104] An ADCC reporter bioassay core kit from Promega was used. Briefly, mTNF α CHO-K1 target cells at 1×10^5 /mL were seeded on white (clear bottom) tissue culture plates, 100 μ L per well. The plates were incubated O/N at 37°C/5% CO₂. On day 2, 95 μ L of assay medium was removed and replaced with 25 μ L of engineered Jurkat effector cells at 3×10^6 /mL. The plates were then incubated for 6 h at 37°C/5% CO₂. The BioGlo™ reagent was prepared towards the end of the incubation. Plates were equilibrated to RT for 10 - 20 min before adding 75 μ L of BioGlo™ reagent per well. After 5 - 10 min of incubation in the dark, luminescence was measured. A 4-PL model was used to fit the data.

Results:

[0105] The results (see Figure 4) showed that all of the anti-TNF α antibodies induced ADCC but with distinct strengths. Compared to the wildtype antibody Ab-wt, Ab-A-AEA-PVA Δ G showed similar ADCC activity, while the other antibody variants showed increased ADCC. Specifically Ab-A-DLE-PVA Δ G had significantly improved ADCC.

Example 7. Induction of regulatory macrophages

Method:

[0106] Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy donors. Cells were isolated through Ficoll gradient centrifugation. Cells of two individual donors were mixed in equal numbers and 2×10^5 cells of the mixture were plated in 96 well plates in a total volume of 100 μ L/well. Cells were incubated for 48 h at 37°C/5% CO₂. After 48 h, anti-TNF α antibody variants or IFX were added to reach a final concentration of 10 μ g/mL. Each compound was added in replicates of five or six. Final volume was 150 μ L/well. Human serum IgG1 (Sigma #I5154) was used as control. After addition of the compounds, mixed lymphocyte reactions (MLRs) were cultured for another 4 days at 37°C/5% CO₂. Afterwards, plates were washed using PBS/5 mM EDTA (PBS/EDTA) and incubated with 50 μ L/well PBS/ EDTA for 20 min at RT. Plates were centrifuged and liquid was flicked out. Antibody was diluted in PBS/EDTA (anti-CD14-PE, anti-CD206-APC, both diluted 1:10). Cells were resuspended in 50 μ L of antibody solution and incubated for 20 min at RT. Afterwards, cells were washed with PBS/EDTA and resuspended in 50 μ L PBS/EDTA. Stained samples were analysed on a FACS Fortessa using FACSDiva software. Analysis was performed using FlowJo software.

Results:

[0107] Induction of regulatory macrophages was analysed in four independent MLRs and was successful in all experiments (comparing IFX to IgG control). The results are shown in Figure 5. The levels of induction by IFX can differ between experiments due to the fact that each experiment was performed using different donors with inter-individual variation. All tested anti-TNF α antibody variants induced CD14⁺CD206⁺ regulatory macrophages with slight variation between the compounds. Ab-A-DLE-PVA Δ G induced more regulatory macrophages than IFX.

Example 8. Inhibition of T-cell proliferation

Method:

[0108] PBMC were isolated from buffy coats of healthy donors. Cells were isolated through Ficoll gradient centrifugation. Cells of two individual donors were mixed in equal numbers and 2×10^5 cells of the mixture were plated in 96 well plates in a total volume of 100 μ L/well. Cells were incubated for 48 h at 37°C/5% CO₂. After 48 h, anti-TNF α antibody variants or IFX were added to reach a final concentration of 10 μ g/mL. Each compound was added in replicates of five or six. Final volume was 150 μ L/well. Human serum IgG1 (Sigma #I5154) was used as control. After addition of the compounds, mixed lymphocyte reactions (MLRs) were cultured for another 2 days at 37°C/5% CO₂. Afterwards, tritiated thymidine (³H thymidine, 0.5 microCurie/well) was added to the cultures. Cultures were further incubated for 18 h at 37°C/5% CO₂. Samples were harvested using a Microbeta Filtermat 96 cell harvester and analysed using a Microbeta MicroplateCounter equipped with a single detector. Samples were counted for 10 seconds/well and converted to counts per minute (cpm).

Results:

[0109] Inhibition of T-cell proliferation was measured in three independent MLRs and was defined as successful if IFX as positive control induced suppression. The levels of suppression by IFX in individual experiments can differ presumably due to the variance in regulatory macrophage induction. In each experiment, the potential of the anti-TNF α antibody variants to suppress T-cell proliferation was calculated relative to the positive control IFX. Antibody Ab-A-DLE-PVA Δ G showed significantly enhanced suppression compared to IFX while suppression by Ab-YTE-DLE-PVA Δ G was comparable to IFX (see Figure 6).

Example 9. Protease stability

Methods:

[0110] Analysis was performed under reducing and non-reducing conditions. The corresponding sample buffer from the PerkinElmer Protein Express Reagent Kit with and without the reducing agent DTT was used to quench the reaction (i.e. as stopping reagents).

[0111] To be able to differentiate between the variants, the amount of protease per IgG was chosen as follows that degradation-time profiles could be obtained in between 30 hours. Analysis was performed using the microchip based electrophoresis system.

IdeS digestion

[0112] To prepare the working solution (ws), one IdeS aliquot was reconstituted in 100 µL Milli-Q water. IdeS ws and samples were combined in a 1:1 (v/v) ratio and homogenized thoroughly. The solution was incubated at 37 °C, samples were pulled after 5, 10, 30, 60 minutes and quenched with one of the stopping reagents. Molar ratio of protease/IgG: 4:1.

GluC digestion

[0113] To prepare the working solution (ws), the GluC stock was diluted with 2X GluC reaction buffer to a concentration of 50 µg/mL. GluC ws and samples were combined in a 1:1 (v/v) ratio and homogenized thoroughly. The solution was incubated at 37 °C, samples were pulled after 2, 6, 24, 30 hours and quenched with one of the stopping reagents. Molar ratio of IgG/protease: 4:1.

MMP-3 digestion

[0114] The chymotrypsin stock was diluted with assay buffer MMP to 50 µg/mL. MMP-3 a 0.186 mg/mL was spiked with the diluted chymotrypsin in a 1:1 (v/v) ratio and incubated at 37 °C for 30 minutes. Activation was stopped with PMSF in a final concentration of 2 mM. To prepare the working solution (ws), activated MMP-3 was diluted in assay buffer MMP to 3.72 µg/mL.

[0115] MMP-3 ws and samples were combined in a 1:1 (v/v) ratio and homogenized thoroughly. The solution was incubated at 37 °C, samples were pulled after 2, 6, 24, 30 hours and quenched with one of the stopping reagents. Molar ratio of IgG/protease: 98:1.

Results:

[0116] The tested antibody variants showed excellent resistance to proteolytic degradation by MMP-3 and IdeS, and good resistance to degradation by GluC (see Figures 7-9).

SEQUENCE LISTING

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 35 40 45

Thr Tyr Ile Tyr Pro Gly Phe Ala Ile Thr Asn Phe Ala Asn Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr Val Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Asp Pro Val Tyr Ala Thr Ser Ser Gly Tyr Phe Asp Leu Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
225 230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
275 280 285

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
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Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
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Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
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Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
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385 390 395 400

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
405 410 415

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
420 425 430

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
435 440 445

Ser Pro Gly Lys
450

<210> 3

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR L1 of clone 16-22-H05

<400> 3

Gln Ala Ser Gln Ser Ile Phe Ser Gly Leu Ala
1 5 10

<210> 4

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR L2 of clone 16-22-H05

<400> 4

Gly Ala Ser Lys Leu Ala Ser
1 5

<210> 5

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR L3 of clone 16-22-H05

<400> 5

Gln Ser Tyr Tyr Tyr Ser Ser Ser Ser Ser Asp Gly Ser Tyr Ala
1 5 10 15

<210> 6

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR H1 of clone 16-22-H05

<400> 6

Gly Ile Asp Phe Asn Asn Tyr Gly Ile Gly
1 5 10

- - -

<210> 7

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR H2 of clone 16-22-H05

<400> 7

Tyr	Ile	Tyr	Pro	Gly	Phe	Ala	Ile	Thr	Asn	Phe	Ala	Asn	Ser	Val	Lys
1				5					10					15	

Gly

<210> 8

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR H3 of clone 16-22-H05

<400> 8

Asp	Pro	Val	Tyr	Ala	Thr	Ser	Ser	Gly	Tyr	Phe	Asp	Leu
1				5					10			

<210> 9

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> VH of humanized IgG of clone 16-22-H05

<400> 9

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	

Ser	Leu	Arg	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Ile	Asp	Phe	Asn	Asn	Tyr
			20						25					30	

Gly	Ile	Gly	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Thr	Tyr	Ile	Tyr	Pro	Gly	Phe	Ala	Ile	Thr	Asn	Phe	Ala	Asn	Ser	Val
	50					55					60				

Lys	Gly	Arg	Phe	Thr	Ile	Ser	Ser	Asp	Asn	Ser	Lys	Asn	Thr	Val	Tyr
65					70				75						80

Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	

Ala Arg Asp Pro Val Tyr Ala Thr Ser Ser Gly Tyr Phe Asp Leu Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 10

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> VL of humanized IgG of clone 16-22-H05

<400> 10

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Phe Ser Gly
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro
65 70 75 80

Ala Asp Phe Ala Thr Tyr Tyr Cys Gln Ser Tyr Tyr Tyr Ser Ser Ser
85 90 95

Ser Ser Asp Gly Ser Tyr Ala Phe Gly Gly Gly Thr Lys Leu Thr Val
100 105 110

Leu Gly

<210> 11

<211> 451

<212> PRT

<213> Artificial Sequence

<220>

<223> Heavy chain of Ab-YTE-DLE-PVAdeltaG (based on clone 16-22-H05)

<400> 11

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Ile Asp Phe Asn Asn Tyr
20 25 30

Gly Ile Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Thr Tyr Ile Tyr Pro Gly Phe Ala Ile Thr Asn Phe Ala Asn Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Val Tyr Ala Thr Ser Ser Gly Tyr Phe Asp Leu Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser

210

215

220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
225 230 235 240

Gly Pro Asp Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Tyr
245 250 255

Ile Thr Arg Glu Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Leu Pro Glu
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 12

<211> 451

<212> PRT

<213> Artificial Sequence

<220>

<223> Heavy chain of Ab-A-DLE-PVAdeltaG (based on clone 16-22-H05)

<400> 12

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Ile Asp Phe Asn Asn Tyr
20 25 30

Gly Ile Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Thr Tyr Ile Tyr Pro Gly Phe Ala Ile Thr Asn Phe Ala Asn Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Val Tyr Ala Thr Ser Ser Gly Tyr Phe Asp Leu Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
 225 230 235 240

Gly Pro Asp Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Leu Pro Glu
 325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
 355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380

Trp Glu Ser Asn Gly Glu Pro Glu Asn Asn Thr Lys Thr Thr Pro Pro

Asp Glu Ser Asn Gly Gln Phe Glu Asn Asn Tyr Lys Thr Thr Phe Phe
 385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 420 425 430

His Glu Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 435 440 445

Pro Gly Lys
 450

<210> 13

<211> 451

<212> PRT

<213> Artificial Sequence

<220>

<223> Heavy chain of Ab-A-AEA-PVA?G (based on clone 16-22-H05)

<400> 13

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Ile Asp Phe Asn Asn Tyr
 20 25 30

Gly Ile Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Thr Tyr Ile Tyr Pro Gly Phe Ala Ile Thr Asn Phe Ala Asn Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr Val Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Asp Pro Val Tyr Ala Thr Ser Ser Gly Tyr Phe Asp Leu Trp
 100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175

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180      185      190
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
180      185      190

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
195      200      205

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
210      215      220

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
225      230      235      240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245      250      255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260      265      270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275      280      285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290      295      300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305      310      315      320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala Ala Leu Pro Ala Pro Glu
325      330      335

Ala Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340      345      350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
355      360      365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370      375      380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385      390      395      400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405      410      415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420      425      430

His Glu Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435      440      445

Pro Gly Lys
450

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<210> 14

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR L1 of clone 17-22-B03

<400> 14

Gln	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr	Leu	Ala
1			5						10	

<210> 15

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR L2 of clone 17-22-B03

<400> 15

Lys	Ala	Ser	Thr	Leu	Ala	Ser
1			5			

<210> 16

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR L3 of clone 17-22-B03

<400> 16

Gln	Cys	Thr	Tyr	Tyr	Glu	Pro	Ser	Tyr	Val	Phe	Arg	Ala
1			5						10			

<210> 17

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR H1 of clone 17-22-B03

<400> 17

Gly	Ile	Asp	Phe	Ser	Ala	Gly	Tyr	Asp	Met	Cys
1			5						10	

<210> 18

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR H2 of clone 17-22-B03

<400> 18

Cys	Ile	Asp	Ser	Arg	Arg	Glu	Glu	Ser	Asp	Tyr	Ala	Ser	Trp	Ala	Lys
1				5					10					15	

Gly

<210> 19

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> CDR H3 of clone 17-22-B03

<400> 19

Gly	Gly	Tyr	Gly	Gly	Asp	Gly	Val	Asp	Gly	Ala	Phe	Asp	Pro
1				5					10				

<210> 20

<211> 124

<212> PRT

<213> Artificial Sequence

<220>

<223> VH of humanized IgG of clone 17-22-B03

<400> 20

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	

Ser	Leu	Arg	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Ile	Asp	Phe	Ser	Ala	Gly
			20						25				30		

Tyr	Asp	Met	Cys	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp
		35					40					45			

Ile	Ala	Cys	Ile	Asp	Ser	Arg	Arg	Glu	Glu	Ser	Asp	Tyr	Ala	Ser	Trp
	50					55					60				

Ala	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Lys	Asp	Ser	Ser	Lys	Asn	Thr	Val
65					70					75					80

Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr
				85					90					95	

Cys	Ala	Arg	Gly	Gly	Tyr	Gly	Gly	Asp	Gly	Val	Asp	Gly	Ala	Phe	Asp
			100					105					110		

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 21

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> VL of humanized IgG of clone 17-22-B03 -(sc08)

<400> 21

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Cys Thr Tyr Tyr Glu Pro Ser
85 90 95

Tyr Val Phe Arg Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

<210> 22

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<223> VL of humanized IgG of clone 17-22-B03 -(sc02)

<400> 22

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Asp Leu Glu Pro
65 70 75 80

Ala Asp Phe Ala Thr Tyr Tyr Cys Gln Cys Thr Tyr Tyr Glu Pro Ser
85 90 95

Tyr Val Phe Arg Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

<210> 23

<211> 219

<212> PRT

<213> Artificial Sequence

<220>

<223> Light chain of humanized IgG of clone 17-22-B03 -(sc08)

<400> 23

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Cys Thr Tyr Tyr Glu Pro Ser
85 90 95

Tyr Val Phe Arg Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser

195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215
 <210> 24
 <211> 219
 <212> PRT
 <213> Artificial Sequence
 <220>
 <223> Light chain of humanized IgG of clone 17-22-B03 -(sc02)
 <400> 24
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Ser Tyr
 20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45
 Tyr Lys Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Asp Leu Glu Pro
 65 70 75 80
 Ala Asp Phe Ala Thr Tyr Tyr Cys Gln Cys Thr Tyr Tyr Glu Pro Ser
 85 90 95
 Tyr Val Phe Arg Ala Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 25

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<223> Heavy chain of Ab-YTE-DLE-PVAdeltaG (based on clone 17-22-B03)

<400> 25

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Ile Asp Phe Ser Ala Gly
20 25 30

Tyr Asp Met Cys Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Ala Cys Ile Asp Ser Arg Arg Glu Glu Ser Asp Tyr Ala Ser Trp
50 55 60

Ala Lys Gly Arg Phe Thr Ile Ser Lys Asp Ser Ser Lys Asn Thr Val
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Gly Gly Tyr Gly Gly Asp Gly Val Asp Gly Ala Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro
225 230 235 240

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Val Ala Gly Pro Asp Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Tyr Ile Thr Arg Glu Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Leu
325 330 335

Pro Glu Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
340 345 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
355 360 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
370 375 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
405 410 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys
450

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<210> 26

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<223> Heavy chain of Ab-A-DLE-PVAdeltaG (based on clone 17-22-B03)

<400> 26

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

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1	5	10	15
Ser Leu Arg 20	Ser Cys Lys 25	Gly Ile Asp 30	Phe Ser Ala Gly
Tyr Asp Met 35	Cys Trp Val Arg 40	Gln Ala Pro Gly Lys 45	Gly Leu Glu Trp
Ile Ala Cys 50	Ile Asp Ser Arg 55	Arg Glu Glu Ser Asp 60	Tyr Ala Ser Trp
Ala Lys Gly 65	Arg Phe Thr Ile Ser 70	Lys Asp Ser Ser Lys 75	Asn Thr Val
Tyr Leu Gln 85	Met Asn Ser Leu Arg 90	Ala Glu Asp Thr Ala 95	Val Tyr Tyr
Cys Ala Arg 100	Gly Gly Tyr Gly Gly 105	Asp Gly Val Asp Gly 110	Ala Phe Asp
Pro Trp Gly 115	Gln Gly Thr Leu Val 120	Thr Val Ser Ser Ala 125	Ser Thr Lys
Gly Pro Ser 130	Val Phe Pro Leu Ala 135	Pro Ser Ser Lys Ser 140	Thr Ser Gly
Gly Thr Ala 145	Ala Leu Gly Cys Leu 150	Val Lys Asp Tyr Phe 155	Pro Glu Pro
Val Thr Val 165	Ser Trp Asn Ser Gly 170	Ala Leu Thr Ser Gly 175	Val His Thr
Phe Pro Ala 180	Val Leu Gln Ser Ser 185	Gly Leu Tyr Ser Leu 190	Ser Ser Val
Val Thr Val 195	Pro Ser Ser Ser Leu 200	Gly Thr Gln Thr Tyr 205	Ile Cys Asn
Val Asn His 210	Lys Pro Ser Asn Thr 215	Lys Val Asp Lys Lys 220	Val Glu Pro
Lys Ser Cys 225	Asp Lys Thr His Thr 230	Cys Pro Pro Cys Pro 235	Ala Pro Pro
Val Ala Gly 245	Pro Asp Val Phe Leu 250	Phe Pro Pro Lys Pro 255	Lys Asp Thr
Leu Met Ile 260	Ser Arg Thr Pro Glu 265	Val Thr Cys Val Val 270	Val Asp Val
Ser His Glu 275	Asp Pro Glu Val Lys 280	Phe Asn Trp Tyr Val 285	Asp Gly Val
Glu Val His 290	Asn Ala Lys Thr Lys 295	Pro Arg Glu Glu Gln 300	Tyr Asn Ser

1999년	1998년	1997년	1996년	1995년	1994년	1993년	1992년	1991년	1990년	1989년	1988년	1987년	1986년	1985년	1984년	1983년	1982년	1981년	1980년	1979년	1978년	1977년	1976년	1975년	1974년	1973년	1972년	1971년	1970년	1969년	1968년	1967년	1966년	1965년	1964년	1963년	1962년	1961년	1960년	1959년	1958년	1957년	1956년	1955년	1954년	1953년	1952년	1951년	1950년	1949년	1948년	1947년	1946년	1945년	1944년	1943년	1942년	1941년	1940년	1939년	1938년	1937년	1936년	1935년	1934년	1933년	1932년	1931년	1930년	1929년	1928년	1927년	1926년	1925년	1924년	1923년	1922년	1921년	1920년	1919년	1918년	1917년	1916년	1915년	1914년	1913년	1912년	1911년	1910년	1909년	1908년	1907년	1906년	1905년	1904년	1903년	1902년	1901년	1900년	1899년	1898년	1897년	1896년	1895년	1894년	1893년	1892년	1891년	1890년	1889년	1888년	1887년	1886년	1885년	1884년	1883년	1882년	1881년	1880년	1879년	1878년	1877년	1876년	1875년	1874년	1873년	1872년	1871년	1870년	1869년	1868년	1867년	1866년	1865년	1864년	1863년	1862년	1861년	1860년	1859년	1858년	1857년	1856년	1855년	1854년	1853년	1852년	1851년	1850년	1849년	1848년	1847년	1846년	1845년	1844년	1843년	1842년	1841년	1840년	1839년	1838년	1837년	1836년	1835년	1834년	1833년	1832년	1831년	1830년	1829년	1828년	1827년	1826년	1825년	1824년	1823년	1822년	1821년	1820년	1819년	1818년	1817년	1816년	1815년	1814년	1813년	1812년	1811년	1810년	1809년	1808년	1807년	1806년	1805년	1804년	1803년	1802년	1801년	1800년	1799년	1798년	1797년	1796년	1795년	1794년	1793년	1792년	1791년	1790년	1789년	1788년	1787년	1786년	1785년	1784년	1783년	1782년	1781년	1780년	1779년	1778년	1777년	1776년	1775년	1774년	1773년	1772년	1771년	1770년	1769년	1768년	1767년	1766년	1765년	1764년	1763년	1762년	1761년	1760년	1759년	1758년	1757년	1756년	1755년	1754년	1753년	1752년	1751년	1750년	1749년	1748년	1747년	1746년	1745년	1744년	1743년	1742년	1741년	1740년	1739년	1738년	1737년	1736년	1735년	1734년	1733년	1732년	1731년	1730년	1729년	1728년	1727년	1726년	1725년	1724년	1723년	1722년	1721년	1720년	1719년	1718년	1717년	1716년	1715년	1714년	1713년	1712년	1711년	1710년	1709년	1708년	1707년	1706년	1705년	1704년	1703년	1702년	1701년	1700년	1699년	1698년	1697년	1696년	1695년	1694년	1693년	1692년	1691년	1690년	1689년	1688년	1687년	1686년	1685년	1684년	1683년	1682년	1681년	1680년	1679년	1678년	1677년	1676년	1675년	1674년	1673년	1672년	1671년	1670년	1669년	1668년	1667년	1666년	1665년	1664년	1663년	1662년	1661년	1660년	1659년	1658년	1657년	1656년	1655년	1654년	1653년	1652년	1651년	1650년	1649년	1648년	1647년	1646년	1645년	1644년	1643년	1642년	1641년	1640년	1639년	1638년	1637년	1636년	1635년	1634년	1633년	1632년	1631년	1630년	1629년	1628
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Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Leu
325 330 335

Pro Glu Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
340 345 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
355 360 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala

370 375 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
405 410 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430

Val Met His Glu Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys
450

<210> 27

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<223> Heavy chain of Ab-A-AEA-PVAdeltaG (based on clone 17-22-B03)

<400> 27

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1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Ile Asp Phe Ser Ala Gly
20 25 30

Tyr Asp Met Cys Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Ala Cys Ile Asp Ser Arg Arg Glu Glu Ser Asp Tyr Ala Ser Trp
50 55 60

Ala Lys Gly Arg Phe Thr Ile Ser Lys Asp Ser Ser Lys Asn Thr Val
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Gly Gly Tyr Gly Gly Asp Gly Val Asp Gly Ala Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro
225 230 235 240

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala Ala Leu Pro Ala
325 330 335

Pro Glu Ala Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
340 345 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
355 360 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala

370 375 380
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 385 390 395 400
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 405 410 415
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 420 425 430
 Val Met His Glu Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser
 435 440 445
 Leu Ser Pro Gly Lys
 450
 <210> 28
 <211> 231
 <212> PRT
 <213> Artificial Sequence
 <220>
 <223> Fc region of Ab-YTE-DLE-PVAdeltaG (including hinge region)
 <400> 28
 Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1 5 10 15
 Pro Pro Val Ala Gly Pro Asp Val Phe Leu Phe Pro Pro Lys Pro Lys
 20 25 30
 Asp Thr Leu Tyr Ile Thr Arg Glu Pro Glu Val Thr Cys Val Val Val
 35 40 45
 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 50 55 60
 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 65 70 75 80
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 85 90 95
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 100 105 110
 Pro Leu Pro Glu Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 115 120 125
 Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
 130 135 140
 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 145 150 155 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
165 170 175

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
180 185 190

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
195 200 205

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
210 215 220

Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 29

<211> 231

<212> PRT

<213> Artificial Sequence

<220>

<223> Fc region of Ab-A-DLE-PVAdeltaG (including hinge region)

<400> 29

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Pro Val Ala Gly Pro Asp Val Phe Leu Phe Pro Pro Lys Pro Lys
20 25 30

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
35 40 45

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
50 55 60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
65 70 75 80

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
85 90 95

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
100 105 110

Pro Leu Pro Glu Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
115 120 125

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
130 135 140

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
145 150 155 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 165 170 175

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 180 185 190

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 195 200 205

Cys Ser Val Met His Glu Ala Leu His Ala His Tyr Thr Gln Lys Ser
 210 215 220

Leu Ser Leu Ser Pro Gly Lys
 225 230

<210> 30

<211> 231

<212> PRT

<213> Artificial Sequence

<220>

<223> Fc region of Ab-A-AEA-PVAdeltaG (including hinge region)

<400> 30

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1 5 10 15

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 20 25 30

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 35 40 45

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 50 55 60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 65 70 75 80

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 85 90 95

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala Ala Leu
 100 105 110

Pro Ala Pro Glu Ala Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 115 120 125

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
 130 135 140

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 145 150 155 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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P a t e n t k r a v

- 5 **1.** IgG₁-antistof, der omfatter et TNF α -bindingsdomæne og et FcRn-bindingssted, hvor aminosyresekvensen af antistoffet omfatter, under henvisning til EU-nummereringssystemet,
- (i) aminosyrerne 233P, 234V, 235A og en deletion ved aminosyreposition 236;
- (ii) aminosyren 434A eller aminosyrerne 252Y, 254T og 256E; og
- (iii) aminosyrerne 239D, 330L og 332E;
- 10 i en Fc-region af et humant IgG; og hvor antistoffet omfatter (i) et V_L-domæne med aminosyresekvensen vist i SEQ ID NO:10 og (ii) et V_H-domæne med aminosyresekvensen som vist i SEQ ID NO:9.
- 15 **2.** Antistof ifølge krav 1, hvor aminosyresekvensen af antistoffet omfatter aminosyrerne 233P, 234V, 235A, 239D, 330L, 332E og 434A og en deletion ved aminosyreposition 236.
- 20 **3.** Antistof ifølge krav 1, hvor aminosyresekvensen af antistoffet omfatter aminosyrerne 233P, 234V, 235A, 239D, 330L, 332E, 252Y, 254T og 256E og en deletion ved aminosyreposition 236.
- 25 **4.** Antistof ifølge et hvilket som helst af de foregående krav, som transporteres over et polariseret celle-monolag fra den apikale side til den basolaterale side i større mængde end et kontrol-antistof omfattende en let kæde med aminosyresekvensen som vist i SEQ ID NO:1 og en tung kæde med aminosyresekvensen som vist i SEQ ID NO:2.
- 30 **5.** Nukleinsyre, der koder for antistoffet ifølge et hvilket som helst af de foregående krav.
- 6.** Antistof ifølge et hvilket som helst af kravene 1 til 4 til anvendelse ved behandling af en inflammatorisk tilstand.

7. Antistof til anvendelse ifølge krav 6, hvor den inflammatoriske tilstand er en inflammatorisk lidelse i mave-tarm-kanalen.

5

8. Antistof til anvendelse ifølge krav 6, hvor behandlingen omfatter oral indgivelse af en virksom mængde af antistoffet.

9. Antistof til anvendelse ifølge krav 6, hvor antistoffet påføres topisk.

10

10. Farmaceutisk sammensætning omfattende antistoffet ifølge et hvilket som helst af kravene 1 til 4.

DRAWINGS

Figure 1

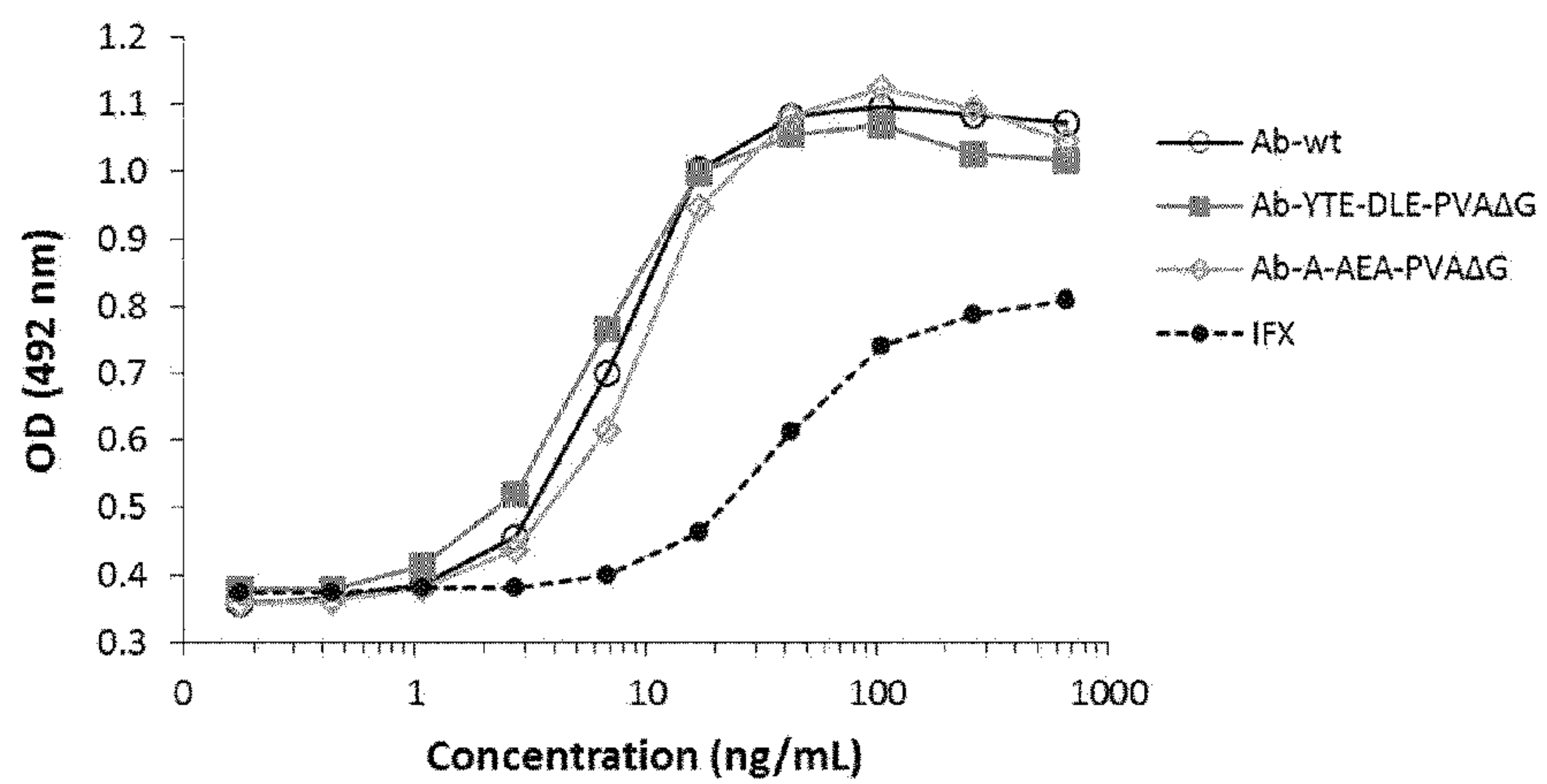
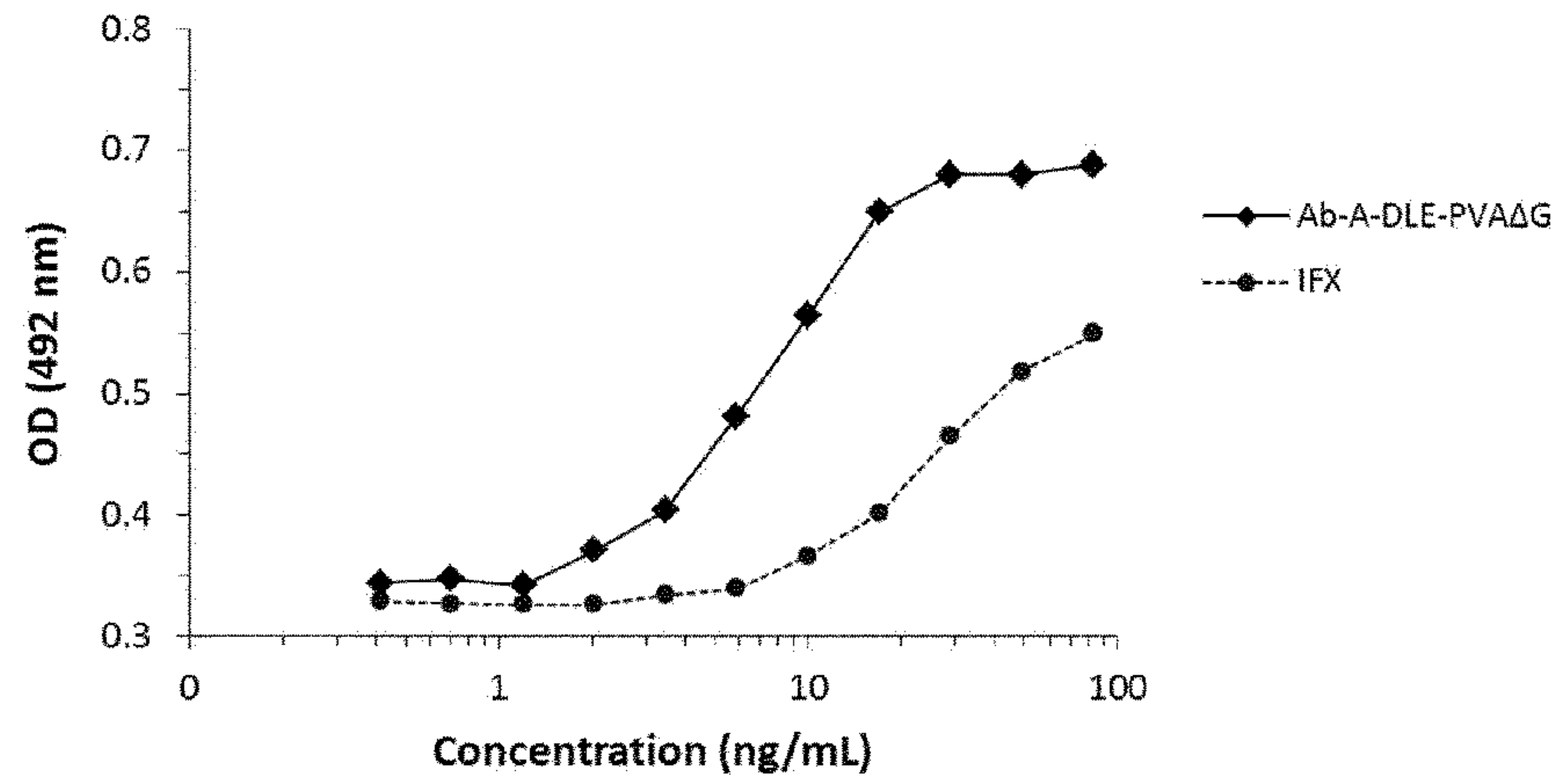


Figure 2

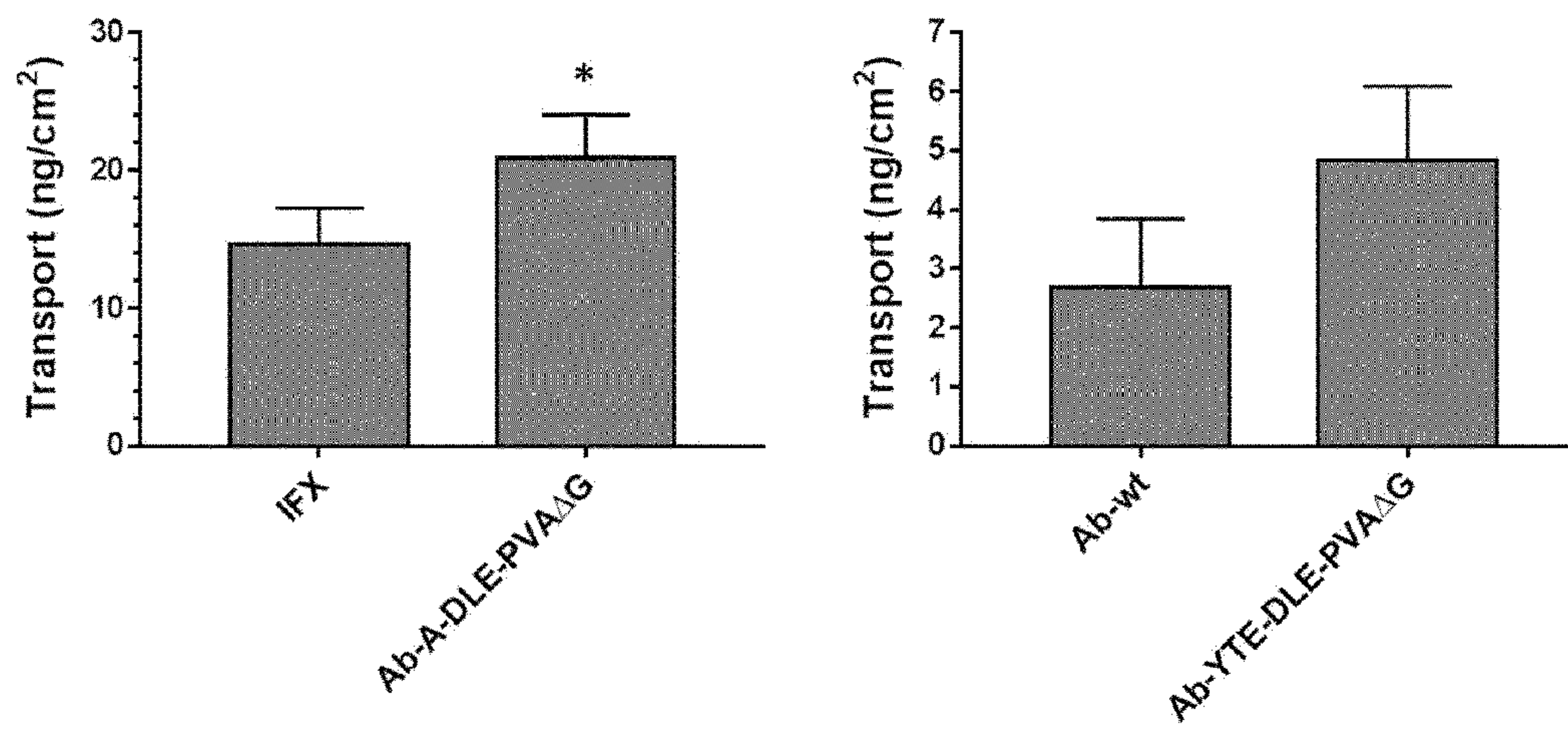


Figure 3

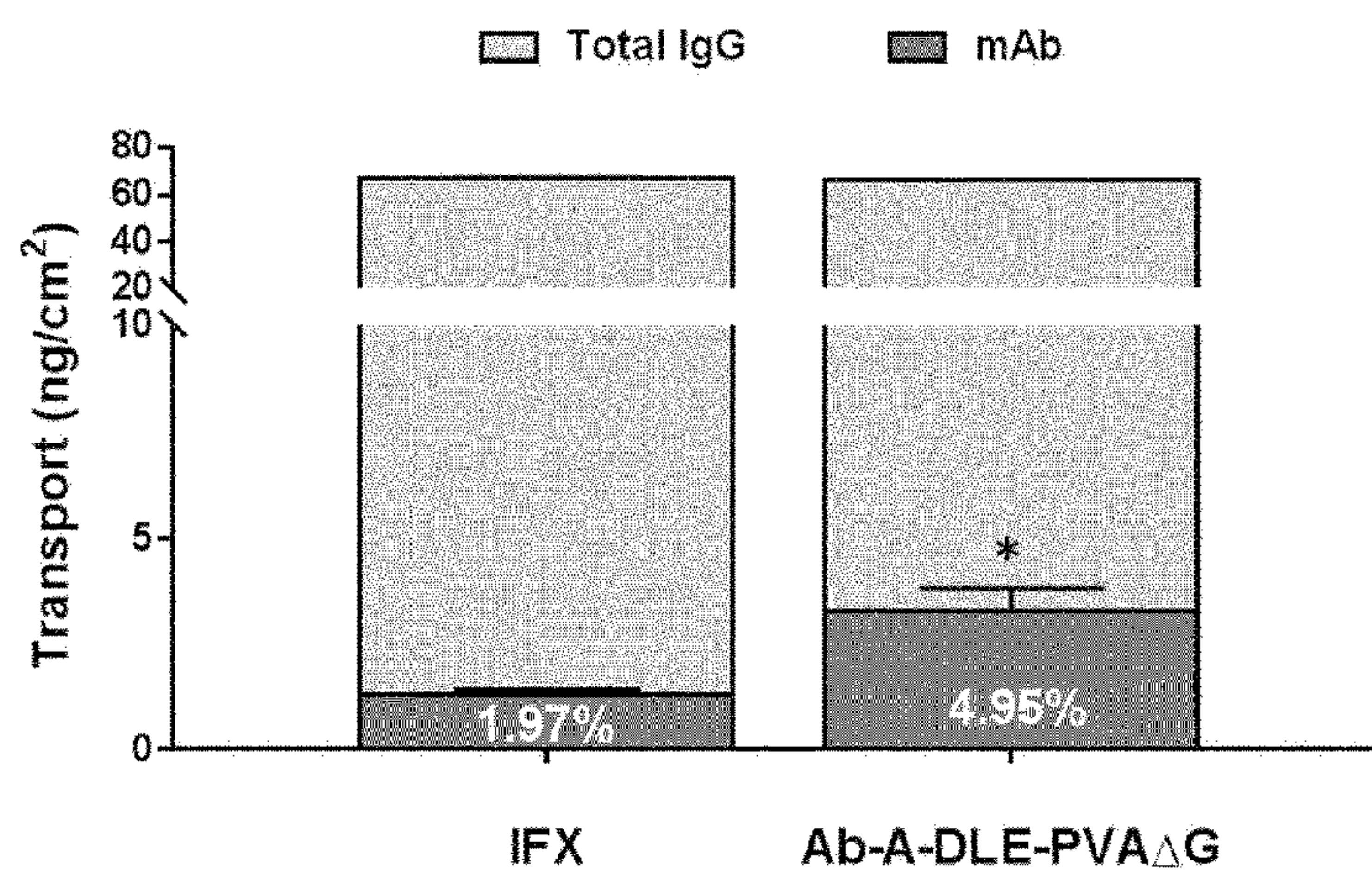


Figure 4

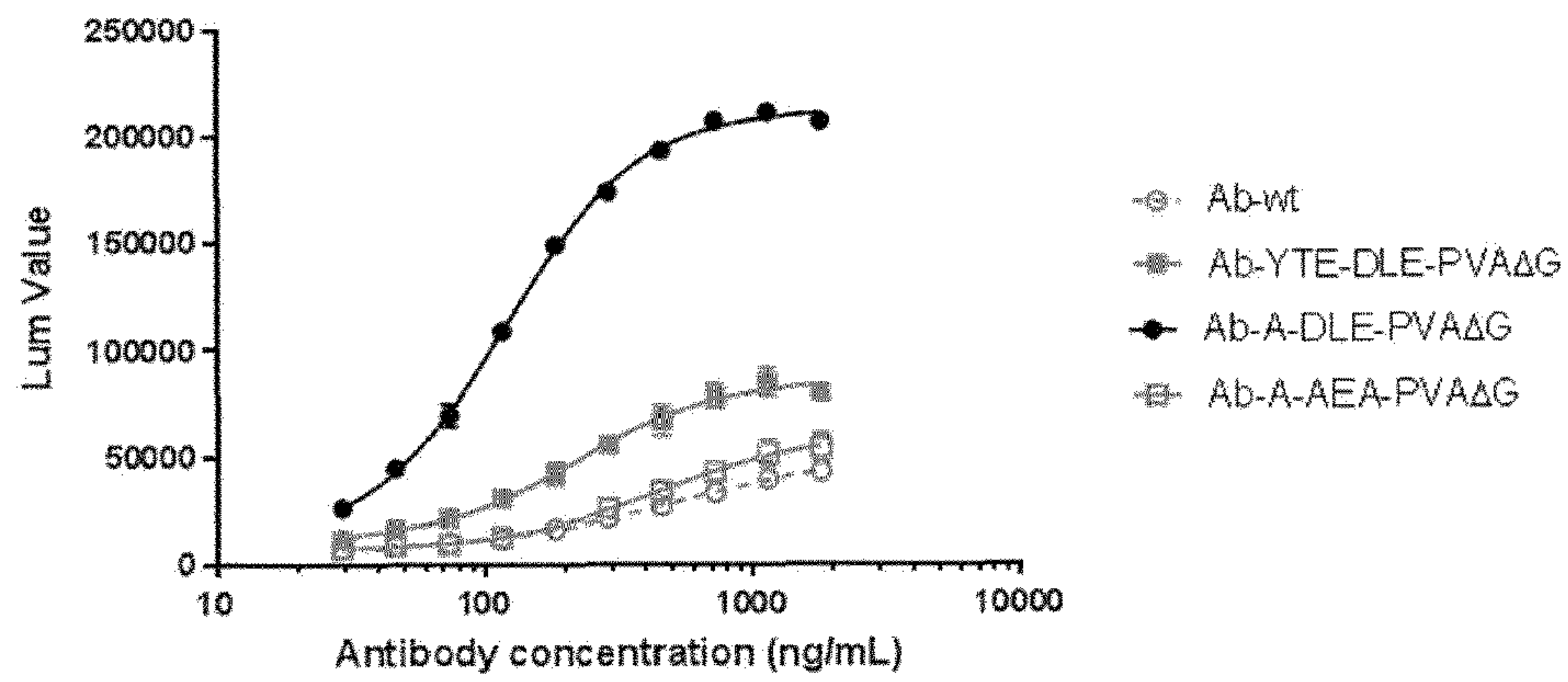


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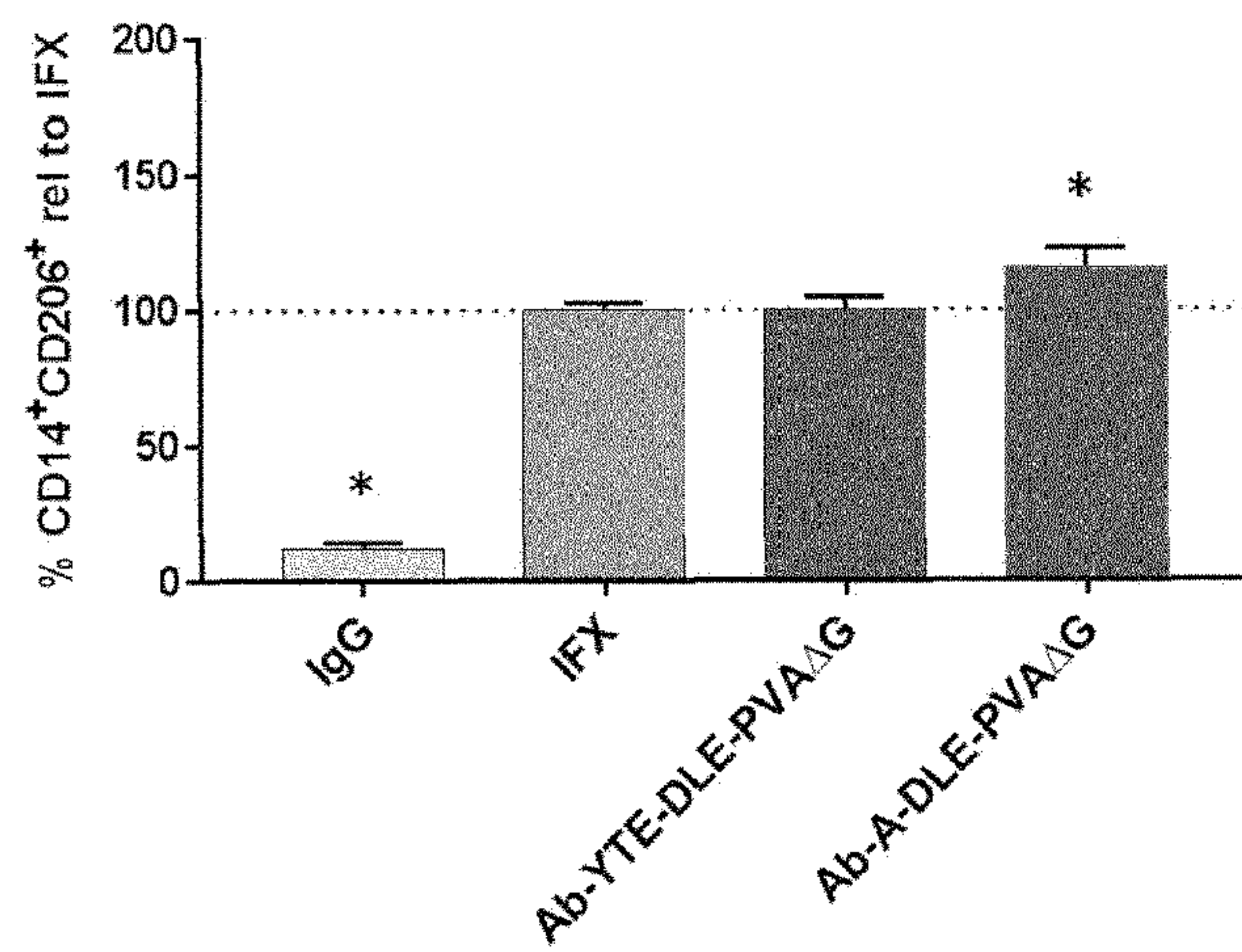


Figure 6

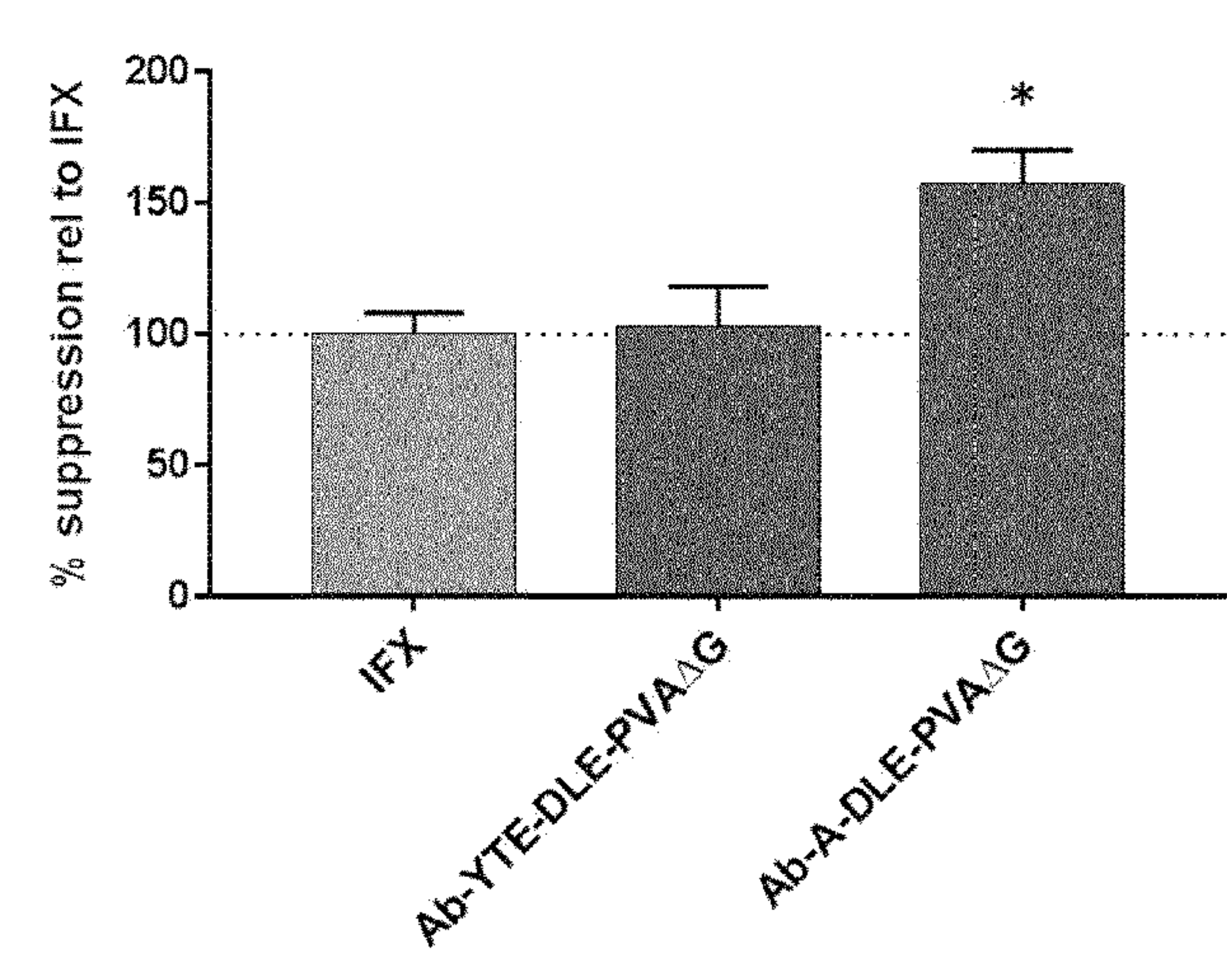


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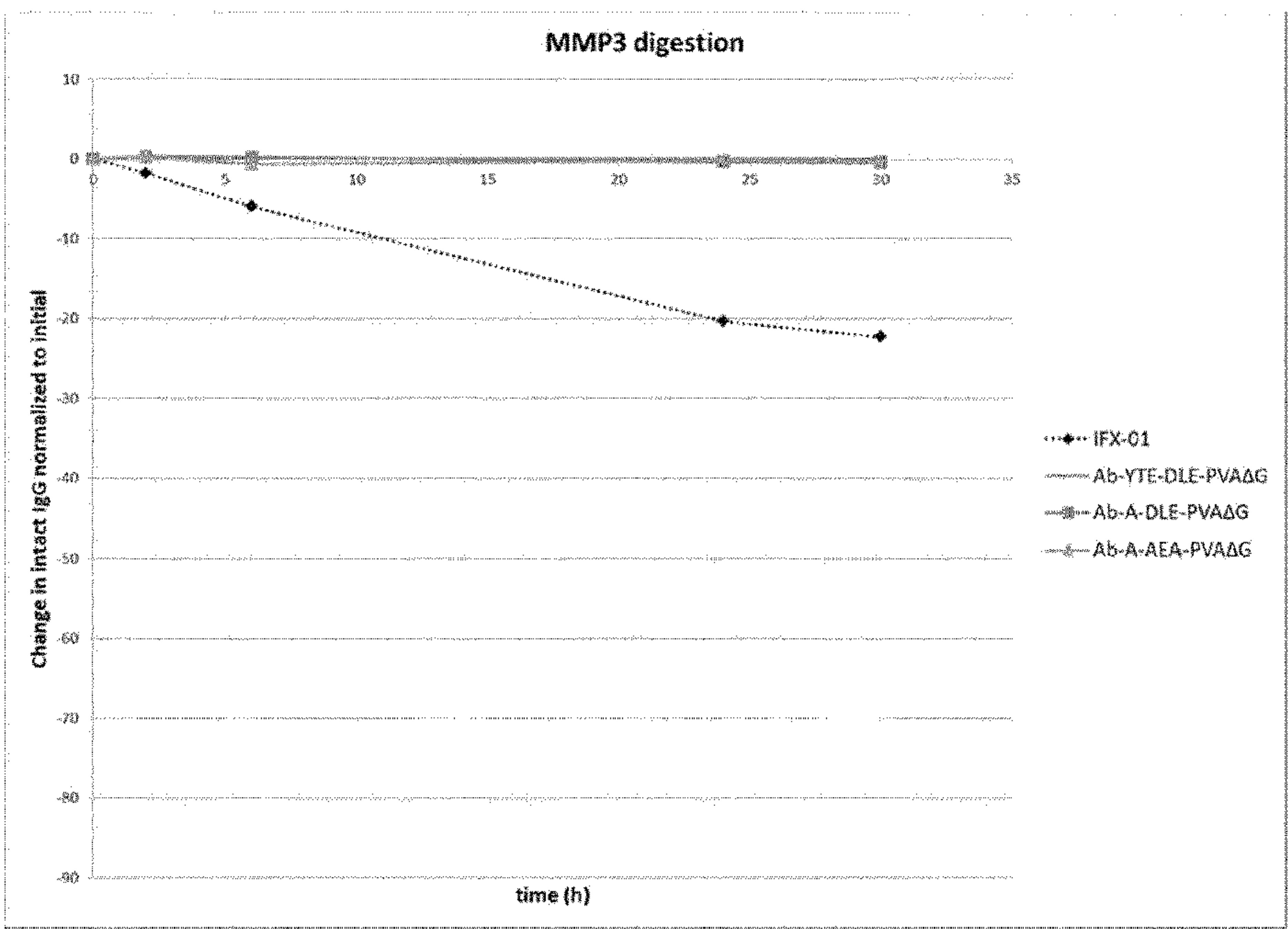


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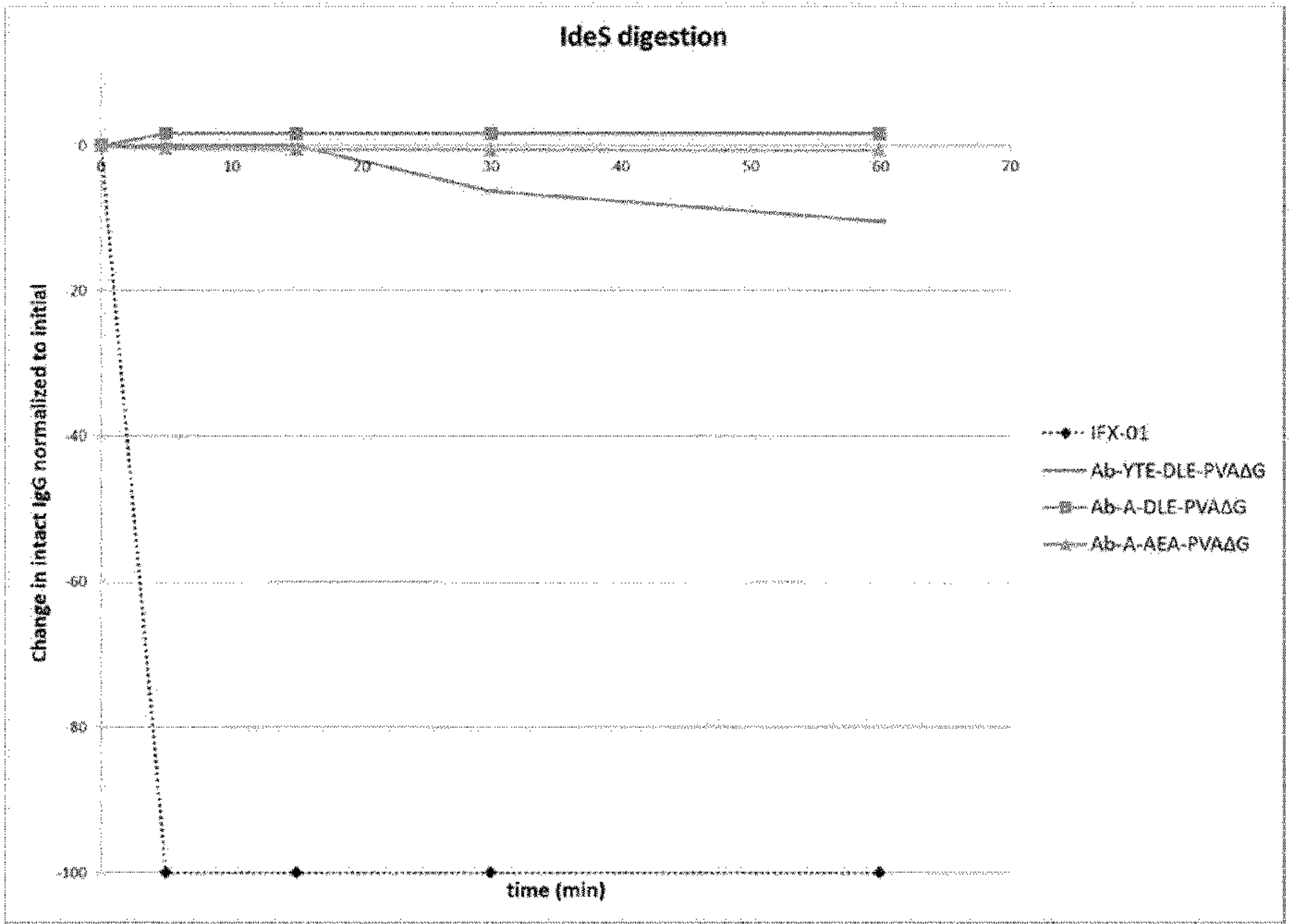


Figure 9

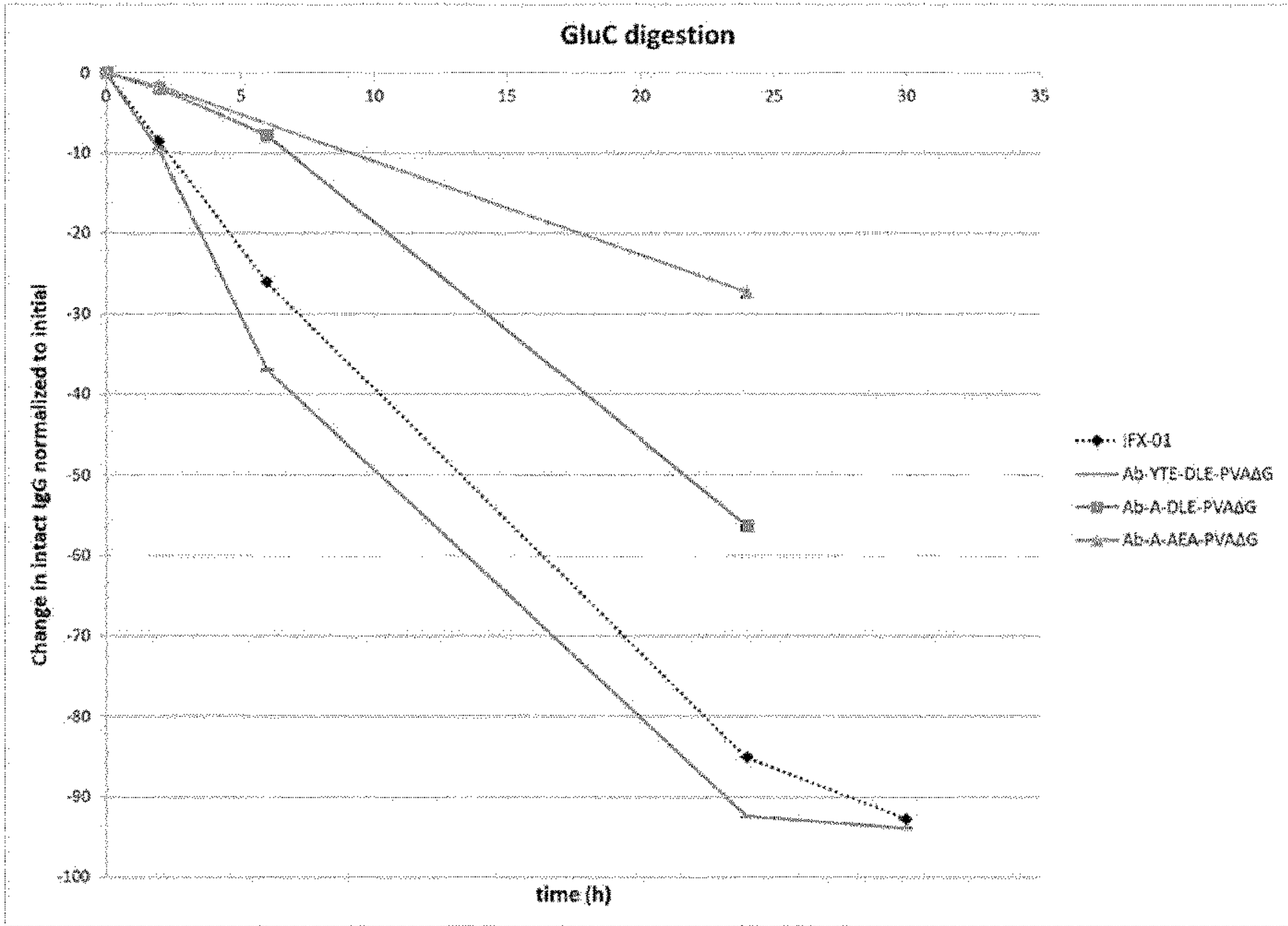


Figure 10

