

CLAIMS

1. A protein comprising a first polypeptide and a second polypeptide, wherein said first and second polypeptide each comprise at least a hinge region, a CH2 region and a CH3 region, respectively, of a human IgG1 immunoglobulin heavy chain, wherein at least one of said first and second polypeptides is modified and comprises a substitution of amino acids corresponding with amino acids at the positions L234, L235 and G236, wherein amino acid positions are as defined by Eu numbering.
2. The protein according to claim 1, wherein the amino acids at positions L234, L235 and G236 in at least one of said first and second polypeptide are substituted with F, E and R, respectively.
3. The protein according to claim 1 or claim 2, wherein one of the first and second polypeptides comprises said substitution of amino acids corresponding with amino acids at positions L234, L235 and G236, and the other is modified and comprises a substitution of amino acids corresponding with amino acids at positions L234, L235, and D265, wherein preferably, said substitutions are F, E and A, respectively.
4. The protein according to claim 1 or claim 2, wherein both first and second polypeptides comprise said substitution of amino acids corresponding with amino acids L234, L235 and G236.
5. The protein according to any one of claims 1-4, wherein each of said first and second polypeptides comprises an immunoglobulin CH1 region.
6. The protein according to any one of claims 1-5, wherein said protein comprises a first and a second binding region.
7. The protein according to claim 6, wherein said first and second binding regions comprise respectively a first immunoglobulin heavy chain variable region and a first immunoglobulin light chain variable region, and wherein said second binding region comprises a second immunoglobulin heavy chain variable region and a second immunoglobulin light chain variable region.

8. The protein according to claim 7, wherein said immunoglobulin heavy and light chain variable regions are human or humanized immunoglobulin heavy and light chain variable regions.
9. The protein according to claim 7 or 8, wherein said first and second polypeptides are immunoglobulin heavy chains, and wherein said first and second polypeptides comprise said respective first and second immunoglobulin heavy chain variable regions.
10. The protein according to any of claims 6-9, wherein said protein comprises a first immunoglobulin light chain constant region and a second immunoglobulin light chain constant region.
11. The protein according to claim 10, wherein said protein comprises first and second immunoglobulin light chains, said immunoglobulin light chains comprising said respective first and second immunoglobulin light chain variable regions and said respective first and second immunoglobulin constant light chain regions.
12. The protein according to claim 11, wherein said first immunoglobulin light chain is connected with said first immunoglobulin heavy chain via disulfide bridges and said second immunoglobulin light chain is connected with said second immunoglobulin heavy chain via disulfide bridges, thereby forming said first binding region and said second binding region, respectively, and wherein said first and second immunoglobulin heavy chains are connected via disulfide bridges.
13. The protein according to any one of claims 1-12, which is an antibody.
14. The protein in accordance with any one of claims 4-12, which is a monospecific antibody.
15. The protein in accordance with claim 4-14, wherein said first and second polypeptide chains have an identical amino acid sequence.
16. The protein in accordance with claim 15, wherein said first and second polypeptide comprise a further amino acid substitution, preferably a substitution of an amino acid selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409, such as F405L or K409R.

17. The protein according to any of claims 14-16, wherein said monospecific antibody binds an antigen selected from the group consisting of cellular targets, cytokines, toxins, pathogens, cancer antigens, plasma proteins.
18. The protein according to any one of claims 1-13, wherein said protein is a bispecific antibody.
19. The bispecific antibody according to claim 18, wherein said first and second polypeptide comprise further substitutions in said respective CH2 and CH3 regions such that the sequences of the respective CH2 and CH3 regions from said first and second polypeptides are different, said substitutions allowing to obtain said polypeptide comprising said first and second polypeptide.
20. The bispecific antibody according to claim 19, wherein in said first polypeptide at least one of the amino acids in the positions corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and in said second polypeptide at least one of the amino acids in the positions corresponding to a position selected from the group consisting of; T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, and wherein said substitutions of said first and said second polypeptides are not in the same positions.
21. The bispecific antibody according to claim 19, wherein the amino acid in the position corresponding to F405 is L in said first polypeptide, and the amino acid in the position corresponding to K409 is R in said second polypeptide, or vice versa.
22. The bispecific antibody according to any one of claims 18-21, wherein said bispecific antibody has modifications in both of said first and second polypeptide consisting of substitutions of the amino acids at positions L234, L235 and G236 with F, E and R, and substitutions of the amino acid at position F405 with is L in said first polypeptide, and at K409 with R in said second polypeptide, or vice versa.
23. The bispecific antibody according to any one of claims 18-22, wherein said first and second polypeptides have substitutions consisting of substitutions as defined in any one of claims 1-4 and 19-22.

24. The protein according to any one of claims 1-17 or bispecific antibody according to any one of claims 18-23, wherein said first and second polypeptides comprise an amino acid sequence in accordance with SEQ ID NO: 1, wherein said amino acid sequence which is comprised in said first and second polypeptides have amino acid substitutions as defined in claims 1-4 and 18-22.
25. The protein or bispecific antibody according to claim 24, wherein said amino acid sequence in accordance with SEQ ID NO:1 does not comprise a terminal lysine.
26. The bispecific antibody according to any one of claims 18-25, wherein one of said binding region binds a cancer antigen.
27. The bispecific antibody according to any one of claims 18-25, wherein one of said binding regions binds an effector cell, such as a T-cell, NK cell, dendritic cell, monocyte, macrophage or neutrophil.
28. The bispecific antibody according to any one of claims 18-25, wherein one of said binding regions binds an effector cell, such as a T-cell, NK cell, dendritic cell, monocyte, macrophage or neutrophil, and the other binding region binds a cancer antigen.
29. A nucleic acid encoding said first or second polypeptide as defined in any one of claims 4-17 and 24-25, wherein said first or second polypeptide comprises said substitution of amino acids corresponding with amino acids L234, L235 and G236, preferably wherein said substitutions of positions L234, L235 and G236 are with F, E and R, respectively.
30. A nucleic acid in accordance with claim 29, wherein said first or second polypeptide is an immunoglobulin heavy chain.
31. A host cell comprising a nucleic acid in accordance with claim 29 or 30.
32. A pharmaceutical composition comprising the protein according to any of claims 1 to 17, or bispecific antibody according to any of claims 18-28 and a pharmaceutical acceptable carrier.
33. The protein according to any of claims 1 to 17, or bispecific antibody according to any of claims 18-28, or the pharmaceutical composition according to claim 32, for use in the treatment of a disease.

34. The protein, bispecific antibody, or pharmaceutical composition for use in accordance with claim 33, wherein said use comprises the treatment of a cancer, an infectious disease, or an autoimmune disease.
35. Use of the protein according to any of claims 1 to 17, or the bispecific antibody according to any of claims 18-28, or the pharmaceutical composition according to claim 32, for the manufacture of a medicament for treatment of a disease selected from a cancer, an infectious disease, and an autoimmune disease.
36. The use according to claim 35, wherein the disease is a cancer.
37. A method of preparing a bispecific antibody comprising
- a) providing a first antibody, comprising
 - a. an immunoglobulin heavy chain comprising at least a hinge region, a CH2 region and a CH3 region, respectively of a human IgG1 immunoglobulin heavy chain, comprising substitutions of amino acids at positions L234, L235 and G236, with F, E and R, respectively,
 - b. an immunoglobulin light chain;
 - b) providing a second antibody, comprising
 - a. an immunoglobulin heavy chain comprising at least a hinge region, a CH2 region and a CH3 region, respectively, of a human IgG1 immunoglobulin heavy chain, comprising substitutions of amino acids at
 - positions L234, L235, and D265, wherein preferably, said substitutions are F, E and A, respectively,or,
 - comprising substitutions of amino acids at positions L234, L235 and G236, with F, E and R, respectively,
 - b. an immunoglobulin light chain;
 - c) wherein the sequences of said first and second CH3 regions of said respective first and second antibodies are different and are such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions;
 - d) incubating said first antibody together with said second antibody under reducing conditions sufficient to allow the cysteines in the hinge regions to undergo disulfide-bond isomerization; and
 - e) obtaining said bispecific antibody comprising said first immunoglobulin heavy chain and said first immunoglobulin light chain of said first antibody and said second

immunoglobulin heavy chain and said second immunoglobulin light chain of said second antibody.

38. The method of claim 37, wherein in step c) said differences in sequence are in accordance with any one of claims 19-25.

39. The method of claim 37 or claim 38, for preparing a bispecific antibody as defined in any of claims 18-28.