CLAIMS

1. An isolated antibody that specifically binds human CD73 at the surface of a cell and that is capable of neutralizing the 5'-ectonucleotidase activity thereof, in which the antibody is characterized by an EC_{50} for neutralization of 5'-ectonucleotidase activity of cellular CD73 of no more than 1 μg/ml, in which neutralization of the enzymatic activity of CD73 is determined by assessing neutralization of 5'-ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine.

2. An isolated antibody according to Claim 1, in which the antibody binds a CD73 polypeptide dimer in bivalent manner.

3. An isolated antibody according to Claim 1, in which the antibody does not induce the internalization of the antibody-CD73 complex.

4. An isolated antibody according to Claim 1 or 2, in which the antibody lacks binding, via an Fc domain, to the human CD16 polypeptide (FcγIII receptor).

5. An isolated antibody that specifically binds human CD73 and acts as an allosteric inhibitor of a human CD73 polypeptide expressed by a cell, in which the antibody is characterized by an EC_{50} for neutralization of 5'-ectonucleotidase activity of cellular CD73 of no more than 1 μg/ml, in which neutralization of the enzymatic activity of CD73 is determined by assessing neutralization of 5'-ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine.

6. An isolated antibody according to Claim 5, in which the antibody is a monoclonal antibody that specifically binds a human CD73 polypeptide without causing intracellular internalization of the CD73 polypeptide.

7. An isolated antibody according to Claim 6, in which the antibody is an antibody lacking binding, via an Fc domain, to the human CD16 polypeptide (FcγIII receptor).

8. An isolated antibody according to any preceding claim, in which the antibody is a full-length antibody.

9. An isolated antibody according to Claim 6 or 7, in which the antibody binds a CD73 polypeptide dimer in bivalent manner.

10. An isolated antibody according to any preceding claim, in which the antibody is capable of causing a decrease in the 5'-ectonucleotidase activity of CD73 expressed by a cell by at least 80%.

11. An isolated antibody according to any preceding claim, in which the antibody is capable of neutralizing the 5'-ectonucleotidase activity of a soluble human CD73 polypeptide.

12. An isolated antibody according to any preceding claim, in which the antibody is a tetrameric antibody comprising two heavy and two light chains, the heavy chains comprising Fc regions of human isotype and which lack binding to human Fcγ receptors CD16A, CD16B, CD32A, CD32B and CD64.

13. An isolated antibody according to any preceding claim, in which the antibody binds to a CD73 polypeptide that is bound to a substrate, optionally in which the substrate adenosine 5'-(α,β-methylene)diphosphate (APCP) or AMP.

14. An isolated antibody according to any preceding claim, in which the antibody is capable of neutralizing the 5'-ectonucleotidase activity of a soluble human dimeric CD73 polypeptide when the antibody is provided at a 10-fold of greater molar excess to CD73 polypeptide dimer.

15. An isolated antibody according to any preceding claim, in which the antibody is characterized by an EC_{50}, as determined by flow cytometry, of no more than 0.5 μg/ml, for binding to cells made to express at their surface a CD73 polypeptide comprising an amino acid sequence of SEQ ID NO: 1.

16. An isolated antibody according to any preceding claim, in which the antibody is capable of causing a decrease in the 5'-ectonucleotidase activity of a human CD73 polypeptide in solution by more than 70%.

17. An isolated monoclonal antibody characterized by:

(a) specifically binding with high affinity to, and neutralizing the 5'-ectonucleotidase activity of, human CD73 polypeptides expressed at the surface of a cell, in which the antibody has a KD of less than 10^{-9} M for binding to a CD73 polypeptide, as determined by surface plasmon resonance, and in which neutralization of the 5'-ectonucleotidase

activity of CD73 is determined by assessing neutralization of 5'-ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine;

(b) capable of specifically binding in bivalent manner to human CD73 polypeptides;

(c) neutralizing the 5'-ectonucleotidase activity of a human CD73 polypeptide in solution;

(d) not inducing the down-modulation and/or internalization of the antibody-CD73 complex; and

(e) not specifically binding to the CD16 human Fcγ receptor.

18. An isolated antibody according to any preceding claim, in which the antibody binds a common antigenic determinant present on CD73 when its active site is not occupied by/bound to a substrate and when the CD73 active site is occupied by/bound to a substrate.

19. An isolated antibody according to any preceding claim, in which the antibody has reduced binding to a mutant CD73 polypeptide comprising the mutations A99S, E129A, K133A, E134N and A135S with reference to SEQ ID NO: 1, relative to binding between the antibody and a wild-type CD73 polypeptide comprising the amino acid sequence of SEQ ID NO: 1.

20. An isolated antibody selected from the group consisting of:

(a) monoclonal antibody comprising (i) a heavy chain comprising CDR 1, 2 and 3 of the heavy chain variable region of SEQ ID NO: 3 and (ii) a light chain comprising CDR 1, 2 and 3 of the light chain variable region of SEQ ID NO: 4;

(b) monoclonal antibody comprising (i) a heavy chain comprising CDR 1, 2 and 3 of the heavy chain variable region of SEQ ID NO: 21 and (ii) a light chain comprising CDR 1, 2 and 3 of the light chain variable region of SEQ ID NO: 22;

(c) monoclonal antibody comprising (i) a heavy chain comprising CDR 1, 2 and 3 of the heavy chain variable region of SEQ ID NO: 28 and (ii) a light chain comprising CDR 1, 2 and 3 of the light chain variable region of SEQ ID NO: 29; and

(d) monoclonal antibody comprising (i) a heavy chain comprising CDR 1, 2 and 3 of the heavy chain variable region of SEQ ID NO: 36 and (ii) a light chain comprising CDR 1, 2 and 3 of the light chain variable region of SEQ ID NO: 37,

in which CDRs are determined according to Kabat numbering.

21. An isolated antibody that binds CD73, in which the antibody has reduced binding to a mutant CD73 polypeptide comprising the mutations A99S, E129A, K133A, E134N and A135S with reference to SEQ ID NO: 1, relative to binding between the antibody and a wild-type CD73 polypeptide comprising the amino acid sequence of SEQ ID NO: 1, and in which the antibody specifically binds human CD73 at the surface of a cell and is capable of neutralizing the 5'-ectonucleotidase activity thereof, in which neutralization of the 5'-ectonucleotidase activity of CD73 is determined by assessing neutralization of 5'-ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine.

22. An isolated antibody according to any preceding claim, in which the antibody binds to an epitope present on a non-human primate CD73 polypeptide, optionally in which the non-human primate is a cynomolgus.

23. An isolated antibody according to any preceding claim, in which the antibody is an IgG4 antibody or an antibody having an Fc domain that is modified to reduce binding between the Fc domain and an Fcγ receptor.

24. An antibody obtained by chimerizing or humanizing an antibody according to any preceding claim.

25. A pharmaceutical composition comprising an antibody according to any preceding claim and a pharmaceutically acceptable carrier.

26. A kit comprising an antibody according to any preceding claim, optionally further comprising a labeled secondary antibody that specifically recognizes the antibody according to any preceding claim.

27. A nucleic acid encoding a heavy and light chain of an antibody according to any of Claims 1 to 24.

28. A hybridoma or recombinant host cell producing the antibody according to any of Claims 1 to 24.

29. An antibody according to any of Claims 1 to 24 or a composition according to Claim 25 for use in a method for the treatment or prevention of a disease in a patient in need thereof.

30. An antibody or composition for use according to Claim 29, in which the disease is cancer.

31. An antibody that binds human CD73 at the surface of a cell and that is capable of neutralizing the 5'-ectonucleotidase activity thereof as determined by assessing neutralization of 5'-ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine, in which the antibody comprises an Fc domain that is modified to reduce binding between the Fc domain and an Fcγ receptor, for use in a method for the treatment or prevention of a cancer in a patient in need thereof.

32. An antibody according to any of Claims 1 to 24 or an antibody or composition for use according to Claim 29, 30 or 31, in which the Fc domain comprises one or more amino acid substitutions that confer a decrease in sensitivity to cleavage by proteases, optionally in which the substitutions comprise the substitution of one, two, three or more residues within residues 233-238 (EU numbering).

33. An antibody according to any of Claims 1 to 24 or an antibody or composition for use according to any of Claims 29 to 32, in which the antibody is an IgG1 and comprises one, two or three substitutions at residues 234, 235 and 331 or at 234, 235 and 322 in accordance with EU numbering.

34. An antibody according to any of Claims 1 to 24, 32 or 33 or a composition according to Claim 25 for use in a method for increasing T cell activity in a subject having a cancer.

35. An antibody according to any of Claims 1 to 24, 32 or 33 or a composition according to Claim 25 for use in a method for relieving adenosine-mediated inhibition of T cell activity in a subject having a cancer.

36. An antibody according to any of Claims 1 to 24, 32 or 33 or a composition according to Claim 25 for use in a method for increasing T cell activity in the tumor microenvironment of a subject.

37. An antibody according to any of Claims 1 to 24, 32 or 33 or a composition according to Claim 25 for use in a method for the treatment or prevention of a cancer in an individual in need thereof, the method comprising:

(a) determining, *in vitro*, the CD73 polypeptide status of cells within the tumor environment, optionally within the tumor and/or within adjacent tissue, optionally tumor cells, and

(b) upon a determination that tumor environment comprises cells that express CD73 polypeptide, optionally at a level that is increased compared to a reference level, administering to the individual the antibody or composition.

38. An antibody or composition for use according to Claim 37, in which determining the CD73 polypeptide status within the tumor environment comprises obtaining from the individual a biological sample that comprises cancer tissue and/or adjacent tissue, bringing the cells into contact with an antibody that binds a CD73 polypeptide, and detecting cells that express CD73.

39. An antibody or composition for use according to any of Claims 29 to 38, in which the anti-CD73 antibody is administered at least once in an amount effective to achieve a concentration in blood serum or a tumor tissue that corresponds to at least the EC_{50} for neutralization of the enzymatic activity of CD73.

40. An anti-CD73 antibody for use in a method for treatment of disease in an individual having a tumour, in which the antibody is characterized by an EC_{50} for neutralization of 5'-ectonucleotidase activity of cellular CD73 of no more than 1 μg/ml, in which neutralization of the enzymatic activity of CD73 is determined by assessing neutralization of 5' ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine, the treatment comprising administering to the individual an anti-CD73 antibody that neutralizes the enzymatic activity of CD73 for at least one administration cycle in which the anti-CD73 antibody is administered at least once in an amount effective to achieve a concentration in blood serum or a tumor tissue that corresponds to at least the EC_{50} for neutralization of the enzymatic activity of CD73.

41. An antibody or composition for use according to any of Claims 31 to 40, in which the tumor or cancer is a solid tumor.

42. An antibody or composition for use according to any of Claims 31 to 40, in which the tumor or cancer is a leukemia, bladder cancer, glioma, glioblastoma, ovarian cancer, melanoma, prostate cancer, thyroid cancer, esophageal cancer or a breast cancer.