

CD73 BLOCKING ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application No. US 62/837,214 filed 23 April 2019, which is incorporated herein by reference in its entirety, including any drawings.

REFERENCE TO SEQUENCE LISTING

10 The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled "CD73-6_ST25", created 1 April 2020, which is 62 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

15 The present invention relates to antibodies, and fragments thereof, that bind and inhibit CD73. The invention also relates to cells producing such compounds; methods of making such compounds, and antibodies, fragments, variants, and derivatives thereof; pharmaceutical compositions comprising the same; methods of using the compounds to diagnose, treat or prevent diseases, e.g., cancer.

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BACKGROUND

CD73 (ecto-5'-nucleotidase) is a 70-kDa glycosylphosphatidylinositol (GPI)-anchored protein normally expressed on endothelial cells and subsets of hematopoietic cells. CD73, together with CD39, regulates adenosine triphosphate (ATP) metabolism. CD39 (NTPDase-1) converts ATP into AMP, with only trace amounts of ADP being released, while CD73 catalyzes the conversion of AMP to adenosine.

25 Adenosine triphosphate (ATP) and its metabolites AMP and adenosine, have important roles in cellular metabolism, signaling and immune homeostasis. The release of extracellular adenosine triphosphates (ATP) in response to cell death or cellular stress acts to activate immune responses. However, its metabolite adenosine has immunosuppressive activity. Extracellular adenosine accumulates in cancerous tissues and constitutes an important mechanism of tumor immune escape. Among other effects, tumor-derived adenosine profoundly inhibits infiltrating effector T cells through adenylyl cyclase-activating A2A receptors.

30 CD73 expression has been reported in a range of tumor cells, including leukemia, bladder cancer, glioma, glioblastoma, ovarian cancer, melanoma, prostate cancer, thyroid

cancer, esophageal cancer and breast cancer. CD73 expression has also been associated with a prometastatic phenotype in melanoma and breast cancer. It has been reported that therapy with an antibody that binds murine CD73 can inhibit breast tumor growth and metastasis in mice (Stagg, et al. (2010) Proc. Natl. Acad. Sci. USA 104:1547-1552). It has been shown that genetic deletion of A2A receptors can induce T cell-dependent tumor rejection (Ohta, et al., (2006) Proc Natl Acad Sci USA 103:13132–13137). Knock-down using siRNA or overexpression of CD73 on tumor cells can modulate tumor growth and metastasis (Beavis et al (2013 Proc. Natl. Acad. Sci. USA 110:14711-716; Stagg et al. (2010), supra; Jin et al. (2010) Cancer Res. 70: 2245-55). CD73^{-/-} mice are protected from transplanted and spontaneous tumors (Stagg et al. (2010) Cancer Res. 71: 2892-2900). In humans, high CD73 expression had been shown to be a negative prognostic for triple negative breast cancer (Loi et al (2013 Proc. Natl. Acad. Sci. USA 110: 11091-11096). However, while CD73 is expressed on tumor cells, it is also expressed on different cells of the immune system, notably CD4 and CD8 T cells, as well as B cells. Additionally, one further complicating factor is that many of the antibodies described in the literature have generally been of murine isotypes that are capable of being bound by Fc γ receptors, making it difficult to separate any potential blocking effect from Fc-mediated effects.

Despite the long-standing interest in CD73 as a therapeutic target, there remains a need for antibodies with higher potency in inhibition of the enzymatic activity of CD73 and that are suitable for use in human therapy.

SUMMARY OF THE INVENTION

The inventors provide antibodies that bind an epitope present on CD73 expressed at the surface of cells, including tumor cells, and that inhibit the enzymatic (ecto-5' nucleotidase) activity of the CD73 enzyme. The antibodies have human-derived framework regions with minimal non-human (e.g. mouse) sequence content. The antibodies can bind CD73 in an intra-dimer mode and can inhibit the enzymatic activity of both soluble CD73 and membrane-bound CD73 protein expressed at the surface of cells. Advantageously, these antibodies can be used as pure CD73 blocking antibodies, e.g., they inhibit the enzymatic activity of membrane-bound CD73 protein expressed at the surface of cells without substantially binding Fc γ receptors and/or without substantially directing ADCC toward a CD73-expressing cell. Optionally, the antibodies retain an Fc domain and retain binding to human FcRn. The antibodies can advantageously have low risk of immunogenicity, e.g. low or decreased (compared to a murine parental antibody) likelihood to induce a human anti-mouse antibody (HAMA) response when administered to a human.

The antibodies bind an epitope present on human CD73 polypeptide expressed at the surface of cells, including but not limited to tumor cells, and inhibit the enzymatic (ecto-5' nucleotidase) activity of the CD73 enzyme. They have a particularly advantageous ability to inhibit the suppression of T cell proliferation mediated by CD73, and consequently can cause an increase in the biological activity, including but not limited to proliferation, of cytotoxic CD4 and/or CD8 T cells, for example as observed in a T cell proliferation assay. In one embodiment, inhibition or neutralization of the enzymatic activity of CD73 is determined by assessing the ability of an antibody or antibody fragment to increase the proliferation of T cells when said T cells are induced to proliferate in vitro (e.g. via TCR co-stimulation) in the presence of AMP (e.g. exogenously added AMP).

In one embodiment, provided is an antibody or antibody fragment, or an antigen binding domain thereof, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 and a light chain variable region comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 33, 34, 35 or 36. In one embodiment, provided is an antibody or antibody fragment, or an antigen binding domain thereof, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 33.

In one embodiment, provided is an antibody or antibody fragment, or an antigen binding domain thereof, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable region comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 43, 44, 45, or 46. In one embodiment, provided is an antibody or antibody fragment, or an antigen binding domain thereof, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 43.

In one embodiment, provided is an anti-CD73 antibody or antibody fragment comprising 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.

In one embodiment, provided is an anti-CD73 antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 47 and a light chain comprising the amino acid sequence of SEQ ID NO: 48.

In one embodiment, provided is an anti-CD73 antigen binding domain, or a protein that comprises such (e.g., an antibody or antibody fragment, a multispecific binding protein, a bispecific antibody, etc.), comprising a heavy chain variable region (VH) comprising a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID

NOS: 2, 3 and 4 and framework FR1, FR2 and FR3 amino acid sequences from the human IGHV1-3 gene (and optionally further framework 4 (FR4) amino acid sequences from the human IGHJ4 gene); and a light chain variable region (VL) CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 5, 6 and 7, and framework FR1, FR2 and FR3 amino acid sequences from the human IGKV1-33 gene (and optionally further framework 4 (FR4) amino acid sequences from the human IGKJ2 gene). Optionally the residue present in the heavy chain variable region at Kabat position 59 (HCDR2) is a leucine or a glutamine residue. Optionally the residue present in the heavy chain variable region at Kabat position 60 (HCDR2) is threonine or a lysine residue. Optionally the residue present in the heavy chain variable region at Kabat position 97 (HCDR3) is a glycine or an asparagine residue. Optionally the residue present in the light chain variable region at Kabat position 30 (LCDR1) is a serine or a threonine residue. Optionally the residue present in the light chain variable region at Kabat position 53 (LCDR2) is a threonine or an asparagine residue.

In one embodiment, provided is an anti-CD73 antigen binding domain, or a protein that comprises such (e.g., an antibody or antibody fragment, a multispecific binding protein, a bispecific antibody, etc.), comprising a heavy chain variable region (VH) comprising a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 8 and 9 and framework FR1, FR2 and FR3 amino acid sequences from the human IGHV1-3 gene (and optionally further framework 4 (FR4) amino acid sequences from the human IGHJ4 gene); and a light chain variable region (VL) CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 10, 11 and 7, and framework FR1, FR2 and FR3 amino acid sequences from the human IGKV1-33 gene (and optionally further framework 4 (FR4) amino acid sequences from the human IGKJ2 gene). In one embodiment, the VH further comprises one, two or three of the amino acid substitutions in the Kabat heavy chain CDRs at Kabat heavy chain positions selected from the group consisting of 59 (HCDR2), 60 (HCDR2) and 97 (HCDR3). Optionally the leucine residue at position 59 is substituted by a glutamine residue. Optionally the threonine residue at position 60 is substituted by a lysine residue. Optionally the glycine residue at position 97 is substituted by an asparagine residue. In one embodiment, the VL further comprises an amino acid substitution in the Kabat light chain CDRs at Kabat light chain positions 30 (LCDR1) and/or 53 (LCDR2), optionally further wherein the serine residue at position 30 is substituted by an threonine residue, optionally further wherein the threonine residue at position 53 is substituted by an asparagine residue.

In one embodiment, provided is an anti-CD73 antigen binding domain, or a protein that comprises such (e.g., an antibody or antibody fragment, a multispecific binding protein,

a bispecific antibody, etc.), comprising a heavy chain variable region (VH) comprising a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 12 and 13 and framework FR1, FR2 and FR3 amino acid sequences from the human IGHV1-3 gene (and optionally further framework 4 (FR4) amino acid sequences from the human IGJH4 gene); and a light chain variable region (VL) CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 14, 15 and 7, and framework FR1, FR2 and FR3 amino acid sequences from the human IGKV1-33 gene (and optionally further framework 4 (FR4) amino acid sequences from the human IGKJ2 gene).

In one aspect of any embodiment herein, a heavy chain variable region (VH) can be characterized by comprising one, two, three, four or five amino acid substitutions at Kabat heavy chain positions selected from the group consisting of 2, 30, 48, 69 and 73, optionally wherein the residue present in a human sequence at the particular position is replaced by the residue present in the murine donor sequence at that particular position; and a light chain variable region (VL) can be characterized by comprising an amino acid substitution at Kabat light chain position 67, optionally wherein the residue present in a human sequence at the particular position is replaced by the residue present in a murine donor sequence at that particular position. In one embodiment, the VL comprises a substitution at Kabat light chain position 67, and optionally further one, two or three amino acid substitutions at Kabat light chain positions selected from the group consisting of 2, 67 and 87, optionally wherein the residue present in a human sequence at the particular position is replaced by the residue present in the murine donor sequence at that particular position. In one embodiment, an antibody or antibody binding domain comprises a heavy chain variable region comprising the amino acid substitutions V2I, T30A, M48I, I69L and T73K and a light chain variable region comprising the amino acid substitution S67Y, wherein numbering is according to Kabat.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 2 is an isoleucine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 30 is an alanine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 48 is an isoleucine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 69 is a leucine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 73 is a lysine.

In one embodiment, a VH comprises an isoleucine residue at Kabat position 2, an alanine at position 30, an isoleucine at position 48, a leucine at position 69 and a lysine at position 73.

5 In one aspect of any embodiment herein, the amino acid at Kabat light chain position 67 is a tyrosine.

In one aspect of any embodiment herein, the amino acid at Kabat light chain position 60 is an aspartic acid.

In one aspect of any embodiment herein, the amino acid at Kabat light chain position 2 is an isoleucine.

10 In one aspect of any embodiment herein, the amino acid at Kabat light chain position 87 is a phenylalanine.

In one embodiment, a VL comprises a tyrosine residue at Kabat position 67; optionally the VL comprises no other substitutions in the Kabat framework by non-human residues and/or has a VL Kabat framework that is fully human other than a substitution at residue 67.

In one embodiment, a VL comprises a tyrosine residue at Kabat position 67 and an aspartic acid at position 60.

In one embodiment, a VL comprises a tyrosine residue at Kabat position 67, an aspartic acid at position 60 and an isoleucine at position 2.

20 In one embodiment, a VL comprises a tyrosine residue at Kabat position 67, an aspartic acid at position 60, an isoleucine at position 2, and a phenylalanine at position 87.

In one embodiment, provided is an anti-CD73 antigen binding domain, or a protein that comprises the antigen binding domain (e.g., an antibody or antibody fragment, a multispecific binding protein, a bispecific antibody, etc.), comprising a heavy chain variable region (VH) comprising an amino acid sequence at least 80%, 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 37, and a light chain variable region (VL) comprising an amino acid sequence at least 80%, 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of any of SEQ ID NOS: 33, 34, 35 or 36. In one embodiment, the VH comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 12 and 13 and the VL comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 14, 15 and 7. In one embodiment, the VH comprises an isoleucine residue at Kabat position 2, an alanine at position 30, an isoleucine at position 48, a leucine at position 69 and a lysine at position 73. In one embodiment, the VL comprises a tyrosine residue at Kabat position 67.

35 In one embodiment, provided is an anti-CD73 antigen binding domain or a protein that comprises the antigen binding domain (e.g., an antibody or antibody fragment, a

multispecific binding protein, a bispecific antibody, etc.), comprising a heavy chain variable region (VH) comprising CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 3 and 4 and human frameworks (e.g., FR1, FR2, FR3 and FR4 of human origin); and a light chain variable region (VL) CDR1, CDR2 and CDR3 comprising the respective amino acid sequences shown in SEQ ID NOS: 5, 6 and 7 and human frameworks (e.g., FR1, FR2, FR3 and FR4 of human origin), wherein the (VH) comprises an amino acid sequence at least 80%, 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NOS: 37 or 42, and a light chain variable region (VL) comprising an amino acid sequence at least 80%, 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of any of SEQ ID NOS: 33-36 or 43-46). Optionally the residue present in the heavy chain variable region at Kabat position 59 (HCDR2) is a leucine or a glutamine residue. Optionally the residue present in the heavy chain variable region at Kabat position 60 (HCDR2) is threonine or a lysine residue. Optionally the residue present in the heavy chain variable region at Kabat position 97 (HCDR3) is a glycine or an asparagine residue. Optionally the residue present in the light chain variable region at Kabat position 30 (LCDR1) is a serine or a threonine residue. Optionally the residue present in the light chain variable region at Kabat position 53 (LCDR2) is a threonine or an asparagine residue. In one embodiment, the VH comprises an isoleucine residue at Kabat position 2, an alanine at position 30, an isoleucine at position 48, a leucine at position 69 and a lysine at position 73. In one embodiment, the VL comprises a tyrosine residue at Kabat position 67.

In any embodiment, the VH can be characterized as comprising a human VH acceptor framework amino acid sequences, and the VL comprises human VL acceptor framework amino acid sequences. In one embodiment, the VH segment of the VH human acceptor framework is from a human IGHV1-3 gene segment and the J-segment is from a human IGHJ4 gene segment. In one embodiment, the VH human acceptor framework is from a human IGHV1-3*01 gene segment. In one embodiment, the VL domain human acceptor framework is from a human IGKV1-33 gene segment, optionally the VL domain human acceptor framework is from a human IGKV1-33*01 gene segment. In one embodiment, the VL domain human acceptor framework comprises the J-segment from a human IGKJ2 gene segment.

In one embodiment, an antibody or antibody fragment comprises a H4+ VH domain and a L1, L2, L3 or L4 VL domain. In one embodiment, the antibody is antibody H4+L1.

In one embodiment, provided is an anti-CD73 antigen binding domain, or a protein that comprises the antigen binding domain (e.g., an antibody or antibody fragment, a multispecific binding protein, a bispecific antibody, etc.), comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 and a light chain variable

region comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO: 33, 34, 35 or 36.

In one embodiment, provided is an antibody or antibody fragment that binds human CD73 and that neutralizes the ATPase activity of CD73, wherein the antibody or antibody
5 fragment comprises a heavy chain variable region and a light chain variable region of antibody H4+L1.

In one embodiment, provided is an antibody or antibody fragment that binds a human CD73 polypeptide and that neutralizes the ATPase activity of CD73, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid
10 sequence of SEQ ID NO: 37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 33.

In one embodiment, provided is an antibody or antibody fragment that binds a human CD73 polypeptide and that neutralizes the ATPase activity of CD73, wherein the antibody or antibody fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID
15 NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In one embodiment, an antibody or antibody fragment comprises a 2H4+ VH domain and a 2L1, 2L2, 2L3 or 2L4 VL domain. In one embodiment, the antibody is antibody 2H4+2L1.

In one embodiment, provided is an anti-CD73 antigen binding domain, or a protein
20 that comprises the antigen binding domain (e.g., an antibody or antibody fragment, a multispecific binding protein, a bispecific antibody, etc.), comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable region comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO: 43, 44, 45 or 46.

In one embodiment, provided is an antibody or antibody fragment that binds human CD73 and that neutralizes the ATPase activity of CD73, wherein the antibody or antibody
25 fragment comprises a heavy chain variable region and a light chain variable region of antibody 2H4+2L1.

In one embodiment, provided is an antibody or antibody fragment that binds a human
30 CD73 polypeptide and that neutralizes the ATPase activity of CD73, wherein the antibody or antibody fragment comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 42 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 43.

In one embodiment, provided is an antibody or antibody fragment that binds a human
35 CD73 polypeptide and that neutralizes the ATPase activity of CD73, wherein the antibody or antibody fragment comprises a heavy chain comprising an amino acid sequence of SEQ ID

NO: 47 and a light chain comprising an amino acid sequence of SEQ ID NO: 48.

In one embodiment, the antibodies are non-depleting and neutralize the enzymatic activity of CD73 in the tumor environment.

In one embodiment, the antibody is a human IgG1, IgG2, IgG3, or IgG4 isotype antibody. For example, the antibody may be an antibody comprising an Fc domain of human IgG4 isotype, or an antibody comprising an Fc domain of any human IgG isotype (e.g., IgG1, IgG2, IgG3, or IgG4) modified to reduce or lack binding between the Fc domain and a human Fc γ receptor (e.g., CD16), optionally modified to reduce binding between the Fc domain and a plurality of human Fc γ receptors, e.g., CD16A, CD16B, CD32A, CD32B and CD64). In one embodiment, the antibodies comprise an Fc domain of human IgG subtype (e.g., IgG1) comprising an amino acid modification that results in a decrease (compared to the antibody comprising a wild-type human IgG1 Fc domain) or substantially complete loss of binding to each of CD16A, CD16B, CD32A, CD32B and CD64.

In any embodiment, an antibody heavy chain comprises a human CH1 constant domain and a modified human Fc domain, optionally of human IgG1 isotype, optionally further comprising an amino acid sequence of any one of SEQ ID NOS: 16, 17, 18 or 19. In any embodiment, an antibody light chain comprises a human light chain constant domain, optionally wherein the constant domain is a human kappa domain.

In one aspect, the antibodies of the disclosure do not induce or increase intracellular internalization of, or more generally down-modulation of, cell surface-expressed CD73 polypeptide and/or do not depend thereupon for their CD73 inhibitory activity. The antibodies of the disclosure can provide greater inhibitory potency (the ability to substantially neutralize CD73 enzymatic activity) than antibodies that inhibit CD73 by causing CD73 internalization. As opposed to antibodies that inhibit soluble CD73 by other mechanisms (e.g., causing CD73-antibody oligomer formation), the antibodies of the disclosure are capable of inhibiting the enzymatic activity of CD73 at high (e.g., 10 fold) excess of antibody:enzyme. Furthermore, unlike antibodies that bind an epitope on recombinant CD73 that may be modified or absent on cell surface CD73 (e.g., antibody 7G2) or with affinity that is too low to translate into efficacy in CD73-expressing cells, the present antibodies bind with high affinity to an epitope that is present and/or remains intact on cell surface CD73, providing the antibodies with the ability to potently neutralize of the enzymatic activity of cellular CD73. The present antibodies inhibit CD73 enzymatic activity in cells but can optionally also inhibit the ecto-5'nucleotidase activity of soluble recombinant CD73 (as observed in a cell-free assay using soluble dimeric CD73 polypeptide).

In one aspect, the antibodies of the disclosure are capable of inhibiting the activity of the human CD73 polypeptide without binding to the enzymatic active site of the CD73

polypeptide, and/or are non-competitive inhibitors of CD73, e.g., they inhibit the activity of the human CD73 polypeptide without detectably reducing binding between the CD73 polypeptide and a natural substrate thereof.

5 In one aspect, the antibodies of the disclosure lose binding to CD73 mutants having a substitution at residue K136. In one aspect, the antibodies of the disclosure have a decrease in binding to CD73 mutants having a substitution at residues K136 (with reference to the sequence of SEQ ID NO: 1; and compared to a wild-type CD73 comprising an amino acid sequence of SEQ ID NO: 1); optionally the antibodies also have a decrease in binding to CD73 mutants having substitutions at residues A99, E129, K133, E134 and A135 (with
10 reference to the sequence of SEQ ID NO: 1; and compared to a wild-type CD73 comprising an amino acid sequence of SEQ ID NO: 1; optionally further the antibodies have decreased binding to CD73 mutants having a substitution at residues K97, E125, Q153 and K330 (with reference to the sequence of SEQ ID NO: 1; and compared to a wild-type CD73 comprising an amino acid sequence of SEQ ID NO: 1.

15 In one embodiment, the antibodies bind to an epitope on each CD73 polypeptide chain within a CD73 dimer (i.e. binds to CD73 in an intra-dimer manner), optionally further wherein the epitope is present on the same face of a CD73 dimer. Antibodies can thus bind bivalently to one CD73 dimer, notably in a position that is more "closed" in which the antibody binding sites are spatially further apart than in "open" position. In view of binding to
20 ligand-bound CD73, the antibodies described herein may be useful for binding to CD73 when bound to AMP, e.g., for treating an individual or cancer characterized by (e.g. known or suspected to be characterized by) a tumor environment where upstream ADP and/or AMP are present at significant levels prior to treatment). The antibodies may be useful for treating an individual or cancer having (e.g. known or suspected to have) a tumor environment
25 characterized by high levels of ADP (e.g., generated by dying cells, taken up by CD73 on stromal and cellular infiltrate (e.g., TReg cells) to yield high levels of AMP), as well as more generally by AMP, by adenosine, by presence or levels of CD73 expression or CD73-expressing cells, by high (e.g. compared to healthy tissue) numbers or frequencies of CD73-expressing cells and/or high levels of CD73 expression on cells (e.g. as assessed by an
30 immunohistochemistry assay), by high (e.g. compared to healthy tissue) soluble CD73 polypeptides (e.g. as assessed by an ELISA or generally any antibody based detection assay), or by presence or levels of adenosine receptor expression or adenosine-receptor expressing cells. CD73 molecules in the tumor environment may be in the substrate-bound conformation, and the ability to bind and inhibit substrate-bound cellular CD73 (e.g., cells
35 expressing CD73 pre-incubated with substrate such as AMP) in addition to non-substrate bound CD73 may provide greater ability to inhibit CD73 in vivo. Optionally, soluble CD73

protein, numbers or frequencies of CD73-expressing cells and/or levels of CD73 expression on cells can be assessed in the tumor environment prior to treatment. The antibodies may have a particular advantage for treatment of an individual having significant levels (e.g., high levels, compared to a reference) of soluble CD73 protein, numbers or frequencies of CD73-expressing cells and/or levels of CD73 expression on cells in the tumor sample.

Accordingly, in one aspect the disclosure provides a humanized antibody or antibody fragment that binds human CD73 polypeptide expressed at the surface of cells and that inhibits the enzymatic (ecto-5' nucleotidase) activity of the CD73 polypeptide, wherein the antibody is capable of binding bivalently to a single CD73 polypeptide dimer (a soluble CD73 polypeptide dimer or a CD73 polypeptide dimer expressed by a cell). Optionally, the antibody binds with a first antigen binding domain to a first CD73 polypeptide within the dimer and with a second antigen binding domain to a second CD73 polypeptide. In one aspect the antibody is an allosteric inhibitor of the CD73 polypeptide.

The epitope on CD73 bound by the antibodies is present on CD73 polypeptides as expressed by a range of cells, e.g., cancer cells, CD4 T cells, CD8 T cells, B cells, transfected cells, and binds with high affinity as determined by flow cytometry. For example, an antibody can be characterized by an EC_{50} , as determined by flow cytometry, of no more than 5 $\mu\text{g/ml}$, optionally no more than 2 $\mu\text{g/ml}$, no more than 1 $\mu\text{g/ml}$, no more than 0.5 $\mu\text{g/ml}$, no more than 0.1 $\mu\text{g/ml}$ or no more than 0.05 $\mu\text{g/ml}$, for binding to cells that express at their surface a CD73 polypeptide. In one embodiment the cells are cells that are made to express CD73 at their surface. In one embodiment the cells are cells that endogenously express CD73 at their surface, e.g., cancer cells, leukemia cells, bladder cancer cells, glioma cells, glioblastoma cells, ovarian cancer cells, melanoma cells, prostate cancer cells, thyroid cancer cells, esophageal cancer cells or breast cancer cells.

In one embodiment, the CD73 neutralizing antibodies can be characterized by being capable of causing a decrease in cells' 5'-ectonucleotidase activity of CD73 by at least 60%, 75% or 80%. In one embodiment, the CD73-neutralizing antibodies can be characterized by an EC_{50} for inhibition of 5'-ectonucleotidase activity of CD73 expressed by a cell of no more than 1 $\mu\text{g/ml}$, optionally no more than 0.5 $\mu\text{g/ml}$, optionally no more than 0.2 $\mu\text{g/ml}$.

Optionally, inhibition of 5'-ectonucleotidase activity of CD73 expressed by a cell is determined by assessing neutralization of 5' ectonucleotidase activity in CD73-expressing cells (e.g. MDA-MB-231 cells) by quantifying hydrolysis of AMP to adenosine.

Optionally, inhibition of 5'-ectonucleotidase activity of CD73 expressed by a cell is determined by assessing the ability of an antibody or antibody fragment to increase the proliferation of T cells when said T cells (e.g. from a healthy human donor) are induced to proliferate in vitro (e.g. via TCR co-stimulation, stimulation of CD3 and CD28 signaling, e.g.,

by contacting T cells with beads functionalized with CD3 agonists and CD28 agonists) in the presence of AMP (e.g. exogenously added AMP). Optionally T cell proliferation is assessed using the assay described in the Examples herein (see Example 5 and Methods).

5 In one aspect, an anti-CD73 antibody binds a common antigenic determinant present on both soluble CD73 and CD73 expressed at the cell surface.

In one aspect, an anti-CD73 antibody binds an antigenic determinant within each CD73 polypeptide chain within a CD73 dimer (in intra-dimer mode), e.g., wherein the antigenic determinants are present on a common face of the CD73 dimer.

10 In one aspect, an anti-CD73 antibody binds an epitope on CD73 comprising residue K136 (with reference to SEQ ID NO: 1).

In one aspect, an anti-CD73 antibody binds an epitope on CD73 comprising one, two, three or four of the residues selected from the group consisting of K97, E125, Q153 and K330 (with reference to SEQ ID NO: 1).

15 In one aspect, an anti-CD73 antibody binds an epitope on CD73 comprising one, two, three, four or five of the residues selected from the group consisting of A99, E129, K133, E134, and A135 (with reference to SEQ ID NO: 1).

In one aspect, an anti-CD73 antibody binds at least partly within a domain or segment of amino acid residues on a human CD73 protein (e.g., a CD73 homodimer protein) comprising the amino acid residues K97, A99, E125, E129, K133, E134, A135, K136, Q153 and K330 (with reference to SEQ ID NO: 1). In one aspect, an anti-CD73 antibody binds an epitope on CD73 comprising at least one, two, three, four or five, or more, of the residues selected from the group consisting of K97, A99, E125, E129, K133, E134, A135, K136, Q153 and K330 (with reference to SEQ ID NO: 1).

20 In one aspect, an anti-CD73 antibody has reduced binding to a CD73 polypeptide having a mutation at a residue K136 (with reference to SEQ ID NO: 1) compared to a wild-type CD73 polypeptide comprising an amino acid sequence of SEQ ID NO: 1; optionally, the mutant CD73 polypeptide has the mutation: K136A.

25 In one aspect, an anti-CD73 antibody has reduced binding to a CD73 polypeptide having a mutation at a residue selected from the group consisting of: K97, E125, Q153 and K330 (with reference to SEQ ID NO: 1) compared to a wild-type CD73 polypeptide comprising an amino acid sequence of SEQ ID NO: 1; optionally, the mutant CD73 polypeptide has the mutations: K97A, E125A, Q153A and/or K330A (e.g., K97A, E125A and K330A; K97A, E125A and/or Q153A).

30 In one aspect, an anti-CD73 antibody has reduced binding to a CD73 polypeptide having a mutation at a residue selected from the group consisting of: A99, E129, K133, E134, and A135 (with reference to SEQ ID NO: 1) compared to a wild-type CD73

polypeptide comprising an amino acid sequence of SEQ ID NO: 1; optionally, the mutant CD73 polypeptide has the mutations: A99S, E129A, K133A, E134N, and A135S.

In one embodiment, provided are methods of using the antibodies in the treatment or prevention of disease, e.g., infectious disease or cancer. In one aspect, the antibodies are administered to an individual having a cancer in an amount and frequency sufficient to neutralize the activity of CD73 in the tumor microenvironment. In one embodiment, the antibodies are administered in an amount and frequency sufficient to decrease the generation and/or concentration of adenosine in the tumor microenvironment. In one embodiment, the antibodies are administered in an amount and frequency sufficient to increase the generation and/or concentration of ATP in the tumor microenvironment. In one embodiment, the antibodies are administered in an amount and frequency sufficient to neutralize the activity of CD73 expressed by tumor cells. In one embodiment, the antibodies are administered in an amount and frequency sufficient to neutralize the activity of CD73 expressed by CD4 T cells, CD8 T cells and/or B cells.

The antibodies will be useful in inhibiting CD73-mediated catabolism of AMP to adenosine, e.g., decreasing the concentration of adenosine in the tumor microenvironment. These antibodies will therefore be useful in reversing the immunosuppressive effect of CD73 and/or adenosine on T cells, B cells and other cells that express adenosine receptors, for example in the treatment of cancer. In one embodiment, the anti-CD73 antibody neutralizes adenosine-mediated inhibition of proliferation, cytokine production, cytotoxicity and/or NF κ B activity in T cells.

Because the CD73-mediated catabolism of AMP to adenosine is irreversible, whereas the catabolism of ATP to ADP and ADP to AMP by CD73 is reversible (by NDK kinase and adenylate kinase, respectively), the antibodies that block the irreversible CD73-mediated catabolism will increase the pool of AMP, thereby being of use in increasing the concentrations of ADP and ATP, e.g., in the tumor microenvironment. The antibodies can be useful to increase the formation of ADP from AMP and the formation of ATP from ADP. Since ATP has immune activating roles, the anti-CD73 antibodies can be useful in activating T cells, for example in the treatment of cancer.

The antibodies will be useful in inhibiting the production, amounts and/or concentrations of adenosine into the tumor microenvironment.

The antibodies that neutralize the activity of a soluble human CD73 polypeptide dimer can further neutralize CD73 in any other suitable context, e.g., in a reporter cell made to express CD73, in a T cell, etc.

Provided is a method for treating an individual, the method comprising administering to an individual (e.g., an individual having a disease, a tumor, etc.) a therapeutically active

amount of any of the anti-CD73 antigen binding compounds described herein. In one aspect provided is a method for treating an individual, the method comprising, consisting essentially of or consisting of: administering to an individual (e.g., an individual having a disease, a tumor, etc.) a therapeutically active amount of an antigen binding compound of the disclosure.

In one aspect provided is a method for decreasing adenosine produced by a CD73-expressing cell (e.g., an immune cell and/or a tumor cell in an individual), or a method for neutralizing of the enzymatic activity of cellular CD73, the method comprising, consisting essentially of or consisting of: bringing the CD73-expressing cell into contact with an antigen binding compound of the disclosure (e.g. an anti-CD73 antibody or antibody fragment, or a composition comprising such). In one embodiment, the step of bringing the CD73-expressing cell into contact with an antigen binding compound of the disclosure comprises administering to an individual a therapeutically active amount of the antigen binding compound. In one embodiment the individual has a cancer.

In one aspect provided is a method for decreasing adenosine present in the tumor environment (e.g., in an individual), the method comprising, consisting essentially of or consisting of: administering to an individual a therapeutically active amount of a compound of the disclosure (e.g. an anti-CD73 antibody or antibody fragment, or a composition comprising such). In one embodiment the individual has a cancer. Optionally the individual is a human having or who is susceptible to having a cancer.

The antibodies are optionally characterized by binding affinity (K_D) for a human CD73 polypeptide (e.g. as CD73 dimer), as determined by SPR (e.g. according to the methods of the Examples) of less than (better than) 10^{-9} M, preferably less than 10^{-10} M, or preferably less than 10^{-11} M, and/or by binding human CD73 polypeptide with an EC_{50} lower than (better binding than) 1 μ g/ml. Optionally, binding affinity can be specified as being bivalent.

In one embodiment, the antibody has an EC_{50} of no more than 0.5 μ g/ml, optionally no more than 0.2 μ g/ml, optionally no more than 0.1 μ g/ml, for binding to cells (e.g., tumor cells, MDA-MB-231 cells) expressing human CD73 at the cell surface.

The antibodies are optionally characterized by an EC_{50} for neutralization of the enzymatic activity of CD73 in CD73-expressing cells (e.g., tumor cells, MDA-MB-231 cells) of less than (better than) 1 μ g/ml, optionally less than 0.5 μ g/ml.

In one embodiment, the antibody is a monoclonal antibody or a fragment thereof that retains binding specificity and ability to neutralize the enzymatic activity of CD73 (e.g. as determined by assessing the ability of an antibody or antibody fragment to increase the proliferation of T cells when said T cells are induced to proliferate in vitro in the presence of AMP (e.g. exogenously added AMP). In one embodiment, the antibody is substantially as

potent or is at least as potent as parental antibody having the respective VH and VL amino acid sequences of SEQ ID NOS: 40 and 41 in neutralizing the enzymatic activity of CD73, optionally wherein the antibody has an EC_{50} for neutralizing the enzymatic activity of CD73 that is within 1-log, 0.5-log of the EC_{50} of parental antibody. In one embodiment, the antibody is more potent than parental antibody having the respective VH and VL amino acid sequences of SEQ ID NOS: 40 and 41 in neutralizing the enzymatic activity of CD73, optionally wherein the antibody has an EC_{50} for neutralizing the enzymatic activity of CD73 that is lower than that of parental antibody having the respective VH and VL amino acid sequences of SEQ ID NOS: 40 and 41.

In one embodiment, the antibody is substantially as potent or is at least as potent as parental antibody having the respective VH and VL amino acid sequences of SEQ ID NOS: 27 and 28 in neutralizing the enzymatic activity of CD73, optionally wherein the antibody has an EC_{50} for neutralizing the enzymatic activity of CD73 that is within 1-log or 0.5-log of the EC_{50} of parental antibody. In one embodiment, the antibody is more potent than parental antibody having the respective VH and VL amino acid sequences of SEQ ID NOS: 27 and 28 in neutralizing the enzymatic activity of CD73, optionally wherein the antibody has an EC_{50} for neutralizing the enzymatic activity of CD73 that is less than that of parental antibody having the respective VH and VL amino acid sequences of SEQ ID NOS: 27 and 28.

Also provided are isolated and/or recombinant nucleic acids (e.g., sets of nucleic acids) encoding an antibody (or a heavy or light chain thereof) or a VH and/or VL domain of the disclosure, a vector or set of vectors comprising such a nucleic acid(s), a cell comprising such a vector, and a method of producing a human anti-CD73 antibody or antibody fragment, comprising culturing such a cell under conditions suitable for expression of the anti-CD73 antibody or antibody fragment. The disclosure also relates to compositions, such as pharmaceutically acceptable compositions and kits, comprising such proteins, nucleic acids, vectors, and/or cells and typically one or more additional ingredients that can be active ingredients or inactive ingredients that promote formulation, delivery, stability, or other characteristics of the composition (e.g., various carriers). The disclosure further relates various new and useful methods making and using such antibodies, nucleic acids, vectors, cells, organisms, and/or compositions, such as in the modulation of CD73-mediated biological activities, for example in the treatment of diseases related thereto, notably cancers.

The disclosure also provides methods of producing or testing an antibody or antibody fragment which binds and neutralizes the enzymatic activity of CD73.

The disclosure also provides a method of potentiating the activity of lymphocytes (e.g., T cells) in a subject in need thereof, or for restoring the activity of lymphocytes (e.g., T

cells), or a method of relieving the adenosine-mediated inhibition of lymphocyte activity (e.g., T cells), which method comprises administering to the subject an effective amount of any of the foregoing compositions. In one embodiment, the subject is a patient suffering from cancer. For example, the patient may be suffering from a solid tumor, e.g., colorectal cancer, renal cancer, ovarian cancer, lung cancer, breast cancer or malignant melanoma. Alternatively, the patient may be suffering from a hematopoietic cancer, e.g., acute myeloid leukaemia, chronic myeloid leukaemia, multiple myeloma, or non-Hodgkin's lymphoma.

These aspects are more fully described in, and additional aspects, features, and advantages will be apparent from, the description provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B show titration curves obtained with humanized variants having H0, H1, H2, H3 and H4 heavy chain variable regions, each combined with each of the L0, L1, L2, L3 and L4 light chain variable regions, and parental murine antibody HPLP, as tested on transfected cell lines expressing human (Figure 1A) or cynomolgus (Figure 1B) CD73 protein.

Figure 2 shows blockade of enzymatic activity of soluble recombinant CD73. The upper left-hand panel (A) shows a high level of luminescence was measured when ATP and CTG substrates were mixed. When AMP was added to the reaction mix, the CTG reaction was inhibited resulting in a decrease of luminescence. In the presence of rhCD73 protein that hydrolyzes AMP, the level of luminescence was restored. Blockade of CD73 enzymatic activity by the different humanized variants is shown.

Figures 3A-3C and 4A-4C show two series of experiments to compare the humanized antibodies in their potency in reversing AMP-mediated inhibition of T cell proliferation. Figures 3A and 4A shows control of T cell proliferation and its inhibition by AMP for each series of experiments. Figures 3B and 4B show efficacy of anti-CD73 antibodies to restore CD4+ and Figure 3C and 4C show efficacy of anti-CD73 antibodies to restore CD8+ T cell proliferation. Cell proliferation is determined by dilution of Cell Trace Violet marker. Data are expressed as means of duplicates +/- standard deviation.

Figures 5A-5C show T cell proliferation is restored by all anti-CD73 Abs. Figure 5A shows control of T cell sub-population proliferation and its inhibition by AMP. Figure 5B shows efficacy of H4+Lx antibodies and parental antibody HPLP to restore CD4+ and CD8+ T cell proliferation. Figure 5C shows efficacy of 2H4+2Lx antibodies (the 2H4+ chain combined with 2L1, 2L2, 2L3 or 2L4 chains) and parental antibody 2HP2LP to restore CD4+ and CD8+ T cell proliferation. Data are expressed as mean of duplicates +/- standard deviation.

Figures 6A and 6B show T cell proliferation restored by humanized variants is reproducible, respectively in two representative human donors. Shown is efficacy of 2H4+2Lx anti-CD73 antibody variants to restore CD4 and CD8 T cell proliferation. Data are expressed as mean of duplicates +/- standard deviation.

5 Figure 7 shows CD4+ T cell proliferation is inhibited by ATP and restored with 2H4+2Lx antibody variants. Control of CD4+ T cell proliferation and inhibition by 100 μ M of ATP, shown as tested for two representative donors, D795 (Figure 7A) and D664 (Figure 7B). Data are expressed as mean of duplicates +/- standard deviation.

10 DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used in the specification, "a" or "an" may mean one or more. As used in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more.

15 Where "comprising" is used, this can optionally be replaced by "consisting essentially of" or by "consisting of".

Human CD73, also known as ecto-5'-nucleotidase and as 5-prime-ribonucleotide phosphohydrolase, EC 3.1.3.5, encoded by the *NT5E* gene, exhibits 5'-nucleotidase, notably AMP-, NAD-, and NMN-nucleosidase, activities. CD73 catalyzes the conversion at neutral pH of purine 5-prime mononucleotides to nucleosides, the preferred substrate being AMP. The enzyme consists of a dimer of 2 identical 70-kD subunits bound by a glycosyl phosphatidyl inositol linkage to the external face of the plasma membrane. The amino acid sequence of Human CD73 preprotein (monomer), including a signal sequence at amino acids 1-26, is shown in Genbank under accession number NP_002517, the entire disclosure

25 of which is incorporated herein by reference, and as follows:

30 MCPRAARAPA TLLLALGAVL WPAAGAWELT ILHTNDVHSR LEQTSSEDSSK CVNASRCMGG
VARLFTKVQQ IRRAEPNVLL LDAGDQYQGT IWFTVYKGAE VAHFMNALRY DAMALGNHEF
DNGVEGLIEP LLKEAKFPIL SANIKAKGPL ASQISGLYLP YKVLPGDEV VGIVGYTSKE
TPFLSNPGTN LVFEDEITAL QPEVDKCLKL NVNKIIALGH SGFEMDKLIA QKVRGVDVVV
GGHSNTFLYT GNPPSKEVPA GKYPFIVTSD DGRKVPVQA YAFGKYLGYL KIEFDERGNV
ISSHGNPILL NSSIPEDPSI KADINKWRIK LDNYSTQELG KTIVYLDGSS QSCRFRECNM
GNLICDAMIN NNLRHTDEMF WNHVSMCILN GGGIRSPIDE RNNGTITWEN LAAVLPGGT
FDLVQLKGST LKKA FEHSVH RYGQSTGEFL QVGGIHVVDYD LSRKPGDRVV KLDVLCTKCR
VPSYDPLKMD EVYKVILPNF LANGGDGFQM IKDELLRHDS GDQDINVVST YISKMKVIYP
35 AVEGRIKFST GSHCHGSFSL IFLSLWAVIF VLYQ (SEQ ID NO: 1).

In the context herein, "neutralize the enzymatic activity of CD73", refers to a process in which the 5'-nucleotidase (5'-ectonucleotidase) activity of CD73 is inhibited. This comprises, notably the inhibition of CD73-mediated generation of adenosine, i.e. the

inhibition of CD73-mediated catabolism of AMP to adenosine. This can be measured for example in a cell-free assay that measures the capacity of a test compound to inhibit the conversion of AMP to adenosine, either directly or indirectly. In one embodiment, an antibody preparation causes at least a 50% decrease in the conversion of AMP to adenosine, at least a 70% decrease in the conversion of AMP to adenosine, or at least a 80% decrease in the conversion of AMP to adenosine, referring, for example, to the assays described herein.

Whenever within this whole specification "treatment of cancer" or the like is mentioned with reference to anti-CD73 binding agent (e.g., antibody), there is meant: (a) method of treatment of cancer, said method comprising the step of administering (for at least one treatment) an anti-CD73 binding agent, (preferably in a pharmaceutically acceptable carrier material) to an individual, a mammal, especially a human, in need of such treatment, in a dose that allows for the treatment of cancer, (a therapeutically effective amount), preferably in a dose (amount) as specified herein; (b) the use of an anti-CD73 binding agent for the treatment of cancer, or an anti-CD73 binding agent, for use in said treatment (especially in a human); (c) the use of an anti-CD73 binding agent for the manufacture of a pharmaceutical preparation for the treatment of cancer, a method of using an anti-CD73 binding agent for the manufacture of a pharmaceutical preparation for the treatment of cancer, comprising admixing an anti-CD73 binding agent with a pharmaceutically acceptable carrier, or a pharmaceutical preparation comprising an effective dose of an anti-CD73 binding agent that is appropriate for the treatment of cancer; or (d) any combination of a), b), and c), in accordance with the subject matter allowable for patenting in a country where this application is filed.

The term "antibody," as used herein, refers to polyclonal and monoclonal antibodies. Depending on the type of constant domain in the heavy chains, antibodies are assigned to one of five major classes: IgA, IgD, IgE, IgG, and IgM. Several of these are further divided into subclasses or isotypes, such as IgG1, IgG2, IgG3, IgG4, and the like. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids that is primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are termed "alpha," "delta," "epsilon," "gamma" and "mu," respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. IgG are the exemplary classes of antibodies

employed herein because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting. Optionally the antibody is a monoclonal antibody. Particular examples of antibodies are humanized, chimeric, human, or otherwise-human-suitable antibodies. "Antibodies" also includes any fragment or derivative of any of the herein described antibodies.

The term "specifically binds to" means that an antibody can bind preferably in a competitive binding assay to the binding partner, e.g., CD73, as assessed using either recombinant forms of the proteins, epitopes therein, or native proteins present on the surface of isolated target cells. Competitive binding assays and other methods for determining specific binding are further described below and are well known in the art.

When an antibody is said to "compete with" a particular monoclonal antibody, it means that the antibody competes with the monoclonal antibody in a binding assay using either recombinant CD73 molecules or surface expressed CD73 molecules. For example, if a test antibody reduces the binding of a reference antibody to a CD73 polypeptide or CD73-expressing cell in a binding assay, the antibody is said to "compete" respectively with the reference antibody.

The term "affinity", as used herein, means the strength of the binding of an antibody to an epitope. The affinity of an antibody is given by the dissociation constant K_d , defined as $[Ab] \times [Ag] / [Ab-Ag]$, where $[Ab-Ag]$ is the molar concentration of the antibody-antigen complex, $[Ab]$ is the molar concentration of the unbound antibody and $[Ag]$ is the molar concentration of the unbound antigen. The affinity constant K_a is defined by $1/K_d$. Methods for determining the affinity of mAbs can be found in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.* 92:589-601 (1983), which references are entirely incorporated herein by reference. One standard method well known in the art for determining the affinity of mAbs is the use of surface plasmon resonance (SPR) screening (such as by analysis with a BIAcore™ SPR analytical device).

Within the context herein a "determinant" designates a site of interaction or binding on a polypeptide.

The term "epitope" refers to an antigenic determinant, and is the area or region on an antigen to which an antibody binds. A protein epitope may comprise amino acid residues directly involved in the binding as well as amino acid residues which are effectively blocked by the specific antigen binding antibody or peptide, *i.e.*, amino acid residues within the "footprint" of the antibody. It is the simplest form or smallest structural area on a complex antigen molecule that can combine with e.g., an antibody or a receptor. Epitopes can be

linear or conformational/structural. The term "linear epitope" is defined as an epitope composed of amino acid residues that are contiguous on the linear sequence of amino acids (primary structure). The term "conformational or structural epitope" is defined as an epitope composed of amino acid residues that are not all contiguous and thus represent separated parts of the linear sequence of amino acids that are brought into proximity to one another by folding of the molecule (secondary, tertiary and/or quaternary structures). A conformational epitope is dependent on the 3-dimensional structure. The term 'conformational' is therefore often used interchangeably with 'structural'.

The term "deplete" or "depleting", with respect to CD73-expressing cells, means a process, method, or compound that results in killing, elimination, lysis or induction of such killing, elimination or lysis, so as to negatively affect the number of such CD73-expressing cells present in a sample or in a subject.

The term "internalization", used interchangeably with "intracellular internalization", refers to the molecular, biochemical and cellular events associated with the process of translocating a molecule from the extracellular surface of a cell to the intracellular surface of a cell. The processes responsible for intracellular internalization of molecules are well-known and can involve, *inter alia*, the internalization of extracellular molecules (such as hormones, antibodies, and small organic molecules); membrane-associated molecules (such as cell-surface receptors); and complexes of membrane-associated molecules bound to extracellular molecules (for example, a ligand bound to a transmembrane receptor or an antibody bound to a membrane-associated molecule). Thus, "inducing and/or increasing internalization" comprises events wherein intracellular internalization is initiated and/or the rate and/or extent of intracellular internalization is increased.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. The term "therapeutic agent" refers to an agent that has biological activity.

For the purposes herein, a "humanized" or "human" antibody refers to an antibody in which the constant and variable framework region of one or more human immunoglobulins is fused with the binding region, e.g., the CDR, of an animal immunoglobulin. Such antibodies are designed to maintain the binding specificity of the non-human antibody from which the binding regions are derived, but to avoid an immune reaction against the non-human antibody. Such antibodies can be obtained from transgenic mice or other animals that have been "engineered" to produce specific human antibodies in response to antigenic challenge (see, e.g., Green et al. (1994) Nature Genet 7:13; Lonberg et al. (1994) Nature 368:856; Taylor et al. (1994) Int Immun 6:579, the entire teachings of which are herein incorporated by reference). A fully human antibody also can be constructed by genetic or chromosomal

transfection methods, as well as phage display technology, all of which are known in the art (see, e.g., McCafferty et al. (1990) Nature 348:552-553). Human antibodies may also be generated by in vitro activated B cells (see, e.g., U.S. Pat. Nos. 5,567,610 and 5,229,275, which are incorporated in their entirety by reference).

5 The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity-determining region" or "CDR" (e.g., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; Kabat et al. 1991) and/or those residues from a "hypervariable loop" (e.g., residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, J. Mol. Biol 1987;196:901-917), or a similar system for determining essential amino acids responsible for antigen binding. Typically, the numbering of amino acid residues in this region is performed by the method described in Kabat et al., supra. Phrases such as "Kabat position", "variable domain residue numbering as in Kabat" and "according to Kabat" herein refer to this numbering system for heavy chain variable domains or light chain variable domains. Using the Kabat numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of CDR H2 and inserted residues (e.g., residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

25 By "framework" or "FR" residues as used herein is meant the region of an antibody variable domain exclusive of those regions defined as CDRs. Each antibody variable domain framework can be further subdivided into the contiguous regions separated by the CDRs (FR1, FR2, FR3 and FR4).

30 The terms "Fc domain," "Fc portion," and "Fc region" refer to a C-terminal fragment of an antibody heavy chain, e.g., from about amino acid (aa) 230 to about aa 450 of human γ (gamma) heavy chain or its counterpart sequence in other types of antibody heavy chains (e.g., α , δ , ϵ and μ for human antibodies), or a naturally occurring allotype thereof. Unless otherwise specified, the commonly accepted Kabat amino acid numbering for immunoglobulins is used throughout this disclosure (see Kabat et al. (1991) Sequences of

Protein of Immunological Interest, 5th ed., United States Public Health Service, National Institute of Health, Bethesda, MD).

5 The terms "isolated", "purified" or "biologically pure" refer to material that is substantially or essentially free from components which normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified.

10 The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

15 The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under
20 expressed or not expressed at all.

Within the context herein, the term antibody that "binds" a polypeptide or epitope designates an antibody that binds said determinant with specificity and/or affinity.

25 The term "identity" or "identical", when used in a relationship between the sequences of two or more polypeptides, refers to the degree of sequence relatedness between polypeptides, as determined by the number of matches between strings of two or more amino acid residues. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to,
30 those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer,
35 Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., SIAM J. Applied Math. 48, 1073 (1988).

Methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer programs. Computer program methods for determining identity between two sequences include the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res. 12, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol. 215, 403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra). The well-known Smith Waterman algorithm may also be used to determine identity.

Production of antibodies

The present invention is based, in part, on the discovery of modified human acceptor framework sequences into which antibody CDRs can be incorporated such that the resulting anti-CD73 variable region has high potency in neutralization of the enzymatic activity of human CD73. The antibodies have the advantage of having low or decreased immunogenicity in humans, e.g. having lower ability or likelihood to cause an unwanted anti-CD73 antibody directed immune response upon administration to a human. Examples of such antibodies of the disclosure include antibodies comprising the H4+ or 2H4+ VH domain combined with a L1, L2, L3, L4, 2L1, 2L2, 2L3 or 2L4 VL domain. Examples of antibodies of the disclosure include antibodies comprising the VH and VL domain pairs of any one of the antibodies H4+L1, H4+L2, H4+L3, H4+L4, 2H4+L1, 2H4+L2, 2H4+L3 or 2H4+L4.

In one aspect, an antibody or antibody fragment that binds a human CD73 polypeptide comprises VH and VL frameworks (e.g., FR1, FR2, FR3 and FR4) of human origin. In one aspect, the antibody or antibody fragment comprises: a HCDR1 (heavy chain CDR1) comprising an amino acid sequence SYNMY as set forth in SEQ ID NO: 2; a HCDR2 (heavy chain CDR2) comprising an amino acid sequence YIDPYNGGSSYNQKFKG as set forth in SEQ ID NO: 12, optionally further wherein the glutamine residue (Q) at position 13 of SEQ ID NO: 12 may be substituted by a leucine residue (L), wherein the lysine residue (K) at position 14 of SEQ ID NO: 12 may optionally be substituted by a threonine residue (T); a HCDR3 (heavy chain CDR3) comprising an amino acid sequence GYNKYKAWFAY as set forth in SEQ ID NO: 13, optionally further wherein the asparagine residue (N) at position 3 of SEQ ID NO: 13 may be substituted by a glycine residue (G); a LCDR1 (light chain CDR1) comprising an amino acid sequence KASQSVTNDVA as set forth in SEQ ID NO: 14, optionally further wherein the threonine residue (T) at position 7 of SEQ ID NO: 14 may be substituted by a serine residue (S); a LCDR2 (light chain CDR2) comprising an amino acid

sequence YASNRYT as set forth in SEQ ID NO: 15, optionally wherein the asparagine residue (N) at position 4 of SEQ ID NO: 15 may be substituted by an threonine residue (T); and a LCDR3 (light chain CDR3) comprising an amino acid sequence QQDYSSLT as set forth in SEQ ID NO: 7.

5 In one embodiment, a HCDR2 comprises an amino acid sequence of Formula I:

$$Y - I - D - P - Y - N - G - G - S - S - Y - N - Xaa_1 - Xaa_2 - F - K - G$$
 (SEQ ID NO: 3), or a subsequence thereof, wherein Xaa₁ is Q (Gln) or L (Leu) and wherein Xaa₂ is K (Lys) or T (Thr).

In one embodiment, a HCDR3 comprises an amino acid sequence of Formula II:
 10
$$G - Y - Xaa_1 - N - Y - K - A - W - F - A - Y$$
 (SEQ ID NO: 4), or a subsequence thereof, wherein Xaa₁ is N (Asp) or G (Gly).

In one embodiment, a LCDR1 comprises an amino acid sequence of Formula III:

$$K - A - S - Q - S - V - Xaa_1 - N - D - V - A$$
 (SEQ ID NO: 5),

or a subsequence thereof, wherein Xaa₁ is T (Thr) or S (Ser).

15 In one embodiment, a LCDR2 comprises an amino acid sequence of Formula IV:

$$Y - A - S - Xaa_1 - R - Y - T$$
 (SEQ ID NO: 6),

or a subsequence thereof, wherein Xaa₁ is T (Thr) or N (Asn).

In one aspect, an isolated antibody that binds a human CD73 polypeptide comprises:
 a HCDR1 comprising an amino acid sequence SYNMY as set forth in SEQ ID NO: 2; a
 20 HCDR2 comprising an amino acid sequence YIDPYNGGSSYNLTFKG as set forth in SEQ ID NO: 8; a HCDR3 comprising an amino acid sequence GYGNYKAWFAY as set forth in SEQ ID NO: 9; a LCDR1 comprising an amino acid sequence KASQSVSNDVA as set forth in SEQ ID NO: 10; a LCDR2 comprising an amino acid sequence YASTRYT as set forth in SEQ ID NO: 11; and a LCDR3 comprising an amino acid sequence QQDYSSLT as set forth in SEQ ID NO: 7.

In one aspect, an isolated antibody that binds a human CD73 polypeptide comprises:
 a HCDR1 comprising an amino acid sequence SYNMY as set forth in SEQ ID NO: 2; a
 HCDR2 comprising an amino acid sequence YIDPYNGGSSYNQKFKG as set forth in SEQ ID NO: 12; a HCDR3 comprising an amino acid sequence GYNKYKAWFAY as set forth in
 30 SEQ ID NO: 13; a LCDR1 comprising an amino acid sequence KASQSVTNDVA as set forth in SEQ ID NO: 14; a LCDR2 comprising an amino acid sequence YASNRYT as set forth in SEQ ID NO: 15; and a LCDR3 comprising an amino acid sequence QQDYSSLT as set forth in SEQ ID NO: 7.

In one embodiment, the antibody comprises a heavy chain framework from the
 35 human subgroup IGHV1-3 (optionally together with IGHJ4), optionally the IGHV1-3 is IGHV1-3*01. In one embodiment, the humanized antibody comprises a light chain

framework from the human subgroup IGKV1-33 (optionally together with IGKJ2), optionally from IGKV1-33*01.

The antibody may further comprise one, two, three, four, five or more amino acid substitutions across the human heavy and/or light chain frameworks, to, e.g., enhance affinity, stability, or other properties of the antibody.

Examples of VH and VL amino acid sequences of anti-CD73 antibodies are shown in Example 6 (showing the H4+ chain, and Table 5 for 2H4+2Lx antibodies), and Table 1 for L1-L4 chains.

In one aspect, the invention provides an antigen binding domain or antibody or antibody fragment that binds a human CD73 polypeptide, comprising:

- (a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:2;
- (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NOS: 3, 8 or 12,
- (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NOS: 4, 9 or 13;
- (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NOS: 5, 10 or 14;
- (e) a CDR-L2 comprising the amino acid sequence of SEQ ID NOS: 6, 11 or 15;
- (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:7; and
- (g) human heavy and light chain framework sequences.

In one embodiment, the antibody comprises a heavy chain framework from the human subgroup IGHV1-3 together with IGHJ4, optionally the antibodies comprises IGHV1-3*01, together with IGHJ4. In one embodiment, the humanized antibody comprises a light chain framework from the human subgroup IGKV1-33, optionally IGKV1-33*01, together with IGKJ2.

Optionally, in any embodiment, the antibody can be specified as being an antibody other than a murine parental antibody, e.g. a murine parental antibody having the respective VH and VL of SEQ ID NOS: 27 and 28), or having the respective VH and VL of SEQ ID NOS: 40 and 41).

Optionally a human framework comprises one or more mutations, e.g., back mutations to introduce a residue present at the particular position in a non-human mammal (e.g., a mouse).

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 2 is an isoleucine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 30 is an alanine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 48 is an isoleucine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain

position 69 is a leucine.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 73 is a lysine.

5 In one embodiment, a VH comprises an isoleucine residue at Kabat position 2, an alanine at position 30, an isoleucine at position 48, a leucine at position 69 and a lysine at position 73.

In one aspect of any embodiment herein, the amino acid at Kabat light chain position 67 is a tyrosine.

10 In one aspect of any embodiment herein, the amino acid at Kabat light chain position 60 is serine or aspartic acid.

In one aspect of any embodiment herein, the amino acid at Kabat light chain position 2 is valine or isoleucine.

In one aspect of any embodiment herein, the amino acid at Kabat light chain position 87 is tyrosine or phenylalanine.

15 In one embodiment, a VL comprises a tyrosine residue at Kabat position 67, a serine at position 60, an isoleucine at position 2, and a tyrosine at position 87.

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 28 is threonine (T).

20 In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 66 is arginine (R).

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 67 is valine (V).

In one aspect of any embodiment herein, the amino acid at Kabat heavy chain position 71 is arginine (R).

25 Positions in the VH and VL domains herein are described using the Kabat numbering system (Kabat et al. (1991) Sequences of Protein of Immunological Interest, 5th ed., United States Public Health Service, National Institute of Health, Bethesda, MD).

30 In one aspect, the anti-CD73 antibody comprises a VH domain having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the VH domain of SEQ ID NOS: 37 or 42.

In one aspect, the anti-CD73 antibody or antibody fragment comprises a VL domain having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the VL domain of any of SEQ ID NOS: 33-36 or 43-46.

35 In one aspect, the anti-CD73 antibody or antibody fragment comprises a VH domain having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the VH domain of SEQ ID NO: 42, and a VL domain

having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the VL domain of any of SEQ ID NOS: 43-46.

In one aspect, the anti-CD73 antibody or antibody fragment comprises a heavy chain having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98%
5 or 99% identity, or 100% identity) to the heavy chain of SEQ ID NO: 38 of the H4+L1 antibody. In one embodiment, the antibody or antibody fragment comprises a light chain having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the light chain of SEQ ID NO: 39 of the H4+L1 antibody.

In one aspect, the anti-CD73 antibody or antibody fragment comprises a heavy chain
10 having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the heavy chain of SEQ ID NO: 47 of the 2H4+2L1 antibody. In one embodiment, the antibody or antibody fragment comprises a light chain having at least about 80% sequence identity (e.g., at least about 85%, 90%, 95%, 97%, 98% or 99% identity, or 100% identity) to the light chain of SEQ ID NO: 48 of the 2H4+2L1
15 antibody.

DNA encoding an antibody can be prepared and placed in an appropriate expression vector for transfection into an appropriate host. The host is then used for the recombinant production of the antibody, or variants thereof, such as a humanized version of that monoclonal antibody, active fragments of the antibody, chimeric antibodies comprising the
20 antigen recognition portion of the antibody, or versions comprising a detectable moiety.

DNA encoding the monoclonal antibodies of the disclosure can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). In one aspect, provided is a nucleic acid encoding a heavy chain or a light chain
25 of an anti-CD73 antibody of any embodiment herein. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Such DNA sequences can be modified for any of a large
30 number of purposes, e.g., for humanizing antibodies, producing fragments or derivatives, or for modifying the sequence of the antibody, e.g., in the antigen binding site in order to optimize the binding specificity of the antibody. In one embodiment, provided is an isolated nucleic acid sequence encoding a light chain and/or a heavy chain of an antibody, as well as a recombinant host cell comprising (e.g., in its genome) such nucleic acid. Recombinant
35 expression in bacteria of DNA encoding the antibody is well known in the art (see, for example, Skerra et al., *Curr. Opin. Immunol.*, 5, pp. 256 (1993); and Pluckthun,

Immunol. 130, p. 151 (1992).

Fragments and derivatives of antibodies (which are encompassed by the term “antibody” or “antibodies” as used in this application, unless otherwise stated or clearly contradicted by context) can be produced by techniques that are known in the art.

5 “Fragments” comprise a portion of the intact antibody, generally the antigen binding site or variable region. Examples of antibody fragments include Fab, Fab', Fab'-SH, F (ab')₂, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a “single-chain antibody fragment” or “single chain polypeptide”); and
10 multispecific (e.g., bispecific) antibodies formed from antibody fragments.

Typically, an anti-CD73 antibody provided herein has an affinity for a CD73 polypeptide (e.g., a CD73 polypeptide as produced in the Examples herein) in the range of about 10^4 to about 10^{11} M⁻¹ (e.g., about 10^8 to about 10^{10} M⁻¹). For example, in a particular aspect the disclosure provides Anti-CD73 antibody that have an average disassociation
15 constant (K_D) of less than 1×10^{-9} M with respect to CD73, as determined by, e.g., surface plasmon resonance (SPR) screening (such as by analysis with a BIAcore™ SPR analytical device). In a more particular exemplary aspect, the disclosure provides anti-CD73 antibodies that have a K_D of about 1×10^{-8} M to about 1×10^{-10} M, or about 1×10^{-9} M to about 1×10^{-11} M, for CD73.

20 Antibodies can be characterized for example by a mean K_D of no more than about (i.e. better affinity than) 100, 60, 10, 5, or 1 nanomolar, preferably sub-nanomolar or optionally no more than about 500, 200, 100 or 10 picomolar. K_D can be determined for example for example by immobilizing recombinantly produced human CD73 proteins on a chip surface, followed by application of the antibody to be tested in solution. In one
25 embodiment, the method further comprises a step (d), selecting antibodies from (b) that are capable of competing for binding to CD73 with control antibody.

In one embodiment, the anti-CD73 antibodies can be prepared such that they do not have substantial binding to human Fcγ receptors, e.g., any one or more of CD16A, CD16B, CD32A, CD32B and/or CD64). Such antibodies may comprise constant regions of various
30 heavy chains that are known to lack or have low binding to Fcγ receptors. Alternatively, antibody fragments that do not comprise (or comprise portions of) constant regions, such as F(ab')₂ fragments, can be used to avoid Fc receptor binding. Fc receptor binding can be assessed according to methods known in the art, including for example testing binding of an antibody to Fc receptor protein in a BIAcore assay. Also, generally any antibody IgG
35 isotype can be used in which the Fc portion is modified (e.g., by introducing 1, 2, 3, 4, 5 or more amino acid substitutions) to minimize or eliminate binding to Fc receptors (see, e.g.,

WO 03/101485, the disclosure of which is herein incorporated by reference). Assays such to assess Fc receptor binding are well known in the art, and are described in, e.g., WO 03/101485.

In one embodiment, the antibody can comprise one or more specific mutations in the Fc region that result in "Fc silent" antibodies that have minimal interaction with effector cells. Silenced effector functions can be obtained by mutation in the Fc region of the antibodies and have been described in the art: N297A mutation, the LALA mutations, (Strohl, W., 2009, Curr. Opin. Biotechnol. Vol. 20(6):685-691); and D265A (Baudino et al., 2008, J. Immunol. 181: 6664-69) see also Heusser et al., WO2012/065950, the disclosures of which are incorporated herein by reference. In one embodiment, an antibody comprises one, two, three or more amino acid substitutions in the hinge region. In one embodiment, the antibody is an IgG1 or IgG2 and comprises one, two or three substitutions at residues 233-236, optionally 233-238 (EU numbering). In one embodiment, the antibody is an IgG4 and comprises one, two or three substitutions at residues 327, 330 and/or 331 (EU numbering). Examples of silent Fc IgG1 antibodies are the LALA mutant comprising L234A and L235A mutation in the IgG1 Fc amino acid sequence. Another example of an Fc silent mutation is a mutation at residue D265, or at D265 and P329 for example as used in an IgG1 antibody as the DAPA (D265A, P329A) mutation (US 6,737,056). Another silent IgG1 antibody comprises a mutation at residue N297 (e.g., N297A, N297S mutation), which results in aglycosylated/non-glycosylated antibodies. Other silent mutations include: substitutions at residues L234 and G237 (L234A/G237A); substitutions at residues S228, L235 and R409 (S228P/L235E/R409K,T,M,L); substitutions at residues H268, V309, A330 and A331 (H268Q/V309L/A330S/A331S); substitutions at residues C220, C226, C229 and P238 (C220S/C226S/C229S/P238S); substitutions at residues C226, C229, E233, L234 and L235 (C226S/C229S/E233P/L234V/L235A; substitutions at residues K322, L235 and L235 (K322A/L234A/L235A); substitutions at residues L234, L235 and P331 (L234F/L235E/P331S); substitutions at residues 234, 235 and 297; substitutions at residues E318, K320 and K322 (L235E/E318A/K320A/K322A); substitutions at residues (V234A, G237A, P238S); substitutions at residues 243 and 264; substitutions at residues 297 and 299; substitutions such that residues 233, 234, 235, 237, and 238 defined by the EU numbering system, comprise a sequence selected from PAAAP, PAAAS and SAAAS (see WO2011/066501).

In one embodiment, the antibody can comprise one or more specific mutations in the Fc region. For example, such an antibody can comprise an Fc domain of human IgG1 origin, comprises a mutation at Kabat residue(s) 234, 235, 237, 330 and/or 331. One example of such an Fc domain comprises substitutions at Kabat residues L234, L235 and P331 (e.g.,

L234A/L235E/P331S or (L234F/L235E/P331S). Another example of such an Fc domain comprises substitutions at Kabat residues L234, L235, G237 and P331 (e.g., L234A/L235E/G237A/P331S). Another example of such an Fc domain comprises substitutions at Kabat residues L234, L235, G237, A330 and P331 (e.g.,

5 L234A/L235E/G237A/A330S/P331S). In one embodiment, the antibody comprises an Fc domain, optionally of human IgG1 isotype, comprising: a L234X₁ substitution, a L235X₂ substitution, and a P331X₃ substitution, wherein X₁ is any amino acid residue other than leucine, X₂ is any amino acid residue other than leucine, and X₃ is any amino acid residue other than proline; optionally wherein X₁ is an alanine or phenylalanine or a conservative

10 substitution thereof; optionally wherein X₂ is glutamic acid or a conservative substitution thereof; optionally wherein X₃ is a serine or a conservative substitution thereof. In another embodiment, the antibody comprises an Fc domain, optionally of human IgG1 isotype, comprising: a L234X₁ substitution, a L235X₂ substitution, a G237X₄ substitution and a P331X₄ substitution, wherein X₁ is any amino acid residue other than leucine, X₂ is any

15 amino acid residue other than leucine, X₃ is any amino acid residue other than glycine, and X₄ is any amino acid residue other than proline; optionally wherein X₁ is an alanine or phenylalanine or a conservative substitution thereof; optionally wherein X₂ is glutamic acid or a conservative substitution thereof; optionally, X₃ is alanine or a conservative substitution thereof; optionally X₄ is a serine or a conservative substitution thereof. In another

20 embodiment, the antibody comprises an Fc domain, optionally of human IgG1 isotype, comprising: a L234X₁ substitution, a L235X₂ substitution, a G237X₄ substitution, G330X₄ substitution, and a P331X₅ substitution, wherein X₁ is any amino acid residue other than leucine, X₂ is any amino acid residue other than leucine, X₃ is any amino acid residue other than glycine, X₄ is any amino acid residue other than alanine, and X₅ is any amino acid

25 residue other than proline; optionally wherein X₁ is an alanine or phenylalanine or a conservative substitution thereof; optionally wherein X₂ is glutamic acid or a conservative substitution thereof; optionally, X₃ is alanine or a conservative substitution thereof; optionally, X₄ is serine or a conservative substitution thereof; optionally X₅ is a serine or a conservative substitution thereof.

30 In the shorthand notation used here, the format is: Wild type residue: Position in polypeptide: Mutant residues, wherein residue positions are indicated according to EU numbering according to Kabat.

In one embodiment, an antibody comprises a heavy chain constant region comprising the amino acid sequence below, or an amino acid sequence at least 90%, 95% or 99% identical thereto but retaining the amino acid residues at Kabat positions 234, 235

35 and 331 (underlined):

31

A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P
 E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V
 V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V E P K S
 C D K T H T C P P C P A P E A E G G P S V F L F P P K P K D T L M I
 5 S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A
 K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C
 K V S N K A L P A S I E K T I S K A K G Q P R E P Q V Y T L P P S R
 E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N
 N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F
 10 S C S V M H E A L H N H Y T Q K S L S L S P G K (SEQ ID NO: 16).

In one embodiment, an antibody comprises a heavy chain constant region comprising the amino acid sequence below, or an amino acid sequence at least 90%, 95% or 99% identical thereto but retaining the amino acid residues at Kabat positions 234, 235 and 331 (underlined):

15 A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P
 E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V
 V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V E P K S
 C D K T H T C P P C P A P E F E G G P S V F L F P P K P K D T L M I
 S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A
 20 K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C
 K V S N K A L P A S I E K T I S K A K G Q P R E P Q V Y T L P P S R
 E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N
 N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F
 S C S V M H E A L H N H Y T Q K S L S L S P G K (SEQ ID NO: 17).

25 In one embodiment, an antibody comprises a heavy chain constant region comprising the amino acid sequence below, or an amino acid sequence at least 90%, 95% or 99% identical thereto but retaining the amino acid residues at Kabat positions 234, 235, 237, 330 and 331 (underlined):

30 A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P
 E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V
 V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V E P K S
 C D K T H T C P P C P A P E A E G A P S V F L F P P K P K D T L M I
 S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A
 K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C
 35 K V S N K A L P S S I E K T I S K A K G Q P R E P Q V Y T L P P S R
 E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N

N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F
S C S V M H E A L H N H Y T Q K S L S L S P G K (SEQ ID NO: 18).

In one embodiment, an antibody comprises a heavy chain constant region comprising the amino acid sequence below, or a sequence at least 90%, 95% or 99% identical thereto but retaining the amino acid residues at Kabat positions 234, 235, 237 and 331 (underlined):

A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P
E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V
V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V E P K S
10 C D K T H T C P P C P A P E A E G A P S V F L F P P K P K D T L M I
S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A
K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C
K V S N K A L P A S I E K T I S K A K G Q P R E P Q V Y T L P P S R
E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N
15 N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F
S C S V M H E A L H N H Y T Q K S L S L S P G K (SEQ ID NO: 19).

Fc silent antibodies result in no or low ADCC activity, meaning that an Fc silent antibody exhibits an ADCC activity that is below 50% specific cell lysis. Preferably an antibody substantially lacks ADCC activity, e.g., the Fc silent antibody exhibits an ADCC activity (specific cell lysis) that is below 5% or below 1 %. Fc silent antibodies can also result in lack of FcγR-mediated cross-linking of CD73 at the surface of a CD73-expressing cell.

In one embodiment, the antibody has a substitution in a heavy chain constant region at any one, two, three, four, five or more of residues selected from the group consisting of: 220, 226, 229, 233, 234, 235, 236, 237, 238, 243, 264, 268, 297, 298, 299, 309, 310, 318, 320, 322, 327, 330, 331 and 409 (numbering of residues in the heavy chain constant region is according to EU numbering according to Kabat). In one embodiment, the antibody comprises a substitution at residues 234, 235 and 322. In one embodiment, the antibody comprises a substitution at residues 234, 235 and 331. In one embodiment, the antibody comprises a substitution at residues 234, 235, 237 and 331. In one embodiment, the antibody comprises a substitution at residues 234, 235, 237, 330 and 331. In one embodiment, the Fc domain is of human IgG1 subtype. Amino acid residues are indicated according to EU numbering according to Kabat.

In one embodiment, the antibody comprises an Fc domain comprising an amino acid substitution that increases binding to human FcRn polypeptides in order to increase the in vivo half-life of the antibody. Exemplary mutations are described in Strohl, W., 2009, Curr. Opin. Biotechnol. Vol. 20(6):685-691, the disclosure of which is incorporated herein by

reference. Examples of substitutions used in antibodies of human IgG1 isotype are substitutions at Kabat residues M252, S254 and T256; substitutions at residues T250 and M428; substitutions at residue N434; substitutions at residues H433 and N434; substitutions at residues T307, E380 and N434; substitutions at residues T307, E380, and N434; substitutions at residues M252, S254, T256, H433, N434 and 436; substitutions at residue I253; substitutions at residues P257, N434, D376 and N434. An antigen-binding compound can at any desired stage be assessed for its ability to inhibit the enzymatic activity of CD73, notably to block the 5'-nucleotidase activity of CD73 and to reduce the production of adenosine by a CD73-expressing cell, and in turn restore the activity of and/or relieve the adenosine-mediated inhibition of lymphocytes.

The ability of an antibody to inhibit the enzymatic activity of CD73 can be tested in a cell-free assay using recombinant soluble human CD73 (as dimers) and AMP, where conversion of AMP to adenosine (and/or inhibition thereof) is detected directly (e.g., by measurement of substrates and products, i.e. AMP, adenosine and/or phosphate), or indirectly. In one example, AMP and/or adenosine are detected via HPLC before and after incubation of the test compound with recombinant CD73.

The CD73-inhibitory activity of an antibody can also be assessed in any of a number of other ways. For example, in an indirect assay, a luciferase-based reagent is used (e.g., CellTiter-Glo® system available from Promega), to detect the disappearance of AMP. The luciferase reaction in the assay is inhibited by AMP. Adding the CD73 enzyme to the reaction degrades the AMP, and relieves the inhibition, producing a detectable signal.

The assays using soluble CD73 will include testing at conditions where the antibodies are provided at a substantial molar excess (e.g., 10-fold, 20-fold, 50-fold, 100-fold, etc.) to the CD73 polypeptide dimers. When provided in molar excess to the enzyme, the anti-CD73 antibodies will no longer be capable of forming multimeric complexes of antibodies and CD73 dimers; antibodies that retain inhibition of the enzymatic activity of CD73 can then be selected.

The ability of an antibody to inhibit the 5'-ectonucleotidase enzymatic activity of CD73 can alternatively or in addition also be tested in a cellular assay (using cells that express CD73). Advantageously, antibodies can be tested or screened first in the cell-free assay to identify antibodies that block the activity of the enzyme to reduce likelihood of selecting antibodies that inhibit CD73 by causing internalization of CD73, and then tested as purified antibody in cellular assays. Cellular assays can be carried out as shown in the Examples herein or as disclosed in PCT publication no. WO2016/055609, the disclosure of which is incorporated herein by reference. For example, CD73-expressing cells (e.g., MDA-MB-231 cell line) are plated in flat-bottom 96 well plates in presence of anti-CD73 antibodies and

incubated, as detailed in WO2016/055609. AMP is added to the cells and incubated at 4°C (to avoid CD73 down-modulation). Plates are then centrifuged and supernatant is transferred to flat bottom 96 well culture plate. Free phosphate produced by the hydrolysis of AMP into adenosine is then quantified. A decrease in hydrolysis of AMP into adenosine in the presence of antibody indicate the antibody inhibits cellular CD73.

In one embodiment, an antibody preparation causes at least a 50% decrease in the enzymatic activity of a CD73 polypeptide, preferably at least a 60%, 70% or 80% decrease in the enzymatic activity of a CD73 polypeptide (e.g., a soluble homodimeric CD73 polypeptide; CD73 expressed by cells).

The activity of an antibody can also be measured in an indirect assay for its ability to modulate the activity of lymphocytes, for example to relieve the adenosine-mediated inhibition of lymphocyte activity, or to cause the activation of lymphocyte activity. This can be addressed, for example, using a cytokine-release assay. In another example, an antibody can be evaluated in an indirect assay for its ability to modulate the proliferation of lymphocytes.

In one embodiment, an antibody neutralizes the 5'-ectonucleotidase activity of a homodimeric human CD73 polypeptide in solution. In one embodiment, the antibody binds and inhibits the enzymatic activity of a soluble human CD73 polypeptide, notably an antibody that neutralizes the CD73-mediated catabolism of AMP to adenosine. In one embodiment, the antibody binds CD73 in bivalent manner. In one embodiment, the antibody is a non-depleting antibody e.g., an Fc silent antibody that substantially lacks binding to human Fcγ receptors. In one embodiment, the antibody neutralizes CD73 in solution without reliance on induction of CD73 polypeptide : anti-CD73 antibody oligomers.

In one embodiment, an antibody specifically binds human CD73 at the surface of a cell and that is capable of neutralizing the 5'-ectonucleotidase activity of a soluble human CD73 polypeptide. In one embodiment, the antibody does not induce the oligomerization of the soluble CD73.

In one embodiment, an antibody specifically binds human CD73 at the surface of a cell and is capable of neutralizing the 5'-ectonucleotidase activity of cellular CD73 (CD73 expressed by cells). In one embodiment, an antibody specifically binds and neutralizes the 5'-ectonucleotidase activity of a human CD73 at the surface of a cell and is not internalized into CD73-expressing cells upon binding to CD73. The antibody does not cause multimerization and subsequent internalization of CD73. In one embodiment, an antibody binds and is capable of inhibiting the enzymatic activity of a recombinant human CD73 polypeptide in solution, wherein said antibody is not internalized into CD73-expressing cells. In one embodiment, the non-internalizing antibody binds CD73 in bivalent manner. In one

embodiment, the antibody is a non-depleting antibody, e.g., an Fc silent antibody. The antibody is capable of neutralizing the 5'-ectonucleotidase activity of a dimeric human CD73 polypeptide in solution, moreover without reliance on induction of CD73 polypeptides : anti-CD73 antibodies oligomers.

5 In one embodiment, an antibody specifically binds bivalently to human CD73 polypeptides and inhibits the enzymatic activity of cellular human CD73 (and optionally further recombinant soluble human CD73), wherein said antibody does not induce or increase intracellular internalization of CD73 in CD73-expressing cells. Preferably, the antibody substantially lacks Fc γ receptor binding (e.g., via its Fc domain).

10 In one aspect, an antibody specifically binds human CD73 at the surface of a cell pre-incubated with AMP, and is capable of neutralizing the 5'-ectonucleotidase activity thereof. Optionally, neutralizing the 5'-ectonucleotidase activity is determined by assessing neutralization of 5' ectonucleotidase activity in CD73-expressing cells (e.g. MDA-MB-231 cells) by quantifying hydrolysis of AMP to adenosine.

15 In one aspect, neutralization of the 5'-ectonucleotidase activity of CD73 expressed by a cell is determined by assessing the ability of an antibody to increase the proliferation of T cells when said T cells are induced to proliferate in vitro (e.g. via TCR co-stimulation, stimulation of CD3 and CD28 signaling, e.g., by contacting T cells with beads functionalized with CD3 agonists and CD28 agonists) in the presence of AMP (e.g. exogenously added AMP). In one aspect, neutralization of the 5'-ectonucleotidase activity assay is assessed using the T cell proliferation assay described in the section titled "Methods" in the Examples.

20 Antibodies can be characterized by their ability to bind to cells transfected with CD73 mutants, compared to the ability of anti-CD73 antibody to bind wild-type CD73 polypeptide (e.g., SEQ ID NO: 1). A reduction in binding between an anti-CD73 antibody and a mutant CD73 polypeptide means that there is a reduction in binding affinity (e.g., as measured by known methods such FACS testing of cells expressing a particular mutant, or by Biacore testing of binding to mutant polypeptides) and/or a reduction in the total binding capacity of the anti- CD73 antibody (e.g., as evidenced by a decrease in B_{max} in a plot of anti-CD73 antibody concentration versus polypeptide concentration). A significant reduction in binding indicates that the mutated residue is directly involved in binding to the anti-CD73 antibody or is in close proximity to the binding protein when the anti-CD73 antibody is bound to CD73.

30 In some embodiments, a significant reduction in binding means that the binding affinity and/or capacity between an anti-CD73 antibody and a mutant CD73 polypeptide is reduced by greater than 40 %, greater than 50 %, greater than 55 %, greater than 60 %, greater than 65 %, greater than 70 %, greater than 75 %, greater than 80 %, greater than 85 %, greater than 90% or greater than 95% relative to binding between the antibody and a wild

type CD73 polypeptide. In certain embodiments, binding is reduced below detectable limits. In some embodiments, a significant reduction in binding is evidenced when binding of an anti-CD73 antibody to a mutant CD73 polypeptide is less than 50% (e.g., less than 45%, 40%, 35%, 30%, 25%, 20%, 15% or 10%) of the binding observed between the anti-CD73 antibody and a wild-type CD73 polypeptide.

In one aspect, the anti-CD73 antibodies have reduced binding to a CD73 polypeptide having a mutation at residue K136 (with reference to SEQ ID NO: 1); optionally, the mutant CD73 polypeptide has the mutation: K136A.

In one aspect, the anti-CD73 antibodies have reduced binding to a CD73 polypeptide having a mutation at a residue selected from the group consisting of: K97, E125, Q153 and K330 (with reference to SEQ ID NO: 1); optionally, the mutant CD73 polypeptide has the mutations: K97A, E125A, Q153A and/or K330A (e.g., K97A, E125A and K330A; K97A, E125A and/or Q153A).

In one aspect, the anti-CD73 antibodies have reduced binding to a CD73 polypeptide having a mutation at a residue selected from the group consisting of: A99, E129, K133, E134, and A135 (with reference to SEQ ID NO: 1); optionally, the mutant CD73 polypeptide has the mutations: A99S, E129A, K133A, E134N, and/or A135S.

Optionally, in one aspect, the anti-CD73 antibodies do not have reduced binding to a CD73 polypeptide having a mutation at a residue selected from the group consisting of: Q70, R73, A74, A107 and R109 (with reference to SEQ ID NO: 1); optionally, the mutant CD73 polypeptide has the mutations: A99S, Q70S, R73A, A74E, A107I and/or R109G.

In one aspect, the anti-CD73 antibodies bind an epitope on CD73 comprising residue K136 (with reference to SEQ ID NO: 1).

In one aspect, the anti-CD73 antibodies bind an epitope on CD73 comprising one, two, three or four of the residues selected from the group consisting of K97, E125, Q153 and K330 (with reference to SEQ ID NO: 1).

In one aspect, the anti-CD73 antibodies bind an epitope on CD73 comprising one, two, three, four or five of the residues selected from the group consisting of A99, E129, K133, E134, and A135 (with reference to SEQ ID NO: 1).

In one aspect, the anti-CD73 antibodies bind an epitope on CD73 comprising one, two, three, four, five or more of the residues selected from the group consisting of K97, A99, E125, E129, K133, E134, A135 and K136 (with reference to SEQ ID NO: 1). In one aspect, the anti-CD73 antibodies bind an epitope on CD73 comprising one, two, three, four, five or more of the residues selected from the group consisting of K97, A99, E125, E129, K133, E134, A135, K136, Q153 and K330 (with reference to SEQ ID NO: 1).

Optionally, in one aspect, the anti-CD73 antibodies do not bind an epitope on CD73 comprising one, two, three, four or five of the residues selected from the group consisting of Q70, R73, A74, A107 and R109 (with reference to SEQ ID NO: 1).

5 An anti-CD73 antibody can be incorporated in a pharmaceutical formulation comprising in a concentration from 1 mg/ml to 500 mg/ml, wherein said formulation has a pH from 2.0 to 10.0. The formulation may further comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizers and surfactants. In one embodiment, the pharmaceutical formulation is an aqueous formulation, i.e., formulation comprising water. Such formulation is typically a solution or a suspension. In a further embodiment, the
10 pharmaceutical formulation is an aqueous solution. The term "aqueous formulation" is defined as a formulation comprising at least 50 %w/w water. Likewise, the term "aqueous solution" is defined as a solution comprising at least 50 %w/w water, and the term "aqueous suspension" is defined as a suspension comprising at least 50 %w/w water.

In another embodiment, the pharmaceutical formulation is a freeze-dried formulation,
15 where to the physician or the patient adds solvents and/or diluents prior to use.

In another embodiment, the pharmaceutical formulation is a dried formulation (e.g., freeze-dried or spray-dried) ready for use without any prior dissolution.

In a further aspect, the pharmaceutical formulation comprises an aqueous solution of such an antibody, and a buffer, wherein the antibody is present in a concentration from 1
20 mg/ml or above, and wherein said formulation has a pH from about 2.0 to about 10.0.

In a another embodiment, the pH of the formulation is in the range selected from the list consisting of from about 2.0 to about 10.0, about 3.0 to about 9.0, about 4.0 to about 8.5, about 5.0 to about 8.0, and about 5.5 to about 7.5.

In a further embodiment, the buffer is selected from the group consisting of sodium
25 acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment.

30 In a further embodiment, the formulation further comprises a pharmaceutically acceptable preservative. In a further embodiment, the formulation further comprises an isotonic agent. In a further embodiment, the formulation also comprises a chelating agent. In a further embodiment the formulation further comprises a stabilizer. In a further embodiment, the formulation further comprises a surfactant. For convenience reference is made to
35 Remington: *The Science and Practice of Pharmacy*, 19th edition, 1995.

It is possible that other ingredients may be present in the peptide pharmaceutical formulation. Such additional ingredients may include wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatine or proteins) and a zwitterion (e.g.,
5 an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation.

Pharmaceutical compositions containing an antibody may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example,
10 skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen. Administration of pharmaceutical compositions may be through several routes of administration, for example, subcutaneous, intramuscular, intraperitoneal, intravenous, lingual, sublingual, buccal, in the
15 mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.

20 **Diagnosis and treatment of malignancies**

Methods of treating an individual, notably a human patient, using an anti-CD73 antibody or antibody fragment as described herein are also provided for. In one embodiment, the disclosure provides for the use of an antibody or antibody fragment as described herein in the preparation of a pharmaceutical composition for administration to a human patient.
25 Typically, the patient suffers from, or is at risk for, cancer or an infectious disease (e.g., viral or bacterial infection).

For example, in one aspect, provided is a method of restoring or potentiating the activity of immune cells (e.g., lymphocytes) in a patient in need thereof, comprising the step of administering a neutralizing anti-CD73 antibody of the disclosure to said patient. In one
30 embodiment, the method is directed at increasing the activity of immune cells (e.g., T cells) in patients having a disease such as a cancer or an infectious disease in which increased immune cell activity and/or infiltration of to a site of disease (e.g., tumor environment or site of infection) is beneficial or which is caused or characterized by immunosuppression, immunosuppressive cells, or, e.g., adenosine generated by CD4 T cells, CD8 T cells, B
35 cells).

The methods will be particularly useful for treating individuals having a tumor in which it is suspected the tumor microenvironment (and CD73-mediated adenosine production therein) may contribute to lack of recognition by the immune system (immune escape). The tumor environment may, for example, be characterized by CD73-expressing immune cells, e.g., CD4 T cells, CD8 T cells, B cells, and/or CD73-expressing tumor cells.

The methods and anti-CD73 antibodies can be utilized for the treatment and/or prevention of a variety of cancers and other proliferative diseases. Because these methods operate by reducing adenosine that inhibits the anti-tumor activity of lymphocytes and possibly additionally by increasing ATP that can increase the anti-tumor activity of lymphocytes, they are applicable to a broad range of cancers, including in particular solid tumors in which adenosine in the tumor microenvironment may play a strong role in suppressing the anti-tumor immune response. In one embodiment, a human patient treated with an anti-CD73 antibody has liver cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, non-small cell lung cancer (NSCLC), castrate resistant prostate cancer (CRPC), melanoma, uterine cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, environmentally induced cancers including those induced by asbestos, hematologic malignancies including, for example, multiple myeloma, B-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, non-Hodgkin's lymphomas, acute myeloid lymphoma, chronic myelogenous leukemia, chronic lymphoid leukemia, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B -lymphoblastic lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, mycosis fungoides, anaplastic large cell lymphoma, T-cell lymphoma, and precursor T-lymphoblastic lymphoma, and any combinations of said cancers. The present disclosure is also applicable to treatment of metastatic cancers. Patients can be tested or selected for one or more of the above described clinical attributes prior to, during or after treatment.

Because the methods and anti-CD73 antibodies operate by reducing adenosine that inhibits the activity and/or infiltration of immune cells (e.g., lymphocytes) and possibly additionally by increasing ATP that can increase the activity of lymphocytes, they are applicable to a broad range of infectious diseases, including preferably any infections caused by infection by viruses, bacteria, protozoa, molds or fungi.

In one embodiment, the anti-CD73 antibody is administered an amount effective to achieve and/or maintain in an individual (e.g., for 1, 2, 3, 4 weeks, and/or until the subsequent administration of antigen binding compound) a blood concentration of at least the EC_{50} , optionally the EC_{70} , optionally substantially the EC_{100} , for neutralization of the enzymatic activity of CD73. In one embodiment, the active amount of anti-CD73 antibody is an amount effective to achieve the EC_{50} , optionally the EC_{70} , optionally substantially the EC_{100} , for neutralization of the enzymatic activity of CD73 in an extravascular tissue of an individual. In one embodiment, the active amount of anti-CD73 antibody is an amount effective to achieve (or maintain) in an individual the EC_{50} , optionally the EC_{70} , optionally substantially the EC_{100} , for inhibition of neutralize the enzymatic activity of CD73.

In one embodiment, the anti-CD73 antibody is administered in an amount effective to achieve and/or maintain (e.g., for 1, 2, 3, 4 weeks, and/or until the subsequent administration of anti-CD73 antibody) in an individual a blood concentration of at least the EC_{50} , optionally the EC_{70} , optionally substantially the EC_{100} , for inhibition of CD73-mediated catabolism of AMP to adenosine (e.g., by assessing neutralization of 5' ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine). In one embodiment, the amount of anti-CD73 antibody is an amount effective to achieve (or maintain) the EC_{50} , optionally the EC_{70} , optionally substantially the EC_{100} , for inhibition of CD73-mediated catabolism of AMP to adenosine in an extravascular tissue of an individual.

In one embodiment, provided is a method for treating or preventing cancer in an individual, the method comprising administering to an individual having disease an anti-CD73 antibody in an amount that achieves or maintains for a specified period of time a concentration in circulation, optionally in an extravascular tissue of interest (e.g., the tumor or tumor environment), that is higher than the concentration required for 50%, 70%, or full (e.g., 90%) receptor saturation CD73-expressing cells in circulation (for example as assessed in PBMC). Optionally the concentration achieved is at least 20%, 50% or 100% higher than the concentration required for the specified receptor saturation.

In one embodiment, provided is a method for treating or preventing cancer in an individual, the method comprising administering to the individual an anti-CD73 antibody in an amount that achieves or maintains for a specified period of time a concentration in circulation, optionally in an extravascular tissue of interest (e.g., the tumor or tumor

environment), that is higher than the EC₅₀, optionally EC₇₀ or optionally EC₁₀₀, for binding to CD73-expressing cells (e.g., as assessed by titrating anti-CD73 antibody on CD73-expressing cells, for example MDA-MB-231 cells). Optionally the concentration achieved is at least 20%, 50% or 100% higher than the EC₅₀, optionally EC₇₀ or optionally EC₁₀₀, for binding to CD73-expressing cells.

In any embodiment, the antibody can for example have an EC₅₀, optionally EC₇₀ or optionally EC₁₀₀, for binding to CD73-expressing cells in human PBMC of between 0.5-100 ng/ml, optionally 1-100 ng/ml, optionally 30-100 ng/ml, e.g., about 30-90 ng/ml, (e.g., as assessed by titrating anti-CD73 antibody on CD73-expressing cells, for example MDA-MB-231 cells).

The EC₅₀ for neutralization of the enzymatic activity of CD73 with the anti-CD73 antibody can be for example between about 0.01 µg/ml and 1 µg/ml, optionally between 0.1 µg/ml and 10 µg/ml, optionally between 0.1 µg/ml and 1 µg/ml. For example the EC₅₀ may be about 0.1 µg/ml, about 0.2 µg/ml or about 0.3 µg/ml. Thus an amount of this anti-CD73 antibody is for example administered so as to achieve and/or maintain a blood concentration of at least 0.1 µg/ml, optionally at least 0.2 µg/ml, optionally at least 1 µg/ml, or optionally at least 2 µg/ml.

When tissues outside of the vasculature are targeted (the tumor environment, e.g., in the treatment of solid tumors), an approximately 10-fold higher dose is typically believed to be needed, compared to the dose that provides the corresponding concentration in circulation. An amount of anti-CD73 antibody administered so as to achieve (and/or maintain) a concentration in circulation (blood) of about 1 µg/ml, 2 µg/ml, 10 µg/ml, or 20 µg/ml is expected to achieve (and/or maintain) an extravascular tissue (e.g., tumor tissue) concentration of about 0.1 µg/ml, 0.2 µg/ml, 1 µg/ml, 2 µg/ml, respectively.

In one embodiment, an anti-CD73 antibody is for example administered in an amount so as to achieve and/or maintain a tissue (e.g., tumor environment) concentration of at least 0.1 µg/ml, optionally at least 0.2 µg/ml, optionally at least 1 µg/ml, or optionally at least 2 µg/ml. The antibody can for example be administered in an amount to achieve and/or maintained a blood concentration of at least about 1 µg/ml, 2 µg/ml, 10 µg/ml, or 20 µg/ml, e.g., between 1-100 µg/ml, 10-100 µg/ml, 1-50 µg/ml, 1-20 µg/ml, or 1-10 µg/ml. The amount administered can be adjusted to as to provide for maintenance of the desired concentration for the duration of a specified period of time following administration (e.g., 1, 2, 3, 4 weeks, etc.).

In some embodiments, an amount of anti-CD73 antibody is administered so as to obtain a concentration in blood (serum) or an extravascular tissue (e.g., tumor environment) that corresponds to at least the EC₇₀ or the EC₁₀₀ for neutralization of the enzymatic activity

of CD73. The antibody can for example be administered in an amount to achieve and/or maintained a blood concentration or an extravascular tissue (e.g., tumor environment) of at least about 1 µg/ml, 2 µg/ml, 10 µg/ml, or 20 µg/ml.

5 The EC₅₀, EC₇₀ or the EC₁₀₀ can be assessed for example in a cellular assay for neutralization of the enzymatic activity of CD73 (e.g., neutralization of 5' ectonucleotidase activity in MDA-MB-231 cells by quantifying hydrolysis of AMP to adenosine). "EC₅₀" with respect to neutralization of the enzymatic activity of CD73, refers to the efficient concentration of anti-CD73 antibody which produces 50% of its maximum response or effect with respect to neutralization of the enzymatic activity). "EC₇₀" with respect to neutralization of the enzymatic activity of CD73, refers to the efficient concentration of anti-CD73 antibody which produces 70% of its maximum response or effect. "EC₁₀₀" with respect to neutralization of the enzymatic activity of CD73, refers to the efficient concentration of anti-CD73 antibody which produces its substantially maximum response or effect with respect to such neutralization of the enzymatic activity.

15 In some embodiments, particularly for the treatment of solid tumors, the concentration achieved is designed to lead to a concentration in tissues (outside of the vasculature, e.g., in the tumor or tumor environment) that corresponds to at least the EC₅₀ for neutralization of the enzymatic activity, optionally at about, or at least about, the EC₁₀₀.

20 In one embodiment, the amount of anti-CD73 antibody is between 1 and 20 mg/kg body weight. In one embodiment, the amount is administered to an individual weekly, every two weeks, monthly or every two months.

25 In one embodiment provided is a method of treating a human individual, comprising administering to the individual an effective amount of an anti-CD73 antibody of the disclosure for at least one administration cycle (optionally at least 2, 3, 4 or more administration cycles), wherein the cycle is a period of eight weeks or less, wherein for each of the at least one cycles, one, two, three or four doses of the anti-CD73 antibody are administered at a dose of 1-20 mg/kg body weight. In one embodiment, the anti-CD73 antibody is administered by intravenous infusion.

30 Suitable treatment protocols for treating a human include, for example, administering to the patient an amount as disclosed herein of an anti-CD73 antibody, wherein the method comprises at least one administration cycle in which at least one dose of the anti-CD73 antibody is administered. Optionally, at least 2, 3, 4, 5, 6, 7 or 8 doses of the anti-CD73 antibody are administered. In one embodiment, the administration cycle is between 2 weeks and 8 weeks.

35 A patient having a cancer can be treated with the anti-CD73 antibody with or without a prior detection step to assess expression of CD73 on cells in the tumor microenvironment

(e.g., on tumor cells, CD4 T cells, CD8 T cells, B cells). Optionally, the treatment methods can comprises a step of detecting a CD73 nucleic acid or polypeptide in a biological sample from a tumor from an individual (e.g., cancer tissue or tissue proximal to or at the periphery of a cancer, cancer adjacent tissue, adjacent non-tumorous tissue or normal adjacent tissue). A determination that a biological sample comprises cells expressing CD73 (e.g.,
5 expressing CD73 at a high level, high intensity of staining with an anti-CD73 antibody, compared to a reference, for example healthy tissue) indicates that the patient has a cancer that may have a strong benefit from treatment with the anti-CD73 antibody. In one embodiment, the method comprises determining the level of expression of a CD73 nucleic
10 acid or polypeptide in a biological sample and comparing the level to a reference level corresponding to a healthy individual. A determination that a biological sample comprises cells expressing CD73 nucleic acid or polypeptide at a level that is increased compared to the reference level (e.g. healthy tissue) indicates that the patient has a cancer that can be advantageously treated with an anti-CD73 antibody of the disclosure. Optionally, detecting a
15 CD73 polypeptide in a biological sample comprises detecting CD73 polypeptide expressed on the surface of a malignant cell, or a CD4 T cell, a CD8 T cell or a B cell.

Determining whether an individual has a cancer characterized by cells that express a CD73 polypeptide can for example comprise obtaining a biological sample (e.g., by performing a biopsy) from the individual that comprises cells from the cancer environment
20 (e.g., tumor or tumor adjacent tissue), bringing said cells into contact with an antibody that binds an CD73 polypeptide, and detecting whether the cells express CD73 on their surface. Optionally, determining whether an individual has cells that express CD73 comprises conducting an immunohistochemistry assay.

The antibody compositions may be advantageously be used in combination with one
25 or more other therapeutic agents, including agents normally utilized for the particular therapeutic purpose for which the antibody is being administered. The additional therapeutic agent will normally be administered in amounts and treatment regimens typically used for that agent in a monotherapy for the particular disease or condition being treated. Such therapeutic agents include, but are not limited to anti-cancer agents and chemotherapeutic
30 agents.

In one embodiment, the anti-CD73 antibodies augment the efficacy of agents that neutralizes the inhibitory activity of human PD-1, e.g., that inhibits the interaction between PD-1 and PD-L1. Accordingly, in one embodiment, the second or additional second
35 therapeutic agent is an antibody or other agent that neutralizes the inhibitory activity of human PD-1.

Programmed Death 1 (PD-1) (also referred to as "Programmed Cell Death 1") is an inhibitory member of the CD28 family of receptors. The complete human PD-1 sequence can be found under GenBank Accession No. U64863. Inhibition or neutralization the inhibitory activity of PD-1 can involve use of a polypeptide agent (e.g., an antibody, a polypeptide fused to an Fc domain, an immunoadhesin, etc.) that prevents PD-L1-induced PD-1 signalling. There are currently at least six agents blocking the PD-1/PD-L1 pathway that are marketed or in clinical evaluation. One agent is BMS-936558 (Nivolumab/ONO-4538, Bristol-Myers Squibb; formerly MDX-1106). Nivolumab, (Trade name Opdivo®) is an FDA-approved fully human IgG4 anti-PD-L1 mAb that inhibits the binding of the PD-L1 ligand to both PD-1 and CD80 and is described as antibody 5C4 in WO 2006/121168, the disclosure of which is incorporated herein by reference. For melanoma patients, the most significant OR was observed at a dose of 3 mg/kg, while for other cancer types it was at 10 mg/kg. Nivolumab is generally dosed at 10 mg/kg every 3 weeks until cancer progression. The terms "reduces the inhibitory activity of human PD-1", "neutralizes PD-1" or "neutralizes the inhibitory activity of human PD-1" refers to a process in which PD-1 is inhibited in its signal transduction capacity resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1 or PD-L2. An agent that neutralizes the inhibitory activity of PD-1 decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. Such an agent can thereby reduce the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes, so as to enhance T-cell effector functions such as proliferation, cytokine production and/or cytotoxicity.

MK-3475 (human IgG4 anti-PD1 mAb from Merck), also referred to as lambrolizumab or pembrolizumab (Trade name Keytruda®) has been approved by the FDA for the treatment of melanoma and is being tested in other cancers. Pembrolizumab was tested at 2 mg/kg or 10 mg/kg every 2 or 3 weeks until disease progression. MK-3475, also known as Merck 3745 or SCH-900475, is also described in WO2009/114335.

MPDL3280A/RG7446 (atezolizumab, trade name Tecentriq™, anti-PD-L1 from Roche/Genentech) is a human anti-PD-L1 mAb that contains an engineered Fc domain designed to optimize efficacy and safety by minimizing FcγR binding and consequential antibody-dependent cellular cytotoxicity (ADCC). Doses of ≤1, 10, 15, and 25 mg/kg MPDL3280A were administered every 3 weeks for up to 1 year. In phase 3 trial, MPDL3280A is administered at 1200 mg by intravenous infusion every three weeks in NSCLC.

AMP-224 (Amplimmune and GSK) is an immunoadhesin comprising a PD-L2 extracellular domain fused to an Fc domain. Other examples of agents that neutralize PD-1

may include an antibody that binds PD-L2 (an anti-PD-L2 antibody) and blocks the interaction between PD-1 and PD-L2.

Pidlizumab (CT-011; CureTech) (humanized IgG1 anti-PD1 mAb from CureTech/Teva), Pidlizumab (CT-011; CureTech) (see e.g., WO2009/101611) is another
5 example; the agent was tested in thirty patients with rituximab-sensitive relapsed FL were treated with 3 mg/kg intravenous CT-011 every 4 weeks for 4 infusions in combination with rituximab dosed at 375 mg/m² weekly for 4 weeks, starting 2 weeks after the first infusion of CT-011.

Further known PD-1 antibodies and other PD-1 inhibitors include AMP-224 (a B7-
10 DC/IgG1 fusion protein licensed to GSK), AMP-514 described in WO 2012/145493, antibody MEDI-4736 (durvalumab, trade name Imfinzi™, an anti-PD-L1 developed by AstraZeneca/Medimmune) described in WO2011/066389 and US2013/034559, antibody YW243.55.S70 (an anti-PD-L1) described in WO2010/077634, MDX-1105, also known as BMS-936559, is an anti-PD-L1 antibody developed by Bristol-Myers Squibb described in
15 WO2007/005874, and antibodies and inhibitors described in WO2006/121168, WO2009/014708, WO2009/114335 and WO2013/019906, the disclosures of which are hereby incorporated by reference. Further examples of anti-PD1 antibodies are disclosed in WO2015/085847 (Shanghai Hengrui Pharmaceutical Co. Ltd.), for example antibodies having light chain variable domain CDR1, 2 and 3 of SEQ ID NO: 6, SEQ ID NO: 7 and/or
20 SEQ ID NO: 8, respectively, and antibody heavy chain variable domain CDR1, 2 and 3 of SEQ ID NO: 3, SEQ ID NO: 4 or SEQ ID NO: 5, respectively, wherein the SEQ ID NO references are the numbering according to WO2015/085847, the disclosure of which is incorporated herein by reference. Antibodies that compete with any of these antibodies for binding to PD-1 or PD-L1 also can be used. In some embodiments, the PD-1 neutralizing
25 agent is an anti-PD-L1 antibody that inhibits the binding of PD-L1 to PD-1. In some embodiments, the PD-1 neutralizing agent is an anti-PD1 antibody that inhibits the binding of PD-1 to PD-L1. An exemplary anti-PD-1 antibody is pembrolizumab (commercialized by Merck & Co. as Keytruda™, see, also WO 2009/114335 the disclosure of which is incorporated herein by reference). An exemplary anti-PD-L1 is antibody MEDI-4736
30 (durvalumab, trade name Imfinzi™, an anti-PD-L1 developed by AstraZeneca/Medimmune

In the treatment methods, the CD73-binding compound and the second therapeutic agent can be administered separately, together or sequentially, or in a cocktail. In some
embodiments, the antigen-binding compound is administered prior to the administration of the second therapeutic agent. For example, the CD73-binding compound can be
35 administered approximately 0 to 30 days prior to the administration of the second therapeutic agent. In some embodiments, an CD73-binding compound is administered from about 30

minutes to about 2 weeks, from about 30 minutes to about 1 week, from about 1 hour to about 2 hours, from about 2 hours to about 4 hours, from about 4 hours to about 6 hours, from about 6 hours to about 8 hours, from about 8 hours to 1 day, or from about 1 to 5 days prior to the administration of the second therapeutic agent. In some embodiments, a CD73-binding compound is administered concurrently with the administration of the therapeutic agents. In some embodiments, a CD73-binding compound is administered after the administration of the second therapeutic agent. For example, a CD73-binding compound can be administered approximately 0 to 30 days after the administration of the second therapeutic agent. In some embodiments, a CD73-binding compound is administered from about 30 minutes to about 2 weeks, from about 30 minutes to about 1 week, from about 1 hour to about 2 hours, from about 2 hours to about 4 hours, from about 4 hours to about 6 hours, from about 6 hours to about 8 hours, from about 8 hours to 1 day, or from about 1 to 5 days after the administration of the second therapeutic agent.

Examples

Methods

Cloning, production and purification of recombinant huCD73

Molecular Biology

The huCD73 protein was cloned from MIAPACA-2 cDNA using the following primers TACGACTCACAAAGCTTGCCGCCACCATGTGTCCCCGAGCCGCGCG (SEQ ID NO: 20) (Forward) and CCGCCCCGACTCTAGAtcaGTGATGGTGTGATGGTGcttgatccgacctcaactg SEQ ID NO: 21) (Reverse). The purified PCR product was then cloned into an expression vector using the InFusion cloning system. A 6xHis tag was added in the C-terminal part of the protein for the purification step.

Amino acid sequence of the cloned huCD73:

MCPRAARAPATLLLLALGAVLWPAAGAWELTILHTNDVHSRLEQTSSESSKCVNASRCMGGVARLFTKV
 QQIRRAEPNVLLLDAGDQYQGTIWFVYKGAEVAHFMMNALRYDAMALGNHEFDNGVEGLIEPLLKEAK
 FPILSANIKAKGPLASQISGLYLPYKVLPGDEVVGIVGYTSKETPFLSNPGTNLVFEDEITALQPEV
 DKLKTLNVNKIIALGHSGFEMDKLIAQKVRGVDVVVGHSNTFLYTGNPPSKEVPAGKYPFIVTSDDG
 RKVPVVQAYAFGKYLGYLKIEFDERGNVISHSNPIILNSSIPEDPSIKADINKWRIKLDNYSTQELG
 KTIVYLDGSSQSCRFRECNMGNLICDAMINNNLRHTDEMFWNHVSMCILNGGGIRSPIDERNNGTITW
 ENLAAVLFPFGGTFDLVQLKGSTLKKAFEHSVHRYGQSTGEFLQVGGIHVVDLSRKPGDRVVKLDVLC

TKCRVPSYDPLKMDDEVYKVI L P N F L A N G G D G F Q M I K D E L L R H D S G D Q D I N V V S T Y I S K M K V I Y P A V E G
RIKHHHHHH (SEQ ID NO: 22).

Expression and purification of the huCD73 proteins

5 After validation of the sequence cloned, cells were nucleofected and the producing pool was then sub-cloned to obtain a cell clone producing the huCD73 protein. Supernatant from the huCD73 clone grown in roller was harvested and purified using Ni-NTA column and eluted using 250 mM imidazole. The purified proteins were then loaded onto a S200 size exclusion chromatography column. The purified protein corresponding to a dimer was
10 formulated in a Tris 20 mM pH 7.5, NaCl 120 mM and CaCl₂ 4 mM buffer for enzyme activity assays, while formulation buffer is supplemented with 20% glycerol.

SPR analysis to assess Ab KD on recombinant CD73 protein

SPR measurements were performed on a Biacore™ T200 apparatus at 25°C.
15 Protein-A (GE Healthcare) was immobilized on a Sensor Chip CM5 (GE Healthcare). The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysuccinimide (Biacore™, GE Healthcare). Protein-A was diluted to 10 µg/mL in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2000 RU). Deactivation of the remaining activated
20 groups was performed using 100 mM ethanolamine pH 8 (Biacore™, GE Healthcare). Affinity study was carried out according to a standard Capture-Kinetic protocol recommended by the manufacturer (Biacore™, GE Healthcare kinetic wizard). Serial dilutions of human recombinant soluble CD73 protein, ranging from 1.23 to 300 nM were sequentially injected over the captured anti-CD73 antibodies and allowed to dissociate for 10
25 min before regeneration. The entire sensorgram sets were fitted using the 1:1 kinetic binding model. Bivalent affinities and kinetic association and dissociation rate constants are calculated.

Flow cytometry analysis to assess anti-CD73 recognition by antibodies

30 10⁵ MDA-MB-231 cells resuspended in FCSB were distributed into round-bottom 96W-microplates (50µL per well). A dose range of anti-CD73 mAbs was added to the plates and cells were incubated for 1h at +5 ± 3°C. Cells were washed three times in FCSB by spinning plates at 400g for 3 min at 4°C. PE-conjugated Goat F(ab')₂ Anti-Human IgG (H+L) secondary Ab diluted in FCSB was added to the cells and plates were incubated for 30
35 additional minutes at +5 ± 3°C. Cells were washed three times as described above and analyzed on a flow cytometer.

Median of fluorescence intensity vs. mAb concentration was plotted on graphs and EC₅₀ was calculated using GraphPad Prism™ program.

In vitro enzymatic assay with recombinant human or cynomolgus CD73

5 Briefly, recombinant human or cynomolgus CD73 was incubated in white 96W flat-bottom microplates in the presence of a dose-range of anti-CD73 or isotype control mAbs. Plates were incubated for 1h at +37 ± 1°C. ATP (12.5 µM) and AMP (125 µM) were added to each well and plates were incubated at +37 ± 1°C for 30 additional minutes. Luciferase/luciferin-containing CellTiter-Glo™ (Promega) was added into wells, plates were
10 incubated for 5 minutes at RT in the dark and emitted light was measured using an Enspire™ apparatus (Perkin Elmer).

- Conditions:
- ATP+AMP: maximal luciferase inhibition (100%)
- CD73+ATP+AMP: no luciferase inhibition (0%)

15 The residual enzyme activity vs. anti-CD73 Ab concentration is was plotted on graphs using GraphPad Prism7™ software.

T Cell proliferation assay

Peripheral blood from healthy donors was obtained and mononuclear cells were
20 isolated on a Ficoll gradient. Lymphocytes were further enriched on a 52% Percoll™ gradient (cell pellets) and stained with a 2 µM CellTrace™ Violet (Thermofisher). 5x10⁴ to 1x10⁵ of stained cells were distributed in 96w round-bottom plates, incubated for 1h at 37°C with anti-CD73 Abs and activated for 3 to 5 days by addition of anti-CD3/anti-CD28-coated beads. Inhibition of T cell proliferation was achieved by addition of 200 µM AMP. T cell
25 proliferation and the ability of Abs to block the immune suppressive effect of AMP were assessed by flow cytometry by quantifying the dye dilution on proliferating T cells. Percentage of proliferating T cells vs. anti-CD73 Ab concentration was plotted on graphs using GraphPad Prism™ software. Some experiments were done on whole PBMC from healthy donors or cancer patients; the protocol was as described above except that T cell
30 suppression was achieved by addition of 0.5 to 1 mM ATP.

In order to compare donors or patients, T cell proliferation was normalized using the following formula:

$$\frac{(\text{Activated cells} + \text{AT(M)P} + \text{Ab}) - (\text{Activated cells} + \text{AT(M)P})}{(\text{Activated cells}) - (\text{Activated cells} + \text{AT(M)P})} \times 100$$

Allogeneic mixed Lymphocyte Reaction (MLR) assay

Mononuclear cells from healthy donors were isolated on a Ficoll gradient and monocytes were purified by immunomagnetic selection using CD14 microbeads (Miltenyi Biotec). Monocytes were differentiated into dendritic cells (MoDC) by 5-7 days of culture in presence of GM-CSF (400 ng/ml) and IL-4 (20 ng/ml). The day of DC recovery, CD4⁺ T cells from allogeneic donors were purified by immunomagnetic depletion of non-CD4⁺ T cells (Miltenyi Biotec) and stained with Cell Trace™ Violet. DC (10⁴ cells/well) and T cells (5x10⁴ cells/well) were mixed in 96W round bottom microplates in presence of a dose-ranges of anti-huCD73 Abs and a fixed dose of ATP. T cell proliferation and Ab ability to reverse ATP-mediated suppression was assessed as described for T cell proliferation assay.

Example 1: Modelling and generation of anti-huCD73 antibodies with human framework sequences

Parental antibody 3C12 described in PCT publication WO2016/055609 having VH and VL amino acid sequences of SEQ ID NOS: 27 and 28, respectively, was modified by the introduction into the VH of heavy chain frameworks (FR1, FR2, FR3) from the human subgroup IGHV1-3*01 together with IGHJ4*01 (FR4), and the introduction into the VL of light chain frameworks (FR1, FR2, FR3) from the human subgroup IGKV1-33*01, together with IGKJ2*01 (FR4).

Three-dimensional models based on different human VH and VL gene segments were superimposed and all amino acid differences were scrutinized one by one. The *in silico* molecular design was challenged using 3D models of both parental chimeric (HPLP) and humanized (H0L0) antibodies. Intrachain and extrachain connections between residues were also assessed in order to identify and to avoid disruption of any important low energy bond.

The VH and VL sequences of each designed domain were cloned into vectors containing the hulgG1-derived constant domains having L234A/L235E/G237A/A330S/P331S substitutions and the huCk constant domain respectively. The two obtained vectors were co-transfected into a CHO cell line. The established pool of cell was used to produce the antibody in the CHO medium. The antibody was then purified using protein A. The heavy and light chain sequences used for modelling a chimeric Fab version of 3C12 with human IgG1 constant regions, including an Fc domain comprising L234A/L235E/G237A/A330S/P331S substitutions (retaining N297-linked glycosylation) were the following:

HP Fab heavy chain (variable domain underlined)

QIQQLQQSGPELVKPGASVKVSCASGYAFASYNMNWWKQSHGKSLDWIGYIDPYNGGSSYNLTFKGKA
TLTVDKSSTTAYMHLNSLTSEDSAVYYCARGYGNYKAWFAYWGQGLTVTVSAASTKGPSVFPLAPSSK
 STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN
 VNHKPSNTKVDK (SEQ ID NO: 23).

5

LP Fab light chain (variable domain underlined)

DVVMQTQPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKLLIYYASTRYTGVPDRFTGSGYG
TDFTFTISTVQAEDLAVYFCQQDYSSLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPV
 TKSEFNRGEC (SEQ ID NO: 24).

10

H0 Fab heavy chain (variable domain underlined)

QVQLVQSGAEVKKPGASVKVSCASGYTFTSYNMYWVRQAPGQRLEWMGYIDPYNGGSSYNLTFKGRV
TITRDTASTAYMELSSLRSEDTAVYYCARGYGNYKAWFAYWGQGLTVTVSSAASTKGPSVFPLAPSSK
 STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN
 VNHKPSNTKVDK (SEQ ID NO: 25).

15

L0 Fab light chain (variable domain underlined)

DIQMTQSPSSLSASVGRVITITCKASQSVSNDVAWYQQKPKAPKLLIYYASTRYTGVPDRFTGSGSG
TDFTFTISSLPEDIATYYCQQDYSSLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPV
 TKSEFNRGEC (SEQ ID NO: 26).

20

The heavy chain variable region and light chain variable region sequences of the
 HPLP parental antibody are shown below:

25

HP VH

QIQQLQQSGPELVKPGASVKVSCASGYAFASYNMNWWKQSHGKSLDWIGYIDPYNGGSSYNLTFKGKA
TLTVDKSSTTAYMHLNSLTSEDSAVYYCARGYGNYKAWFAYWGQGLTVTVSA (SEQ ID NO: 27).

30

LP VL

DVVMQTQPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKLLIYYASTRYTGVPDRFTGSGYG
TDFTFTISTVQAEDLAVYFCQQDYSSLTFGAGTKLELK (SEQ ID NO: 28).

35

Heavy chain design

In order to investigate whether residue I2 (also position 2 according to Kabat numbering) forms a hydrophobic bond with CDR_{H3}-Y109 and plays a role in CDR_{H3}

positioning or flexibility, the valine residue at position 2 was substituted by an isoleucine. V2 and I2 side chains are not coincident in the two models. V2 also forms a hydrophobic bond with H0-Y109 (Kabat Y108) but the Y109 position is slightly modified and H0-Y109 forms h-bond with LC-Y55 (Kabat Y54). Y55 is a CDR_L2 residue. The V2 substitution may influence the position of both CDR_H3 and CDR_L2.

In order to investigate whether the residues at positions 28 and 30 (framework 1, also positions 28 and 30 according to Kabat,) influence binding to antigen, these positions were substituted by an alanine. The parental antibody has the sequence YAFA compared to YTFT of the H0L0 antibody. Residues A28 and A30 are exposed at the molecular surface close to the paratope. It appears likely that a threonine at position 28 would likely negatively influence binding to antigen. On the other hand, A30 appeared unlikely to be critical.

In order to investigate whether the residue at position 44 (also Kabat 44) plays a role in the VH/VL interface, this residue was back-mutated thereby conserving the serine present in parental antibody. The parental antibody has the sequence GKS compared to GQR of the H0L0 antibody. R44 is located at the VH/VL interface, very close to L45 side chain.

In order to investigate whether the residue at position 48 (also Kabat 48) is important, this residue was back-mutated thereby conserving the residue present in parental antibody. The parental antibody has the sequence WIG compared to WMG of the H0L0 antibody. I48 and M48 appear to occupy critical positions just below the CDR_H2 and are involved in a complex hydrophobic network.

In order to investigate whether the importance of the residues at positions 67 (Kabat position 66) and 68 (Kabat position 67), these residues were back-mutated thereby conserving the residues present in parental antibody. The parental antibody has the sequence KAT compared to RVT in the H0L0 antibody. The loop between F64 (Kabat F63) and K67/R67 (Kabat K66/R66) shows very different conformations in the two models. A68 (Kabat 67) is a Vernier residue.

In order to investigate the role of the residue at Abnum position 70 (Kabat position 69), this residue was back-mutated thereby conserving the leucine present in parental antibody. The parental antibody has the sequence TLT compared to TIT of the H0L0 antibody. L70 and I70 (Kabat L69 and I69) are involved in a complex hydrophobic network.

In order to investigate the role of the residue at position 72 (Kabat 71), this residue was back-mutated thereby conserving the serine present in parental antibody. The parental antibody has the sequence TVD compared to TRD of the H0L0 antibody. V72 and R72 (Kabat V71 and R71) occupy critical positions just below the CDR_H2. The distal hydrophilic side chain of residue V72 is projected at the molecular surface and forms an h-bond with N55 (Kabat N54). N55 occupies the same position in both models. However, the h-bond with

R72 (Kabat R71) may lower the flexibility of the corresponding loop. Consequently, the impact on antigen binding of replacing parental valine with an arginine in the H0L0 cannot be predicted.

5 In order to investigate whether the residue at position 74 (Kabat 73) influences binding to antigen, this residue was back-mutated thereby conserving the serine present in parental antibody. The parental antibody has the sequence DKS compared to DTS in the H0L0 antibody. K74 and T74 (Kabat K73 and T73) are exposed at the molecular surface close to the paratope and they may be involved in antigen binding.

10 A first heavy chain variant (H1) having the amino acid sequence shown in SEQ ID NO: 29 had a V2I and a T73K substitution. A second heavy chain (H2) variant having the amino acid sequence shown in SEQ ID NO: 30 had the substitutions V2I, T28A, R71V and T73K. A third heavy chain variant (H3) having the amino acid sequence shown in SEQ ID NO: 31 had the substitutions V2I, T28A, M48I, R66K, V67A, R71V and T73K. A fourth heavy chain variant (H4) having the amino acid sequence shown in SEQ ID NO: 32 had the
15 substitutions V2I, T28A, T30A, M48I, R66K, V67A, I69L, R71V and T73K. Numbering of substitutions is according to Kabat.

Light chain design

20 The parental antibody has the sequence DVVM compared to DIQM of the H0L0 antibody. Residue V2 forms a hydrophobic bond with A25. Residue I2 may be exposed at the paratope surface but impact on paratope organisation cannot be predicted. I2 does not form hydrophobic bond with A25, and this may slightly modify the position of CDR-L1. The isoleucine at residue 2 was therefore substituted by a valine.

25 The parental antibody has the sequence PDR compared to PSR in the H0L0 antibody. Residue R61 occupies rotameric positions. The loops seem to be oriented the same way but are not superimposed (lateral translation corresponding to the global translation of the entire domain). In the parental antibody, D60 forms a salt bridge and a h-bond with CDR-L2-R54. In the H0L0 antibody, residue S60 does not contact R54. R54 occupies rotameric positions. Contact with D60 may be critical to impose a specific and
30 suitable position to CDR-L2 residues. The aspartic acid residue at position 60 in the H0L0 antibody was therefore substituted by a serine.

The parental antibody has the sequence GYG compared to GSG in the H0L0 antibody. To investigate whether residue Y67 contributes to the paratope the serine in the H0L0 antibody was replaced by a tyrosine.

35 The parental antibody has the sequence YFC compared to YYC in the H0L0 antibody. While Y87 does not appear to form h-bond with adjacent residues, the role of this

residue is to be investigated and the tyrosine at position 87 in the H0L0 was substituted by a phenylalanine.

A first light chain variant (L1) having the amino acid sequence shown in SEQ ID NO: 33 had the substitution S67Y. A second light chain (L2) variant having the amino acid sequence shown in SEQ ID NO: 34 had the substitutions S60D and S67Y. A third light chain variant (L3) having the amino acid sequence shown in SEQ ID NO: 35 had the substitutions I2V, S60D and S67Y. A fourth light chain variant (L4) having the amino acid sequence shown in SEQ ID NO: 36 had the substitutions I2V, S60D, S67Y and Y87F.

The amino acid sequences of respective heavy ("H" chains in Table 1) and light ("L" chains in Table 1) chain variable regions are shown in the Table 1 below.

Table 1

Chain	SEQ ID NO	Sequence (amino acid substitutions in bold, Kabat CDRs underlined)
H1	29	QIQLVQSGAEVKKPGASVKVSCASGYFTSYNMYWVRQAPGQRLEWMGYIDPYNGGSSYN LTFKGRVTITRD K SASTAYMELSSLRSEDVAVYYCARGYGN K AWFAYWGQGLVTVSS
H2	30	QIQLVQSGAEVKKPGASVKVSCASGY A FTSYNMYWVRQAPGQ S LEWMGYIDPYNGGSSYN LTFKGRVTIT V D K SASTAYMELSSLRSEDVAVYYCARGYGN K AWFAYWGQGLVTVSS
H3	31	QIQLVQSGAEVKKPGASVKVSCASGY A FTSYNMYWVRQAPGQ S LEW I GYIDPYNGGSSYN LTFK G K ATIT V D K SASTAYMELSSLRSEDVAVYYCARGYGN K AWFAYWGQGLVTVSS
H4	32	QIQLVQSGAEVKKPGASVKVSCASGY A F A SYNMYWVRQAPGQ S LEW I GYIDPYNGGSSYN LTFK G K AT L T V D K SASTAYMELSSLRSEDVAVYYCARGYGN K AWFAYWGQGLVTVSS
L1	33	DIQMTQSPSSLSASVGDRVTITCKASQSVSNDAWYQQKPGKAPKLLIYYASTRYTGVPSPR FSGSGYGTDFFTFTISLQPEDATYYCQQDYSSLTFTGQGTKLEIK
L2	34	DIQMTQSPSSLSASVGDRVTITCKASQSVSNDAWYQQKPGKAPKLLIYYASTRYTGVPDR FSGSGYGTDFFTFTISLQPEDATYYCQQDYSSLTFTGQGTKLEIK
L3	35	DVQMTQSPSSLSASVGDRVTITCKASQSVSNDAWYQQKPGKAPKLLIYYASTRYTGVPDR FSGSGYGTDFFTFTISLQPEDATYYCQQDYSSLTFTGQGTKLEIK
L4	36	DVQMTQSPSSLSASVGDRVTITCKASQSVSNDAWYQQKPGKAPKLLIYYASTRYTGVPDR FSGSGYGTDFFTFTISLQPEDATY F CQQDYSSLTFTGQGTKLEIK

The antibodies having the heavy and light chain combinations shown in Table 2 below were produced.

Table 2

Light chains				
L0	L1	L2	L3	L4

Heavy chains	H0	H0L0	H0L1	H0L2	H0L3	H0L4
	H1	H1L0	H1L1	H1L2	H1L3	H1L4
	H2	H2L0	H2L1	H2L2	H2L3	H2L4
	H3	H3L0	H3L1	H3L2	H3L3	H3L4
	H4	H4L0	H4L1	H4L2	H4L3	H4L4

Example 2: Binding to soluble CD73 protein by SPR

The binding of the antibodies in Table 2 of Example 1 were tested for binding to human soluble dimeric CD73 proteins and were cloned, produced and purified. Affinity and association and dissociation constants of humanized variants were assessed by SPR analysis. Table 3 summarizes all the calculated constants.

The H0L0 antibody with an entirely human IGHV1-3*01 and IGHJ4*01 heavy chain framework and entirely human IGKV1-33*01 and IGKJ2*01 light chain framework resulted in a KD of 65 nM. As shown in Table 3, humanized variants HxL0 (H1L0, H2L0, H3L0 and H4L0) exhibited an association constant equivalent to other variants but have a faster dissociation profile. KD of the HxL0 variants was, accordingly, higher than those observed for all other variants (Table 3). Introduction of the full human IGKV1-33*01 and IGKJ2*01 light chain amino acid sequences in the humanized variants therefore appears to be detrimental to the affinity for the humanized Ab to CD73.

However, all other humanized variants had similar affinity for CD73 as the chimeric parental 3C12 antibody (0.68 nM). Notably, all heavy chains, including the fully human unmodified IGHV1-3*01 and IGHJ4*01 heavy chain framework, provided high affinity binding to CD73.

Table 3

humanized variants	KD (nM)	ka (1/Ms)	kd (1/s)
H0L0	65	1.36E+05	8.82E-03
H0L1	0.9	1.44E+05	1.27E-04
H0L2	0.7	1.68E+05	1.19E-04
H0L3	0.6	1.70E+05	9.49E-05
H0L4	0.6	1.37E+05	8.65E-05
H1L0	28.7	1.03E+05	2.96E-03
H1L1	0.8	1.00E+05	8.43E-05
H1L2	0.6	1.35E+05	8.38E-05
H1L3	0.8	9.26E+04	7.20E-05
H1L4	0.7	9.82E+04	6.77E-05
H2L0	5.6	2.38E+05	3.20E-03
H2L1	1.4	8.37E+04	1.18E-04

H2L2	1.3	1.18E+05	1.55E-04
H2L3	1	1.20E+05	1.23E-04
H2L4	0.9	1.20E+05	1.06E-04
H3L0	29.4	2.71E+05	7.97E-03
H3L1	1.8	7.89E+04	1.40E-04
H3L2	1.4	8.66E+04	1.22E-04
H3L3	1.1	9.57E+04	1.05E-04
H3L4	1	9.04E+04	8.91E-05
H4L0	7.8	1.58E+05	1.23E-03
H4L1	1.7	1.06E+05	1.79E-04
H4L2	0.9	7.75E+04	7.12E-05
H4L3	0.7	1.10E+05	7.69E-05
H4L4	1.2	6.01E+04	7.14E-05

Example 3: Binding to cells expressing human CD73 by flow cytometry

The binding of the humanized antibodies in Table 2 were tested for binding to CD73-expressing cells by flow cytometry. As shown in **Figure 1**, all tested Abs recognized human CD73 proteins. The binding efficacy on huCD73 was similar between humanized variants and the chimeric format, except for H0L0, H2L0 and H3L0 which were less efficient than the parental antibody. These observations are in accordance with SPR data that showed lower affinity for HxL0 variants (H1L0, H2L0, H3L0 and H4L0). Consequently, all heavy chains, including the unmodified fully human IGHV1-3*01 and IGHJ4*01 heavy chain framework, provided excellent binding to CD73-expressing cells, comparable to the parental antibody.

Example 4: *In vitro* enzymatic assay with recombinant soluble human CD73 protein

The efficacy of the humanized variants were compared to the parental antibody to inhibit the enzymatic activity of the recombinant CD73 protein (**Figure 2**).

As shown in Figure 2 (panel A), high levels of luminescence was measured when ATP and CTG substrates were mixed. When AMP was added to the reaction mix, the CTG reaction was inhibited resulting in a decrease of luminescence. In the presence of rhCD73 protein that hydrolyzes AMP, the level of luminescence was restored. Blockade of CD73 enzymatic activity by the humanized variants is shown in Figure 2.

Results showed that with the exception of the L0 variants (H0L0, H1L0, H2L0, H3L0 and H4L0) which had decreased activity compared to the parental antibody, all humanized variants showed strong inhibition of enzymatic activity of the soluble CD73 protein. However, surprisingly, for most antibodies the potency was somewhat lower than that of the parental

antibody. Only the H1L4 and all H4 variants blocked the enzymatic activity with similar potency as the parental antibody.

These experiments showed that independently of the number of back-mutations on the heavy chain, the absence of mutation on the light chain when using the IGKV1-33*01 and IGKJ2*01 sequences as acceptor framework leads to a significant decrease in the ability to inhibit the activity of the soluble CD73 protein.

The best efficacy of CD73 protein activity blockade was achieved with H1L4 and H4Lx variants other than H4L0 (i.e. H4L1, H4L2, H4L3 and H4L4).

10 **Example 5: T Cell proliferation assay**

The concentration of AMP able to inhibit T cell proliferation was tested in two series of experiments in order to establish a concentration of AMP that induces a high suppression effect and that permits restoration of proliferation in presence of parental 3C12 antibody. Enriched lymphocyte fraction was incubation for 3 days in presence of anti-CD3/anti-CD28 beads and a dose range of AMP (from 50 μ M to 1.6 mM to 50 μ M). The best percentage of restoration of CD4+ and CD8+ T cells are was observed with 0.2 to 0.8mM and 0.2 to 1mM of AMP, respectively, in the two series of experiments.

The ability of humanized variants and parental antibody were assessed in the two series of experiments to compare their ability to restore T cell proliferation inhibited by 0.8mM AMP.

As shown in **Figure 3**, with the exception of the HxL0 variants, the chimeric and the humanized Abs are were able to restore T cell proliferation (for both CD4+ and CD8+ sub-populations). Despite their ability to restore the proliferation, the potency of the majority of humanized variants was lower as compared to the parental antibody. The H0L4, H1L4 and H4L4 variants demonstrated a similar level of potency of the parental antibody, although without achieving the full potency of the parental antibody.

A second experiment was performed with the same experimental conditions except that L0 variants were replaced by isotype control. The results are presented in **Figure 4**.

Similarly to that shown in Figure 3, with the exception of the HxL0 variants, the chimeric and the humanized Abs are were able to restore T cell proliferation (for both CD4+ and CD8+ sub-populations). However, despite their ability to restore the proliferation, the potency of the majority of humanized variants was again somewhat lower as compared to the parental antibody. The H0L4, H1L4 and H4L4 variants most closely approached the efficiency of the parental antibody.

35 **Example 6: Design of new framework and CDR variants**

Surprisingly, the preceding examples showed that there was no direct correlation between flow cytometry titration and potency in inhibition of the enzymatic activity of CD73. Interestingly, while H1, H2, H3 and H4 variants were essentially indistinguishable based on binding affinity to CD73 recombinant protein in SPR assays and in flow cytometry on CD73-expressing cells, the H2 and H3 variants were less potent in the functional assays than H4 variants in the ability to restore T cell proliferation in Example 5. One possibility is that the mutations introduced into the H2 and H3 frameworks which were detrimental to potency in inhibiting CD73 (but were not detrimental to binding affinity for CD73) were compensated by mutations introduced into the H4 variant frameworks. Consequently, new variants with substitutions in heavy chain framework and/or CDR residues were designed. Based on these observations, the substitutions made in the H2, H3 and H4 chains were classified as either residues that are believed to be critical for function, residues likely not to affect function, residues that negatively impact function, and residues that restore function.

A new heavy chain variant, referred to as "H4+", having the amino acid sequence shown below (SEQ ID NO: 37; amino acid substitutions in bold, Kabat CDRs underlined), was designed and were combined with L1, L2, L3 and L4 light chains (of SEQ ID NOS: 33, 34, 35 and 36, respectively) to generate four new antibodies, H4+L1, H4+L2, H4+L3 and H4+L4.

H4+ VH:

QIQLVQSGAEVKKPGASVKVSCASGYTF**AS**SYNMYWVRQAPGQRLEWIGYIDPYNGGSSYNLTFKGRV
 TLTRDKSASTAYMELSSLRSED**TAVYYCARGY**GNYKAWFAYWGQTLTVSS (SEQ ID NO: 37).

The amino acid sequence of the full H4+ heavy chain comprising a human IgG1 Fc domain with L234A/L235E/G237A/A330S/P331S substitutions is shown below:

QIQLVQSGAEVKKPGASVKVSCASGYTF**AS**SYNMYWVRQAPGQRLEWIGYIDPYNGGSSYNLTFKGRV
 TLTRDKSASTAYMELSSLRSED**TAVYYCARGY**GNYKAWFAYWGQTLTVSSASTKGPSVFPPLAPSSK
 STSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICN
 VNHKPSNTKVDKRVPEPKSCDKHTCTPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTIIS
 KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF
 LYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
 (SEQ ID NO: 38).

The H4+L1 antibody contained a L1 light chain comprising a human Ckappa domain, the full sequence of which is shown below:

DIQMTQSPSSLSASVGDRVTITCKASQSVSNDAWYQQKPGKAPKLLIYYASTRYTGVPSR

FSGSGYGTDFFTTISSLQPEDATYYCQQDYSSLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC (SEQ ID NO: 39).

In order to investigate how the same framework substitutions would impact an antibody having differences in its heavy and light chain CDRs, a further series of four antibodies was produced having a common heavy chain and one of four different light chains. The common heavy chain designated "2H4+" had a glutamine at Kabat position 59 (Q59) in HCDR2 (i.e. L59Q substitution, compared to the H4+ chain), a lysine at Kabat residue 60 (K60) in HCDR2 (i.e. a T60K substitution, compared to the H4+ chain), and an asparagine at Kabat position 97 (N97) in HCDR3 (i.e. a G97N substitution, compared to the H4+ chain). The four light chains designated 2L1, 2L2, 2L3 and 2L4 had the same framework residues as L1, L2, L3 and L4 chains, respectively, and differed in their CDRs from L1-L4 chains by presence of a threonine at Kabat position 30 (T30) in LCDR1 (i.e. a S30T substitution, compared to the L1-L4 chains), and an asparagine at Kabat residue 53 (N53) in LCDR2 (i.e. a T53N substitution, compared to the L1-L4 chains). The resulting four new antibodies are designated 2H4+2L1, 2H4+2L2, 2H4+2L3 and 2H4+2L4 (see Table 4), below. A parental antibody sharing the CDRs in murine frameworks was produced with heavy and light chain variable regions designated 2HP and 2LP (amino acid sequences shown in Table 5, below).

As in Example 1, all antibodies were produced as human IgG1 isotype with L234A/L235E/G237A/A330S/P331S substitutions to reduce Fc receptor binding.

Table 4

mAb reference	VH	VL
2H4+L1	2H4+ (SEQ ID NO: 42)	2L1 (SEQ ID NO: 43)
2H4+L2	2H4+ (SEQ ID NO: 42)	2L2 (SEQ ID NO: 44)
2H4+L3	2H4+ (SEQ ID NO: 42)	2L3 (SEQ ID NO: 45)
2H4+L4	2H4+ (SEQ ID NO: 42)	2L4 (SEQ ID NO: 46)
2HP2LP	2HP (SEQ ID NO: 40)	2LP (SEQ ID NO: 41)

Table 5

Chain	SEQ ID NO	Variable region amino acid sequence (amino acid substitutions in bold, Kabat CDRs underlined)
2HP	40	EIQLQQSGPELVKPGASVKVSCKASGYAFTSYNMYWVKQSHGKRLEWIGYIDPYNG GSSYNQKFKGKATLTVDKSSSTAYMHLNNLTSEDSAVYYCARGYNNYKAWFAYWGQ GTLVTVSA
2LP	41	DVVMTQTPKFLVLSAGDRVITITCKASQSVTNDVAWYQQKPGQSPKLLIYYASNRYT GVPD RFTGSGYGTDFTFITISTMQAEDLAVYFCQQDYSSLTFGAGTKLEIK
2H4+	42	QIQLVQSGAEVKKPGASVKVSCKASGYTFASYNMYWVRQAPGQRLEWIGYIDPYNG GSSYNQKFKGRVTLTRDKSASTAYMELSSLRSEDTAVYYCARGYNNYKAWFAYWGQ GTLVTVSS
2L1	43	DIQMTQSPSSLSASVGDRVITITCKASQSVTNDVAWYQQKPGKAPKLLIYYASNRYT GVPSRFSGSGYGTDFTFITISLQPEDATYYCQQDYSSLTFGQGTKLEIK
2L2	44	DIQMTQSPSSLSASVGDRVITITCKASQSVTNDVAWYQQKPGKAPKLLIYYASNRYT GVPDRFSGSGYGTDFTFITISLQPEDATYYCQQDYSSLTFGQGTKLEIK
2L3	45	DVQMTQSPSSLSASVGDRVITITCKASQSVTNDVAWYQQKPGKAPKLLIYYASNRYT GVPDRFSGSGYGTDFTFITISLQPEDATYYCQQDYSSLTFGQGTKLEIK
2L4	46	DVQMTQSPSSLSASVGDRVITITCKASQSVTNDVAWYQQKPGKAPKLLIYYASNRYT GVPDRFSGSGYGTDFTFITISLQPEDATYFCQQDYSSLTFGQGTKLEIK

The amino acid sequence of the full 2H4+ heavy chain comprising a human IgG1 Fc domain with L234A/L235E/G237A/A330S/P331S substitutions is shown below:

5 QIQLVQSGAEVKKPGASVKVSCKASGYTFASYNMYWVRQAPGQRLEWIGYIDPYNGGSSYNQKFKGRV
 TLTRDKSASTAYMELSSLRSEDTAVYYCARGYNNYKAWFAYWGQGTTLVTVSSASTKGPSVFPPLAPSSK
 STSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
 VNHKPSNTKVDKRVPEPKSCDKHTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVDVSHE
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTI
 10 KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFF
 LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 47).

The amino acid sequence of the full 2L1 light chain of the antibody comprising a human Ckappa domain is shown below:

15 DIQMTQSPSSLSASVGDRVITITCKASQSVTNDVAWYQQKPGKAPKLLIYYASNRYTGVPSRFSGSGYG
 TDFTFITISLQPEDATYYCQQDYSSLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPV
 TKSFNRGEC (SEQ ID NO: 48).

Example 7: Study of new variants in vitro enzymatic assay with recombinant CD73 protein

The efficacy of the humanized variants were compared to the parental antibody to inhibit the enzymatic activity of the recombinant CD73 protein, using the methods described in Example 4. All of the new humanized variants produced in Example 6 potentially inhibited the activity of the CD73 protein.

The H4+Lx antibodies were as efficient as their parental counterpart. Furthermore, all of the 2H4+2Lx (2H4+L1, 2H4+L2, 2H4+L3 and 2H4+L4) variants showed an increase in potency in CD73 inhibition compared to the H4+Lx variants. The 2H4+2Lx variants inhibited the activity of CD73 with an efficacy similar to that of the parental 2HP2LP antibody.

An additional experiment was performed with different concentrations of the CD73 protein (50, 100, 200, 400 ng/mL). The aim of this experiment was to study the ability of the humanized antibodies to block the activity of high amounts of CD73 protein. Again, the 2H4+2Lx variants were as potent as the parental 2HP2LP antibody.

Example 8: Study of new variants for efficacy on cynomolgus CD73 protein

An experiment on recombinant rec cyCD73 protein was performed with 2H1Lx and 2H4+2Lx variants. Experimental conditions were the same as in experiments on human protein except for the concentration of cyCD73 protein that was used (400 ng/mL).

The efficacy of the humanized variants to block enzymatic activity of cyCD73 protein was the same as that of the parental (chimeric) antibody.

Example 9: Study of new variants in T Cell proliferation assay

Humanized variants of the different antibodies were tested for their ability to restore T cell proliferation inhibited by AMP.

Figure 5A shows the inhibitory effect of AMP on T cell proliferation. All humanized variants potentially blocked inhibitory effect of AMP on T cell proliferation (**Figures 5B and C**). While most humanized variants generally appear to be as efficient as their chimeric parental counterpart in reversing AMP-mediated T cell suppression, the 2H4+2Lx variants were the most potent among all variants, and surprisingly they were even more potent than the parental 2HP2LP antibody to block the suppressive effects of AMP.

Example 10: Comparative study of new variants in T cell proliferation assay in two human donors

According to results previously obtained, the 2H4+2Lx humanized variants were further characterized in further series of T cell proliferation experiments.

Figures 6A and 6B shows results obtained in a T cell proliferation assay using cells from two healthy donors, respectively. As observed previously, 2H4+2Lx variants were each more potent than the parental 2HP2LP to restore T cell proliferation (Figure 6A and B, right panels). No clear differences among the different 2H4+2Lx variants were observed. Thus, once again, the 2H4+2Lx variants were more potent than the parental 2HP2LP antibody to block the suppressive effects of AMP.

Taken together, the results obtained on T cell proliferation assay indicate that the humanized variants having the frameworks of the H4+ and H4+ chains are the most potent antibody variants, and that the 2H4+2Lx variants overall have the greatest potency amongst all the antibodies.

Example 11: Study of new variants in allogeneic Mixed Lymphocyte Reaction (MLR) assay

The 2H4+2Lx antibody variant was tested in an allogenic MLR in order to confirm the results obtained previously in T cell proliferation assays. T cell proliferation was inhibited by adding 100 μ M of ATP (which is degraded into ADP and AMP by CD39), shown in Figure 7 for two human donor samples.

As shown in Figure 7, left panel, T cell proliferation was inhibited by addition of ATP. T cell proliferation was restored in the presence of the anti-CD73 antibodies (middle and right hand panels). All of the 2H4+2Lx humanized variants were able to restore T cell proliferation with comparable or better efficacy comparable to or better than the parental 2HP2LP antibody. All 2H4+2Lx variants were more potent than the parental 2HP2LP to restore T cell proliferation in these settings. These results were in accordance with those obtained in T cell proliferation assay using AMP as inhibitor.

In summary, the substitutions introduced in the H4+ and 2H4+ variable regions together with the substitutions introduced into the L1 and 2L1 chains (and the L2, L3, L4, 2L2, 2L3, 2L4 chains) appear to restore (and even improve on) the important functional properties of their parental murine antibodies, yet have human framework regions and therefore lower risk of immunogenicity in humans. The 2H4+2Lx antibodies are the most potent of all.

One possible explanation for the surprising finding that binding affinity for CD73 did not directly correlate with potency in functional inhibition of CD73 activity may be related to the fact that these antibodies bind to CD73 act as allosteric inhibitors. The antibodies bind to CD73 in an intra-dimer binding mode in a 1:1 stoichiometry between an intact full-length antibody and a CD73 dimer. Previous structural studies of CD73 have shown that its enzymatic activity requires extensive N-terminal domain rotation defining open (inactive) and

closed (active, substrate-bound) states of the enzyme (Knapp et al., 2012 Structure 20(12):2161-73). Based on the crystal structure of parental anti-CD73 F(ab) in complex with the ectodomain of CD73, and taking into consideration the 1:1 stoichiometry between an intact antibody and a CD73 dimer, it is anticipated that steric hindrance would make it unlikely that the intact antibody could bind to CD73 open conformers. Instead, the intact antibody constraints CD73 in an intermediate state in which AMP cannot be hydrolysed. Since CD73 is therefore bound by the antibodies while in an energetically unstable conformation, small changes that modify the rigidity of the antibody structure here may be affecting the ability of the antibody to adapt to the changing conformation of the CD73 dimer. These changes consequently may impact the ability of the antibody to inhibit the activity of CD73 in the presence of substrate. However these small changes in antibody structure would not be observed in SPR or other binding assays, that furthermore are carried out in the absence of substrate. The heavy and light chain variable regions described herein appear to be able to capture (and even improve on) the full CD73 interaction dynamics of the parental antibodies, while incorporating human frameworks.

All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference in their entirety and to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein (to the maximum extent permitted by law), regardless of any separately provided incorporation of particular documents made elsewhere herein.

The use of the terms "a" and "an" and "the" and similar references are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

The description herein of any aspect or embodiment herein using terms such as "comprising", "having," "including," or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment herein that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

CLAIMS

1. An antibody or antibody fragment capable of specifically binding to a human CD73 polypeptide, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 42 (2H4+ chain), and a light chain variable region (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 43 (2L1 chain), 44 (2L2 chain), 45 (2L3 chain) and 46 (2L4 chain).
2. The antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 42 (2H4+ chain), and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 43 (2L1 chain).
3. The antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 47, and a light chain comprising the amino acid sequence of SEQ ID NO: 48.
4. An antibody or antibody fragment capable of specifically binding a human CD73 polypeptide, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 37 (H4+ chain), and a light chain variable region (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 33 (L1 chain), 34 (L2 chain), 35 (L3 chain) and 36 (L4 chain).
5. The antibody or antibody fragment of claim 4, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 37 (H4+ chain), and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 33 (L1 chain).
6. The antibody or antibody fragment of claim 4, 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.
7. The antibody or antibody fragment of any of the above claims, wherein the antibody or antibody fragment is capable of neutralizing the 5'-ectonucleotidase activity of a CD73 polypeptide.
8. An antibody or antibody fragment that is capable of specifically binding a human CD73 polypeptide at the surface of a cell and neutralizing the 5'-ectonucleotidase activity of the cell surface CD73 polypeptide, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOS: 42, and a light chain variable

region (VL) comprising an amino acid sequence at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 43.

9. An antibody or antibody fragment that is capable of specifically binding a human CD73 polypeptide at the surface of a cell and neutralizing the 5'-ectonucleotidase activity of the cell surface CD73 polypeptide, wherein the antibody or antibody fragment comprises a heavy chain comprising an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NOS: 47, and a light chain comprising an amino acid sequence at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 48.

10. The antibody or antibody fragment of claims 8 or 9, wherein the VH comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 3 and 4; and a the VL comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 5, 6 and 7.

11. The antibody or antibody fragment of any one of claims 8 to 10, wherein the VH comprises framework FR1, FR2 and FR3 amino acid sequences from the human IGHV1-3 gene and the VL comprises framework FR1, FR2 and FR3 amino acid sequences from the human IGKV1-33 gene.

12. The antibody or antibody fragment of any one of claims 8-11, wherein the VH comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 8 and 9; and a the VL comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 10, 11 and 7.

13. The antibody or antibody fragment of claim 12, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NOS: 37, and a light chain variable region (VL) comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 33.

14. The antibody or antibody fragment of claim 12, wherein the antibody or antibody fragment comprises a heavy chain comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NOS: 38, and a light chain comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 39.

15. The antibody or antibody fragment of any one of claims 8-11, wherein the VH comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 2, 12 and 13; and a the VL comprises a CDR1, CDR2 and CDR3 having the respective amino acid sequences shown in SEQ ID NOS: 14, 15 and 7.

16. The antibody or antibody fragment of claim 15, wherein the antibody or antibody fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NOS: 42, and a light chain variable region (VL) comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 43.

17. The antibody or antibody fragment of claim 15, wherein the antibody or antibody fragment comprises a heavy chain comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NOS: 47, and a light chain comprising an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 48.

18. The antibody or antibody fragment of any one of the above claims, wherein: the amino acid at Kabat heavy chain position 2 is an isoleucine.

19. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat heavy chain position 30 is an alanine.

20. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat heavy chain position 48 is an isoleucine.

21. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat heavy chain position 69 is a leucine.

22. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat heavy chain position 73 is a lysine.

23. The antibody or antibody fragment of any one of the above claims, wherein the heavy chain comprises an isoleucine residue at Kabat position 2, an alanine at position 30, an isoleucine at position 48, a leucine at position 69 and a lysine at position 73.

24. The antibody or antibody fragment of any one of the above claims, wherein amino acid at Kabat light chain position 67 is a tyrosine.

25. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat light chain position 60 is serine or aspartic acid.

26. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat light chain position 2 is valine or isoleucine.

27. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat light chain position 87 is tyrosine or phenylalanine.

28. The antibody or antibody fragment of any one of the above claims, wherein the light chain comprises a tyrosine residue at Kabat position 67, a serine at position 60, an isoleucine at position 2, and a tyrosine at position 87.

29. The antibody or antibody fragment of any one of the above claims, wherein the amino acid at Kabat heavy chain position 28 is threonine (T), the amino acid at Kabat heavy chain position 66 is arginine (R), the amino acid at Kabat heavy chain position 67 is valine (V), and the amino acid at Kabat heavy chain position 71 is arginine (R).

30. The antibody or antibody fragment of any of the above claims, wherein the antibody or antibody fragment specifically binds to a human CD73 polypeptide at the surface of a cell and that is capable of neutralizing the 5'-ectonucleotidase activity thereof, wherein the antibody or antibody fragment inhibits the activity of the human CD73 polypeptide without detectably reducing binding between the CD73 polypeptide and a substrate thereof.

31. The antibody or antibody fragment of any one of the above claims wherein the antibody or antibody fragment has reduced binding to a mutant CD73 polypeptide comprising the mutation K136A and/or the mutations A99S, E129A, K133A, E134N, and A135S (with reference to SEQ ID NO: 1), relative to binding between the antibody or antibody fragment and a wild-type CD73 polypeptide comprising the amino acid sequence of SEQ ID NO: 1.

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

33. The antibody or antibody fragment of any one of the above claims, wherein the antibody or antibody fragment comprises a human IgG1 Fc domain comprising amino acid substitutions at residues 234, 235 and 331 or comprising amino acid substitutions at residues 234, 235 and 322 (EU numbering according to Kabat).

34. The antibody of any of the above claims, wherein the antibody is a full-length antibody.

35. The antibody or antibody fragment of any of the above claims, wherein the antibody is an antibody fragment.

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

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

38. A nucleic acid or set of nucleic acids encoding a heavy and/or light chain of an antibody or antibody fragment of any one of claims 1 to 35.

39. A recombinant host cell producing the antibody or antibody fragment of any one of claims 1 to 35.

40. A method for the treatment or prevention of a disease in a patient in need thereof, the method comprising administering to said patient an effective amount of an antibody, antibody fragment or a composition of any one of claims 1-36.

41. The method of claim 40, wherein said disease is cancer or infectious disease.

42. The method of any one of claims 40 or 41, wherein the disease or cancer is a leukemia, bladder cancer, glioma, glioblastoma, ovarian cancer, melanoma, prostate cancer, thyroid cancer, stomach cancer, esophageal cancer, pancreatic cancer or a breast cancer.