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(54) Title: ANTIBODIES HAVING SPECIFICITY TO MYOSIN 18A AND USES THEREOF

(57) Abstract: The present disclosure relates to antibodies having specificity to myosin 18A and uses thereof. In particular, the present disclosure relates to an antibody having specificity to myosin 18A comprising a heavy chain comprising i) a H-CDR1 having at least 90% of identity with the H-CDR1 of DY12, ii) a H-CDR2 having at least 90% of identity with the H-CDR2 of DY12 and iii) a H-CDR3 having at least 90% of identity with the H-CDR3 of DY12 and a light chain comprising i) a L- CDR1 having at least 90% of identity with the L-CDR1 of DY12, ii) a L- CDR2 having at least 90% of identity with the L-CDR2 of DY12 and iii) a L-CDR3 having at least 90% of identity with the L-CDR3 of DY12.

ANTIBODIES HAVING SPECIFICITY TO MYOSIN 18A AND USES THEREOF

FIELD OF THE INVENTION:

The present invention relates to antibodies having specificity to myosin 18A and uses thereof.

BACKGROUND OF THE INVENTION:

Natural Killer (NK) cells were identified over 40 years ago as a subset of lymphocytes able to spontaneously kill tumor cells in the absence of pre-stimulation (1–4). Present in most mammalian and avian species, NK cells play a critical role in the anti-tumor and anti-infectious immunity (5,6) and in reproduction (7). In humans, NK lymphocytes are phenotypically characterized by the expression of CD56, an isoform of the neural cell adhesion molecule, and by the absence of CD3 (8). NK cell cytotoxicity is tightly controlled by a balance between signals from the engagement of activating and inhibitory receptors (6,9). Upon contact with a target cell, integrins on the NK cell surface bind to adhesion molecules on the target cell and stabilize the cell-to-cell interaction (10). Binding of the integrin Lymphocyte Function-associated Antigen 1 (LFA-1) to Intercellular Adhesion Molecule 1 (ICAM-1) on target cells initiate an early signaling cascade in NK cells through activation of the guanine nucleotide exchange factor (GEF) domain-containing Vav1 and of p21-activated kinases (PAK) (11). This leads to cytoskeleton reorganization and aggregation of activating NK receptors (NKR) at the NK-target cell interface (12), referred to as the NK immune synapse (NKIS) (13). Engagement of activating NKRs leads to the recruitment of immunoreceptor tyrosine activation motifs (ITAM)-bearing adapter proteins. The two tyrosines in the ITAM are phosphorylated by Src-kinase family members, and phosphorylated ITAM form a binding site for the Src-homology domain 2 (SH2) domains of tyrosine kinases (14), triggering a signalling cascade responsible for granule polarization, degranulation, and cytolysis of the target cell. Activating NKR include members of the family of natural cytotoxicity receptors (such as CD245 (15), NKp44 (16), and NKp30 (17)), of the NKG2 family of C-type lectin receptors (NKG2D) (18), of the killer cell Ig-like receptors (KIRs) (19,20), of the Ig-like signaling lymphocytic activation molecule (SLAM) family (2B4) (21), and others such as CD160 (22,23). 4-1BB (CD137) is a costimulatory receptor expressed on T, B and NK cells (24) whose expression is triggered by engagement of Fc receptors on the NK cell surface, as is the case during antibody-dependent cell cytotoxicity (25). Stimulation

of CD137 increases cetuximab-, rituximab-, and trastuzumab-dependent NK cell cytotoxicity in different cancer models (26–28). NK cell activation is dominantly suppressed if the inhibitory NKR bind to major histocompatibility complex (MHC) class I molecules on target cells (29). In humans, these receptors mainly belong to C-type lectin receptors, as the NKG2A heterodimer (30), or to the KIR superfamily of receptors (19,20). Unlike the activating KIRs, the inhibitory KIRs carry a long cytoplasmic tail bearing immunoreceptor tyrosine inhibition motifs (ITIM) sequences (31). The latter provide a specific binding site for the tandem SH2 domains of Src homology region 2-containing protein tyrosine phosphatase-1 (SHP-1) or SHP-2. SHP recruitment at the NKIS is able to block many of the key steps in the signalling cascade leading to cytolysis (32). CD245 was previously described as a unique surface antigen on the surface of human peripheral blood lymphocytes, recognized by the monoclonal antibody DY12 (33). However CD245 molecular and functional characteristics remain largely unknown.

SUMMARY OF THE INVENTION:

The present invention relates to antibodies having specificity to myosin 18A and uses thereof. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to antibodies having specificity to myosin 18A and uses thereof. In particular, the present invention provides antibodies that derive from the DY12 antibody. The inventors have indeed characterized the DY12 antibody and demonstrate that said antibody is capable of binding to myosin 18A (i.e. CD245) and enhancing NK cell cytotoxicity.

As used herein, the term “myosin 18A” denotes the unconventional myosin 18A, unconventional myosin-XVIIIa or Myo18A. Exemplary human nucleic acid sequences are referenced in under NCBI Reference Numbers NM_078471.3 and NM_203318.1. Exemplary human amino acid sequences are referenced in under NCBI Reference Numbers NP_510880.2 and NP_976063.1. A further exemplary amino acid sequence includes SEQ ID NO:5 (Figure 1B).

As used herein the term "antibody" or "immunoglobulin" have the same meaning, and will be used equally in the present invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds

an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (1) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CHI, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from non-hypervariable or framework regions (FR) can participate to the antibody binding site or influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L- CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, typically includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs. The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat et al. This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al."). This numbering system is used in the present specification. The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues in SEQ ID sequences. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a

structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence. The CDRs of the heavy chain variable domain are located at residues 31-35B (H-CDR1), residues 50-65 (H-CDR2) and residues 95-102 (H-CDR3) according to the Kabat numbering system. The CDRs of the light chain variable domain are located at residues 24-34 (L-CDR1), residues 50-56 (L-CDR2) and residues 89-97 (L-CDR3) according to the Kabat numbering system.

As used herein, the term "specificity" refers to the ability of an antibody to detectably bind an epitope presented on an antigen, such as a myosin 18A, while having relatively little detectable reactivity with non-myosin 18A proteins or structures (such as other proteins presented on NK cells, or on other cell types). Specificity can be relatively determined by binding or competitive binding assays, using, e.g., Biacore instruments, as described elsewhere herein. Specificity can be exhibited by, e.g., an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules (in this case the specific antigen is a myosin 18A polypeptide). 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 Kd, defined as [Ab] x [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 Ka is defined by 1/Kd. Preferred 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 preferred and standard method well known in the art for determining the affinity of mAbs is the use of Biacore instruments.

According to the present invention, the VH region of the DY12 antibody consists of the sequence of SEQ ID NO:1. Accordingly, the H-CDR1 of DY12 is defined by the sequence ranging from the amino acid residue at position 31 to the amino acid residue at position 35 in SEQ ID NO:1. Accordingly, the H-CDR2 of DY12 is defined by the sequence ranging from the amino acid residue at position 50 to the amino acid residue at position 66 in SEQ ID NO:1. Accordingly, the H-CDR3 of DY12 is defined by the sequence ranging from

the amino acid residue at position 99 to the amino acid residue at position 109 in SEQ ID NO:1.

SEQ ID NO:1: VH region of DY12 antibody FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

QVQLQQPGAELVRPGASVMLSCKASGYTFT**NYWIN**WVRQRPGQGLEWIG**NIYPSDSYTNYNQ**
KFKDKATLTVDNSSSTAYMQFSSPTSEDSAVYFCTR**LTTVGAGAMDY**WGQGTSVTVSS

According to the present invention, the VL region of the DY12 antibody consists of the sequence of SEQ ID NO:2. Accordingly, the L-CDR1 of DY12 is defined by the sequence ranging from the amino acid residue at position 24 to the amino acid residue at position 34 in SEQ ID NO:2. Accordingly, the L-CDR2 of DY12 is defined by the sequence ranging from the amino acid residue at position 50 to the amino acid residue at position 56 in SEQ ID NO:2. Accordingly, the L-CDR3 of DY12 is defined by the sequence ranging from the amino acid residue at position 89 to the amino acid residue at position 97 in SEQ ID NO:2.

SEQ ID NO:2: VL region of DY12 antibody FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

DIQMTQSSSYLSVSLGGRVTITC**KASDHINNWLA**WYQQKPGNAPRLLIS**GATSLET**GVPSRF
SGSGSGKDYTLSIFSLQSEDVATYYC**QQYWSTPFT**FGSGTKLEIK

The present invention thus provides antibodies comprising functional variants of the VL region, VH region, or one or more CDRs of DY12. A functional variant of a VL, VH, or CDR used in the context of a human monoclonal antibody of the present invention still allows the antibody to retain at least a substantial proportion (at least about 50%, 60%, 70%, 80%, 90%, 95% or more) of the affinity/avidity and/or the specificity/selectivity of the parent antibody (i.e. DY12 antibody) and in some cases such a human monoclonal antibody of the present invention may be associated with greater affinity, selectivity and/or specificity than the parent Ab. Such functional variants typically retain significant sequence identity to the parent Ab. The sequence of CDR variants may differ from the sequence of the CDR of the parent antibody sequences through mostly conservative substitutions; for instance at least about 35%, about 50% or more, about 60% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, (e.g., about 65-95%, such as about 92%, 93% or 94%) of the substitutions in the variant are conservative amino acid residue replacements. The sequences of CDR variants may differ from the sequence of the CDRs of the parent antibody sequences through mostly conservative substitutions; for instance at least 10, such as at least 9, 8, 7, 6, 5, 4, 3, 2 or 1 of the substitutions in the variant are conservative amino acid residue replacements. In the context of the present invention,

conservative substitutions may be defined by substitutions within the classes of amino acids reflected as follows:

Aliphatic residues I, L, V, and M

Cycloalkenyl-associated residues F, H, W, and Y

Hydrophobic residues A, C, F, G, H, I, L, M, R, T, V, W, and Y

Negatively charged residues D and E

Polar residues C, D, E, H, K, N, Q, R, S, and T

Positively charged residues H, K, and R

Small residues A, C, D, G, N, P, S, T, and V

Very small residues A, G, and S

Residues involved in turn A, C, D, E, G, H, K, N, Q, R, S, P, and formation T

Flexible residues Q, T, K, S, G, P, D, E, and R

More conservative substitutions groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Conservation in terms of hydropathic/hydrophilic properties and residue weight/size also is substantially retained in a variant CDR as compared to a CDR of DY12. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8) ; phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophane (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). The retention of similar residues may also or alternatively be measured by a similarity score, as determined by use of a BLAST program (e.g., BLAST 2.2.8 available through the NCBI using standard settings BLOSUM62, Open Gap= 11 and Extended Gap= 1). Suitable variants typically exhibit at least about 70% of identity to the parent peptide. According to the present invention a first amino acid sequence having at least 70% of identity with a second amino acid sequence means that the first sequence has 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; or 100% of identity with the second amino acid sequence. According to the present invention a first amino acid sequence having at least 90%

of identity with a second amino acid sequence means that the first sequence has 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; or 100% of identity with the second amino acid sequence.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain comprising i) a H-CDR1 having at least 90% of identity with the H-CDR1 of DY12, ii) a H-CDR2 having at least 90% of identity with the H-CDR2 of DY12 and iii) a H-CDR3 having at least 90% of identity with the H-CDR3 of DY12.

In some embodiments, the antibody of the present invention is an antibody comprising a light chain comprising i) a L-CDR1 having at least 90% of identity with the L-CDR1 of DY12, ii) a L-CDR2 having at least 90% of identity with the L-CDR2 of DY12 and iii) a L-CDR3 having at least 90% of identity with the L-CDR3 of DY12.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain comprising i) a H-CDR1 having at least 90% of identity with the H-CDR1 of DY12, ii) a H-CDR2 having at least 90% of identity with the H-CDR2 of DY12 and iii) a H-CDR3 having at least 90% of identity with the H-CDR3 of DY12 and a light chain comprising i) a L-CDR1 having at least 90% of identity with the L-CDR1 of DY12, ii) a L-CDR2 having at least 90% of identity with the L-CDR2 of DY12 and iii) a L-CDR3 having at least 90% of identity with the L-CDR3 of DY12.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain comprising i) the H-CDR1 of DY12, ii) the H-CDR2 of DY12 and iii) the H-CDR3 of DY12.

In some embodiments, the antibody of the present invention is an antibody comprising a light chain comprising i) the L-CDR1 of DY12, ii) the L-CDR2 of DY12 and iii) the L-CDR3 of DY12.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain comprising i) the H-CDR1 of DY12, ii) the H-CDR2 of DY12 and iii) the H-CDR3 of DY12 and a light chain comprising i) the L-CDR1 of DY12, ii) the L-CDR2 of DY12 and iii) the L-CDR3 of DY12.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain having at least 70% of identity with SEQ ID NO:1

In some embodiments, the antibody of the present invention is an antibody comprising a light chain having at least 70 of identity with SEQ ID NO:2.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain having at least 70% of identity with SEQ ID NO:1 and a light chain having at least 70 %of identity with SEQ ID NO:2.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain which is identical to SEQ ID NO:1

In some embodiments, the antibody of the present invention is an antibody comprising a light chain identical to SEQ ID NO:2.

In some embodiments, the antibody of the present invention is an antibody comprising a heavy chain identical to SEQ ID NO:1 and a light chain identical to SEQ ID NO:2.

In some embodiments, the antibody of the present invention is a chimeric antibody, typically a chimeric mouse/human antibody. The term "chimeric antibody" refers to a monoclonal antibody which comprises a VH domain and a VL domain of an antibody derived from a non-human animal, a CH domain and a CL domain of a human antibody. As the non-human animal, any animal such as mouse, rat, hamster, rabbit or the like can be used. In particular, said mouse/human chimeric antibody may comprise the heavy chain and the light chain of the DY12 antibody.

In some embodiments, the antibody of the present invention is a humanized antibody which comprises the CDRs of the DY12 antibody. As used herein the term "humanized antibody" refers to antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR from a donor immunoglobulin of different specificity as compared to that of the parent immunoglobulin.

In some embodiments, the antibody of the present invention is selected from the group of Fab, F(ab')2, Fab' and scFv. As used herein, the term “Fab” denotes an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papaine, are bound together through a disulfide bond. The term “F(ab')2” refers to an antibody fragment having a molecular weight of about 100,000 and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin. The term “Fab' “ refers to an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab')2. A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. The human scFv fragment of the invention includes CDRs that are held in appropriate conformation, preferably by using gene recombination techniques.

The antibodies of the present invention are produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique,

either alone or in combination. Typically, knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said antibodies, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions. Alternatively, antibodies of the present invention can be synthesized by recombinant DNA techniques well-known in the art. For example, antibodies can be obtained as DNA expression products after incorporation of DNA sequences encoding the antibodies into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired antibodies, from which they can be later isolated using well-known techniques.

Accordingly, a further object of the invention relates to a nucleic acid molecule encoding an antibody according to the invention. More particularly the nucleic acid molecule encodes a heavy chain or a light chain of an antibody of the present invention. More particularly the nucleic acid molecule comprises a nucleic acid sequence having 70% of identity with SEQ ID NO:3 or SEQ ID NO:4.

SEQ ID NO:3 Heavy chain: DNA sequence FR1-**CDR1**-FR2-**CDR2**-FR3-**CDR3**-FR4

CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTGGTGAGGCCTGGGGCTTCAGTGATGCTGTC
CTGCAAGGCTTCTGGCTACACCTTCACC**AACTACTGGATAAA**CTGGGTGAGGCAGAGGCCTG
GACAAGGCCTTGAGTGGATCGGA**AATATTTATCCTTCTGATAGTTATACCAACTACAATCAA**
AAGTTCAAGGACAAGGCCACTTTGACTGTAGACAATTCATCCAGCACAGCCTACATGCAGTT
CAGCAGCCCGACATCTGAGGACTCTGCGGTCTATTTCTGTACAAGG**TTAACTACGGTGGGGG**
CTGGTGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

SEQ ID NO:4 Light chain: DNA sequence FR1-**CDR1**-FR2-**CDR2**-FR3-**CDR3**-FR4

GACATCCAGATGACACAATCTTCATCCTACTTGTCTGTATCTCTAGGAGGCAGAGTCACCAT
TACTTGC**AAGGCAAGTGACCACATTAATAATTGGTTAGCC**TGGTATCAGCAGAAACCAGGAA
ATGCTCCTAGGCTCTTAATATCT**GGTGCAACCAGTTTGGAAACT**GGGGTTCCTTCAAGATTT
AGTGGCAGTGGATCTGGAAAGGATTACACTCTCAGCATTTTTAGTCTTCAGAGTGAAGATGT
TGCTACTTATTACTGT**CAACAGTATTGGAGTACTCCATTCACG**TTCGGCTCGGGGACAAAGC
TGGAAATAAAA

Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector. As used herein, the terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and

translation) of the introduced sequence. So, a further object of the invention relates to a vector comprising a nucleic acid of the invention. Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said antibody upon administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40, LTR promoter and enhancer of Moloney mouse leukemia virus, promoter and enhancer of immunoglobulin H chain and the like. ny expression vector for animal cell can be used, so long as a gene encoding the human antibody C region can be inserted and expressed. Examples of suitable vectors include pAGE107, pAGE103, pHSG274, pKCR, pSG1 beta d2-4 and the like. Other examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

A further object of the present invention relates to a host cell which has been transfected, infected or transformed by a nucleic acid and/or a vector according to the invention. As used herein, the term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA bas been "transformed".

The nucleic acids of the invention may be used to produce an antibody of the present invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell. Common expression systems include E. coli host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include E.coli, Kluyveromyces or Saccharomyces yeasts, mammalian cell lines (e.g., Vero cells, CHO cells,

3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.). Examples also include mouse SP2/0-Ag14 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G et al; 1980), rat YB2/3HL.P2.G11.16Ag.20 cell (ATCC CRL1662, hereinafter referred to as "YB2/0 cell"), and the like. The present invention also relates to a method of producing a recombinant host cell expressing an antibody according to the invention, said method comprising the steps of: (i) introducing in vitro or ex vivo a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing in vitro or ex vivo the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete said antibody. Such recombinant host cells can be used for the production of antibodies of the present invention.

Antibodies of the present invention are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

In some embodiments, the human chimeric antibody of the present invention can be produced by obtaining nucleic sequences encoding VL and VH domains as previously described, constructing a human chimeric antibody expression vector by inserting them into an expression vector for animal cell having genes encoding human antibody CH and human antibody CL, and expressing the coding sequence by introducing the expression vector into an animal cell. As the CH domain of a human chimeric antibody, it may be any region which belongs to human immunoglobulin, but those of IgG class are suitable and any one of subclasses belonging to IgG class, such as IgG1, IgG2, IgG3 and IgG4, can also be used. Also, as the CL of a human chimeric antibody, it may be any region which belongs to Ig, and those of kappa class or lambda class can be used. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art (See Morrison SL. et al. (1984) and patent documents US5,202,238; and US5,204,244).

The humanized antibody of the present invention may be produced by obtaining nucleic acid sequences encoding CDR domains, as previously described, constructing a humanized antibody expression vector by inserting them into an expression vector for animal cell having genes encoding (i) a heavy chain constant region identical to that of a human antibody and (ii) a light chain constant region identical to that of a human antibody, and

expressing the genes by introducing the expression vector into an animal cell. The humanized antibody expression vector may be either of a type in which a gene encoding an antibody heavy chain and a gene encoding an antibody light chain exists on separate vectors or of a type in which both genes exist on the same vector (tandem type). In respect of easiness of construction of a humanized antibody expression vector, easiness of introduction into animal cells, and balance between the expression levels of antibody H and L chains in animal cells, humanized antibody expression vector of the tandem type is preferred. Examples of tandem type humanized antibody expression vector include pKANTEX93 (WO 97/10354), pEE18 and the like. Methods for producing humanized antibodies based on conventional recombinant DNA and gene transfection techniques are well known in the art (See, e. g., Riechmann L. et al. 1988; Neuberger MS. et al. 1985). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan EA (1991); Studnicka GM et al. (1994); Roguska MA. et al. (1994)), and chain shuffling (U.S. Pat. No.5,565,332). The general recombinant DNA technology for preparation of such antibodies is also known (see European Patent Application EP 125023 and International Patent Application WO 96/02576).

The Fab of the present invention can be obtained by treating an antibody which specifically reacts with AMH with a protease, papaine. Also, the Fab can be produced by inserting DNA encoding Fab of the antibody into a vector for prokaryotic expression system, or for eukaryotic expression system, and introducing the vector into a procaryote or eucaryote (as appropriate) to express the Fab.

The F(ab')2 of the present invention can be obtained treating an antibody which specifically reacts with AMH with a protease, pepsin. Also, the F(ab')2 can be produced by binding Fab' described below via a thioether bond or a disulfide bond.

The Fab' of the present invention can be obtained treating F(ab')2 which specifically reacts with AMH with a reducing agent, dithiothreitol. Also, the Fab' can be produced by inserting DNA encoding Fab' fragment of the antibody into an expression vector for prokaryote, or an expression vector for eukaryote, and introducing the vector into a prokaryote or eukaryote (as appropriate) to perform its expression.

The scFv of the present invention can be produced by obtaining cDNA encoding the VH and VL domains as previously described, constructing DNA encoding scFv, inserting the DNA into an expression vector for prokaryote, or an expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote (as appropriate) to express

the scFv. To generate a humanized scFv fragment, a well known technology called CDR grafting may be used, which involves selecting the complementary determining regions (CDRs) from a donor scFv fragment, and grafting them onto a human scFv fragment framework of known three dimensional structure (see, e. g., W098/45322; WO 87/02671; US5,859,205; US5,585,089; US4,816,567; EP0173494).

Engineered antibodies of the present invention include those in which modifications have been made to framework residues within VH and/or VL, e.g. to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis. Such "backmutated" antibodies are also intended to be encompassed by the invention. Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell -epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr et al.

In some embodiments, the antibody of the present invention comprises human heavy chain constant regions sequences but will not deplete NK cells to which they are bound and preferably do not comprise an Fc portion that induces antibody dependent cellular cytotoxicity (ADCC). As used herein, the term “depleting”, with respect to myosin 18A-expressing cells means a process, method, or compound that can kill, eliminate, lyse or induce such killing, elimination or lysis, so as to negatively affect the number of myosin 18A expressing cells present in a sample or in a subject. 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 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). In some embodiments the antibody of the present invention does not lead, directly or indirectly, to the depletion of NK cells expressing myosin 18A polypeptides (e.g. do not lead to a 10%, 20%, 50%, 60% or greater elimination or decrease in number of myosin 18A+ NK cells). In some embodiments, the antibody of the present invention does not comprise an Fc domain capable of substantially binding to a FcγRIIIA (CD16) polypeptide. In some embodiments, the antibody of the present invention lacks an Fc domain (e.g. lacks a CH2 and/or CH3 domain) or comprises an Fc domain of IgG2 or IgG4 isotype. In some embodiments, the antibody of the present invention consists of or comprises a Fab, Fab', Fab'-SH, F (ab') 2, Fv, a diabody, single-chain antibody fragment, or a multispecific antibody comprising multiple different antibody fragments. In some embodiments, the antibody of the present invention is not linked to a toxic moiety. In some embodiments, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C2q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by ldusogie et al.

Another modification of the antibodies herein that is contemplated by the invention is pegylation. An antibody can be pegylated to, for example, increase the biological (e.g., serum) half-life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. The pegylation can be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (DY12- DY120) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the present invention. See for example, EP O 154 316 by Nishimura et al. and EP 0 401 384 by Ishikawa et al.

Accordingly, one object of the present invention relates to a method of enhancing NK cell killing activities in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an antibody of the present invention.

As used herein, "NK cells" refers to a sub-population of lymphocytes that is involved in non-conventional immunity. NK cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including CD56 and/or CD16 for human NK cells, the absence of the alpha/beta or gamma/delta TCR complex on the cell surface, the ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic machinery, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response ("NK cell killing activities"). Any subpopulation of NK cells will also be encompassed by the term NK cells. Within the context of this invention "active" NK cells designate biologically active NK cells, including NK cells having the capacity of lysing target cells or enhancing the immune function of other cells. For instance, an "active" NK cell can be able to kill cells that express a ligand for an activating NK receptor and/or fail to express MHC/HLA antigens recognized by a KIR on the NK cell.

The ability of the antibody of the present invention to enhance NK cell killing activities may be determined by any assay well known in the art. Typically said assay is an *in vitro* assay wherein NK cells are brought into contact with target cells (e.g. target cells that are recognized and/or lysed by NK cells). For example, the compound can be selected for the ability to increase specific lysis by NK cells by more than about 20%, preferably with at least about 30%, at least about 40%, at least about 50%, or more of the specific lysis obtained at the same effector: target cell ratio with NK cells or NK cell lines that are contacted by the antibody of the present invention,. Examples of protocols for classical cytotoxicity assays are described, for example, in Pessino et al, J. Exp. Med, 1998, 188 (5): 953-960; Sivori et al, Eur J Immunol, 1999. 29:1656-1666; Brando et al, (2005) J. Leukoc. Biol. 78:359-371; El-Sherbiny et al, (2007) Cancer Research 67(18):8444-9; and Nolte-'t Hoen et al, (2007) Blood 109:670-673). Typically, NK cell cytotoxicity is determined by any assay described in the EXAMPLE. NK cell cytotoxicity may be measured by a classical in vitro chromium release test of cytotoxicity. Effector cells are typically fresh PB-NK from healthy donors. The target cells are typically the murine mastocytoma P815 cells or EBV-infected B cell lines. Accordingly, the antibody of the present invention is selected if it causes an increase in the reactivity or cytoxicity of NK cells toward target cells (infected cells, tumor cells, pro-inflammatory cells, etc.), increased activation, activation markers (e.g. CD107 expression) and/or IFNgamma production in NK cells, and/or increased the frequency in vivo of such activated, reactive, cytotoxic and/or activated NK cells.

In some embodiments, the subject suffers from a cancer or an infectious disease. Accordingly, a further object of the present invention relates to a method of treating a cancer or an infectious disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an antibody of the present invention.

As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (e.g., preventing or delaying the worsening of the disease), preventing or delaying the spread (e.g., metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of cancer. The methods of the present invention contemplate any one or more of these aspects of treatment.

As used herein, the term "cancer" has its general meaning in the art and includes, but is not limited to, solid tumors and blood borne tumors The term cancer includes diseases of the skin, tissues, organs, bone, cartilage, blood and vessels. The term "cancer" further encompasses both primary and metastatic cancers. Examples of cancers that may treated by methods and compositions of the invention include, but are not limited to, cancer cells from the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In addition, the cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma;

basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous; adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; and roblastoma, malignant; Sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malign melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; Hodgkin's disease; Hodgkin's lymphoma; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast

cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

As used herein the term "infectious disease" includes any infection caused by viruses, bacteria, protozoa, molds or fungi. In some embodiments, the viral infection comprises infection by one or more viruses selected from the group consisting of *Arenaviridae, Astroviridae, Birnaviridae, Bromoviridae, Bunyaviridae, Caliciviridae, Closteroviridae, Comoviridae, Cystoviridae, Flaviviridae, Flexiviridae, Hepevirus, Leviviridae, Luteoviridae, Mononegavirales, Mosaic Viruses, Nidovirales, Nodaviridae, Orthomyxoviridae, Picobirnavirus, Picornaviridae, Potyviridae, Reoviridae, Retroviridae, Sequiviridae, Tenuivirus, Togaviridae, Tombusviridae, Totiviridae, Tymoviridae, Hepadnaviridae, Herpesviridae, Paramyxoviridae or Papillomaviridae viruses. Relevant taxonomic families of RNA viruses include, without limitation, Astroviridae, Birnaviridae, Bromoviridae, Caliciviridae, Closteroviridae, Comoviridae, Cystoviridae, Flaviviridae, Flexiviridae, Hepevirus, Leviviridae, Luteoviridae, Mononegavirales, Mosaic Viruses, Nidovirales, Nodaviridae, Orthomyxoviridae, Picobirnavirus, Picornaviridae, Potyviridae, Reoviridae, Retroviridae, Sequiviridae, Tenuivirus, Togaviridae, Tombusviridae, Totiviridae*, and *Tymoviridae* viruses. In some embodiments, the viral infection comprises infection by one or more viruses selected from the group consisting of adenovirus, rhinovirus, hepatitis, immunodeficiency virus, polio, measles, Ebola, Coxsackie, Rhino, West Nile, small pox, encephalitis, yellow fever, Dengue fever, influenza (including human, avian, and swine), lassa, lymphocytic choriomeningitis, junin, machuppo, guanarito, hantavirus, Rift Valley Fever, La Crosse, California encephalitis, Crimean-Congo, Marburg, Japanese Encephalitis, Kyasanur Forest, Venezuelan equine encephalitis, Eastern equine encephalitis, Western equine encephalitis, severe acute respiratory syndrome (SARS), parainfluenza, respiratory syncytial, Punta Toro, Tacaribe, pachindae viruses, adenovirus, Dengue fever, influenza A and influenza B (including human, avian, and swine), junin, measles, parainfluenza, Pichinde, punta toro, respiratory syncytial, rhinovirus, Rift Valley Fever, severe acute respiratory syndrome (SARS), Tacaribe, Venezuelan equine encephalitis, West Nile and yellow fever viruses, tick-borne encephalitis virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley virus, Powassan virus, Rocio virus, louping-ill virus, Banzi virus, Ilheus virus, Kokobera virus, Kunjin virus, Alfuy virus, bovine diarrhea virus, and Kyasanur forest disease. Bacterial infections that can be treated according to this invention include, but are not limited

to, infections caused by the following: *Staphylococcus; Streptococcus*, including *S. pyogenes*; *Enterococcl; Bacillus*, including *Bacillus anthracis*, and *Lactobacillus; Listeria; Corynebacterium diphtheriae; Gardnerella* including *G. vaginalis; Nocardia; Streptomyces; Thermoactinomyces vulgaris; Treponerna; Camplyobacter, Pseudomonas* including *aeruginosa; Legionella; Neisseria including N.gonorrhoeae and N.meningitides; Flavobacterium* including *F. meningosepticum and F. odoraturn; Brucella; Bordetella* including *B. pertussis and B. bronchiseptica; Escherichia* including *E. coli, Klebsiella; Enterobacter, Serratia including S. marcescens* and *S. liquefaciens; Edwardsiella; Proteus* including *P. mirabilis* and *P. vulgaris; Streptobacillus; Rickettsiaceae* including R. *fickettsfi, Chlamydia* including *C. psittaci and C. trachornatis; Mycobacterium* including *M. tuberculosis, M. intracellulare, M. folluiturn, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare*, and *M. lepraernurium; and Nocardia*. Protozoa infections that may be treated according to this invention include, but are not limited to, infections caused by leishmania, kokzidioa, and trypanosoma. A complete list of infectious diseases can be found on the website of the National Center for Infectious Disease (NCID) at the Center for Disease Control (CDC) (World Wide Web (www) at cdc.gov/ncidod/diseases/), which list is incorporated herein by reference. All of said diseases are candidates for treatment using the compositions according to the invention.

In some embodiments, the antibody of the present invention is particularly suitable for treating pulmonary diseases associated with deficiency in pulmonary surfactant. For instance, both quantitative and qualitative deficiencies in pulmonary surfactant are associated with neonatal respiratory distress, adult respiratory distress syndrome, congenital deficiencies of surfactant protein B, and allergic asthma. In some embodiments, deficiency in pulmonary surfactant may contribute to the increased susceptibility of some individuals to microbial challenge, especially in the setting of inadequate or impaired specific immunity. These disorders as well as some disorders associated with increased risk of pneumonia (cystic fibrosis, asthma, prematurity, chronic bronchitis, diffuse alveolar damage) may also be associated with acquired defects or deficiency in collectin function. In some embodiments, the pulmonary disease is selected from the group consisting of cystic fibrosis, emphysema, infectious diseases, inflammatory diseases, and transplantation rejection. In some embodiments, the pulmonary disease is a pulmonary infection. In some embodiments, the pulmonary infection is bacterial, viral or fungal pneumonia. In some embodiments, the pulmonary viral disease is selected from the group consisting of influenza A, rhinovirus, coronavirus, Respiratory Syncytial Virus (RSV), chickenpox, human parvovirus B19,

parainfluenza virus types 1-3, cytomegalovirus, adenovirus, hantavirus and rubella. In some embodiments, the subject who suffers from a pulmonary disease that is associated with deficiency in pulmonary surfactant is immunocompromised, has immature lung development, is elderly, or has a chronic lung disease.

The present invention also provides for therapeutic applications where an antibody of the present invention is used in combination with at least one further therapeutic agent, e.g. for treating cancer. Such administration may be simultaneous, separate or sequential. For simultaneous administration the agents may be administered as one composition or as separate compositions, as appropriate. The further therapeutic agent is typically relevant for the disorder to be treated. Exemplary therapeutic agents include other anti-cancer antibodies, cytotoxic agents, chemotherapeutic agents, anti-angiogenic agents, anti-cancer immunogens, cell cycle control/apoptosis regulating agents, hormonal regulating agents, and other agents described below.

In some embodiments, the second agent is a natural ligand of an NK cell activating or an antibody that binds and activates an NK cell activating receptor other than myosin 18A. In some embodiments, the agent is an agent that increases the presence of a natural ligand of an NK cell activating receptor on the surface of a target cell (e.g., infected cells, or tumor cells). NK cell activating receptors include, for example, NKG2D or activating KIR receptors (KIR2DS receptors, KIR2DS2, KIR2DS4). As used herein, the term "activating NK receptor" refers to any molecule on the surface of NK cells that, when stimulated, causes a measurable increase in any property or activity known in the art as associated with NK activity, such as cytokine (for example IFN-γ and TNF-α) production, increases in intracellular free calcium levels, the ability to target cells in a redirected killing assay as described, *e.g.* elsewhere in the present specification, or the ability to stimulate NK cell proliferation. The term "activating NK receptor" includes but is not limited to activating forms or KIR proteins (for example KIR2DS proteins), NKG2D, IL-2R, IL-12R, IL-15R, IL-18R and IL-21R. Examples of ligands that act as agonists at activating receptors include, e.g. IL-2, IL-15, IL-21 polypeptides. In some embodiments, the second antibody is specific for CD137. As used herein the term "CD137" has its general meaning in the art and may also be referred to as Ly63, ILA or 4-1BB. CD137 is a member of the tumor necrosis factor (TNF) receptor family. Members of this receptor family and their structurally related ligands are important regulators of a wide variety of physiologic processes and play an important role in the regulation of immune responses. CD137 is expressed by activated NK cells, T and B lymphocytes and monocytes/macrophages. The gene encodes a 255-amino acid protein with 3 cysteine-rich

motifs in the extracellular domain (characteristic of this receptor family), a transmembrane region, and a short N- terminal cytoplasmic portion containing potential phosphorylation sites. Expression in primary cells is strictly activation dependent. The ligand for the receptor is TNFSF9. Human CD137 is reported to bind only to its ligand. Agonists include the native ligand (TNFSF9), aptamers (see McNamara et al. (2008) J. Clin. Invest. 1 18: 376-386), and antibodies.

In some embodiments, the antibody of the present invention is used in combination with a chemotherapeutic agent. The term "chemotherapeutic agent" refers to chemical compounds that are effective in inhibiting tumor growth. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaorarnide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a carnptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estrarnustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimus tine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin (11 and calicheamicin 211, see, e.g., Agnew Chem Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, canninomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idanrbicin, marcellomycin, mitomycins, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptomgrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin,

methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophospharnide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pento statin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogennanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylarnine; trichothecenes (especially T-2 toxin, verracurin A, roridinA and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobromtol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.].) and doxetaxel (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-1 1 ; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; capecitabine; and phannaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are antihormonal agents that act to regulate or inhibit honnone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and phannaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the antibody of the present invention is used in combination with a targeted cancer therapy. Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Targeted cancer therapies are sometimes called "molecularly targeted drugs," "molecularly targeted therapies," "precision medicines," or similar names. In some embodiments, the targeted therapy consists of administering the subject with a tyrosine kinase inhibitor. The term “tyrosine kinase

inhibitor" refers to any of a variety of therapeutic agents or drugs that act as selective or non-selective inhibitors of receptor and/or non-receptor tyrosine kinases. Tyrosine kinase inhibitors and related compounds are well known in the art and described in U.S Patent Publication 2007/0254295, which is incorporated by reference herein in its entirety. It will be appreciated by one of skill in the art that a compound related to a tyrosine kinase inhibitor will recapitulate the effect of the tyrosine kinase inhibitor, e.g., the related compound will act on a different member of the tyrosine kinase signaling pathway to produce the same effect as would a tyrosine kinase inhibitor of that tyrosine kinase. Examples of tyrosine kinase inhibitors and related compounds suitable for use in methods of embodiments of the present invention include, but are not limited to, dasatinib (BMS-354825), PP2, BEZ235, saracatinib, gefitinib (Iressa), sunitinib (Sutent; SU11248), erlotinib (Tarceva; OSI-1774), lapatinib (GW572016; GW2016), canertinib (CI 1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), imatinib (Gleevec; STI571), leflunomide (SU101), vandetanib (Zactima; ZD6474), MK-2206 (8-[4-aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-f][1,6]naphthyridin-3(2H)-one hydrochloride) derivatives thereof, analogs thereof, and combinations thereof. Additional tyrosine kinase inhibitors and related compounds suitable for use in the present invention are described in, for example, U.S Patent Publication 2007/0254295, U.S. Pat. Nos. 5,618,829, 5,639,757, 5,728,868, 5,804,396, 6,100,254, 6,127,374, 6,245,759, 6,306,874, 6,313,138, 6,316,444, 6,329,380, 6,344,459, 6,420,382, 6,479,512, 6,498,165, 6,544,988, 6,562,818, 6,586,423, 6,586,424, 6,740,665, 6,794,393, 6,875,767, 6,927,293, and 6,958,340, all of which are incorporated by reference herein in their entirety. In some embodiments, the tyrosine kinase inhibitor is a small molecule kinase inhibitor that has been orally administered and that has been the subject of at least one Phase I clinical trial, more preferably at least one Phase II clinical, even more preferably at least one Phase III clinical trial, and most preferably approved by the FDA for at least one hematological or oncological indication. Examples of such inhibitors include, but are not limited to, Gefitinib, Erlotinib, Lapatinib, Canertinib, BMS-599626 (AC-480), Neratinib, KRN-633, CEP-11981, Imatinib, Nilotinib, Dasatinib, AZM-475271, CP-724714, TAK-165, Sunitinib, Vatalanib, CP-547632, Vandetanib, Bosutinib, Lestaurtinib, Tandutinib, Midostaurin, Enzastaurin, AEE-788, Pazopanib, Axitinib, Motasenib, OSI-930, Cediranib, KRN-951, Dovitinib, Seliciclib, SNS-032, PD-0332991, MKC-I (Ro-317453; R-440), Sorafenib, ABT-869, Brivanib (BMS-582664), SU-14813, Telatinib, SU-6668, (TSU-68), L-21649, MLN-8054, AEW-541, and PD-0325901.

In some embodiments, the antibody of the present invention is used in combination with an immunotherapeutic agent. The term "immunotherapeutic agent," as used herein, refers to a compound, composition or treatment that indirectly or directly enhances, stimulates or increases the body's immune response against cancer cells and/or that decreases the side effects of other anticancer therapies. Immunotherapy is thus a therapy that directly or indirectly stimulates or enhances the immune system's responses to cancer cells and/or lessens the side effects that may have been caused by other anti-cancer agents. Immunotherapy is also referred to in the art as immunologic therapy, biological therapy biological response modifier therapy and biotherapy. Examples of common immunotherapeutic agents known in the art include, but are not limited to, cytokines, cancer vaccines, monoclonal antibodies and non-cytokine adjuvants. Alternatively the immunotherapeutic treatment may consist of administering the subject with an amount of immune cells (T cells, NK, cells, dendritic cells, B cells…). Immunotherapeutic agents can be non-specific, i.e. boost the immune system generally so that the human body becomes more effective in fighting the growth and/or spread of cancer cells, or they can be specific, i.e. targeted to the cancer cells themselves immunotherapy regimens may combine the use of non-specific and specific immunotherapeutic agents. Non-specific immunotherapeutic agents are substances that stimulate or indirectly improve the immune system. Non-specific immunotherapeutic agents have been used alone as a main therapy for the treatment of cancer, as well as in addition to a main therapy, in which case the non-specific immunotherapeutic agent functions as an adjuvant to enhance the effectiveness of other therapies (e.g. cancer vaccines). Non-specific immunotherapeutic agents can also function in this latter context to reduce the side effects of other therapies, for example, bone marrow suppression induced by certain chemotherapeutic agents. Non-specific immunotherapeutic agents can act on key immune system cells and cause secondary responses, such as increased production of cytokines and immunoglobulins. Alternatively, the agents can themselves comprise cytokines. Non-specific immunotherapeutic agents are generally classified as cytokines or non-cytokine adjuvants. A number of cytokines have found application in the treatment of cancer either as general non-specific immunotherapies designed to boost the immune system, or as adjuvants provided with other therapies. Suitable cytokines include, but are not limited to, interferons, interleukins and colony-stimulating factors. Interferons (IFNs) contemplated by the present invention include the common types of IFNs, IFN-alpha (IFN-α), IFN-beta (IFN-β) and IFN-gamma (IFN-γ). IFNs can act directly on cancer cells, for example, by slowing their growth, promoting their development into cells with more normal behavior and/or increasing their

production of antigens thus making the cancer cells easier for the immune system to recognise and destroy. IFNs can also act indirectly on cancer cells, for example, by slowing down angiogenesis, boosting the immune system and/or stimulating natural killer (NK) cells, T cells and macrophages. Recombinant IFN-alpha is available commercially as Roferon (Roche Pharmaceuticals) and Intron A (Schering Corporation). Interleukins contemplated by the present invention include IL-2, IL-4, IL-11 and IL-12. Examples of commercially available recombinant interleukins include Proleukin® (IL-2; Chiron Corporation) and Neumega® (IL-12; Wyeth Pharmaceuticals). Zymogenetics, Inc. (Seattle, Wash.) is currently testing a recombinant form of IL-21, which is also contemplated for use in the combinations of the present invention. Colony-stimulating factors (CSFs) contemplated by the present invention include granulocyte colony stimulating factor (G-CSF or filgrastim), granulocyte-macrophage colony stimulating factor (GM-CSF or sargramostim) and erythropoietin (epoetin alfa, darbepoietin). Treatment with one or more growth factors can help to stimulate the generation of new blood cells in subjects undergoing traditional chemotherapy. Accordingly, treatment with CSFs can be helpful in decreasing the side effects associated with chemotherapy and can allow for higher doses of chemotherapeutic agents to be used. Various-recombinant colony stimulating factors are available commercially, for example, Neupogen® (G-CSF; Amgen), Neulasta (pelfilgrastim; Amgen), Leukine (GM-CSF; Berlex), Procrit (erythropoietin; Ortho Biotech), Epogen (erythropoietin; Amgen), Arnesp (erytropoietin). Combination compositions and combination administration methods of the present invention may also involve "whole cell" and "adoptive" immunotherapy methods. For instance, such methods may comprise infusion or re-infusion of immune system cells (for instance tumor-infiltrating lymphocytes (TILs), such as CC2+ and/or CD8+ T cells (for instance T cells expanded with tumor-specific antigens and/or genetic enhancements), antibody-expressing B cells or other antibody-producing or -presenting cells, dendritic cells (e.g., dendritic cells cultured with a DC-expanding agent such as GM-CSF and/or Flt3-L, and/or tumor-associated antigen-loaded dendritic cells), anti-tumor NK cells, so-called hybrid cells, or combinations thereof. Cell lysates may also be useful in such methods and compositions. Cellular "vaccines" in clinical trials that may be useful in such aspects include Canvaxin™, APC-8015 (Dendreon), HSPPC-96 (Antigenics), and Melacine® cell lysates. Antigens shed from cancer cells, and mixtures thereof (see for instance Bystryn et al., Clinical Cancer Research Vol. 7, 1882-1887, July 2001), optionally admixed with adjuvants such as alum, may also be components in such methods and combination compositions.

In some embodiments, the antibody of the present invention is used in combination with radiotherapy. Radiotherapy may comprise radiation or associated administration of radiopharmaceuticals to a patient. The source of radiation may be either external or internal to the patient being treated (radiation treatment may, for example, be in the form of external beam radiation therapy (EBRT) or brachytherapy (BT)). Radioactive elements that may be used in practicing such methods include, e.g., radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodide-123, iodide-131, and indium-111.

In some embodiments, the antibody of the present invention is used in combination with an antibody that is specific for a costimulatory molecule. Examples of antibodies that are specific for a costimulatory molecule include but are not limited to anti-CTLA4 antibodies (e.g. Ipilimumab), anti-PD1 antibodies, anti-PDL1 antibodies, anti-TIMP3 antibodies, anti-LAG3 antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies or anti-B7H6 antibodies.

In some embodiments, the second agent is an agent that induces, via ADCC, the death a cell expressing an antigen to which the second agent binds. In some embodiments, the agent is an antibody (e.g. of IgG1 or IgG3 isotype) whose mode of action involves induction of ADCC toward a cell to which the antibody binds. NK cells have an important role in inducing ADCC and increased reactivity of NK cells can be directed to target cells through use of such a second agent. In some embodiments, the second agent is an antibody specific for a cell surface antigens, *e.g.,* membrane antigens. In some embodiments, the second antibody is specific for a tumor antigen as described above (*e.g.,* molecules specifically expressed by tumor cells), such as CD20, CD52, ErbB2 (or HER2/Neu), CD33, CD22, CD25, MUC-1, CEA, KDR, $\alpha V\beta 3$, etc., particularly lymphoma antigens (*e.g.,* CD20). Accordingly, the present invention also provides methods to enhance the anti-tumor effect of monoclonal antibodies directed against tumor antigen(s). In the methods of the invention, ADCC function is specifically augmented, which in turn enhances target cell killing, by sequential administration of an antibody directed against one or more tumor antigens, and an antibody of the present invention.

Accordingly, a further object relates to a method of enhancing NK cell antibody-dependent cellular cytotoxicity (ADCC) of an antibody in a subject in need thereof comprising administering to the subject the antibody, and administering to the subject an antibody of the present invention.

A further object of the present invention relates to a method of treating cancer in a subject in need thereof comprising administering to the subject a first antibody selective for a cancer cell antigen, and administering to the subject an antibody of the present invention.

A number of antibodies are currently in clinical use for the treatment of cancer, and others are in varying stages of clinical development. Antibodies of interest for the methods of the invention act through ADCC, and are typically selective for tumor cells, although one of skill in the art will recognize that some clinically useful antibodies do act on non-tumor cells, e.g. CD20. There are a number of antigens and corresponding monoclonal antibodies for the treatment of B cell malignancies. One popular target antigen is CD20, which is found on B cell malignancies. Rituximab is a chimeric unconjugated monoclonal antibody directed at the CD20 antigen. CD20 has an important functional role in B cell activation, proliferation, and differentiation. The CD52 antigen is targeted by the monoclonal antibody alemtuzumab, which is indicated for treatment of chronic lymphocytic leukemia. CD22 is targeted by a number of antibodies, and has recently demonstrated efficacy combined with toxin in chemotherapy-resistant hairy cell leukemia. Monoclonal antibodies targeting CD20, also include tositumomab and ibritumomab. Monoclonal antibodies useful in the methods of the invention, which have been used in solid tumors, include without limitation edrecolomab and trastuzumab (herceptin). Edrecolomab targets the 17-1 A antigen seen in colon and rectal cancer, and has been approved for use in Europe for these indications. Its antitumor effects are mediated through ADCC, CDC, and the induction of an anti-idiotypic network. Trastuzumab targets the HER- 2/neu antigen. This antigen is seen on 25% to 35% of breast cancers. Trastuzumab is thought to work in a variety of ways: downregulation of HER-2 receptor expression, inhibition of proliferation of human tumor cells that overexpress HER-2 protein, enhancing immune recruitment and ADCC against tumor cells that overexpress HER-2 protein, and downregulation of angiogenesis factors. Alemtuzumab (Campath) is used in the treatment of chronic lymphocytic leukemia; colon cancer and lung cancer; Gemtuzumab (Mylotarg) finds use in the treatment of acute myelogenous leukemia; Ibritumomab (Zevalin) finds use in the treatment of non-Hodgkin's lymphoma; Panitumumab (Vectibix) finds use in the treatment of colon cancer. Cetuximab (Erbitux) is also of interest for use in the methods of the invention. The antibody binds to the EGF receptor (EGFR), and has been used in the treatment of solid tumors including colon cancer and squamous cell carcinoma of the head and neck (SCCHN).

As used herein, the term "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of the antibody of the present invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody of the present invention to elicit a desired response in the individual. A

therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. The efficient dosages and dosage regimens for the antibody of the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art. A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician could start doses of the antibody of the present invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above. For example, a therapeutically effective amount for therapeutic use may be measured by its ability to stabilize the progression of disease. Typically, the ability of a compound to inhibit cancer may, for example, be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition may be evaluated by examining the ability of the compound to induce cytotoxicity by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, about 3 mg/kg, about 5 mg/kg or about 8 mg/kg. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the present invention is 0.02-100 mg/kg, such as about 0.02-30 mg/kg, such as about 0.05-10 mg/kg or 0.1-3 mg/kg, for example about 0.5-2 mg/kg. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target. Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. In some embodiments, the efficacy of the treatment is monitored during the therapy, e.g. at

predefined points in time. In some embodiments, the efficacy may be monitored by visualization of the disease area, or by other diagnostic methods described further herein, e.g. by performing one or more PET-CT scans, for example using a labeled antibody of the present invention, fragment or mini-antibody derived from the antibody of the present invention. If desired, an effective daily dose of a pharmaceutical composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In some embodiments, the human monoclonal antibodies of the present invention are administered by slow continuous infusion over a long period, such as more than 24 hours, in order to minimize any unwanted side effects. An effective dose of an antibody of the present invention may also be administered using a weekly, biweekly or triweekly dosing period. The dosing period may be restricted to, e.g., 8 weeks, 12 weeks or until clinical progression has been established. As non-limiting examples, treatment according to the present invention may be provided as a daily dosage of an antibody of the present invention in an amount of about 0.1-100 mg/kg, such as 0.2, 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

Typically, the antibody of the present invention is administered to the subject in the form of a pharmaceutical composition which comprises a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene- block polymers, polyethylene glycol and wool fat. For use in administration to a patient, the composition will be formulated for administration to the patient. The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via

an implanted reservoir. The used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Sterile injectable forms of the compositions of this invention may be aqueous or an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono-or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation. The compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include, e.g., lactose. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added. Alternatively, the compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols. The compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are

readily prepared for each of these areas or organs. For topical applications, the compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Patches may also be used. The compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents. For example, an antibody present in a pharmaceutical composition of this invention can be supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for IV administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5. An exemplary suitable dosage range for an antibody in a pharmaceutical composition of this invention may between about 1 mg/m^2 and 500 mg/m^2. However, it will be appreciated that these schedules are exemplary and that an optimal schedule and regimen can be adapted taking into account the affinity and tolerability of the particular antibody in the pharmaceutical composition that must be determined in clinical trials. A pharmaceutical composition of the invention for injection (e.g., intramuscular, i.v.) could be prepared to contain sterile buffered water (e.g. 1 ml for intramuscular), and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an anti-myosin 18A antibody of the invention.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. Human NK cells express the long (α) and short (β) isoforms of myosin 18A

A. DY12 recognizes a unique 220-240 kDa protein at the cell surface of YT2C2 NK cells. Biotinylated YT2C2 leukemic cell lines were immunoprecipitated with DY12 mAb or control IgG1 (D6212) antibody. After revelation with horse radish peroxidase (HRP)-conjugated streptavidin, the immunoprecipitate was found to be a unique 220-240 kDa cell surface protein.

B. The sequence of the protein recognized by DY12 at the cell surface of YT2C2 NK cells corresponds to that of myosin 18A. Aminoacid sequence of the immunoprecipitate as determined by mass spectrometry (MS) analysis. Underlined are the sequences common to that of myosin 18A. In the list of 239 mass of tryptic peptides, 59 corresponded to those of myosin 18A, with a difference lower than 36 ppm from corresponding theoretical mass.

C. The protein recognized by DY12 is a target of anti-myosin 18A antibodies. YT2C2 cell lysates were immunoprecipitated using DY12 mAb or IgG1 control isotype and the immunoprecipitate was subjected to immunoblotting using polyclonal anti-myosin 18A antibodies. DY12 was shown to recognize the 230 kDa (myosin 18Aα, L-Myo18A) and 190 kDa (myosin 18Aβ, S-Myo18A) isoforms of myosin 18A.

D. DY12 recognizes the short isoform of myosin 18A at the cell surface of human peripheral blood lymphocytes. Fresh peripheral blood mononuclear cells (PBMCs) from healthy subjects were lysed, immunoprecipitated with DY12 or control IgG1 antibody, and immunoblotted using the anti-myosin18A polyclonal antibodies followed by HRP-conjugated goat anti-mouse antibodies. Whole PBMCs lysates were used as positive controls. The protein immunoprecipitated by DY12 in PBMCs from healthy subjects was the short isoform of myosin 18A (S-Myo18A).

E. DY12 recognizes the short and long isoforms of myosin 18A on fresh human lung lysates

Fresh, healthy, lung tissue from a human subject were lysed, immunoprecipitated with DY12 or control IgG1 antibody, and immunoblotted using the anti-myosin18A polyclonal antibodies followed by HRP-conjugated goat anti-mouse antibodies. Whole PBMCs lysates were used as positive controls. The proteins immunoprecipitated by DY12 were the short and long isoforms of myosin 18A (S-Myo18A).

Figure 2. The recruitment of myosin 18A increases NK cell cytotoxicity

A, B, C, D, E, F. Myosin 18A-induced reverse cytotoxicity towards P815 mastocytoma cell lines. Cytotoxicity assays were performed according to a standard ^{51}Cr-release method. Effector cells were freshly isolated (A, C, E) or IL-2 activated (B, D, F) PB-NK from healthy donors. The target cells were the murine mastocytoma P815 cells. P815 were preincubated with DY12 or control mAb, and anti-CD335 (NKp46) or anti-CD337 (NKp30) at 10 μg/ml. Assays at various Effector:Target (E:T) cell ratios with 10^3 target cells were performed in triplicate.

G. The recruitment of myosin 18A increases the lymphokine-activated killer activity of human NK cells. Cytotoxicity assays were performed according to a standard ^{51}Cr-release method. Effector cells were freshly IL-2 activated PB-NK from healthy donors. The target cells were the Epstein-Barr Virus (EBV)-infected B cell lines. Target cells were preincubated with DY12 control F(ab')2 or control F(ab')2 Ab at 10 μg/ml. Assays at various Effector:Target (E:T) cell ratios with 10^3 target cells were performed in triplicate.

H. The recruitment of myosin 18A increases NK cell degranulation in the presence of target tumor cells.

Effector cells were freshly isolated PB-NK from healthy donors incubated for 1h with DY12 or control IgG1 antibody at a concentration of 10 μg/ml, followed by crosslinking with rabbit anti-mouse IgG 10 μg/ml. The target cells were then added in a final volume of 100 μl/well at various effector/target ratios. After 4 h of culture at 37°C in presence of PE-Cy7 anti-CD107a antibody, cells were washed and CD107a expression was measured on live $CD3^-$ $CD56^+$ NK cells by flow cytometry.

Figure 3. Myosin 18A-induced NK cell cytotoxicity is 4-1BB (CD137)-dependent

Blocking the CD137-CD137L interaction with human CD137L polyclonal antibodies completely abrogates the CD245-induced NK cell degranulation in the presence of RAJI cells. Effector cells were freshly isolated PB-NK from healthy donors incubated for 1h with DY12 or control IgG1 antibody at a concentration of 10 μg/ml, followed by crosslinking with rabbit anti-mouse IgG 10 μg/ml. The target cells were then added in a final volume of 100 μl/well at various effector/target ratios, in the presence or not of human CD137L polyclonal antibodies 10 μg/ml. After 4 h of culture at 37°C in presence of PE-Cy7 anti-CD107a antibody, cells were washed and CD107a expression was measured on live $CD3^-CD56^+$ NK cells by flow cytometry.

EXAMPLE:

Methods

Cells

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood obtained from healthy donors by density gradient centrifugation over lymphocytes separation medium (PAA Laboratories / GE Healthcare Europe, Vélizy-Villacoublay, France). Fresh NK cells from the peripheral blood (PB-NK) cells were isolated by magnetic-activated cell sorting (MACS) using the NK cell isolation kit according to the manufacturers' recommendations (Miltenyi Biotec, Bergisch Gladbach, Germany). PB-NK cell purity was shown to be >90% as assessed by flow cytometry. YT2C2 NK cell lines (purchased from ATCC, Manassas, USA), Epstein-Barr Virus (EBV)-infected B cell lines (locally produced (34)) and RAJI cells (a Burkitt-lymphoma B-cell line, ATCC) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with penicillin/streptomycin, L-glutamine, and 10% heat-inactivated fetal calf serum (FCS) (Perbio Science, Villebon-sur-Yvette, France).

Flow cytometry analysis of CD245 expression by PBMCs

The monoclonal antibodies (mAbs) to human antigens used in flow cytometry assays in this study were the following: anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-CD56, anti-CD279 (Programmed cell Death (PD)-1), anti-CD197 (C-C chemokine receptor type 7 (CCR7)), anti-γδ T-cell receptor mAb (Milteniy), and anti-CD245 mAb (DY12, mouse IgG1k, locally produced). Irrelevant isotype-matched Abs were used as negative controls. Fluorescein isothiocyanate (FITC), allophycocyanin (APC)- or R-phycoerythrin (RPE)-conjugated goat anti-mouse IgG or IgM (Beckman Coulter, Brea, USA) were used as secondary reagents. Cells were phenotyped by indirect immunofluorescence. Briefly, the cells were incubated with the specific mAb for 30 min at 4°C, washed twice in phosphate buffer saline (PBS) (Life Technologies, Carlsbad, USA), and further incubated with the appropriated FITC- or RPE-labeled secondary Abs. Cells were washed and analyzed by flow cytometry on a FC 500 analyzer (Beckman Coulter). In some experiments, whole PBMC were stimulated with recombinant human IL-2 100 IU/ml (Peprotech France, Neuilly-sur-Seine, France) 72h before cell labeling for flow cytometry analysis.

Immunohistochemistry

Formalin-fixed and paraffin-embedded lung biopsies and PB-NK from the peripheral blood of healthy subjects were analyzed for CD245 expression using a standard peroxidase method. PB-NK were pre-incubated or not with recombinant human IL-15 10 ng/ml overnight. Mouse anti-human CD245 antibody (DY12, locally produced), or monoclonal

mouse anti-human granzyme B (clone GrB-7, DAKO) was used as the primary antibody followed by peroxidase-conjugated anti-mouse antibody revealed with the avidin-streptavidin peroxidase (LSAB kit, Dako, Les Ulis, France). The peroxidase reaction was then developed using 3-amino-9-ethyl carbazole substrate for 5 to 8 minutes.

Cell surface biotinylation

Cells were biotinylated by a sulfosuccinimidobiotin (Sulfo-NHS-biotin, Pierce, Rockford, USA) procedure. Briefly, after three washes in PBS, cells were suspended at 10 × 10^6/ml in PBS) and 1 mg/ml of Sulfo-NHS-biotin. After a 30-min incubation at 4°C, cells were washed three times with complete medium.

Immunoprecipitation and Western Blot

YT2C2 biotinylated cells were lysed and incubated with DY12 or D6212 control IgG1 Ab followed by protein G-Sepharose beads. The precipitated proteins were washed, separated by SDS-8% PAGE and blotted onto a nitrocellulose membrane (Millipore, Bedford, USA). The membrane was blocked for 1h with 5% dried milk in PBS plus 0.05% Tween-20, and the protein bands were developed with horseradish peroxidase (HRP)-conjugated streptavidin and enhanced chemiluminescent (ECL) reagents (Amersham Biosciences, GE Healthcare Europe).

Immunoblotting

Immunoprecipitation using DY12 or D6212 control Ab was performed on YT2C2 cell lysates, freshly isolated human NK cells or fresh human lung, as described above. The immunoprecipitates were resolved by 8% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), blotted onto a nitrocellulose membrane and subjected to immunoblot analysis using rabbit polyclonal anti-myosin 18A (Protein Tech Group, Manchester, UK), anti-SHP2 or anti-PAK2 polyclonal Abs (Cell Signaling Technologies, Beverly, USA). HRP-conjugated goat anti-rabbit Abs (Jackson ImmunoResearch Laboratories, West Grove, USA) were used as secondary Abs, and the immunoreactive proteins were visualized using an ECL kit (Amersham Biosciences). Whole YT2C2 cell lysates were used as positive controls.

Mass spectrometry (MS)

After immunoprecipitation with DY12 mAb, the band was cut with a scalpel from the nitrocellulose. The piece of blot was then processed for MS analysis without chemical treatment as previously described (35,36). The band was digested with trypsin and MS analysis was carried out using a MALDI TOF/TOF ABI 4800 (Applied Biosystems, Foster City, USA). The masses obtained by MS-MALDI were analyzed using the Expasy database

and software [http://www.expasy.org] and a local Visual Basic for Applications (VBA) software (Microsoft Excel, Microsoft, Redmond, USA).

Cytotoxicity assays

Cytotoxicity assays were performed according to a standard ^{51}Cr-release method. Effector cells were fresh PB-NK from healthy donors. The target cells were the murine mastocytoma P815 cells or EBV-infected B cell lines. Target cells were labeled with 100 µCi of Na51CrO4 for 90 min at 37°C, and washed three times in RPMI 1640 medium containing 10% FCS. The target cells were then plated in 96-well V-bottom microtiter plates (Greiner BioOne, Courtaboeuf, France).

In redirected cytotoxicity assays against P815 cell lines, PB-NK cells were pre-activated or not by 72h culture in the presence of recombinant human IL-2 (100 international units (IU)/ml). P815 were then preincubated with DY12 or control mAb, and anti-CD335 (NKp46) or anti-CD337 (NKp30) at 10 µg/ml. Assays at various Effector:Target (E:T) cell ratios with 10^3 target cells were performed in triplicate.

In lymphokine-activated killer assays against EBV-infected B cell lines, PB-NK were preactivated for 24h or not in the presence of recombinant human IL-15 10 ng/ml (Peprotech). The effector cells were then added in a final volume of 150 µl/well in the presence of DY12 mAb or control IgG1 antibody (10 µg/ml).

After 4 h of culture at 37°C, the plates were spun down and 100 µl of the cell supernatant were collected from each well. The ^{51}Cr release was quantified using a gamma-counter (Packard Instrument Company, Meriden, USA). The percentage of specific lysis was calculated as follows: % Specific lysis = [(Sample cpm - Spontaneous Lysis Control cpm)/Maximum Lysis Control cpm - Spontaneous Lysis Control cpm)] x 100. The lysis was considered significant if >10% of the maximum cell lysis.

Flow cytometry analysis of NK cell activating receptors expression

The expression of NK cell activating receptors was assessed on freshly purified NK cell lines after 1h *in vitro* stimulation of Myo18A with DY12 or control IgG1 antibody at a concentration of 10 µg/ml, washing and crosslinking with rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories) 10 µg/ml. Cells were then washed and labeled with Fixable *Viability Stain 450 (Becton Dickinson*, Franklin Lakes, USA*)* and the following antibodies to human cell surface antigens: APC-conjugated anti-CD137, PE-conjugated anti-NKG2D, FITC-conjugated anti-DNAX Accessory Molecule-1 (DNAM-1, CD226), PE-conjugated anti-CD160 (Becton Dickinson), PE-conjugated anti-NKp30 (CD337), anti-NKp44 (CD336),

and anti-NKp46 (CD335) (Beckman-Coulter). Cells were washed and analyzed on a Canto II Cytometer (Becton Dickinson).

CD137L expression by target B cell lines

RAJI and EBV-infected B cell lines (34) were cultured as described above, washed and stained with Fixable *Viability Stain 450 (Becton Dickinson)* and PE-conjugated anti-CD137L (Becton Dickinson) for flow cytometry analysis.

Cytotoxicity against RAJI, EBV and Blocking of the CD137/CD137L interaction

Freshly isolated PB-NK cells were pre-activated or not by 12h culture in the presence of recombinant human IL-15 10 ng/ml (Peprotech), washed, and incubated for 1h with DY12 or control IgG1 antibody at a concentration of 10 µg/ml, washed again and crosslinked with rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories) 10 µg/ml. The target cells were then added in a final volume of 100 µl/well at various effector/target ratios. After 4 h of culture at 37°C in presence of PE-Cy7 anti-CD107a (Becton Dickinson), cells were washed and prepared for flow cytometry analysis. In some experiments, human 4-1BB Ligand/TNFSF9 Affinity Purified Polyclonal Ab (RnD systems, Minneapolis, USA) was added to the culture at a final concentration of 10 µg/ml to block the CD137/CD137L interaction.

Analysis

Flow cytometry analysis were carried out using FlowJo software. All values are expressed as means. Values are plotted with their mean and standard deviation and compared between groups with Prism software (Graph Pad) by two-tailed Mann-Whitney U test to compare continuous variables in 2 sample groups. $p \leq 0.05$ was considered statistically significant.

Results

Human NK cells express the long (α) and short (β) isoforms of myosin 18A

We previously described CD245 as a surface antigen expressed by human hematopoietic cells, recognized by the monoclonal antibody DY12 (33). To determine the molecular characteristics of CD245, we immunoprecipitated biotinylated YT2C2 leukemic cell lines with DY12 mAb or control IgG1 antibody. As shown in **Fig. 1A**, after revelation with HRP-conjugated streptavidin, the immunoprecipitate was found to be a unique 220-240 kDa cell surface protein. To further characterize this cell surface protein, the band was cut with a scalpel from the nitrocellulose, digested with trypsin and then processed for mass

spectrometry (MS) analysis as previously described (36). In the list of 239 masses of tryptic peptides, 59 corresponded to those of myosin 18A, with a difference lower than 36 ppm from corresponding theoretical mass (**Fig. 1B**). To confirm that CD245 expressed at the cell surface of YT2C2 cell lines was the unconventional myosin 18A, YT2C2 cell lysates were immunoprecipitated using DY12 mAb or IgG1 control isotype and the immunoprecipitate was subjected to immunoblotting using polyclonal anti-myosin 18A antibodies. DY12 was shown to recognize the 230 kDa (myosin 18Aα) and 190 kDa (myosin 18Aβ) isoforms of myosin 18A (**Fig. 1C**). Thus, CD245 expressed at the cell surface of human YT2C2 NK cell line is the *bona fide* myosin 18A. In order to further investigate if CD245 expressed *in vivo* on human hematopoietic cells was myosin 18A, fresh PBMCs from healthy subjects were lysed, immunoprecipitated with DY12 or control IgG1 antibody, and immunoblotted using the anti-myosin18A polyclonal antibodies followed by HRP-conjugated goat anti-mouse antibodies. Whole PBMCs lysates were used as positive controls. The protein immunoprecipitated by DY12 in PBMCs from healthy subjects was the short isoform of myosin 18A (**Fig. 1D**). In conclusion, these results confirm that CD245 expressed on human hematopoietic cells is the unconventional myosin 18A. NK cells expressed the p190 and p230 isoforms. p230 was shown to be the main isoform expressed at the NK cell surface of YT2C2 cell lines. By contrast, p190, not p230, was found in whole human PBMC lysates. These data are consistent with previous studies in mice that showed that myosin 18Aα (p230) and β (p190) had different subcellular localizations, the former colocalizing with the endoplasmic reticulum and Golgi structures (37). We confirmed these results on fresh human lung lysates, showing that DY12 recognized the 2 isoforms of myosin 18A in the human lung (**Fig. 1E**).

Myosin 18A/CD245 expression at the cell surface of peripheral blood lymphocytes is constitutive and increased by activation

To investigate the expression of Myo18A/CD245 on various subsets of peripheral blood lymphocytes *in vivo,* live PBMCs from healthy subjects were isolated and subjected to flow cytometry analysis using fluorochrome-conjugated DY12 mAb and anti-CD3, CD4, CD8, CD20, CD56, γδ TCR (T-cell receptor) mAbs. All peripheral blood lymphocyte subsets expressed Myo18A/CD245 at various degrees. Most $CD3^+$ T cells, γδ T cells, $CD56^+$ (i.e., NK cells, but also the numerically minor γδ T and NKT peripheral blood lymphocyte subsets), and half of the $CD20^+$ B cells expressed Myo18A/CD245. CD245 expression was associated, although to a lesser extent, with that of CCR7, a chemokine receptor expressed in T, B cells and $CD56^{bright}$ NK cells (38) involved in lymph node homing (39). $CD56^{bright}$ cells expressed CD245 at higher levels than $CD56^{dim}$ cells. After IL-2 activation of whole PBMCs, nearly all

lymphocytes expressed CD245. CD245 mean fluorescence intensity increased between 3 and 8-fold after IL-2. By contrast, using a polyclonal antibody against surfactant protein A-receptor (SP-R)-210 that was previously shown to detect myosin 18A (40), Samten *et al.* found that myosin 18A was expressed by a very small fraction of peripheral blood CD3+ lymphocytes. The percentage of $CD3^+$ cells expressing myosin 18A was increased five to 10 fold in 48h *M. tuberculosis*-stimulated PBMCs (41).

Recruitment of myosin 18A on peripheral blood NK cells increases NK cell reverse cytotoxicity towards mastocytoma cell lines

To confirm the expression of CD245 by human NK cells from the peripheral blood, freshly isolated PB-NK from healthy donors were assessed for CD245 expression by immunohistochemistry using the DY12 mAb. Anti-granzyme B antibodies were used as positive control. PB-NK expressed myosin 18A at the cell membrane and in the cytoplasm in steady state.

SP-A, a ligand for Myo18A, has previously been shown to stimulate the anti-tumor immunity *in vivo* in a NK cell-dependent manner (42). We investigated whether the stimulation of Myo18A was able to modulate the NK cell cytotoxicity against tumor cells. As shown in **Fig. 2A, B, C, D, E, F**, NK cells stimulated with DY12 alone exhibited poor cytotoxic activity. By contrast, DY12 stimulation strongly enhanced NKp46- and NKp30-induced cell cytotoxicity against the P815 murine mastocytoma cell lines. On average, stimulation with DY12 increased NKp46- and NKp30-induced cell cytotoxicity by 69% and 283%, respectively in freshly isolated NK cells; and by 75% and 39% in IL-2 activated NK cells. These data identify CD245 as a strong activator of NK cell anti-tumor activity triggered by natural cytotoxicity receptors.

Myo18A recruitment increases the IL-2 activated NK cell cytotoxicity towards Epstein Barr Virus (EBV)-infected B cells

Because (i) NK cells play a critical and first-line role in the antiviral immune defense (5), (ii) human NK cells express high levels of myosin 18A, (iii) myosin 18A cell surface expression is induced by IL-2 in NK cells and (iv) myosin 18A has been shown to be a receptor for SP-A (40), that is involved in viral clearance in the human lung (43), we asked whether engagement of Myo18A on the NK cell surface was able to regulate their IL-2-activated killer activity against virally infected cells. Engagement of Myo18A on the surface of IL-2 activated PB-NK from healthy subjects increased their cytotoxic activity against EBV-infected B cell lines on average by 110% (83-158%) (**Fig. 2G**). The recruitment of Myo18A did not significantly increase the formation of conjugates between EBV-infected B

cells and NK cells (data not shown), but increased PB-NK cell degranulation in the presence of RAJI cells by 25% in the 50/1 ratio (**Fig. 2H**). These data suggest that Myo18A plays a role in the lymphokine-activated killer cell activity and antiviral function of human NK cells.

NK cell cytotoxicity induced by the recruitment of myosin 18A is 4-1BB-dependent

To further understand the mechanism by which recruitment of CD245 increases NK cell cytotoxicity against tumor cell lines, we studied the expression of activating NK cell receptors after engagement of CD245 by DY12 mAb or control isotype. CD245 recruitment did not induce any significant change in the expression of NKp30, NKp44, NKp46 and NKG2D. Nor did it have significantly impact on the expression of DNAM1 or CD160 (data not shown). By contrast, CD245 stimulation increased the expression of CD137 (4.1BB) by on average 94% (56-156%). As shown above, CD245 stimulation increased the NK cell cytotoxicity against EBV-infected B cell lines (**Fig. 2G**) and RAJI B cells (**Fig. 2H**). B-cell lymphoma cells have previously been shown to express the CD137 ligand, CD137L (44). We confirmed that the target cells, EBV-infected B cells and RAJI cells, expressed CD137L. Blocking the CD137-CD137L interaction with human 4-1BB ligand polyclonal Ab completely abrogated the CD245-induced NK cell degranulation in the presence of RAJI cells (**Fig. 3**). By contrast, no significant increase in the NK cell degranulation was induced by DY12 in presence of Sezary cells that do not express CD137L (4-1BBL) (data not shown). Altogether, these data suggest that NK cell cytotoxicity induced by the recruitment of myosin 18A is 4-1BB-dependent.

Myosin 18A interacts with PAK-2 and SHP-2, two key signal transducers of the NK cell activation and degranulation

As shown previously, recruitment of Myo18A enhanced NK cell cytotoxicity against tumor cells and virally infected cell lines. NK cell cytotoxicity is dependent on cytoskeleton reorganization, to create the NK immune synapse first (13), and then to allow the polarization and exocytosis of the cytolytic granules (45,46). Myo18A has been shown to play a role in the cytoskeleton organization and to interact with PAK-2 in epithelial cell lines (47). To further elucidate the mechanisms by which CD245 stimulation increased NK cell degranulation and lysis of target cells, we immunoprecipitated YT2C2 NK cell lines with DY12 or control IgG1 isotype and subjected the immunoprecipitate to immunoblotting using anti-PAK2 antibody. Whole YT2C2 were used as positive controls.PAK2 immunoprecipitated with Myo18A in YT2C2 cells. These data show, for the first time, that Myo18A interacts with PAK2 in NK

cells, and identify the PAK2 kinase as a potential signal transducer of the Myo18A-induced NK cell cytotoxicity.

As previously demonstrated (33), CD245 was shown to exhibit spontaneous phosphatase activity in the NK YT2C2 cell line. Among the main phosphatases involved in the signal transduction from NK receptors are the Src-homology domain-containing phosphatases (SHP) that, through their SH2 domains, interact with phosphorylated tyrosine residues on other proteins (32,48–50). In particular, SHP-2 regulates NK cell function (50). We thus investigated whether Myo18A was able to recruit SHP-2.immunoprecipitation of YT2C2 cell lysates with DY12 antibody and further immunoblotting using anti-SHP-2 Ab revealed the association of Myo18A with the phosphatase SHP-2. Thus, SHP-2 may be involved in the signal transduction from the Myo18A activating receptor.

Discussion

In the present work, we identified CD245, a human cell surface antigen expressed on peripheral blood lymphocytes, as the unconventional myosin 18A (Myo18A), a highly conserved motor enzyme involved in cytoskeleton organization and Golgi budding (51–54). We identified Myo18A/CD245 as a crucial human NK cell activating receptor, whose cell surface expression is induced by IL-2. Myo18A stimulation was able to increase NK cell degranulation and cytotoxicity towards virally infected and tumor B cells. We found that Myo18A stimulation was able to induce CD137 expression at the NK cell surface and that the Myo18A-induced NK cell cytotoxicity was dependent on the CD137/CD137L interaction. Last, Myo18A was able to interact with SHP-2, a phosphatase with a key role in the signal transduction of NK cell receptors (50) and PAK-2, a serine/threonine kinase that controls the cytoskeletal organization. These entirely novel molecular and functional data on a newly described NK cell activating receptor have broad potential applications.

The unconventional myosin 18A (Myo18A) is a member of the myosin superfamily of motor enzymes. Myosins generally contain a conserved catalytic head that catalyzes ATP hydrolysis and binds F-actin, thus promoting motility. The first myosin, M2, was discovered in muscle extracts and is referred to as conventional myosin, whereas other classes, including class 18, are called unconventional myosins (55). Myosin 2A is required for cytolytic granule exocytosis in human NK cells (56). Class 18 myosins have been involved in fundamental tissular processes in mammalians, including epithelial cell migration (47), stromal cell differentiation (57) and tumor suppression (58–60). In humans and mice, myosin 18A is expressed in hematopoietic cells as two splice variants, referred to as α (230 kDa) and β (190

kDa). Myosin-18Aα contains a lysine- and glutamic acid-rich (KE-rich) sequence at the extreme N terminus, followed by a PDZ domain (37,57). Myosin-18Aβ lacks the KE-rich sequence and the PDZ domain and, instead, has a short leading sequence upstream of the motor (37). Both isoforms have a predicted canonical IQ calmodulin-binding motif followed by a coiled-coil tail, analogous to the tail of myosin-2. A third isoform of Myo18A, p110 myosin, was identified in macrophages, which may come about through post-translational processing of Myo18Aα or β via the phosphorylation of tyrosine residues after the induction of macrophage differentiation by macrophage colony-stimulating factor-1 (CSF-1) treatment (61). At the cell level, myosin 18A participates in cytoskeleton organization (51), Golgi budding (52,53) and DNA-damaged-induced Golgi dispersion by its association with F-actin and Golgi Phosphoprotein 3 (GOLPH3) in epithelial cells (54) but its specific role in NK cells was not shown yet.

Myosin 18A was reported as a receptor for the surfactant protein A (SP-A) (40), a collectin present in human lung (62), blood (63), intestinal tract (64) and skin (65), that participates in the elimination of pathogens (43). SP-A has also been shown to strongly stimulate the anti-tumor immunity in a xenograft mouse model (42). Tumor cells transduced with SP-A grew slower than those transduced with vector alone. This anti-tumor effect of SP-A was entirely dependent on NK cells *in vivo* (42) although the exact mechanism remained unknown. Our data support the hypothesis that this major anti-tumor effect of SP-A *in vivo* is mediated by its interaction with Myo18A on NK cells.

The use of monoclonal antibodies modulating the NK cell antitumor function is a fastgrowing field of research. On the one side, monoclonal antibodies able to induce antibody-dependent cell cytotoxicity by targeting both the cancer cell and the FcγRIIIA/CD16 activating receptor present on NK cells have revolutionized the management of lymphoma (66–69) and human cancer (70,71). On the other, not all malignancies have an identified druggable target, and some cancers with an identified target escape therapeutic antibodies. In this setting, strategies aimed at increasing the efficacy of monoclonal antibodies are promising. Stimulation of the CD137 (4-1BB) receptor present on NK cells has been shown able to increase the efficacy of cetuximab (26), trastuzumab (27) and rituximab (28) in both *in vitro* and *in vivo* models of human cancer. The use of monoclonal antibodies that modulate the expression of CD137 at the NK cell surface, such as DY12 as shown in the present work, could be interesting in this setting. In conclusion, the NK cell activating receptor Myo18A appears as a very promising target in the field of the immunotherapy of human cancer and hematological malignancies.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. An antibody having specificity to myosin 18A comprising a heavy chain comprising i) a H-CDR1 having at least 90% of identity with the H-CDR1 of DY12, ii) a H-CDR2 having at least 90% of identity with the H-CDR2 of DY12 and iii) a H-CDR3 having at least 90% of identity with the H-CDR3 of DY12 and a light chain comprising i) a L-CDR1 having at least 90% of identity with the L-CDR1 of DY12, ii) a L-CDR2 having at least 90% of identity with the L-CDR2 of DY12 and iii) a L-CDR3 having at least 90% of identity with the L-CDR3 of DY12 wherein the H-CDR1 of DY12 is defined by the sequence ranging from the amino acid residue at position 31 to the amino acid residue at position 35 in SEQ ID NO:1; the H-CDR2 of DY12 is defined by the sequence ranging from the amino acid residue at position 50 to the amino acid residue at position 66 in SEQ ID NO:1; the H-CDR3 of DY12 is defined by the sequence ranging from the amino acid residue at position 99 to the amino acid residue at position 109 in SEQ ID NO:1, the L-CDR1 of DY12 is defined by the sequence ranging from the amino acid residue at position 24 to the amino acid residue at position 34 in SEQ ID NO:2, the L-CDR2 of DY12 is defined by the sequence ranging from the amino acid residue at position 50 to the amino acid residue at position 56 in SEQ ID NO:2 and the L-CDR3 of DY12 is defined by the sequence ranging from the amino acid residue at position 89 to the amino acid residue at position 97 in SEQ ID NO:2.

2. The antibody of claim 1 comprising a heavy chain comprising i) the H-CDR1 of DY12, ii) the H-CDR2 of DY12 and iii) the H-CDR3 of DY12.

3. The antibody of claim 1 comprising a light chain comprising i) the L-CDR1 of DY12, ii) the L-CDR2 of DY12 and iii) the L-CDR3 of DY12.

4. The antibody of claim 1 comprising a heavy chain comprising i) the H-CDR1 of DY12, ii) the H-CDR2 of DY12 and iii) the H-CDR3 of DY12 and a light chain comprising i) the L-CDR1 of DY12, ii) the L-CDR2 of DY12 and iii) the L-CDR3 of DY12.

5. The antibody of claim 1 comprising a heavy chain having at least 70% of identity with SEQ ID NO:1

6. The antibody of claim 1 comprising a light chain having at least 70 of identity with SEQ ID NO:2.

7. The antibody of claim 1 comprising a heavy chain having at least 70% of identity with SEQ ID NO:1 and a light chain having at least 70 % of identity with SEQ ID NO:2.

8. The antibody of claim 1 comprising a heavy chain which is identical to SEQ ID NO:1

9. The antibody of claim 1 comprising a light chain identical to SEQ ID NO:2.

10. The antibody of claim 1 which is a chimeric or a humanized antibody.

11. The antibody of claim 1 which does not comprise an Fc portion that induces antibody dependent cellular cytotoxicity (ADCC).

12. The antibody of claim 11 which does not comprise an Fc domain capable of substantially binding to a FcgammaRIIIA (CD16) polypeptide.

13. The antibody of claim 11 which lacks an Fc domain or comprises an Fc domain of IgG2 or IgG4 isotype.

14. A nucleic acid molecule which encodes a heavy chain and/or a light chain of the antibody of claim 1.

15. The nucleic acid molecule of claim 14 which comprises a nucleic acid sequence having 70% of identity with SEQ ID NO:3 or SEQ ID NO:4.

16. A host cell which has been transfected, infected or transformed by the nucleic acid of claim 14.

17. A method of enhancing NK cell killing activities in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody of claim 1.

18. A method of treating a cancer or an infectious disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody of claim 1.

19. A method of treating a pulmonary disease associated with deficiency in pulmonary surfactant comprising administering to the subject with a therapeutically effective amount of the antibody of claim 1.

20. A method of enhancing NK cell antibody-dependent cellular cytotoxicity (ADCC) of an antibody in a subject in need thereof comprising administering to the subject the antibody, and administering to the subject the antibody of claim 1.

21. A method of treating cancer in a subject in need thereof comprising administering to the subject a first antibody selective for a cancer cell antigen, and administering to the subject the antibody of claim 1.

22. A pharmaceutical composition comprising the antibody of claim 1.

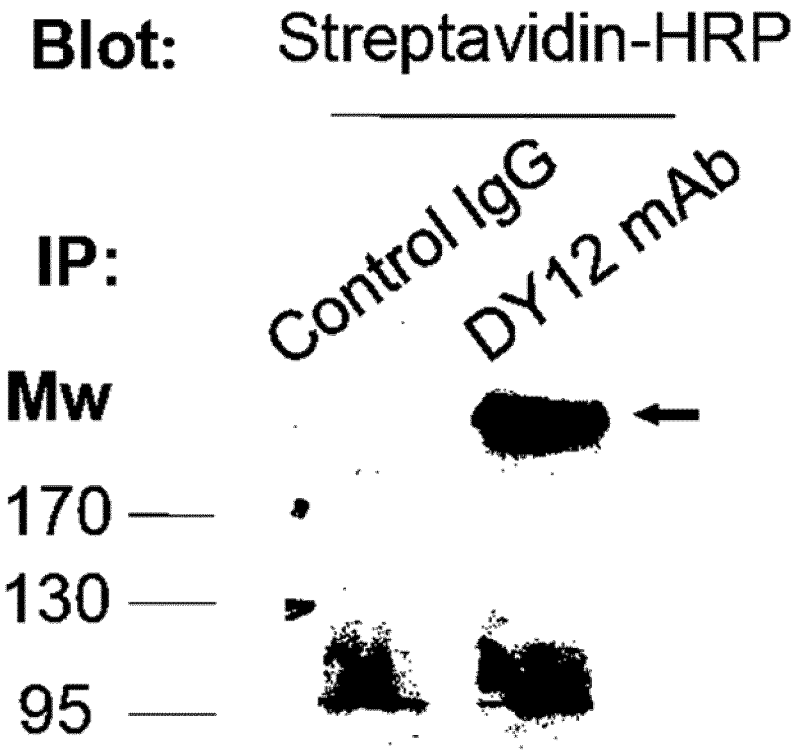

Figure 1A

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321   LRSGEGPRREPSDAKteeqiaaeeawnetekvwlvhrdgfslasqlkseelnlpegkvrvkldhdgaildvdeddvekan
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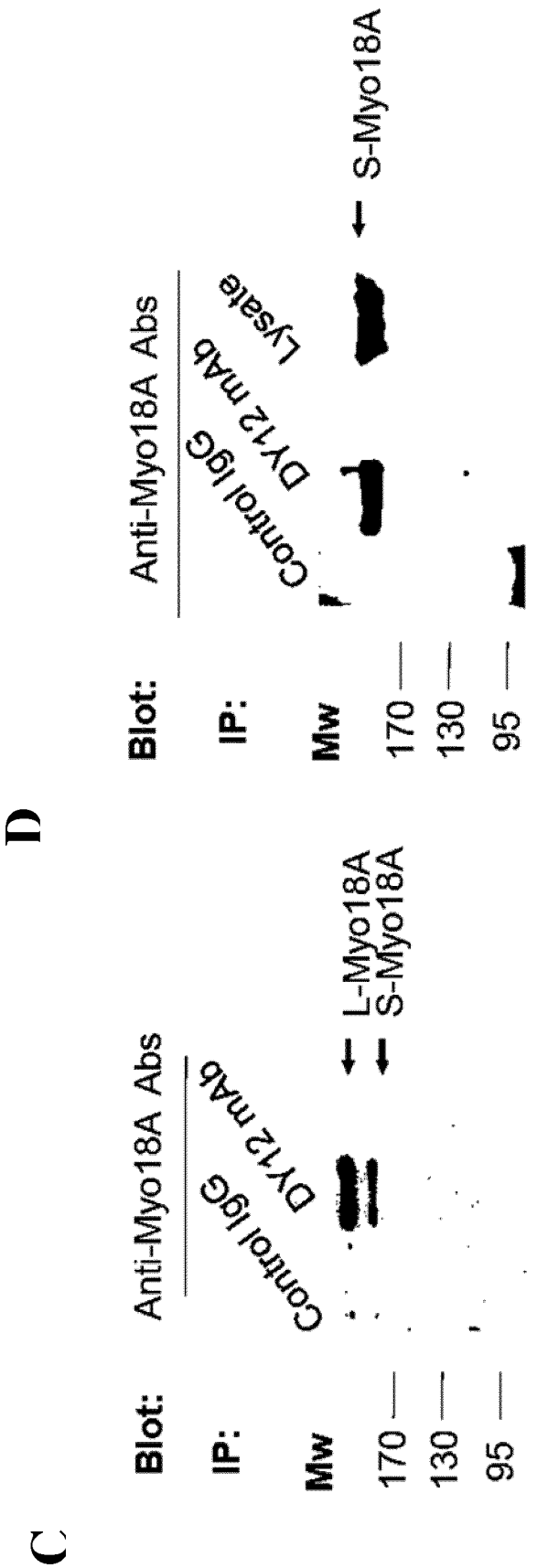
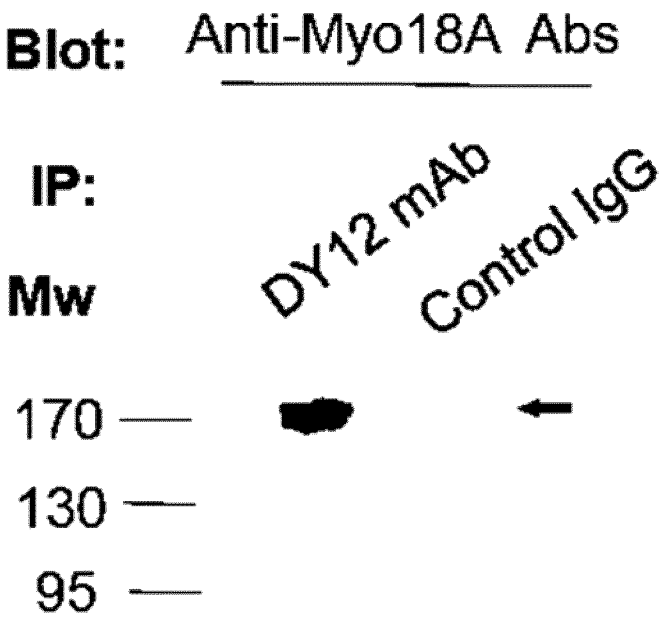
Figure 1B



Figures 1C and 1D



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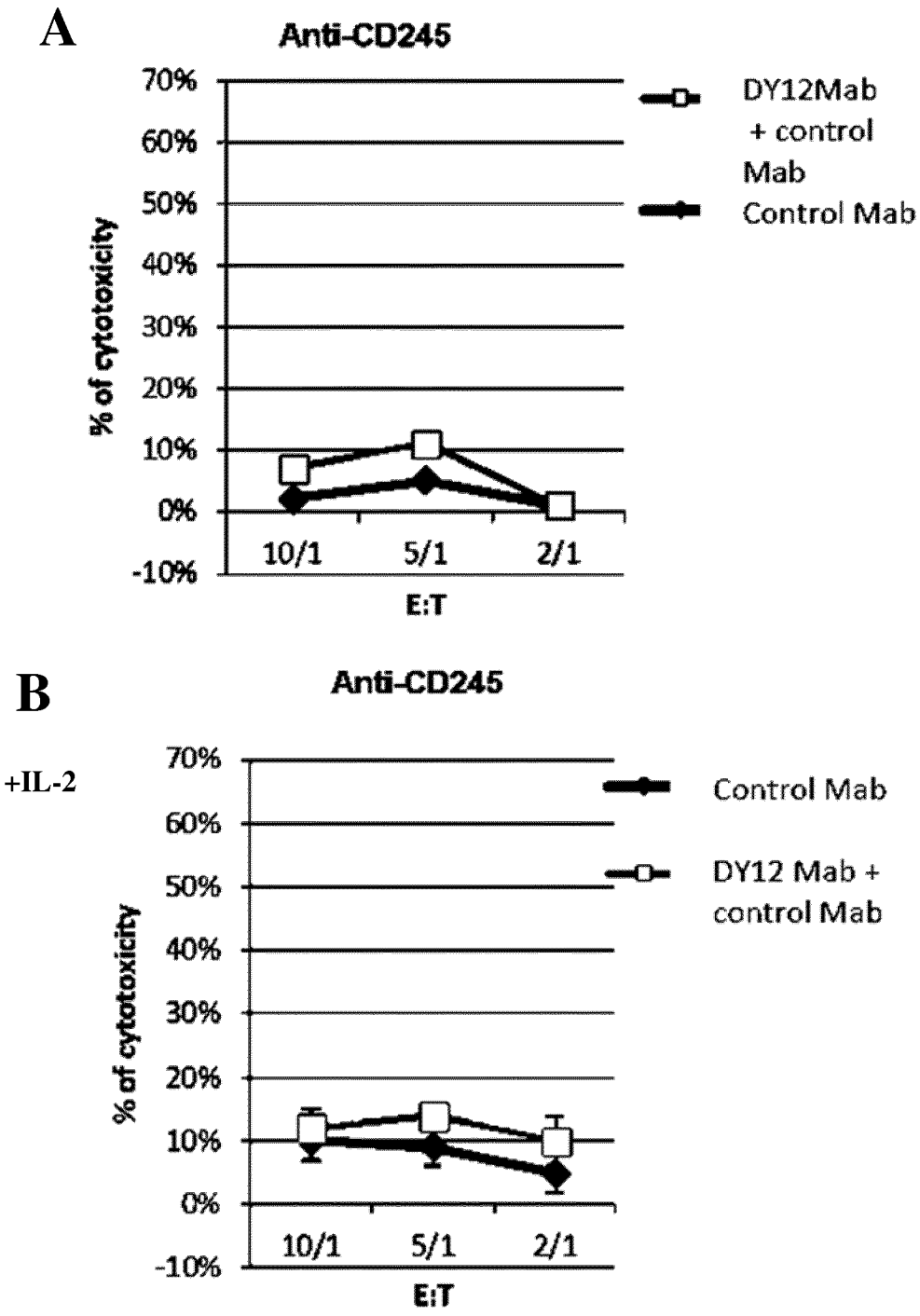

Figures 2A and 2B

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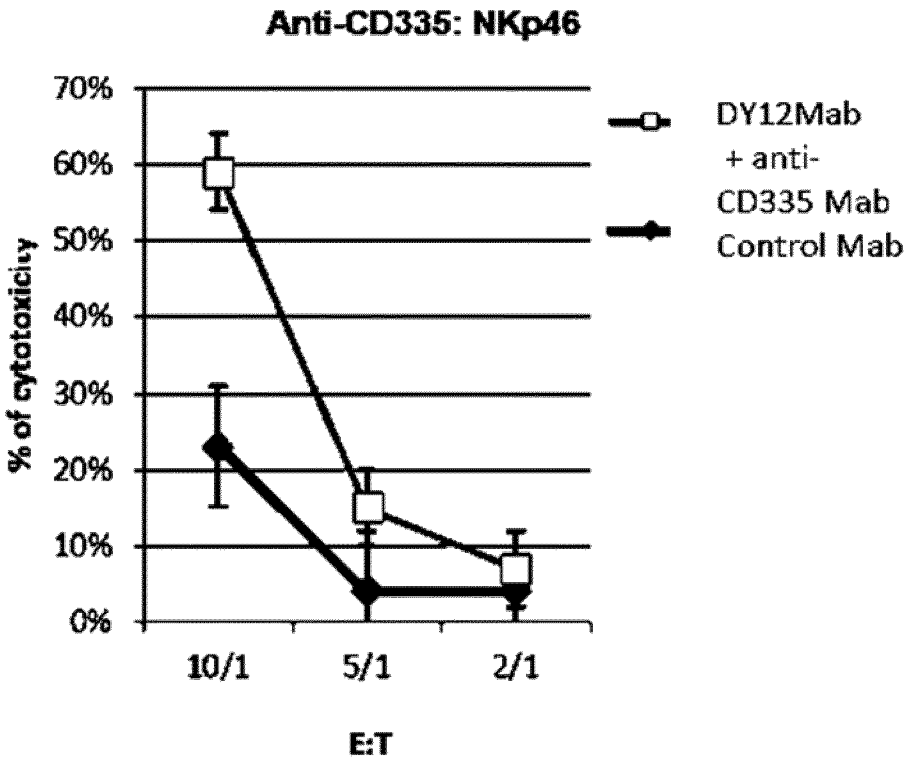

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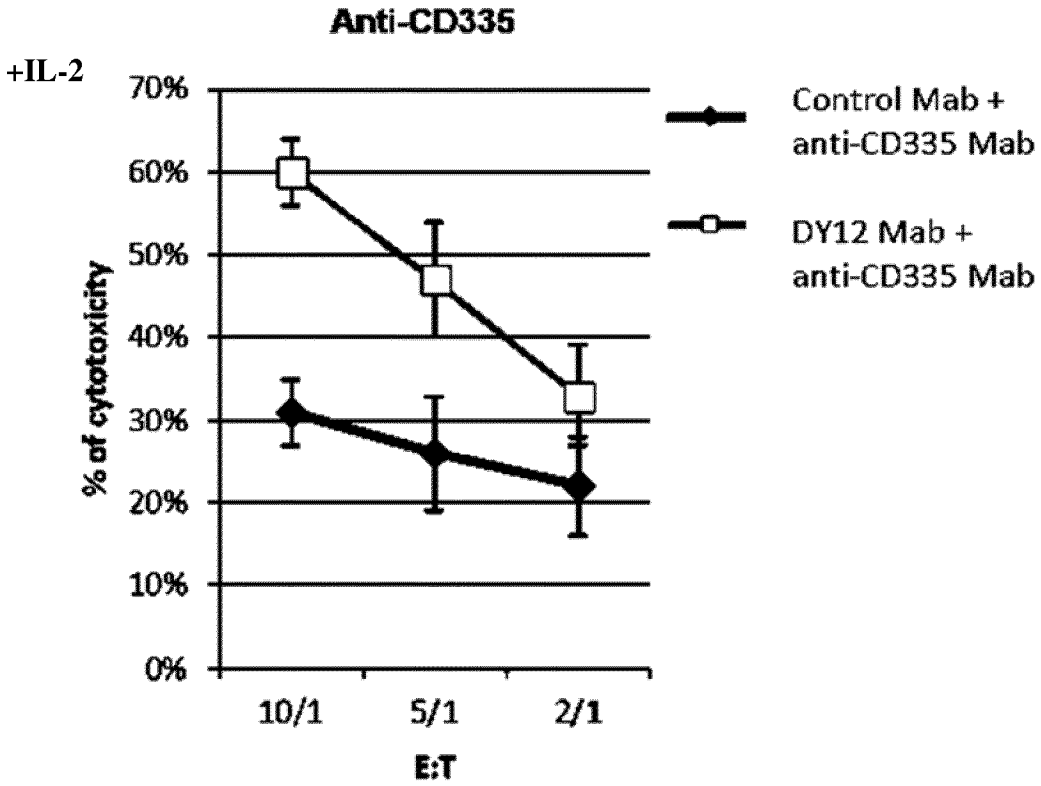
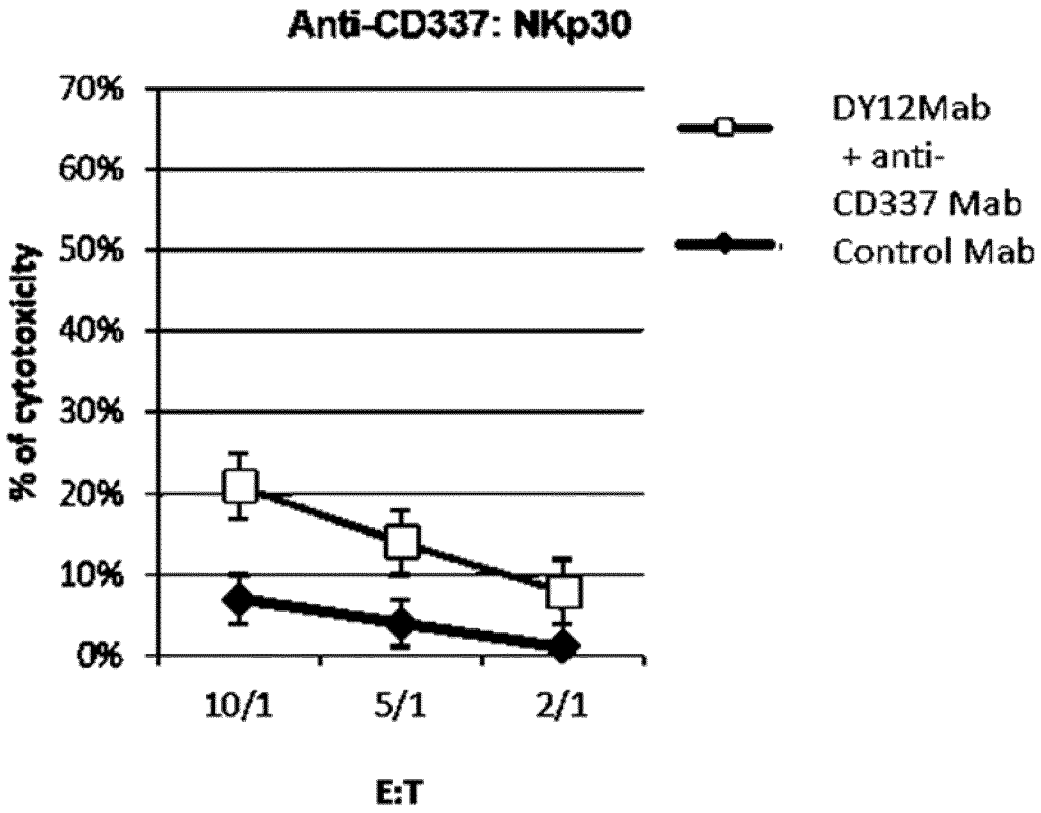


Figures 2C and 2D

E



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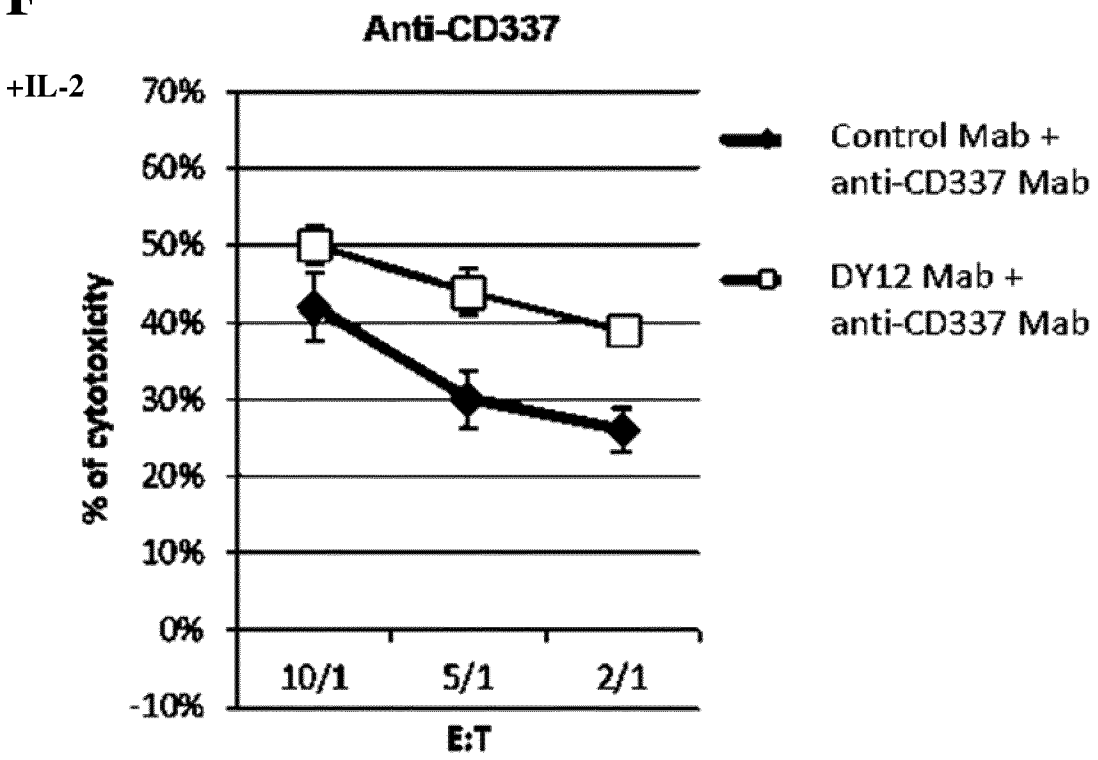
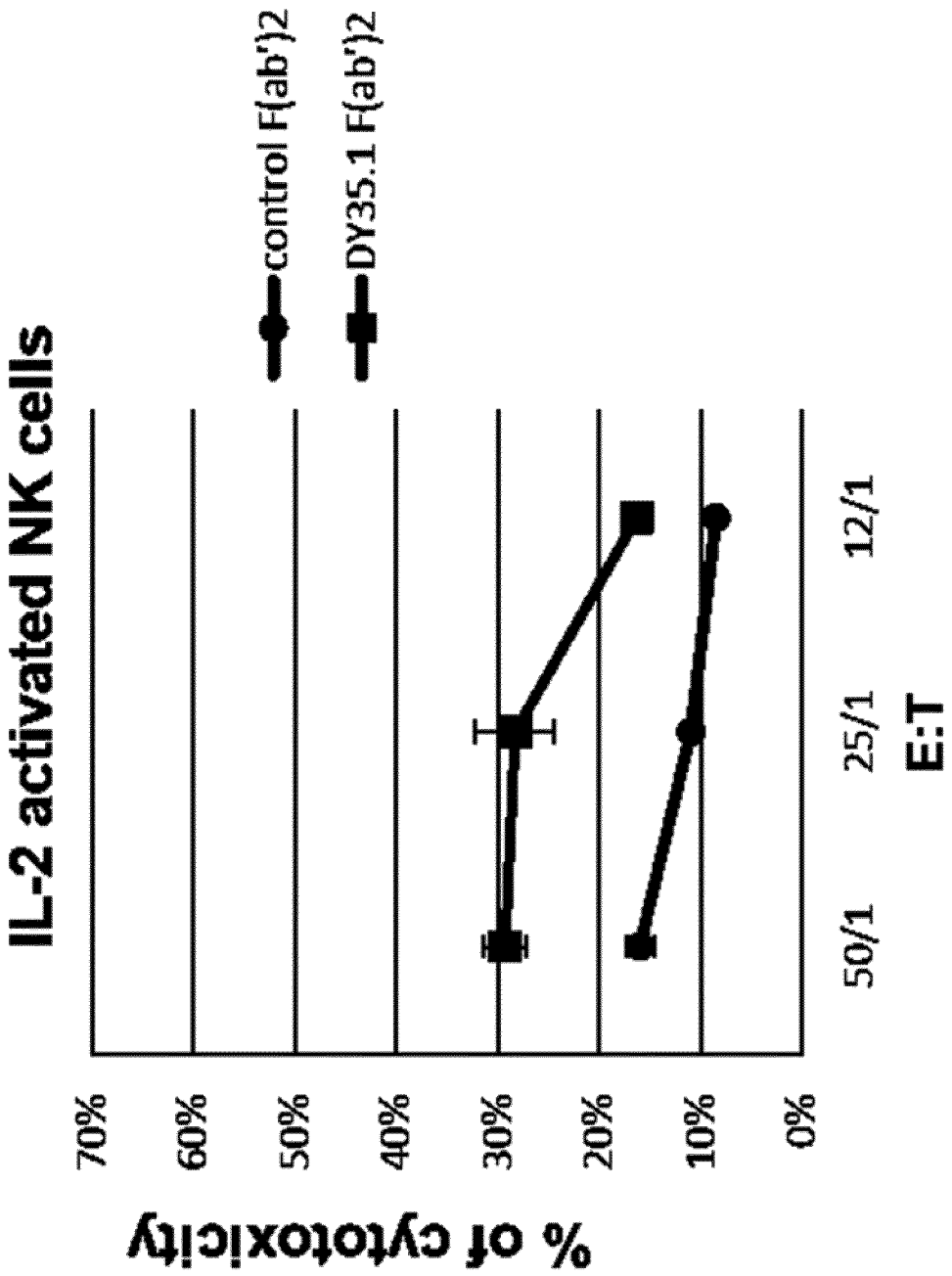


Figures 2E and 2F


IL-2 activated NK cells
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DY35.1 F(ab')2
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50%
40%
30%
20%
10%
0%
50/1
25/1
12/1
E:T

Figure 2G

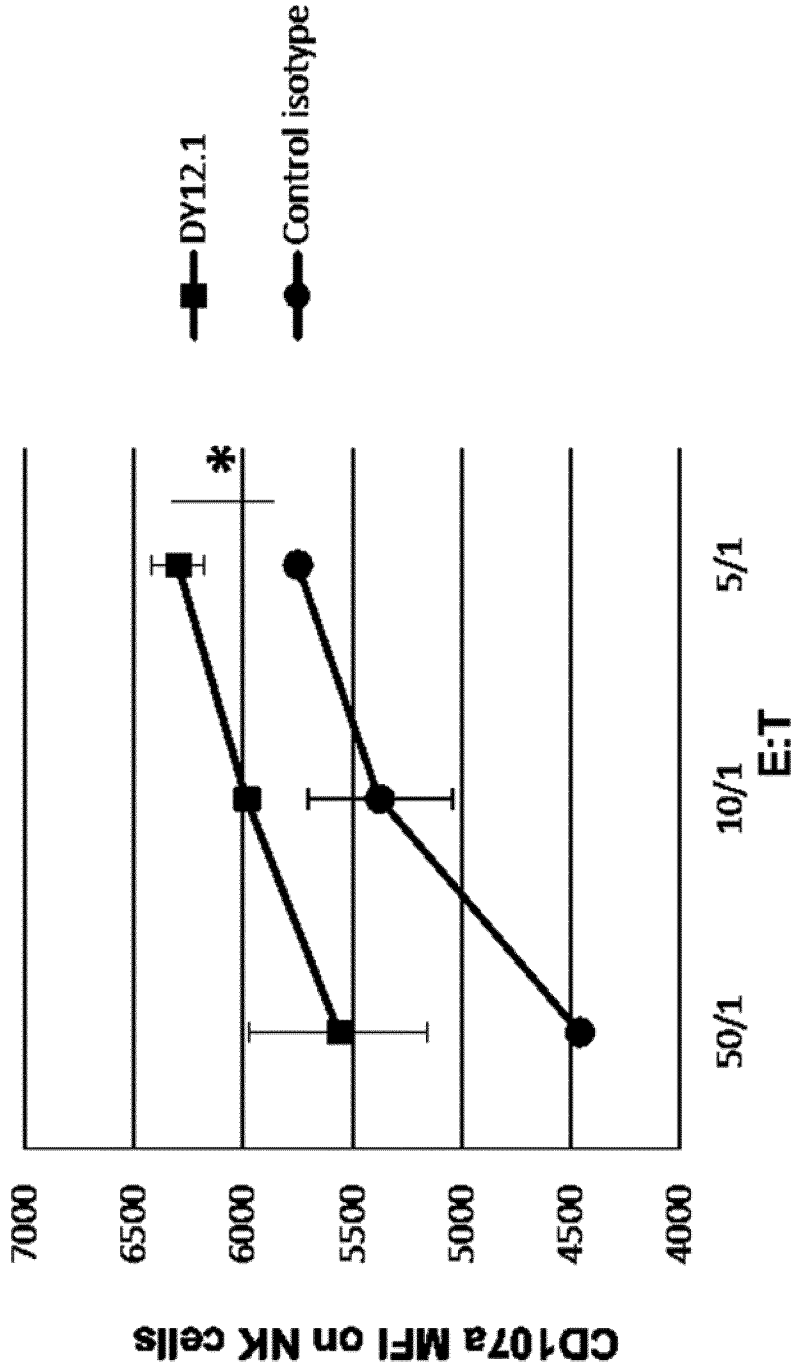
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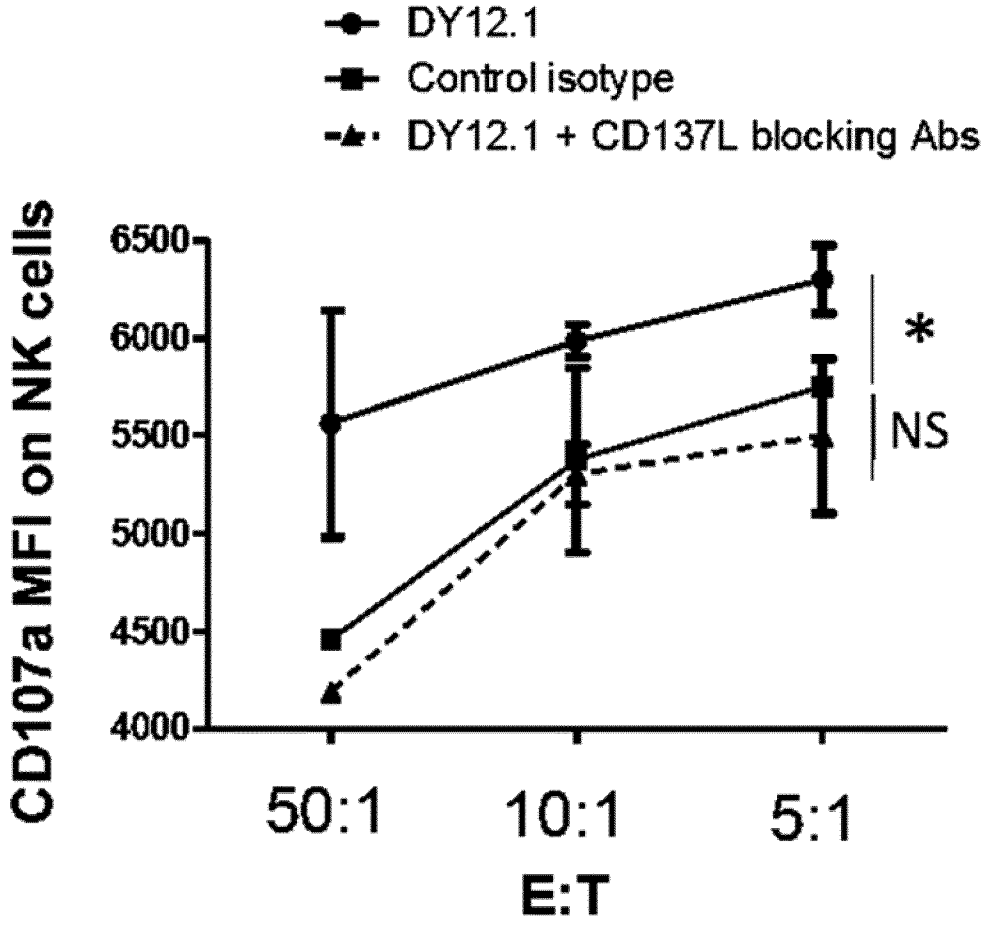
Figure 2H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5:1
E:T

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066101

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MADDALENA CROSS ET AL: "A novel 110 kDa form of myosin XVIIIA (MysPDZ) is tyrosine-phosphorylated after colony-stimulating factor-1 receptor signalling", BIOCHEMICAL JOURNAL, vol. 380, no. 1, 15 May 2004 (2004-05-15), pages 243-253, XP055223223, GB ISSN: 0264-6021, DOI: 10.1042/bj20031978 page 245, left-hand column, paragraph 4 ----- -/--	1-3, 5-16,22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2016	31/08/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TADASHI FURUSAWA ET AL: "Isolation of a Novel PDZ-Containing Myosin from Hematopoietic Supportive Bone Marrow Stromal Cell Lines", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 270, no. 1, 1 April 2000 (2000-04-01) , pages 67-75, XP055223216, US ISSN: 0006-291X, DOI: 10.1006/bbrc.2000.2377 page 68, left-hand column, paragraph; figure 5	1-3, 5-16,22
X	Rae-Mann Hsu ET AL: "Identification of MYO18A as a Novel Interacting Partner of the PAK2/?PIX/GIT1 Complex and Its Potential Function in Modulating Epithelial Cell Migration", Molecular Biology of the Cell, 15 January 2010 (2010-01-15), pages 287-301, XP055223222, DOI: 10.1091/mbc.E09 Retrieved from the Internet: URL:http://www.molbiolcell.org/content/21/2/287.full.pdf page 288, left-hand column, last paragraph	1-3, 5-16,22
X	Santa Cruz Biotechnology: "Myosin XVIIIa (H-10): sc-365328", SANTA CRUZ BIOTECHNOLOGY CATALOGUE, 30 December 2013 (2013-12-30), page 1, XP055222642, Retrieved from the Internet: URL:http://datasheets.scbt.com/sc-365328.pdf [retrieved on 2015-10-21] the whole document	1-3, 5-16,22

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