

CLAIMS

1. An antibody variant binding to human CD38, the antibody variant comprising
 - (a) an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, and
 - (b) a variant Fc region comprising a mutation in one or more amino acid residues selected from the group of amino acid residues corresponding to E430, E345 and S440 of a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index, wherein said mutation in the one or more amino acid residues comprises E430G.
2. The antibody variant according to claim 1, comprising
 - (a) a variable heavy chain (VH) region comprising SEQ ID NO:1 or an amino acid sequence having at least 80% identity, such as 90%, or 95%, or 97%, or 98%, or 99%, to SEQ ID NO:1; and/or
 - (b) a variable light chain (VL) region comprising SEQ ID NO:5 or an amino acid sequence having at least 80% identity, such as 90%, or 95%, or 97%, or 98%, or 99%, to SEQ ID NO:5.
3. The antibody variant according to claim 2, wherein
 - (a) the VH differs from SEQ ID NO:1 by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues; and/or
 - (b) the VL differs from SEQ ID NO:5 by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.
4. The antibody variant according to any one of the preceding claims, comprising a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5.
5. The antibody variant according to any one of the preceding claims, wherein the mutation in the one or more amino acid residues consists of E430G.

6. The antibody variant according to any one of the preceding claims, wherein the variant Fc region comprises one or more further mutations which do not reduce complement-dependent cytotoxicity (CDC) and/or antibody-dependent cell-mediated cytotoxicity (ADCC) induced by the antibody variant without the one or more further mutations.
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7. The antibody variant according to claim 6, wherein the one or more further mutations are 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.
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8. The antibody variant according to any one of the preceding claims, wherein the variant Fc region is, except for the recited mutation, a human IgG1, IgG2, IgG3 or IgG4 isotype or a mixed isotype thereof.
9. The antibody variant according to any one of the preceding claims, wherein the variant Fc region is, except for the recited mutation, a human IgG1 Fc region.
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10. The antibody variant according to any one of the preceding claims, wherein the variant Fc region is, except for the recited mutations, a human IgG1m(f), IgG1m(a), IgG1m(x), IgG1m(z) allotype or a mixed allotype of any two or more thereof.
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11. The antibody variant according to any one of the preceding claims, which is a bivalent antibody.
12. The antibody variant according to any one of the preceding claims, which is a full-length antibody.
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13. The antibody variant according to any one of the preceding claims, wherein the antibody variant is, except for the recited mutations, a human antibody.
14. The antibody variant according to any one of the preceding claims, which is a monoclonal antibody.
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15. The antibody variant according to any one of the preceding claims, wherein the antibody variant is, except for the recited mutations, an IgG1 antibody.
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16. The antibody variant according to any one of the preceding claims, wherein the antibody variant is, except for the recited mutations, a human monoclonal full-length bivalent IgG1m(f), κ antibody.

17. An antibody variant binding to human CD38, the antibody variant comprising
- 5 (a) a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, the amino acid residues being numbered according to the EU index, wherein said mutation in the one or more amino acid residues comprises E430G;
- 10 (b) a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7.
18. An antibody variant binding to human CD38, the antibody variant comprising
- 15 (a) a heavy chain comprising a VH region comprising SEQ ID NO:1 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, wherein the amino acid residue numbering is according to the EU index, wherein said mutation in the one or more amino acid residues comprises E430G, and
- 20 (b) a light chain comprising a VL comprising SEQ ID NO:5.
19. The antibody variant according to claim 17 or claim 18, wherein the mutation consists of E430G.
20. The antibody variant according to any one of claims 17 to 19, wherein the human
- 25 IgG1 CH region is a human IgG1m(f), IgG1m(a), IgG1m(x) and IgG1m(z) allotype, or a mixed allotype of any two or more thereof.
21. The antibody variant according to any one of claims 17 to 20, wherein the CH comprises, except for the recited mutation, the sequence of SEQ ID NO:19, SEQ ID NO:20,
- 30 SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 or SEQ ID NO: 45.
22. The antibody variant according to claim 21, wherein the CH comprises one or more further mutations.
23. The antibody variant according to claim 22, wherein said one or more further mutations are 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.

24. The antibody variant according to claim 23, wherein Lys (K) at position 447 according to Eu numbering is deleted.
25. The antibody variant according to any one of claims 17 to 21, wherein the CH region
5 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:24 and SEQ ID NO: 46.
26. The antibody variant according to claim 25, wherein the CH region comprises SEQ ID
10 NO:24 or SEQ ID NO: 46, and wherein the light chain comprises a CL comprising SEQ ID NO:37.
27. The antibody variant according to any of the preceding claims comprising a variable heavy chain (VH) region comprising SEQ ID NO:1, a variable light chain (VL) region comprising SEQ ID NO:5, a CH region comprising SEQ ID NO: 46 and a CL comprising SEQ
15 ID NO:37.
28. The antibody variant according to any one of claims 17 to 27, which is a bivalent antibody.
29. The antibody variant according to any one of the preceding claims, wherein the
20 antibody variant is a monospecific or bispecific antibody.
30. The antibody variant according to any one of the preceding claims, which
25 (a) has an inhibitory effect on the cyclase activity of human CD38;
(b) induces apoptosis in the presence, but not in the absence, of an Fc-cross-linking antibody; and/or
(c) induces CDC, ADCC, antibody-dependent cell-phagocytosis (ADCP), trogocytosis, or any combination thereof, of cells expressing human CD38.
- 30 31. The antibody variant according to claim 30, which inhibits the cyclase activity of human CD38 by at least 40%, such as at least 50%, such as at least 60%, optionally wherein the inhibition of cyclase activity is determined by an assay comprising the steps of:
35 (a) seeding 200,000 Daudi or Wien133 cells in 100 μ L 20 mM Tris-HCL per well; or seeding 0.6 μ g/mL His-tagged soluble human CD38 (SEQ ID NO:39) in 100 μ L 20 mM Tris-HCL per well in a multi-well plate;
(b) adding 1 μ g/mL CD38 antibody and 80 μ M NGD to each well;
(c) measuring fluorescence until a plateau is reached (e.g.; 5, 10 or 30 minutes); and

(d) determining the percentage inhibition as compared to a control, such as a well incubated with an isotype control antibody.

32. The antibody variant according to any one of the preceding claims, wherein the antibody variant induces CDC of cells expressing human CD38.

33. The antibody variant according to any one of the preceding claims, wherein the antibody variant induces CDC against Daudi cells (ATCC No. CCL-213) or Ramos cells (ATCC No. CRL-1596) resulting in a maximum lysis at least 50%, such as at least 60%, such as at least 70% higher than that obtained with a reference antibody variant differing only in the absence of the one or more mutations in the Fc region.

34. The antibody variant according to claim 33, wherein the CDC is determined by an assay comprising the steps of:

- (a) plating 100,000 CD38-expressing cells in 40 μ L culture medium supplemented with 0.2% BSA per well in a multi-well plate;
- (b) preincubating cells for 20 minutes with 40 μ L of serially diluted CD38 antibody (0.0002-10 μ g/mL);
- (c) incubating each well for 45 minutes at 37°C with 20 percent of pooled normal human serum;
- (d) adding a viability dye and measuring the percentage of cell lysis on a flow cytometer;
- (e) determining the maximum lysis using non-linear regression.

35. An antibody variant according to any one of the preceding claims, wherein the antibody variant is conjugated to a cytotoxic agent, a radioisotope or a drug.

36. An isolated nucleic acid encoding the antibody variant according to any one of the preceding claims.

37. An expression vector comprising the nucleic acid according to claim 36.

38. A nucleic acid encoding a heavy chain of an antibody variant according to any one of claims 1-35.

39. A nucleic acid according to claim 38, wherein said heavy chain comprises a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430,

E345 and S440, wherein said mutation in the one or more amino acid residues comprises E430G, the amino acid residues being numbered according to the EU index.

40. A nucleic acid encoding an antibody variant according to any one of claims 1-35.
41. A combination of nucleic acids encoding an antibody variant according to any one of claims 1-35.
42. A delivery vehicle comprising the nucleic acid(s) according to any one of claims 38-41.
43. A delivery vehicle according to claim 42, wherein said delivery vehicle is a particle.
44. A delivery vehicle according to claim 43, wherein said particle is a lipid nanoparticle (LNP).
45. A delivery vehicle according to claim 44, wherein said LNP comprises lipids, ionizable aminolipids, PEG-lipids, cholesterol or any combination thereof.
46. A recombinant host cell which produces an antibody variant as defined in any one of claim 1 to 35, optionally wherein the host cell comprises the nucleic acid of claim 36 or the expression vector of claim 37.
47. The recombinant host cell according to claim 46, which is a eukaryotic or prokaryotic cell.
48. A method of producing an antibody variant according to any one of claims 1 to 35, comprising cultivating the recombinant host cell of claim 46 in a culture medium and under conditions suitable for producing the antibody variant and, optionally, purifying or isolating the antibody variant from the culture medium.
49. A method of increasing at least one effector function of a parent antibody comprising an Fc region and an antigen-binding region binding to CD38, which method comprises introducing into the Fc region a mutation in one or more amino acid residues selected from the group of amino acid residues corresponding to E430, E345, and S440 of the Fc region of a human IgG1 heavy chain, wherein said mutation in the one or more amino acid residues comprises E430G, wherein the amino acid residues are numbered according to the EU index; wherein the antigen-binding region comprises a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as

set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7.

50. A method of producing a variant of a parent antibody comprising an Fc region and an antigen-binding region binding to CD38, the variant having an increased effector function as compared to the parent antibody, which method comprises
- (a) introducing into the Fc region a mutation in one or more amino acid residues selected from the group of a mutation in amino acid residues corresponding to E430, E345, and S440 of the Fc region of a human IgG1 heavy chain to obtain a variant antibody, wherein said mutation in the one or more amino acid residues comprises E430G,
- (b) selecting any variant antibody having an increased effector function as compared to the parent antibody, and
- (c) producing said variant antibody in a recombinant host cell,
- wherein the antigen-binding region comprises a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7.
51. The method according to claim 49 or claim 50, wherein the effector function is CDC, trogocytosis, or both.
52. The method according to any one of claims 49 to 51, wherein the mutation in the one or more amino acid residue(s) consists of E430G.
53. The method according to any one of claims 49 to 52, wherein the Fc region of the parent antibody is a human IgG1, IgG2, IgG3 or IgG4 Fc region, or an isotype mixture thereof.
54. The method according to any one of claims 49 to 53, wherein the Fc region of the parent antibody is a human IgG1 Fc region.
55. The method according to claim 54, wherein the parent antibody is a human full-length IgG1 antibody, optionally a human monoclonal full-length bivalent IgG1, κ antibody.
56. The method according to any one of claims 49 to 55, wherein the Fc region of the parent antibody comprises one or more further mutations.

57. The method according to any one of claims 49 to 56, wherein the parent antibody is a monospecific or bispecific antibody.
58. An antibody obtained or obtainable by the method according to any one of claims 49 to 57.
59. A composition comprising an antibody variant according to any one of claims 1 to 35 or 58, a nucleic acid according to any one of claims 36 or 40, a combination of nucleic acids according to claim 41, an expression vector according to claim 37, a delivery vehicle according to any one of claims 42-45, or a host cell according to any one of claims 46-47.
60. A pharmaceutical composition comprising an antibody variant as defined in any one of claims 1 to 35 or 58, a nucleic acid according to any one of claims 36 or 40, a combination of nucleic acids according to claim 41, an expression vector as defined in claim 37, or a delivery vehicle according to any one of claims 42-45, and a pharmaceutically acceptable carrier.
61. An (i) antibody variant according to any one of claims 1 to 35 or 58, (ii) a nucleic acid according to any one of claims 36 or 40, (iii) a combination of nucleic acids according to claim 41, (iv) an expression vector according to claim 37, (v) a delivery vehicle according to any of claims 42-45 comprising nucleic acid(s) according to any one of claims 36, 40 or 41, or (vi) a composition according to any one of claims 59-60 comprising any one of (i) to (v) or comprising a host cell comprising any one of (i) to (iv) for use as a medicament.
62. Use of
- i. an antibody variant according to any one of claims 1 to 35 or 58
 - ii. a nucleic acid according to any one of claims 36 or 40
 - iii. a combination of nucleic acids according to claim 41,
 - iv. an expression vector according to claim 37,
 - v. a delivery vehicle according to any one of claims 42-45 comprising nucleic acid(s) according to any one of claims 36, 40 or 41, or
 - vi. a composition according to claim 59 or claim 60 comprising any one of i. to v. or comprising a host cell comprising any one of i. to iv.
- in the manufacture of a medicament for:
- a) the treatment of a disease involving cells expressing CD38, wherein said disease is selected from the group consisting of cancer, inflammatory disease, autoimmune disease or metabolic disorder involving cells expressing CD38 ;
 - b) inducing a CDC-response against a tumor comprising cells expressing CD38;
 - c) treating or preventing a cancer in a subject comprising cells expressing CD38;
 - d) treating a cancer refractory to a prior therapy comprising a CD38 antibody;

e) treating a cancer relapsed after a prior therapy comprising a CD38 antibody.

63. The use according to claim 62, wherein the CD38 antibody is daratumumab.

5 64. The use according to claim 62 or claim 63, wherein the cancer is a hematological cancer.

65. The use, according to claim 64, wherein the hematological cancer is selected from the group consisting of multiple-myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (adults) (AML), mantle cell lymphoma, follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL).

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66. The use according to claim 64, wherein the cancer is:

- a) MM
- 15 b) CLL;
- c) mantle cell lymphoma;
- d) DLBCL;
- e) FL; or
- f) acute myelogenous leukemia (adults) (AML).

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67. The use according to any one of claims 62 to 66, wherein the cancer comprises a solid tumor.

68. The use according to claim 67, wherein the solid tumor is melanoma, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, prostate cancer, castration-resistant prostate cancer, stomach cancer, ovarian cancer, gastric cancer, liver cancer, pancreatic cancer, thyroid cancer, squamous cell carcinoma of the head and neck, carcinoma of the esophagus or gastrointestinal tract, breast cancer, fallopian tube cancer, brain cancer, urethral cancer, genitourinary cancer, endometrial cancer, cervical cancer, lung adenocarcinoma, renal cell carcinoma (RCC) (e.g., a kidney clear cell carcinoma or a kidney papillary cell carcinoma), mesothelioma, nasopharyngeal carcinoma (NPC), a carcinoma of the esophagus or gastrointestinal tract, or a metastatic lesion of any one thereof.

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35 69. The use, according to claim 67, wherein the solid tumor is:

- a) lung cancer;
- b) squamous non-small cell lung cancer (NSCLC);
- c) non-squamous NSCLC;

- d) melanoma;
 - e) colorectal cancer;
 - f) prostate cancer;
 - g) castration-resistant prostate cancer;
 - 5 h) stomach cancer;
 - i) ovarian cancer;
 - j) gastric cancer;
 - k) liver cancer;
 - l) pancreatic cancer;
 - 10 m) thyroid cancer;
 - n) squamous cell carcinoma of the head and neck;
 - o) carcinoma of the esophagus or gastrointestinal tract;
 - p) breast cancer;
 - q) fallopian tube cancer;
 - 15 r) brain cancer;
 - s) urethral cancer;
 - t) genitourinary cancer;
 - u) endometrial cancer; or
 - v) cervical cancer.
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70. The use according to any one of claims 67 to 69, wherein the solid tumor lacks detectable CD38 expression.
71. The use according to any one of claims 62 to 70, wherein the cancer is in a patient
- 25 comprising T regulatory cells expressing CD38.
72. Use of an antibody variant according to any one of claims 1 to 35 in the manufacture of a medicament for treating or preventing rheumatoid arthritis.