

CLAIMS

1. An antibody or antibody fragment that binds a human CD39 polypeptide and that is capable of inhibiting the ATPase activity of a soluble extracellular domain human CD39 polypeptide, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NOS: 31 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

2. The antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment comprises a heavy chain comprising an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NOS: 38 and a light chain comprising an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 39 or 40.

3. The antibody or antibody fragment of claims 1 or 2, wherein the antibody or antibody fragment comprises heavy chain framework FR1, FR2 and FR3 amino acid sequences from the human IGHV1-3 gene; and light chain framework FR1, FR2 and FR3 amino acid sequences from the human IGKV4-1 gene.

4. An antibody or antibody fragment that binds a human CD39 polypeptide and is capable of binding and inhibiting the ATPase activity of a soluble extracellular domain human CD39 protein, wherein the antibody or antibody fragment comprises: a heavy chain variable region (VH) comprising a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 8, 9 and 10 and framework FR1, FR2 and FR3 amino acid sequences from the human IGHV1-3 gene; and a light chain variable region (VL) comprising a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 17, 12 and 13, and framework FR1, FR2 and FR3 amino acid sequences from the human IGKV4-1 gene.

5. The antibody or antibody fragment of claim 4, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NOS: 31, and a light chain variable region (VL) comprising an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 36 and 37.

6. The antibody or antibody fragment of any one of the above claims, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36.

7. An antibody or antibody fragment, wherein the antibody or antibody fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

8. The antibody or antibody fragment of any one of claims 1-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 37.

9. An antibody or antibody fragment, wherein the antibody or antibody fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 40.

10. The antibody or antibody fragment of any one of claims 4-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

11. The antibody or antibody fragment of any one of claims 4-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30 and a light chain variable region comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

12. The antibody or antibody fragment of any one of claims 4-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 and a light chain variable region comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

13. The antibody or antibody fragment of any one of claims 4-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino

acid sequence of SEQ ID NO: 32 and a light chain variable region comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

14. The antibody or antibody fragment of any one of claims 4-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

15. The antibody or antibody fragment of any one of claims 4-5, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 34 and a light chain variable region comprising the amino acid sequence of SEQ ID NOS: 36 or 37.

16. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of inhibiting the ATPase activity of soluble extracellular domain human CD39 protein produced by human tumor cells.

17. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of inhibiting the ATPase activity of sCD39 protein shed in cell culture supernatants from human CD39-expressing tumor cells.

18. The antibody or antibody fragment of any one of the above claims, wherein the tumor cells are Ramos tumor cells.

19. The antibody or antibody fragment of any one of the above claims, wherein the antibody inhibits the ATPase activity of the CD39 protein in the presence of exogenously added ATP, optionally wherein exogenously added ATP is provided at a concentration of 20 μ M.

20. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of binding human CD39 protein at the surface of a cell and that is capable of inhibiting the ATPase activity of said CD39 protein at the surface of a cell.

21. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of causing a decrease in the ATPase activity of sCD39 protein shed

in cell culture supernatants from human CD39-expressing tumor cells by more than 50%, optionally more than 70%, optionally more than 80%.

22. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of causing a decrease in the extracellular ATPase activity by a CD39-expressing cell by at least 80%.

23. The antibody or antibody fragment any one of the above claims, wherein the antibody is characterized by an EC_{50} for inhibition of ATPase activity of CD39 of no more than 1 $\mu\text{g/ml}$, wherein inhibition of the enzymatic activity of CD39 is determined by assessing neutralization of ATPase activity in Ramos cells by quantifying ATP.

24. The antibody or antibody fragment of any one of the above claims, wherein the antibody is characterized by an EC_{50} , as determined by flow cytometry, of no more than 2 $\mu\text{g/ml}$, optionally no more than 1 $\mu\text{g/ml}$, no more than 0.5 $\mu\text{g/ml}$, no more than 0.1 $\mu\text{g/ml}$, for binding to Ramos cells.

25. The antibody or antibody fragment of any one of the above claims, wherein the antibody competes for binding to a CD39 polypeptide of SEQ ID NO: 1 with an antibody comprising the respective heavy and light chain variable region amino acid sequences of SEQ ID NOS: 31 and 36.

26. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of increasing the activation of dendritic cells in the presence of ATP.

27. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of causing an increase in expression of a cell surface marker of activation in a monocyte-derived dendritic cells, when such moDC are incubated *in vitro* with the antibody and ATP.

28. The antibody or antibody fragment of claims 26 or 27, wherein exogenously added ATP is provided at 0.125 mM, 0.25 mM or 0.5mM.

29. The antibody or antibody fragment of claims 26-28, wherein an increase in expression of a cell surface marker of activation is assessed by incubating moDC in presence of ATP for 24 hours and analyzing cell surface expression of CD80, CD83 and/or HLA-DR on moDC by flow cytometry.

30. The antibody or antibody fragment of claims 27-29, wherein the increase in expression of a cell surface marker is at least 40%, 50%, 75% or 80%, compared to a negative control (e.g., medium).

31. The antibody or antibody fragment of any one of the above claims, wherein the antibody is capable of increasing T cell proliferation, when T cells co-cultured *in vitro* with CD39-expressing DC cells, in the presence of ATP.

32. The antibody or antibody fragment of any one of the above claims, wherein the antibody has reduced binding to a mutant CD39 polypeptide comprising the mutations R138A, M139A and E142K (with reference to SEQ ID NO: 1), in each case relative to binding between the antibody and a wild-type CD39 polypeptide comprising the amino acid sequence of SEQ ID NO: 1.

33. The antibody or antibody fragment of any one of the above claims, wherein the antibody is a naked antibody, optionally an antibody not bound to a cytotoxic agent.

34. The antibody or antibody fragment of any one of the above claims, wherein the antibody wherein the antibody is an antibody fragment, optionally an antibody fragment lacking an Fc domain.

35. The antibody or antibody fragment of any one of the above claims, wherein the antibody is an antibody having a human Fc domain that is modified to reduce binding between the Fc domain and a human Fc γ receptor.

36. The antibody or antibody fragment of any of the above claims, wherein the antibody substantially lacks binding to human CD16, CD32a, CD32b and/or CD64 polypeptides.

37. The antibody or antibody fragment of any of the above claims, wherein the antibody comprises an Fc domain, optionally wherein the Fc domain comprises an amino acid modification compared to a wild-type Fc domain, that has reduced or abolished binding to one or more, or all of, the human Fc γ receptors CD16A, CD16B, CD32A, CD32B and/or CD64.

38. The antibody or antibody fragment of any one of the above claims, wherein the antibody is a full-length antibody.

39. The antibody or antibody fragment of any one of the above claims, wherein the antibody or antibody fragment comprises a modified human IgG1 Fc domain comprising N-linked glycosylation at Kabat residue N297 and comprising an amino acid substitution at Kabat residue(s) 234 and 235, optionally further at Kabat residue 331, optionally at Kabat residues 234, 235, 237 and at Kabat residues 330 and/or 331, optionally wherein the Fc domain comprises L234A/L235E/P331S substitutions, L234F/L235E/P331S substitutions, L234A/L235E/G237A/P331S substitutions, or L234A/L235E/G237A/A330S/P331S substitutions.

40. A pharmaceutical composition comprising an antibody according to any one of the above claims, and a pharmaceutically acceptable carrier.

41. A kit comprising the antibody or antibody fragment of any one of the above claims, optionally further comprising a labeled secondary antibody or antibody fragment that specifically recognizes the antibody or antibody fragment of any one of the above claims.

42. A nucleic acid encoding a heavy and/or light chain of an antibody or antibody fragment of any one of claims 1 to 39.

43. A recombinant host cell producing the antibody or antibody fragment of any one of claims 1 to 39.

44. A method for the treatment or prevention of cancer in an individual in need thereof, the method comprising administering to the individual an effective amount of an antibody or antibody fragment of any one of claims 1-39 or a composition of claim 40.

45. A method for reducing the ATPase activity of soluble CD39 protein in an individual, the method comprising administering to said patient an effective amount of an antibody or antibody fragment of any one of claims 1-39 or a composition of claim 40.

46. The method of claim 45 wherein the individual has cancer.

47. A method for increasing T, NK and/or B cell activity in a subject having a cancer, and/or for relieving adenosine-mediated inhibition of T, NK and/or B cell activity in a subject having a cancer, the method comprising administering to said subject an effective amount of an antibody or antibody fragment of any one of claims 1-39 or a composition of claim 40.

48. A method for increasing the activation of dendritic cells in a subject having a cancer, and/or for restoring ATP-mediated activation of DC cells in a subject having a cancer, the method comprising administering to said subject an effective amount of an antibody or antibody fragment of any one of claims 1-39 or a composition of claim 40.

49. The method of claims 44-48, wherein the individual has detectable soluble CD39 protein.

50. The method of claim 49 wherein the individual has detectable soluble CD39 protein in circulation, in tumor tissue and/or in tumor adjacent tissue.

51. A method for the treatment or prevention of a cancer in an individual in need thereof, the method comprising:

a) detecting soluble CD39 protein in circulation and/or in the tumor environment, and

b) upon a determination that soluble CD39 protein is comprised in circulation and/or the tumor environment, optionally at a level that is increased compared to a reference level, administering to the individual an antibody or antibody fragment of any one of claims 1-39 or a composition of claim 40.

52. The method of claim 51, wherein detecting soluble CD39 protein comprises obtaining from the individual a biological sample, bringing said sample into contact with an antibody that binds soluble CD39 protein, and detecting antibody bound to soluble CD39 protein.

53. The method of any one of claims 44-52, wherein the anti-CD39 antibody is administered at least once in an amount effective to achieve a concentration in blood (serum) and/or a tumor tissue that corresponds to at least the EC₅₀ for neutralization of the enzymatic activity of soluble CD39 protein.

54. The method of any of the above claims, wherein neutralization of the enzymatic activity of soluble CD39 protein is determined by assessing neutralization of ATPase activity of a CD39 extracellular domain protein in solution, by quantifying the reduction in ATP hydrolyzed when the CD39 protein is incubated with an anti-CD39 antibody or antibody fragment.

55. The method of any one of claims 44-51, wherein the tumor or cancer is a solid tumor.

56. The method of claim 55, wherein the tumor or cancer is a head and neck squamous cell carcinoma, bladder cancer, ovarian cancer, colorectal carcinoma, melanoma, stomach cancer, esophageal cancer or a breast cancer.