CLAIMS

1. A method of increasing complement-dependent cytotoxicity (CDC) of a parent polypeptide comprising an Fc domain of an immunoglobulin and a binding region, comprising the steps of:

i. introducing a mutation to the parent polypeptide in an amino acid residue selected from the group corresponding to E430G, E430S, E430F and E430T in the Fc region of a human IgG1 heavy chain,

ii. evaluating the CDC-response induced by the variant when bound to the surface of an antigen-expressing cell, in the presence of effector cells or complement, as compared to the parent polypeptide,

iii. evaluating the binding of the variant to neonatal Fc receptor (FcRn) compared to the parent polypeptide without the mutation,and

iv. selecting the mutation of any variant having an increased CDC-response but not an altered binding to FcRn.

2. The method according to claim 1, wherein the parent polypeptide is a parent antibody comprising an Fc domain of an immunoglobulin and an antigen-binding region.

3. The method according to claim 1 or claim 2, said method further comprising:
introducing a mutation
(i) in each of the amino acid residues corresponding to K439 and S440 in the Fc region of a human IgG1 heavy chain, with the proviso that the mutation in S440 is not S440Y or S440W,
(ii) in each of the amino acid residues corresponding to K447 and 448 in the Fc region of a human IgG1 heavy chain, such as K447K/R/H and 448E/D in the Fc region of a human IgG1 heavy chain, preferably K447K and 448E in the Fc region of a human IgG1 heavy chain, or
(iii) in each of the amino acid residues corresponding to K447, 448 and 449 in the Fc region of a human IgG1 heavy chain, such as K447D/E, 448K/R/H and 449P in the Fc region of a human IgG1 heavy chain, preferably K447E, 448K and 449P in the Fc region of a human IgG1 heavy chain.

4. The method according to claim 3, wherein the mutation in the position corresponding to K439 in the Fc region of a human IgG1 heavy chain is K439D/E, and/or the mutation in the position corresponding to S440 in the Fc region of a human IgG1 heavy chain is S440K/R.

5. The method according to any one of the preceding claims, wherein the method does not alter antibody dependent cell-mediated cytotoxicity (ADCC) of the parent polypeptide or parent antibody.

6. The method according to any one of the preceding claims, wherein the method does not
a) alter binding of the parent polypeptide or parent antibody to neonatal Fc receptor (FcRn) as determined by the method disclosed in Example 34 and/or
b) increase or decrease binding of the parent polypeptide or parent antibody to neonatal Fc receptor (FcRn) by more than 30%, such as of more than 20%, 10% or 5% as measured by a change in the absorbance at OD405 nm as determined by the method disclosed in Example 34 and/or
c) increase the apparent affinity of the parent polypeptide or parent antibody to mouse neonatal Fc receptor (FcRn) by more than a factor 0.5 or does not decrease the apparent affinity of the parent polypeptide or parent antibody to mouse FcRn by more than a factor 2 as determined by the method disclosed in Example 34 and/or
d) alter the plasma clearance rate of the parent polypeptide or parent antibody as determined by the method disclosed in Example 37 and/or
e) increase or decrease the plasma clearance rate of the parent polypeptide or parent antibody by more than a factor 3.0, such as by more than a factor 2.5, factor 2.0, factor 1.5 or factor 1.2 as determined by the method disclosed in Example 37 and/or
f) alter target independent fluid phase complement activation of the variant as determined by the method as determined by the method disclosed in Example 36 and/or
g) alter the plasma half-life of the parent polypeptide or parent antibody.

7. A method for producing in a host cell, a variant of a parent polypeptide comprising an Fc domain of an immunoglobulin and a binding region, wherein the variant comprises at least the Fc region of a heavy chain, the method comprising the following steps:
i. providing a nucleotide construct encoding the Fc region of the variant, wherein the nucleotide construct comprises a mutation selected from the group corresponding to E430G, E430S, E430F and E430T in the Fc region of a human IgG1 heavy chain,
ii. expressing the nucleotide construct in a host cell,
iii. recovering the variant from the cell culture of the host cell, and
iv. screening the variant for increased complement-dependent cytotoxicity (CDC) and no altered binding of the variant to neonatal Fc receptor (FcRn) compared to the parent polypeptide.

8. The method or variant according to claim 7, wherein
a) the variant does not contain any further mutations in the Fc domain which alter antibody dependent cell-mediated cytotoxicity (ADCC) of the variant and/or
b) the variant does not contain any further mutations in the Fc domain which alter the plasma clearance rate of the variant as determined by the method disclosed in Example 37 and/or
c) the variant does not contain any further mutations in the Fc domain which increase or decrease the plasma clearance rate of the variant by more than a factor 3.0, such as by by more than a factor 2.5, factor 2.0, factor 1.5 or factor 1.2 as determined by the method disclosed in Example 37 and/or
d) the variant does not contain any further mutations in the Fc domain which increase or decrease the binding of the variant to neonatal Fc receptor (FcRn) by more than 30%, such as of more than 20%, 10% or 5% as measured by a change in the absorbance at OD405 nm as determined by the method disclosed in Example 34 and/or
e) the variant does not contain any further mutations in the Fc domain which alter the serum half-life of the variant and/or
f) the variant does not contain any further mutations in the Fc domain which alter target independent fluid phase complement activation of the variant as determined by the method disclosed in Example 36 and/or
g) the variant does not contain any further mutations in the Fc domain.
h) the variant comprises only one mutation.

9. The variant according to claim 8, wherein the variant comprises a combination of two mutations in the amino acid residues selected from the group corresponding to E345X/E430G,S,F,T, E430G,S,F,T/S440Y, and E430G,S,F,T/S440W in the Fc region of a human IgG1 heavy chain , wherein X is K, Q, R or Y.

10. The variant according to any one of claims 7 to 9, wherein the parent polypeptide is a parent antibody comprising an Fc domain of an immunoglobulin and an antigen-binding region.

11. The method or variant according to claims 7 to 10, wherein the variant is
a) conjugated to a drug, toxin or radiolabel, such as wherein the variant is conjugated to a toxin via a linker and/or
b) part of a fusion protein.

12. The variant according to claims 8to 11, wherein the variant or the first and the second antibody is each a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgM, or IgE antibody, optionally each a human full-length antibody, such as each a human full-length IgG1 antibody.

13. The method according to claims 1 to 4 or 7, the variant according to claims 8to 9, wherein the parent antibody such as the first or second parent antibody, the variant or the first and the second antibody is each selected from a monospecific, bispecific or multispecific antibody.

14. A composition comprising the composition according to any one of claim 11 or claim 13 and a pharmaceutically acceptable carrier.

15. A kit-of-parts comprising a first variant and a second variant as defined in claims 8 to 11, for simultaneous, separate or sequential use in therapy.

16. The variant, composition or kit-of-parts according to any one of claims 8 to 15 for use in the treatment of a disease.

17. Use of the variant, the composition or the kit-of-parts according to any one of claims 8 to 15 in the manufacture of a medicament for the treatment of cancer.

18. The variant, composition or kit-of-parts according to any one of claims 8 to 15, for use in imaging at least a part of the body of a human or other mammal.