Example 34

FcRn binding of IgG1-005 mutants compared to wild-type IgG1-005

The neonatal Fc receptor (FcRn) is responsible for the long plasma half-life of IgG by protecting IgG from degradation. After internalization of the antibody, FcRn binds to antibody Fc regions in endosomes, where the interaction is stable in the mildly acidic environment (pH 6.0). Upon recycling to the plasma membrane, where the environment is neutral (pH 7.4), the interaction is lost and the antibody is released back into the circulation. This influences the plasma half-life of IgG.

The capability of the IgG1-005 mutants E345K, E345Q, E345R, E345Y, E430F, E430G, E430S, E430T, S440Y, K439E and S440K to interact with FcRn from mouse, cynomolgous monkey and human was tested in an ELISA. In the mouse FcRn ELISA mutants P247G and I253D were also tested. I253D was used as a negative control for binding to FcRn. All incubations were done at room temperature. 96-well plates were coated with 5 µg/mL (100 µL/well) recombinantly produced biotinylated extracellular domain of FcRn (mouse, human or cynomolgous) (FcRnECDHis-B2M- BIO), diluted in PBST plus 0.2% BSA, and incubated for 1 hour. Plates were washed 3 times with PBST, and 3-fold serially diluted (in PBST/0.2% BSA, pH 6.0) wild-type IgG1-005 or 005 mutants were added, and the plates were incubated for 1 hour. The plates were washed with PBST/0.2% BSA, pH 6.0. Goat-anti-human IgG(Fab'2)-HRP (Jackson Immuno Research, cat no: 109-035-097) diluted in PBST/0.2% BSA, pH 6.0 was added, and the plates were incubated for 1 hour. After washing, ABTS was added as substrate and plates were incubated in the dark for 30 minutes.

Absorbance was read at 405 nm, using an EL808 ELISA reader. The data generated in the mouse FcRn ELISA were analyzed using best-fit values of a non-linear agonist dose-response fit using log -transformed concentrations in GraphPad PRISM 5 and the apparent affinity (EC50) was calculated (**Table 20**). The experiment shows that FcRn binding was not altered by any of the IgG1-005 mutants compared to the wild-type IgG1-005.

Table 20 Apparent affinity (EC50) in µg/ml of IgG1-005 and mutants to mouse FcRn

Tested variants	005-WT	005-P247G	005-E345K	005-E345N	005-E345Q	005-E345R	005-E345Y	005-E430F	
Apparent affinity	0.14	0.28	0.1	0.11	0.12	0.09	0.13	0.11	ug/mL
Apparent affinity	0.93	1.87	0.67	0.73	0.80	0.60	0.87	0.73	nM

Tested variants	005-E440Y	005-E430G	005-E430H	005-E430S	005-E430T	005-E440K	005-E439E		
Apparent affinity	0.15	0.13	0.11	0.14	0.15	0.11	0.31		ug/mL
Apparent affinity	1.00	0.87	0.73	0.93	1.00	0.73	2.07		nM

Figure 29 shows that wild-type IgG1-005 and all tested mutants of IgG1-005 bound well to mouse, human and cynomolgus FcRn at pH 6.0. No significant binding to FcRn was detected at pH 7.4 (data not shown).

Example 35

Enhanced CDC by different mutations in rituximab in B cell lines Ramos and SU-DHL-4

As described in Example 19, oligomerization and CDC activity of the anti- CD38 antibody IgG1-005 may be stimulated by single mutations at specific residues on or at the periphery of the Fc: Fc interface. Oligomerization may also be indirectly stimulated by another type of mutations at residues away from the Fc: Fc interface that allosterically strengthens Fc: Fc interactions. This was also tested for the IgG1 anti-CD20 antibody rituximab on two B cell lines (Ramos and SU-DHL-4). The following mutations were tested : E345K, E345Q, E345R, E345Y, E430G, E430S, E430T, and S440Y (essentially as described in Example 19).

For the CDC assay, 0.1 x 10^6 of cells (Ramos or SU-DHL-4) were pre- incubated in round Bottom 96-well plates with a saturating antibody concentration series (0.0001-10.0 µg/mL in 3-fold dilutions) in a total volume of 100 µL for 15 min on a shaker at RT. Next, 25 µL normal human serum was added as a source of complement (20% final concentration) and incubated in a 37°C incubator for 45 min. The reaction was stopped by putting the plates on ice. 10 µL propidium iodide was added and cell lysis was determined by FACS. The data were analyzed using best-fit values of a non-linear agonist dose-response fit using log-transformed concentrations in GraphPad PRISM 5. **Figure 30** shows that all tested rituximab mutants were able to increase CDC efficacy in both B-cell lines.

Example 36

Target independent fluid phase complement activation: IgG1-005 mutants compared to wild-type IgG1-005

CLAIMS

1. A method of increasing complement-dependent cytotoxicity (CDC) of a variant antibody of a parent antibody comprising an Fc domain of an immunoglobulin and an antigen-binding region, wherein the variant antibody of the parent antibody has an apparent affinity to mouse neonatal Fc receptor (FcRn) form 0.87 nM to 1 nM, said method, comprising the step of: introducing a mutation to the parent antibody in an amino acid residue selected from the group corresponding to E430G, E430S and E430T in the Fc region of a human IgG1 heavy chain.

2. The method according to claim 1, wherein the method does not alter antibody dependent cell-mediated cytotoxicity (ADCC) of the variant antibody of theparent antibody.

3. The method according to claim 1 or claim 2, wherein the method does not
b) alter the plasma half-life of the variant antibody of the parent antibody.

4. A method for producing in a host cell, a variant antibody of a parent antibody comprising an Fc domain of an immunoglobulin and an antigen-binding region, wherein the variant antibody 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 antibody, wherein the nucleotide construct comprises a mutation selected from the group corresponding to E430G, E430S 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 antibody from the cell culture of the host cell, and
iv. screening the variant antibody for increased complement-dependent cytotoxicity (CDC) compared to the parent antibody and an apparent affinity to mouse neonatal Fc receptor (FcRn) form 0.87 nM to 1 nM.

5. The method or variant antibody according to claim 4, wherein
a) the variant antibody does not contain any further mutations in the Fc domain which alter antibody dependent cell-mediated cytotoxicity (ADCC) of the variant antibody and/or
b) the variant antibody does not contain any further mutations in the Fc domain which alter the plasma clearance rate of the variant antibody as determined by the method disclosed in Example 37 and/or
c) the variant antibody does not contain any further mutations in the Fc domain which increase or decrease the plasma clearance rate of the variant antibody 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 antibody 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 antibody does not contain any further mutations in the Fc domain which alter the serum half-life of the variant antibody and/or

f) the variant antibody does not contain any further mutations in the Fc domain which alter target independent fluid phase complement activation of the variant antibody as determined by the method disclosed in Example 36 and/or

g) the variant antibody does not contain any further mutations in the Fc domain.

h) the variant antibody comprises only one mutation

i) the variant antibody has an apparent affinity to mouse neonatal Fc receptor (FcRn) of about 0.87 nM,

j) the variant antibody has an apparent affinity to mouse neonatal Fc receptor (FcRn) of about 0.93 nM,

k) the variant antibody has an apparent affinity to mouse neonatal Fc receptor (FcRn) of about 1 nM.

6. The method or variant antibody according to claim 4 or claim 5, wherein the variant antibody 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.

7. The variant antibody according to claim 5 or claim 6, wherein the variant antibody is a human IgG1, such as a human full-length IgG1 antibody.

8. The method according to any one of claims 1 to 4, the variant antibody according to claims 5 or claim 6, wherein the parent antibody is a monospecific, bispecific or multispecific antibody.

9. A composition comprising the variant antibody according to claim 4 or claim 8, and a pharmaceutically acceptable carrier.

10. The variant antibody or composition according to any one of claims 5 to 9, for use in the treatment of a disease.

11. Use of the variant antibody or, the composition according to any one of claims 5 to 9 in the manufacture of a medicament for the treatment of cancer.

12. The variant antibody or composition according to any one of claims 5 to 9, for use in imaging at least a part of the body of a human or other mammal.