(19) DANMARK

(10) **DK/EP 1648507 T3**

Patent- og Varemærkestyrelsen

(12) # Oversættelse af europæisk patentskrift

(51) Int.Cl.: ***C 07 K 16/28 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2017-05-01**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2017-01-25**

(86) Europæisk ansøgning nr.: **04744267.8**

(86) Europæisk indleveringsdag: **2004-07-23**

(87) Den europæiske ansøgnings publiceringsdag: **2006-04-26**

(86) International ansøgning nr.: **IB2004002636**

(87) Internationalt publikationsnr.: **WO2005009465**

(30) Prioritet: **2003-07-24 US 489489 P**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR**

(73) Patenthaver: **Innate Pharma S.A., 117 Avenue de Luminy, 13009 Marseille, Frankrig**

(72) Opfinder: **VELARDI, Andrea, Via Pieve di Campo 73, 06154 Perugia, Italien**
ROMAGNE, François, Avenue Bellevue, 8 Lot des 3 Citernes, 13600 La Ciotat, Frankrig

(74) Fuldmægtig i Danmark: **Marks & Clerk (Luxembourg) LLP, 44 rue de la Vallée, B.P. 1775, L-1017 Luxembourg, Luxembourg**

(54) Benævnelse: **FREMGANGSMÅDER OG SAMMENSÆTNINGER TIL ØGNING AF EFFEKTIVITETEN AF TERAPEUTISKE ANTISTOFFER VED ANVENDELSE AF FORBINDELSER, DER POTENSERER NK-CELLER**

(56) Fremdragne publikationer:
EP-A- 1 023 906
WO-A-02/34290
US-A1- 2003 026 804
FARAG SHERIF S ET AL: "New directions in natural killer cell-based immunotherapy of human cancer." EXPERT OPINION ON BIOLOGICAL THERAPY. APR 2003, vol. 3, no. 2, April 2003 (2003-04), pages 237-250, XP009040774 ISSN: 1471-2598
SONDEL P M ET AL: "Combination therapy with interleukin-2 and antitumor monoclonal antibodies" CANCER JOURNAL FROM SCIENTIFIC AMERICAN 1997 UNITED STATES, vol. 3, no. SUPPL. 1, 1997, pages S121-S127, XP009040777 ISSN: 1081-4442
KOH CRYSTAL Y ET AL: "Augmentation of antitumor effects by NK cell inhibitory receptor blockade in vitro and in vivo" BLOOD, vol. 97, no. 10, 15 May 2001 (2001-05-15), pages 3132-3137, XP002308582 ISSN: 0006-4971
MORETTA A ET AL: "Novel surface molecules involved in human NK cell activation and triggering of the lytic machinery." INTERNATIONAL JOURNAL OF CANCER. SUPPLEMENT = JOURNAL INTERNATIONAL DU CANCER. SUPPLEMENT. 1992, vol. 7, 1992, pages 6-10, XP001203978 ISSN: 0898-6924
SIVORI SIMONA ET AL: "P46, a novel natural killer cell-specific surface molecular that mediates cell activation"

Fortsættes ...

JOURNAL OF EXPERIMENTAL MEDICINE, vol. 186, no. 7, 1997, pages 1129-1136, XP002308583 ISSN: 0022-1007
CAMPBELL PETER ET AL: "Monoclonal antibody therapy for lymphoma." BLOOD REVIEWS, vol. 17, no. 3, September 2003 (2003-09), pages 143-152, XP002308584 ISSN: 0268-960X
MORETTA A ET AL: "P58 MOLECULES AS PUTATIVE RECEPTORS FOR MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS I MOLECULES IN HUMAN NATURAL KILLER (NK) CELLS. ANTI-P58 ANTIBODIES RECONSTITUTE LYSIS OF MHC CLASS I-PROTECTED CELLS IN NK CLONES DISPLAYING DIFFERENT SPECIFICITIES" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 178, no. 2, August 1993 (1993-08), pages 597-604, XP009005922 ISSN: 0022-1007

DESCRIPTION

Field of the Invention

[0001] The present invention relates, generally, to methods and compositions for increasing the efficiency of therapeutic antibodies. More particularly, the invention relates to the use of a therapeutic antibody in combination with a compound that blocks an inhibitory receptor or stimulates an activating receptor of natural killer (NK) cells, thereby allowing a potentiation of NK cell cytotoxicity in mammalian subjects in order to enhance the efficiency of the treatment in human subjects, particularly through an increase of the ADCC mechanism.

Background of the Invention

[0002] Various therapeutic strategies in human beings are based on the use of therapeutic antibodies. These include, for instance, the use of therapeutic antibodies developed to deplete target cells, particularly diseased cells such as virally-infected cells, tumor cells or other pathogenic cells. Such antibodies are typically monoclonal antibodies, of IgG species, typically with human IgG1 or IgG3 Fc portions. These antibodies can be native or recombinant antibodies, and are often "humanized" mice antibodies (i.e., comprising functional domains from various species, typically an Fc portion of human or non-human primate origin, and with a variable region or complementary determining region (CDR) of mouse origin). Alternatively, the monoclonal antibody can be fully human through immunization in transgenic mice having the human Ig locus, or obtained through cDNA libraries derived from human cells.

[0003] A particular example of such therapeutic antibodies is rituximab (Mabthera®, Rituxan®), which is a chimeric anti-CD20 monoclonal antibody made with human γ1 and κ constant regions (therefore with human IgG1 Fc portion) linked to murine variable domains conferring CD20 specificity. In the past few years, rituximab has considerably modified the therapeutical strategy against B lymphoproliferative malignancies, particularly non-Hodgkin's lymphomas (NHL). Other examples of humanized IgG1 antibodies include alemtuzumab (Campath-1H®), which is used in the treatment of B cell malignancies, and trastuzumab (Herceptin®), which is used in the treatment of breast cancer. Additional examples of therapeutic antibodies under development are disclosed in the art.

[0004] The mechanism of action of therapeutic antibodies is still a matter of debate. Injection of antibodies leads to depletion of cells bearing the antigen specifically recognized by the antibody. This depletion can be mediated through at least three mechanisms: antibody mediated cellular cytotoxicity (ADCC), complement dependant lysis, and direct antitumor inhibition of tumor growth through signals given via the antigen targeted by the antibody.

[0005] While these antibodies represent a novel and efficient approach to human therapy, particularly for the treatment of tumors, they do not always exhibit a strong efficacy. For instance, while rituximab, alone or in combination with chemotherapy, was shown to be effective in the treatment of both low-intermediate and high-grade NHL, 30% to 50% of patients with low grade NHL have no clinical response to rituximab. It has been suggested that the level of CD20 expression on lymphoma cells, the presence of high tumor burden at the time of treatment, or low serum rituximab concentrations may explain the lack of efficacy of rituximab in some patients. Nevertheless, the actual causes of treatment failure remain largely unknown.

[0006] Further, the use of therapeutic antibodies can be limited by side effects caused by their administration. For example, side effects such as fever, headaches, nausea, hypotension, wheezing, rashes, infections, and numerous others can appear in patients, potentially limiting the possible amount or frequency with which the antibodies can be administered.

[0007] Thus, it would be very interesting to increase the efficacy of therapeutic antibodies, or to be able to achieve therapeutic efficacy using reduced doses of the antibodies that are less likely to produce side effects. The present invention addresses these and other needs.

Summary of the Invention

[0008] The present invention concerns the subject matter of the claims.

[0009] The present application discloses novel approaches to enhance the efficacy of therapeutic antibodies. Without being limited by the following theory, it is believed that the surprising results achieved using the present methods stem from their ability to enhance the ADCC mechanism in vivo, when therapeutic antibodies are injected. Indeed, the present invention provides novel compositions and methods that overcome the current difficulty related to the efficacy of therapeutic antibodies. It is shown in the present application that NK cells from an individual can have poor therapeutic mAb (monoclonal antibody)-mediated ADCC because of a lack of activation of NK cells, e.g., by an inhibition of inhibitory receptors on NK cells. Preferably, an increase of the ADCC mechanism is achieved by the administration of compounds that block an inhibitory receptor on NK cells, thereby promoting a potentiation of NK cell cytotoxicity in mammalian subjects. Preferably, the compound is an antibody or a fragment thereof. Said antibodies react with an inhibitory receptor of NK cells, e,g., Killer inhibitory receptor (KIR) molecules on NK cells, thereby neutralizing the inhibition of the cells and increasing their ADCC activity.

[0010] More specifically, the application discloses methods of treatment of a subject in which an antibody or a fragment thereof that blocks an inhibitory receptor of an NK cell is co-administered with the therapeutic antibody to the subject. The application demonstrates that the efficiency of a therapeutic antibody can be greatly enhanced by the co-administration, e.g., co-injection, of such antibody or a fragment thereof that overcomes the inhibition of NK cells, e.g., by blocking the inhibitory receptor of an NK cell.

[0011] The application also discloses pharmaceutical compositions comprising a therapeutic antibody and an antibody or a fragment thereof that blocks an inhibitory receptor of an NK cell. The application also discloses kits comprising a therapeutic antibody an antibody or a fragment thereof that blocks an inhibitory receptor of an NK cell.

[0012] The application also discloses the use of an antibody or a fragment thereof that blocks the inhibitory receptor of an NK cell for increasing the efficiency of a treatment with a therapeutic antibody or for increasing ADCC in a subject submitted to a treatment with a therapeutic antibody.

[0013] The application also discloses the use of an antibody or a fragment thereof that blocks the inhibitory receptor of an NK cell and a therapeutic antibody for the preparation of a drug for treating a disease. More particularly, the treatment of the disease requires the depletion of the targeted cells, preferably the diseased cells such as virally-infected cells, tumor cells or other pathogenic cells. Preferably, the disease is a cancer, infectious disease or immune disease. More preferably, the disease is selected from the group consisting of a cancer, an auto-immune disease, an inflammatory disease, and a viral disease. The disease also concerns a graft rejection, more particularly allograft rejection, and graft versus host disease (GVHD).

[0014] The present application also discloses a method for reducing the dosage of a therapeutic antibody, e.g., an antibody that is bound by an Fcγ receptor, preferably CD 16 (FcγRIIIa). For example, co-administration of a therapeutic antibody and an antibody that blocks an inhibitory receptor on NK cells allows a lower dose of the therapeutic antibody to be used. Such antibodies can be used at a 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or lower dose than the recommended dose in the absence of the compound.

[0015] In addition, the present application discloses a pharmaceutical composition comprising a therapeutic antibody, e.g., that can be bound by CD16, an antibody that blocks an inhibitory receptor of NK cells, and a pharmaceutically acceptable carrier. In another aspect, the present application discloses a kit comprising a therapeutic antibody, e.g., that can be bound by CD16, and an antibody that blocks an inhibitory receptor of NK cells.

[0016] For any of the above-mentioned methods, compositions, or kits, in one embodiment the therapeutic antibody has a human IgG1 or human IgG3 Fc portion. In another aspect, the therapeutic antibody is a monoclonal antibody or fragment thereof. In another aspect, the therapeutic antibody is not conjugated with a radioactive or toxic moiety. In another aspect, the antibody inhibits an inhibitory receptor of an NK cell. In one aspect, the therapeutic antibodies or compounds can be antibody fragments or derivatives such as, inter alia, a Fab fragment, a Fab'2 fragment, a CDR and a ScFv.

[0017] In one aspect, the therapeutic antibody is a human antibody, a humanized antibody, a chimeric antibody, or a fragment thereof. In another aspect, the therapeutic antibody is rituximab or Campath. In another aspect, the antibody is rituximab, and said antibody is administered at a dosage of less than 375 mg/m^2 per week. In another aspect, the antibody is Campath, and the antibody is administered at a dosage of less than 90mg per week.

[0018] The antibody binds to a common determinant of KIR2DL1, KIR2DL2, and KIR2DL3 human receptors and inhibits KIR2DL1-, KIR2DL2-, and KIR2DL3-mediated inhibition of NK cell cytotoxicity. In another embodiment, the antibody binds to the same epitope as monoclonal antibody DF200 produced by hybridoma DF200. In another embodiment, the compound competes with monoclonal antibody DF200 produced by hybridoma DF200 for binding to a KIR receptor at the surface of a human NK cell. In another embodiment, the compound is monoclonal antibody DF200 produced by hybridoma DF200 or a fragment thereof.

[0019] In another aspect, the present application discloses a method of increasing the efficiency of a treatment involving the administration of a therapeutic antibody that can be bound by CD16 in a subject, said method comprising administering to said subject prior to, simultaneously with, or after the administration of said therapeutic antibody, a therapeutically-effective amount of an antibody that blocks an inhibitory receptor of an NK cell. In one aspect, the antibody increases the efficiency of the treatment by enhancing ADCC in said subject.

Legend to the Figures

[0020]

Figure 1 : Monoclonal antibody DF200 binds a common determinant of various human KIR2DL receptors.

Figure 2 : Reconstitution of lysis with anti-KIR2DL mAb (monoclonal antibody) on C1R Cw4 target at effector /target ratio of 4/1. Monoclonal antibody DF200 inhibits KIR2DL-mediated inhibition of KIR2DL1 positive NK cell cytotoxicity (reconstitute lysis) on Cw4 positive target cells.

Figure 3 : Enhancement of ADCC mediated by Rituxan of an KIR2DL1 positive NK clone on a Cw4 positive EBV cell line by blocking KIR / HLA interaction. NK clone cytolysis bearing KIR2DL1 is tested against a Cw4 positive EBV transformed (CD20 positive) target cell line at various effector/target ratio (from 1 to 4) in the presence of 5 µg/ml anti CD20 antibody (Rutixan) and 10 µg/ml EB6 antibody (anti KIR2DL1); Rituxan alone; EB6 alone; or without any antibody. ADCC is greatly enhanced in the presence of anti KIR2DL1 antibody (EB6).

Figure 4 : Enhancement of ADCC mediated by Campath of an KIR2DL1 positive NK clone on a Cw4 positive EBV cell line by blocking KIR / HLA interaction. NK clone cytolysis bearing KIR2DL1 is tested against a Cw4 positive EBV transformed (CD20 positive) target cell line in the presence of Campath and 100 µg/ml EB6 antibody (anti KIR2DL1); Campath alone; EB6 alone; or without any antibody. ADCC is greatly enhanced in the presence of the anti KIR2DL1 antibody (EB6).

Detailed Description of the Invention

[0021] The present application discloses a method for increasing the efficiency of therapeutic antibodies. The application more specifically discloses that the use of an antibody or a fragment thereof that potentiates NK cells by blocking an inhibitory receptor of an NK cell can significantly increase the efficiency of therapeutic antibodies. Indeed, the inventors demonstrate that the efficiency of multiple therapeutic antibodies can be greatly enhanced by the co-administration of an antibody directed against an inhibitory NK cell receptor.

[0022] Therefore, the application discloses a method of treatment of a disease in a subject in need thereof comprising:

1. a) administering to said subject an antibody or a fragment thereof that blocks an inhibitory receptor of a NK cell; and,
2. b) administering to said subject a therapeutic antibody.

[0023] Said therapeutic antibody can be bound by CD16 of NK cells, preferably through its Fc region.

[0024] Preferably, said therapeutic antibody has a human IgG1 or human IgG3 Fc portion, particularly a monoclonal antibody or a fragment thereof further preferably a humanized antibody, a human antibody, a chimeric antibody, or a fragment thereof, for instance rituximab.

[0025] It is intended that antibodies or a fragment thereof that block the inhibitory receptor of a NK cell can be administered to the subject before, simultaneously with, or after the administration of the therapeutic antibody. The way of administration of the different antibodies depends on their bioavailability and phamacokinetics. Preferably, the therapeutic antibody is administrated within a week of the administration of the antibodies or a fragment thereof that block the inhibitory receptor of a NK cell, more preferably within 5 days or 2 days. Preferably, the therapeutic antibody is administrated before or simultaneously with the antibodies or a fragment thereof that block the inhibitory receptor of a NK cell.

[0026] In a further aspect, the application discloses a method of increasing ADCC in a subject receiving a therapeutic antibody treatment, said method comprising administering to said subject prior to, simultaneously with, or after the administration of said therapeutic antibody an amount sufficient to increase ADCC of an antibody or a fragment thereof that blocks the inhibitory receptor of a NK cell. Said therapeutic antibody can be bound by CD16 on NK cells, preferably through its Fc region. Preferably, said therapeutic antibody has a human IgG1 or human IgG3 Fc portion, particularly a monoclonal antibody or a fragment thereof, further preferably a human antibody, a humanized antibody, a chimeric antibody, or a fragment thereof, for instance rituximab.

[0027] In an additional aspect, the application discloses a method of increasing the efficiency of a therapeutic antibody treatment in a subject, said method comprising administering to said subject prior to, simultaneously with, or after the administration of said therapeutic antibody an amount of an antibody or a fragment thereof that blocks the inhibitory receptor of a NK cell, sufficient to increase the efficiency of said therapeutic antibody. Said therapeutic antibody can be bound by CD16, preferably through its Fc region. Preferably, said therapeutic antibody has a human IgG1 or IgG3 Fc portion, particularly a monoclonal antibody or a fragment thereof, further preferably a human antibody, a humanized antibody, a chimeric antibody, or a fragment thereof, for instance rituximab.

DEFINITIONS

[0028] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0029] As used herein, "NK" cells refers to a sub-population of lymphocytes that is involved in nonconventional immunity. NK cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including CD16, CD56 and/or CD57, 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 enzymes, 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. Any of these characteristics and activities can be used to identify NK cells using methods well known in the art.

[0030] The term "antibody," as used herein, refers to polyclonal and monoclonal antibodies. Depending on the type of constant domain in the heavy chains, antibodies are assigned to one of five major classes: IgA, IgD, IgE, IgG, and IgM. Several of these are further divided into subclasses or isotypes, such as IgG1, IgG2, IgG3, IgG4, and the like. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids that is primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are termed "alpha," "delta," "epsilon," "gamma" and "mu," respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. IgG and/or IgM are the preferred classes of antibodies employed in this invention, with IgG being particularly preferred, because they are the most common antibodies in the physiological situation, because they are most easily made in a laboratory setting, and because IgGs are specifically recognized by Fc gamma receptors. Preferably, the antibody of this invention is a monoclonal antibody. Particularly preferred are humanized, chimeric, human, or otherwise-human-suitable antibodies.

[0031] Within the context of this invention, the term "therapeutic antibody or antibodies" designates more specifically any antibody that functions to deplete target cells in a patient. In particular, therapeutic antibodies specifically bind to antigens present on the surface of the target cells, e.g., tumor specific antigens present predominantly or exclusively on tumor cells. Preferably, therapeutic antibodies include human Fc portions, or are capable of interacting with human Fc receptors. Therapeutic antibodies can target cells by any means, e.g., ADCC or otherwise, and can be "naked," i.e., with no conjugated moieties, or they can be conjugated with compounds such as radioactive labels or toxins.

[0032] The term "specifically binds to" means that an antibody can bind preferably in a competitive binding assay to the binding partner, e.g., an activating NK receptor such as NKp30, NKp44, or NKp46, or a human Fc gamma receptor, as assessed using either recombinant forms of the proteins, epitopes therein, or native proteins present on the surface of isolated NK or relevant target cells. Competitive binding assays and other methods for determining specific binding are further described below and are well known in the art.

[0033] A "human-suitable" antibody refers to any antibody, derivatized antibody, or antibody fragment that can be safely used in humans for, e.g., the therapeutic methods described herein. Human-suitable antibodies include all types of humanized, chimeric, or fully human antibodies, or any antibodies in which at least a portion of the antibodies is derived from humans or otherwise modified so as to avoid the immune response that is provoked when native non-human antibodies are used.

[0034] By "immunogenic fragment", it is herein meant any polypeptidic or peptidic fragment which is capable of eliciting an immune response such as (i) the generation of antibodies binding said fragment and/or binding any form of the molecule comprising said fragment, including the membrane-bound receptor and mutants derived therefrom, (ii) the stimulation of a T-cell response involving T-cells reacting to the bi-molecular complex comprising any MHC molecule and a peptide derived from said fragment, and (iii) the binding of transfected vehicles such as bacteriophages or bacteria expressing genes encoding mammalian immunoglobulins. Alternatively, an immunogenic fragment also refers to any construction capable of eliciting an immune response as defined above, such as a peptidic fragment conjugated to a carrier protein by covalent coupling, a chimeric recombinant polypeptide construct comprising said peptidic fragment in its amino acid sequence, and specifically includes cells transfected with a cDNA of which sequence comprises a portion encoding said fragment.

[0035] For the purposes of the present invention, a "humanized" antibody refers to an antibody in which the constant and variable framework region of one or more human immunoglobulins is fused with the binding region, e.g., the CDR, of an animal immunoglobulin. Such humanized antibodies are designed to maintain the binding specificity of the non-human antibody from which the binding regions are derived, but to avoid an immune reaction against the non-human antibody.

[0036] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced, or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. In preferred embodiments of the present invention, the chimeric antibody nevertheless maintains the Fc region of the immunoglobulin, preferably a human Fc region, thereby allowing interactions with human Fc receptors on the surface of target cells.

[0037] Within the context of this invention, "potentiated," "active," or "activated" NK cells designate biologically active NK cells, more particularly NK cells having the capacity of lysing target cells. For instance, an "active" NK cell is able to kill cells that express an NK activating receptor-ligand and fails to express "self" MHC/HLA antigens (KIR-incompatible cells). Examples of suitable target cells for use in redirected killing assays are P815 and K562 cells, but any of a number of cell types can be used and are well known in the art (see, e.g., Sivori et al. (1997) J. Exp. Med. 186: 1129-1136; Vitale et al. (1998) J. Exp. Med. 187: 2065-2072; Pessino et al. (1998) J. Exp. Med. 188: 953-960; Neri et al. (2001) Clin. Diag. Lab. Immun. 8:1131-1135). "Potentiated," "active," or "activated" cells can also be identified by any other property or activity known in the art as associated with NK activity, such as cytokine (e.g., IFN-γ and TNF-α) production of increases in free intracellular calcium levels. For the purposes of the present invention, "potentiated," "active," or "activated" NK cells refer particularly to NK cells in vivo that are not inhibited via stimulation of an inhibitory receptor, or in which such inhibition has been overcome, e.g., via stimulation of an activating receptor.

[0038] As used herein, the term "inhibiting" or "inhibitory" NK receptor refers to any molecule on the surface of NK cells that, when stimulated, causes a measurable decrease in any property or activity known in the art as associated with NK activity, such as cytokine (e.g., IFN-γ and TNF-α) production, increases in intracellular free calcium levels, or the ability to lyse target cells in a redirected killing assay as described, e.g., elsewhere in the present specification. Examples of such receptors include KIR2DL1, KIR2DL2/3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3, LILRB1, NKG2A, NKG2C, NKG2E, and LILRB5. Methods of determining whether an NK cell is active or not are described in more detail below and are well known to those of skill in the art.

[0039] In the present invention, the term "block an inhibitory receptor" refers to the ability of certain antibodies, fragments, or derivatives thereof to directly interact with at least one inhibitory NK cell receptor, e.g., KIR, NKG2A/C, and others listed herein, and neutralizing inhibitory signals of the receptor (in the case of inhibitory receptors). With inhibitory receptors, preferably the antibody or a fragment thereof, is able to block the interaction between HLA and the receptor. The antibodies may by polyclonal or, preferably, monoclonal. They may be produced by hybridomas or by recombinant cells engineered to express the desired variable and constant domains. The antibodies may be single chain antibodies or other antibody derivatives retaining the antigen specificity and the lower hinge region or a variant thereof such as a Fab fragment, a Fab'2 fragment, a CDR, and a ScFv. These may be polyfunctional antibodies, recombinant antibodies, humanized antibodies, or variants thereof.

[0040] Within the context of this disclosure a "common determinant" designates a determinant or epitope that is shared by several members of a group of related receptors, e.g., the human KIR2DL receptor group. The determinant or epitope may represent a peptide fragment or a conformational epitope shared by said members. In a specific embodiment, the common determinant comprises an epitope recognized by monoclonal antibody DF200, NKVSF1, or EB6.

[0041] Within the context of this disclosure, the term antibody that "binds" a common determinant designates an antibody that binds said determinant with specificity and/or affinity, e.g., that essentially does not bind with high affinity or with specificity other unrelated motifs or determinant or structures at the surface of human NK cells. More particularly, the binding of a monoclonal antibody according to this invention to said determinant can be discriminated from the binding of said antibody to another epitope or determinant.

[0042] Antibodies capable of binding to NK cell inhibitory receptors and prevent their stimulation are thus "neutralizing" or "inhibitory" compounds, preferably antibodies, in the sense that they block, at least partially, the inhibitory signaling pathway mediated by an NK cells inhibitory receptor, i.e., KIR or NKG2A/C receptors. More importantly, this inhibitory activity can be displayed with respect to several types of KIR or NKG2A/C receptors, so that these compounds, preferably antibodies, may be used in various subjects with high efficacy.

[0043] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein, or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (nonrecombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed, or not expressed at all.

[0044] Within the context of the present invention, a subject or patient includes any mammalian subject or patient, more preferably a human subject or patient.

THERAPEUTIC ANTIBODIES

[0045] The present application provides for the use of NK cell potentiating compounds in conjunction with therapeutic antibodies as claimed. Any of a large variety of therapeutic antibodies can be used in the present invention. Essentially, any therapeutic antibody, whether "naked" or conjugated with a radiolabel, toxin, or other moiety, or whether full length or a fragment; or whether a true antibody or a modified derivative of an antibody, can be used. Preferably, the methods are used to enhance the efficacy of therapies in which NK cell activity plays a role-not necessarily exclusive-in the efficacy of administered therapeutic antibodies, and also preferably the antibodies or fragments will naturally include, or will be modified to include, a human Fc region or other domain that allows specific recognition of the antibody by human Fc receptors, e.g., Fc gamma receptors.

[0046] The present antibodies can be used to enhance the ability of therapeutic antibodies to deplete target cells that express an antigen that is specifically recognized by the therapeutic antibodies. Accordingly, any disease or condition that is caused or exacerbated at least in part by cells that can be targeted by a therapeutic antibody can be treated using the herein-described methods. Specific examples of target cells include tumor cells, virus-infected cells, allogenic cells, pathological immunocompetent cells (e.g., B lymphocytes, T lymphocytes, antigen-presenting cells, etc.) involved in allergies, autoimmune diseases, allogenic reactions, etc., or even healthy cells (e.g., endothelial cells in an anti-angiogenic therapeutic strategy). Most preferred target cells within the context of this invention are tumor cells and virus-infected cells. The therapeutic antibodies may, for instance, mediate a cytotoxic effect or cell lysis, particularly by antibody-dependent cell-mediated cytotoxicity (ADCC).

[0047] ADCC requires leukocyte receptors for the Fc portion of IgG (FcγR), whose function is to link the IgG-sensitized antigens to FcγR-bearing cytotoxic cells and to trigger the cell activation machinery. Therefore, the therapeutic antibody is capable of forming an immune complex. For example, an immune complex can be a tumor target covered by therapeutic antibodies. More particularly, the antibody can be bound by CD16, preferably through its Fc region. Determining whether a therapeutic antibody binds an Fcγ receptor, such as CD16, can be assessed by any suitable manner, for example by determining binding to a recombinantly produced CD16 polypeptide or fragment thereof, optionally immobilized on a support, or for example by determining binding of the therapeutic antibody to a cell which known or suspected to express CD16.

[0048] The therapeutic antibodies may by polyclonal or, preferably, monoclonal. They may be produced by hybridomas or by recombinant cells engineered to express the desired variable and constant domains. The antibodies may be single chain antibodies or other antibody derivatives retaining the antigen specificity and the lower hinge region or a variant thereof. These may be polyfunctional antibodies, recombinant antibodies, humanized antibodies, fragments or variants thereof. Said fragment or a derivative thereof is preferably selected from a Fab fragment, a Fab'2 fragment, a CDR, and a ScFv. Preferably, a fragment is an antigen-binding fragment. Therapeutic antibodies which comprise an antibody fragment may also include but are not limited to bispecific antibodies; one example a suitable bispecific antibody comprises an antigen binding region specific for CD 16 and an antigen binding region specific for a tumor antigen. Other antibody formats comprising fragments include recombinant bispecific antibody derivatives combining the binding regions of two different antibodies on a single polypeptide chain, also referred to as BiTE™ (Kufer P, et al TRENDS in Biotechnology 2004; 22 (5): 238-244; and Baeuerle et al, Current Opinion in Molecular Therapeutics 2003; 5(4): 413-419.

[0049] Therapeutic antibodies are generally specific for surface antigens, e.g., membrane antigens. Most preferred therapeutic antibodies are specific for tumor antigens (e.g., molecules specifically expressed by tumor cells), such as CD20, CD52, ErbB2 (or HER2/Neu), CD33, CD22, CD25, MUC-1, CEA, KDR, αVβ3, etc., particularly lymphoma antigens (e.g., CD20). The therapeutic antibodies have preferably human or non-human primate IgG1 or IgG3 Fc portion, more preferably human IgG1.

[0050] In one embodiment, the antibodies will include modifications in their Fc portion that enhances the interaction of the antibody with NK cells during ADCC. Such modified therapeutic antibodies ("altered antibodies") generally comprise modifications, preferably in the Fc region, that modify the binding affinity of the antibody to one or more FcγR. Methods for modifying antibodies with modified binding to one or more FcγR are known in the art, see, *e.g.*, PCT Publication Nos. WO 2004/016750 (International Application PCT/US2003/025399), WO 99/158572, WO 99/151642, WO 98/123289, WO 89/107142, WO 88/107089, and U.S. Patent Nos. 5,843,597 and 5,642,821.

[0051] Therapeutic antibodies identified herein, such as D2E7 (Cambridge Antibody Technology Group, plc (Cambridge, UK)/BASF (Ludwigshafen, Germany)) used to treat rheumatoid arthritis, or Infliximab (Centocor, Inc., Malvern, PA; used to treat Crohn's disease and rheumatoid arthritis), or the antibodies disclosed in International Patent Application WO 2004/016750 can be modified as taught in the above and below identified applications and used for the treatment of diseases for which such antibodies are typically used. In some embodiments, the invention provides altered antibodies that have altered affinity, either higher or lower affinity, for an activating FcγR, *e.g.*, FcγRIII. In certain preferred embodiments, altered antibodies having higher affinity for FcγR are provided. Preferably such modifications also have an altered Fc-mediated effector function.

[0052] Modifications that affect Fc-mediated effector function are well known in the art (See, e.g., U.S. 6,194,351). The amino acids that can be modified include but are not limited to proline 329, proline 331, and lysine 322. Proline 329 and/or 331 and lysine 322 can, preferably, be replaced with alanine, however, substitution with any other amino acid is also contemplated. See International Publication No.: WO 00/142072 and U.S. 6,194,551.

[0053] Thus, modification of the Fc region can comprise one or more alterations to the amino acids found in the antibody Fc region. Such alterations can result in an antibody with an altered antibody-mediated effector function, an altered binding to other Fc receptors (e.g., Fc activation receptors), an altered ADCC activity, an altered Clq binding activity, an altered complement dependent cytotoxicity activity, or any combination thereof.

[0054] In one embodiment, the antibody is specifically recognized by an Fc gamma receptor such as FCGR3A (also called CD16, FCGR3, Immunoglobulin G Fc Receptor III; IGFR3, Receptor for Fc Fragment of IgG, Low Affinity IIIa; see, e.g. OMIM 146740), FCGR2A (also called CD32, CDw32, Receptor for Fc Fragment of IgG, Low Affinity IIa, FCG2, Immunoglobulin G Fc Receptor II; see, e.g. OMIM 146790); FCGR2B (also called CD32, Receptor for Fc Fragment of IgG, Low Affinity IIb; FCGR2B, FC-Gamma-RIIB; see, e.g. OMIM 604590), FCG1RA (also called CD64; Receptor for Fc Fragment of IgG, High affinity Ia; IGFR1; see, e.g., OMIM 146760); FCGR1 fragment of IgG, High affinity Ic, Immunoglobulin G Fc receptor IC, IGFRC; see, e.g., OMIM 601503); or FCGR1B (also called CD64, Receptor for Fc Fragment of IgG, High affinity Ib; Immunoglobulin G Fe Receptor IB, IGFRB; see, e.g., OMIM 601502).

[0055] Typical examples of therapeutic antibodies of this invention are rituximab, alemtuzumab, and trastuzumab. Such antibodies may be used according to clinical protocols that have been authorized for use in human subjects. Additional specific examples of therapeutic antibodies include, for instance, epratuzumab, basiliximab, daclizumab, cetuximab, labetuzumab, sevirumab, tuvurimab, palivizumab, infliximab, omalizumab, efalizumab, natalizumab, clenoliximab, etc. Optionally, when a compound that stimulates an activating receptor of a NK cell is a cytokine, the therapeutic antibody is an antibody other than rituximab or herceptin, or optionally other than an anti-CD20 or anti-HER2/neu antibody. Other examples of preferred therapeutic antibodies for use in accordance with the invention include anti-ferritin antibodies (US Patent Publication No. 2002/0106324), anti-p140 and anti-sc5 antibodies (WO 02/50122), and anti-KIR (killer inhibitory receptor) antibodies (The KIR receptors are described in Carrington and Norman, The KIR Gene Cluster, May 3, 2003, available at: http://www.ncbi.nlm.nih.gov/books). Other examples of therapeutic antibodies are listed in the following table, any of which (and others) can be used in the present methods. It will be appreciated that, regardless of whether or not they are listed in the following table or described elsewhere in the present specification, any antibody that can deplete target cells, preferably by ADCC, can benefit from the present methods, and that the following Table 1 is non exhaustive, neither with respect to the antibodies listed therein, nor with respect to the targets or indications of the antibodies that are listed.

Table 1: Therapeutic antibodies

Ab specificity	DCI	Commercial name	Typical Indications
Anti-CD20	rituximab	MabThera®, Rituxan®	NHL B
Anti-CD20		Zevalin	NHL
Anti-CD20		Bexocar	NHL
Anti-CD52	alemtuzumab	CAMPATH-1H®	CLL, allograft
Anti-CD33		SMART-M195	AML
Anti-CD33		Zamyl™	Acute myeloid Leukemia
Anti-HLA-DR antigen		SMART-ID 10	NHL
Anti-HLA-DR		Remitogen™	NHL B
Anti-CD22	epratuzumab	LymphoCide™	NHL B
Anti-HER2		MDX-210	Prostate and other cancers
Anti-erbB2 (HER-2/neu)	trastuzumab	Herceptin®,	Metastatic breast cancer
Anti-CA125		OvaRex	Ovarian cancer
Anti-MUC1		TriAb	Metastatic breast cancer
Anti-MUC1		BravaRex	Metastatic cancers
Anti-PEM antigen		Theragyn, Therex	Ovarian cancer, breast cancer
Anti-CD44	bivatuzumab		Head and neck cancer
Anti-gp72	MAb, idiotypic 105AD7		colorectal cancer
Anti-EpCAM	Anti-EpCAM; MT201	IS-IL2	cancer
Anti-VEGF	MAb-VEGF		metastatic NSCLC, colorectal cancer
Anti-CD18	AMD Fab		age-related macular degeneration
Anti-CD18	Anti-CD 18		Myocardial infarction
Anti-VEGF receptor	IMC-1cl l		colorectal cancer
anti-nuC242	nuC242-DMI		Colorectal, gastric, and pancreatic cancer
Anti-EGFR	MAb425		cancer
Anti-EGFR	ABX-EGF		Cancer
Anti-EGFR	cetuximab		ENT and colorectal Cancers
(HER-1, erbB1)			
Anti-MUC-1		Therex®	Breast and epithelial cancers
Anti-CEA		CEAVac	Colorectal cancer
Anti-CEA	labetuzumab	CEA-Cide™	Solid tumors
Anti-αVβ3		Vitaxin	Leiomyosarcoma, colorectal and other cancers (anti-angiogenic)
Anti-KDR (VEGFR2)			Cancers (anti-angiogenic)
anti-VRS fusion protein	palivizumab	Synagis®	Viral diseases
Idem		Numax™	Idem
CMV	sevirumab	Protovir	CMV Infection
HBs	tuvirumab	Ostavir™	Hepatitis B

Ab specificity	DCI	Commercial name	Typical Indications
Anti-CD25	basiliximab	Simulect®	Prevention/treatment allograft rejection
Anti-CD25	daclizumab	Zenapax®	Prevention/treatment allograft rejection
anti-TNF-α	infliximab	Remicade™	Crohn disease, rheumatoid arthritis
anti-CD80	IDEC-114		psoriasis
anti-IgE		E-26	Allergic asthma and rhinitis
anti-IgE	omalizumab	Xolair™	Asthma
anti-IgE	Rhu-mAb E25		Allergy/asthma
anti-integrin αL (CDI la, LFA-1)	efalizumab	Xanelim™	psoriasis
Anti-beta 2 integrin	LDP-01		Stroke, allograft rejection
anti-integrin αL (CD11a, LFA-1)	anti-CD11a		psoriasis
anti-CD4	keliximab siplizumab MEDI-507		GVHD, psoriasis
Anti-CD4	OKT4A		Allograft rejection
Anti-CD3	OKT3		Allograft rejection
Anti-CD3	SMART-aCD3		Autoimmune disease, allograft rejection, psoriasis
Anti-CD64			anemia
anti-CD 147			GvHD
anti-integrin α4 (α4β1-α4β7)	natalizumab	Antegren®	Multiple Sclerosis, Crohn
Anti-integrin β7			Crohn, ulcerative colitis
Alpha 4 beta 7	LDP-02		Ulcerative colitis
Anti-HLA-DR10 beta		Oncolym	NHL
Anti-CD3		Nuvion	T cell malignancies
Anti-GD2 ganglioside		Trigem	Metastatic melanoma and small cell lung cancer
Anti-SK-1 antigen			Colorectal and pancreatic carcinoma
anti-CD4*	clenoliximab		
anti-IL-8	ABX-IL8		psoriasis
Anti-VLA-4		Antegren	MS
Anti-CD40L		Antova	SLE, allograft rejection
Anti-CD40L	IDEC-131		MS, SLE
Anti-E-selectin	CDP850		psoriasis
Anti-CD11/CD18	Hu23F2G		MS, stroke
Anti-ICAM-3	ICM3		psoriasis
Anti-CBL	ABX-CBL		GVHD
Anti-CD 147			
Anti-CD23	IDEC-152		Asthma, allergies
Anti-CD25		Simulect	Allograft rejection
Anti-T1-ACY	ACY-110		Breast cancer
Anti-TTS	TTS-CD2		Pancreatic, renal cancer
Anti-TAG72	AR54		Breast, ovarian, lung cancer
Anti-CA19.9	GivaRex		Colorectal, pancreatic, gastric

Ab specificity	DCI	Commercial name	Typical Indications
Anti-PSA	ProstaRex		Prostate cancer
Anti-HMFG1	R1550		Breast, gastric cancer
	pemtumomab	Theragyn	Gastric, ovarian cancer
Anti-hCG	CTP-16, CTP-21		Mutiple cancers
Anti collagen Types 1-V	HU177; HUIV26; XL313		Multiple cancers
Anti-CD46		Crucell/J&J	Mutiple cancers
Anti-17A-1	Edrecolomab	Panorex	Colorectal cancer
Anti-HM1.24	AHM		Multiple myeloma
Anti-CD38	Anti-CD38		Multiple myeloma
Anti-IL15 Receptor	HuMax lymphoma		Lymphoma
Anti-IL6	B-E8		Lymphoma
Anti-TRAIL-R1	TRM-1		Mutiple cancers
Anti-VEGF2			Mutiple cancers
Anti-BlyS	Lymphostat		Mutiple cancers
Anti-SCLC, CEA and DTPA	Pentacea		Lung cancer
Anti-CD52	CAMPATH		Leukemia, Lymphoma
Anti-Lewis Y antigen	IGN311		Epithelial cancers
Anti-VE cadherin	E4G10		Mutiple cancers
Anti-CD56	BB10901, huN901DC1		Colorectal, lung cancer
Anti-mertansine/mucine	Cantuzumab		Colorectal, lung, pancreatic cancer
Anti-AFP	AFP-cide		Liver cancer
Anti-CSAp	Mu-9		Colorectal cancer
Anti-CD30	MDX-060		Melanoma, Hodgkins Disease
Anti-PSMA	MDX-070		Prostate cancer
Anti-CD 15	MDX-11		Leukemia
Anti-TAG72	MDX-020		Colorectal cancer
Anti-CD 19,CD3 bispecific	MT103		Lymphoma
Anti-mesothelin antigen	SS1-PE38		Brain and overian cancer, mesothelioma
Anti-DNA and histones	Cotara		Colorectal, pancreatic, sarcoma, brain and other cancers
Anti-a5B1 integrin	Anti-a5 B1		Multiple cancers
Anti-p97	SGN17/19		Melanoma
Anti-CD5	Genimune		Leukemia, lymphoma

COMPOUNDS REGULATING NK CELL ACTIVITY

[0056] NK cell activity is regulated by a complex mechanism that involves both stimulating and inhibitory signals. Accordingly, effective NK cell-mediated therapy can be achieved both by a stimulation of these cells or a neutralization of inhibitory signals. It will also be appreciated that the mechanism by which the receptors are blocked is not critical to the advantages provided by the invention. The antibodies can bind directly to the receptors and inhibit them (in the case of inhibitory receptors). The critical parameter is the effect that the antibodies have on the ability of therapeutic antibodies to deplete their target cells in vivo.

[0057] NK cells are negatively regulated by major histocompatibility complex (MHC) class I-specific inhibitory receptors (Kärre et al., 1986; Öhlén et al, 1989). These specific receptors bind to polymorphic determinants of major histocompatibility complex (MHC) class I molecules or HLA and inhibit natural killer (NK) cell lysis. In humans, a family of receptors termed killer Ig-like receptors (KIRs) recognize groups of HLA class I alleles.

[0058] There are several groups of KIR receptors, including KIR2DL, KIR2DS, KIR3DL, and KIR3DS. KIR receptors having two Ig domains (KIR2D) identify HLA-C allotypes: KIR2DL2 (formerly designated p58.1) or the closely related gene product KIR2DL3 recognizes an epitope shared by group 2 HLA-C allotypes (Cw1, 3, 7, and 8), whereas KIR2DL1 (p58.2) recognizes an epitope shared by the reciprocal group 1 HLA-C allotypes (Cw2, 4, 5, and 6). The recognition by KIR2DL1 is dictated by the presence of a Lys residue at position 80 of HLA-C alleles. KIR2DL2 and KIR2DL3 recognition is dictated by the presence of a Asn residue at position 80. Importantly the great majority of HLA-C alleles have either an Asn or a Lys residue at position 80. One KIR with three Ig domains, KIR3DL1 (p70), recognizes an epitope shared by HLA-Bw4 alleles. Finally, a homodimer of molecules with three Ig domains KIR3DL2 (p140) recognizes HLA-A3 and -A11.

[0059] Although KIRs and other class-I inhibitory receptors (Moretta et al, 1997; Valiante et al, 1997; Lanier, 1998) may be co-expressed by NK cells, in any given individual's NK repertoire, there are cells that express a single KIR and, thus, the corresponding NK cells are blocked only by cells expressing a specific class I allele group. Accordingly, as described infra, when inhibitory receptors are targeted, the present methods will often involve the administration of antibodies that target multiple inhibitory receptors, thereby ensuring a broad-based effect that reaches a maximum range of NK cells.

[0060] Preferably, an antibody or a fragment thereof that blocks the inhibitory receptor of a NK cell is an antibody or a fragment thereof that neutralizes the inhibitory signal of KIR2DL2, KIR2DL 3, and KIR2DL1.

[0061] The application also discloses the use of a combination of several antibodies or a fragment thereof that block different inhibitory receptors of NK cells. Preferably, antibodies or a fragment thereof that block inhibitory receptors of NK cells are specific of an inhibitory receptor selected from KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1, KIR3DL2, NKG2A, and NKG2C and are able to inhibit the related KIR- or NKG2A/C-mediated inhibition of NK cell cytotoxicity.

[0062] In a preferred aspect, monoclonal antibodies, as well as fragments and derivatives thereof are used, wherein said antibody, fragment or derivative cross reacts with several KIR at the surface of NK cells as claimed and neutralizes their inhibitory signals.

[0063] In one embodiment, a monoclonal antibody that binds to a common determinant of human KIR2DL receptors as claimed and inhibits the corresponding inhibitory pathway is used. In a specific embodiment, the monoclonal antibody binds to KIR2DL1 and KIR2DL2/3 receptors at the surface of human NK cells and inhibits KIR2DL1- and KIR2DL2/3-mediated inhibition of NK cell cytotoxicity. The antibody specifically inhibits binding of HLA-C molecules to KIR2DL1 and KIR2DL2/3 receptors. More preferably, the antibody facilitates NK cell activity in vivo. Because KIR2DL1 and KIR2DL3 (or KIR2DL2) are sufficient for covering most of the HLA-C allotypes, respectively group 1 HLA-C allotypes and group 2 HLA-C allotypes, such antibodies may be used to increase the efficiency of a therapeutic antibody in most human individuals, typically in about 90% of human individuals or more. In such an embodiment, any of the antibodies described in PCT Patent Application WO 2005/003172 filed July 1, 2004, titled "Compositions and methods for regulating NK cell activity" can be used in accordance with the invention.

[0064] In a particular aspect of this disclosure, the antibody that blocks the inhibitory receptor of a NK cell is a monoclonal antibody, wherein said antibody binds to a common determinant of KIR2DL human receptors and inhibits KIR2DL-mediated inhibition of NK cell cytotoxicity. The antibody more specifically binds to the same epitope as monoclonal antibody DF200 produced by hybridoma DF200 and/or competes with monoclonal antibody DF200 produced by hybridoma DF200, for binding to a KIR receptor at the surface of a human NK cell. As discussed, examples of antibodies, functional assays and assays to determine whether antibodies compete for binding with said antibodies are described in PCT Patent Application WO 2005/003172.

[0065] In a specific aspect, the monoclonal antibody is monoclonal antibody DF200 produced by hybridoma DF200. In other aspects, the antibody is a fragment or derivative of antibody DF200. The hybridoma producing antibody DF200 has been deposited at the CNCM culture collection, as Identification no. "DF200", registration no. CNCM I-3224, registered 10 June 2004, Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25, Rue du Docteur Roux, F-75724 Paris Cedex 15, France.

[0066] The binding of any compound to any of the herein-described NK cell receptors can be detected using any of a variety of standard methods. For example, colorimetric ELISA-type assays can be used, as can immunoprecipitation and radioimmunoassays. Competition assays may be employed, e.g., to compare the binding of a test compound to a compound known to bind to an NK cell receptor, in which the control (e.g., BAB281, which specifically binds to NKp46) and test compounds are admixed (or pre-adsorbed) and applied to a sample containing the epitope-containing protein, e.g., NKp46 in the case of BAB281. Protocols based upon ELISAs, radioimmunoassays, Western blotting, and the use of BIACORE are suitable for use in such simple competition studies and are well known in the art.

[0067] Inhibition of KIR- or NKG2A/C-mediated inhibition of NK cell cytotoxicity of NK cells can be assessed by various assays or tests, such as binding, cytotoxicity, or other molecular or cellular assays.

[0068] In a specific embodiment, inhibitory activity is illustrated by the capacity of said antibody to reconstitute the lysis of KIR positive NK clones on HLA-C positive targets. In another specific embodiment, the antibody is defined as inhibiting the binding of HLA-C molecules to KIR2DL1 and KIR2DL3 (or the closely related KIR2DL2) receptors, further preferably as its capacity to alter the binding of a HLA-C molecule selected from Cw1, Cw3, Cw7, and Cw8 (or of a HLA-C molecule having an Asn residue at position 80) to KIR2DL2/3; and the binding of a HLA-C molecule selected from Cw2, Cw4, Cw5 and Cw6 (or of a HLA-C molecule having a Lys residue at position 80) to KIR2DL1.

[0069] The inhibitory or potentiating activity of an antibody can be assessed in any of a number of ways, e.g., by its effect on intracellular free calcium as described, e.g., in Sivori et al. (1997) J. Exp. Med. 186:1129-1136. NK cell activity can also be assessed using a cell based cytotoxicity assays, e.g., measuring chromium release, such as assessing the ability of the antibody to stimulate NK cells to kill target cells such as P815, K562 cells, or appropriate tumor cells as disclosed in Sivori et al. (1997) J. Exp. Med. 186: 1129-1136; Vitale et al. (1998) J. Exp. Med. 187: 2065-2072; Pessino et al. (1998) J. Exp. Med. 188: 953-960; Neri et al. (2001) Clin. Diag. Lab. Immun. 8:1131-1135); Pende et al. (1999) J. Exp. Med. 190: 1505-1516). Suitable cytotoxicity assays are also provided in the examples section of the present specification. In a preferred embodiment, the antibodies cause at least a 10% augmentation in NK cytotoxicity, preferably at least a 40% or 50% augmentation in NK cytotoxicity, or more preferably at least a 70% augmentation in NK cytotoxicity.

[0070] NK cell activity can also be addressed using a cytokine-release assay, wherein NK cells are incubated with the antibody to stimulate the NK cells' cytokine production (for example IFN-γ and TNF-α production). In an exemplary protocol, IFN-γ production from PBMC is assessed by cell surface and intracytoplasmic staining and analysis by flow cytometry after 4 days in culture. Briefly, Brefeldin A (Sigma Aldrich) is added at a final concentration of 5 μg/ml for the last 4 hours of culture. The cells are then incubated with anti-CD3 and anti-CD56 mAb prior to permeabilization (IntraPrep™; Beckman Coulter) and staining with PE-anti-IFN-γ or PE-IgG1 (Pharmingen). GM-CSF and IFN-γ production from polyclonal activated NK cells are measured in supernatants using ELISA (GM-CSF: DuoSet Elisa, R&D Systems, Minneapolis, MN; IFN-γ: OptE1A set, Pharmingen).

[0071] In a preferred aspect, the ability of the antibody to activate human NK cells is assessed, where an ability to activate human NK cells at least as well as non-human NK cells indicates that the compounds are suitable for use according to present disclosure. In particular, the ability of the compound to enhance the ability of therapeutic antibodies to direct the depletion of suitable target cells by NK cells in vitro or in vivo can be assessed.

[0072] The antibodies may exhibit partial inhibitory activity, e.g., partially reduce the KIR2DL-mediated inhibition of NK cell cytotoxicity. Most preferred antibodies are able to inhibit at least 20%, preferably at least 30%, 40% or 50% or more of the activity of the NK cell, e.g., as measured in a cell toxicity assay, in comparison to cells in the absence of the antibody. Also preferred, the antibody can provide an increase of depletion of target cells by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 1000%, or more relative to the depletion level in the absence of the antibody. Alternatively, antibodies are able to induce the lysis of matched or HLA compatible or autologous target cell population, i.e., cell population that would not be effectively lysed by NK cells in the absence of said antibody. Accordingly, antibodies of this disclosure may also be defined as facilitating NK cell activity in vivo.

[0073] The application also relates to aspects in which antibodies that block the inhibitory receptor of a NK cell, are fragments of such a monoclonal antibody having substantially the same antigen specificity, including, without limitation, a Fab fragment, a Fab'2 fragment, a CDR, and a ScFv. Furthermore, the monoclonal antibody may be humanized, human, or chimeric (e.g., a bispecific or functionalised antibody). Derivatives, e.g., with modified sequences or with conjugated heterologous functional groups or other compounds, can be used for any of the antibodies described herein.

[0074] The antibodies that block the inhibitory receptor of an NK cell according to the disclosure may be produced by a variety of techniques known in the art. Typically, they are produced by immunization of a non-human animal with an immunogen comprising a KIR polypeptide, or immunogenic fragment of the polypeptide, and collection of spleen cells (to produce hybridomas by fusion with appropriate cell lines). Methods of producing monoclonal antibodies from various species are well known in the art (see, e.g., Harlow et al., "Antibodies: A laboratory Manual," CSH Press, 1988; Goding, "Monoclonal Antibodies: Principles and Practice," Academic Press, 1986). More specifically, these methods comprise immunizing a non-human animal with the antigen, followed by a recovery of spleen cells which are then fused with immortalized cells, such as myeloma cells. The resulting hybridomas produce the monoclonal antibodies and can be selected by limiting dilutions to isolate individual clones. Antibodies may also be produced by selection of combinatorial libraries of immunoglobulins, as disclosed for instance in Ward et al (1989).

[0075] Preferred antibodies that block the inhibitory receptor of a NK cell according to the disclosure are prepared by immunization with an immunogen comprising an inhibiting NK cell receptor, e.g., a KIR2DL polypeptide, more preferably a human KIR2DL polypeptide. The KIR2DL polypeptide may comprise the full length sequence of a human KIR2DL polypeptide, or a fragment or derivative thereof, typically an immunogenic fragment, i.e., a portion of the polypeptide comprising an epitope, preferably a T or B cell epitope. Such fragments typically contain at least 7 consecutive amino acids of the mature polypeptide sequence, even more preferably at least 10 consecutive amino acids thereof. They are essentially derived from the extra-cellular domain of the receptor. In a preferred embodiment, the immunogen comprises a wild-type human KIR2DL in a lipid membrane, typically at the surface of a cell. In a specific embodiment, the immunogen comprises intact NK cells, particularly intact human NK cells, optionally treated or lysed.

[0076] While the therapeutic antibodies may have Fc regions modified so as to enhance their binding by receptors, such as CD16, in certain embodiments NK cell potentiating antibodies will have Fc regions altered so as to reduce their affinity for Fc receptors, thereby reducing the likelihood that NK cells bound by the antibodies will themselves be bound and lysed.

[0077] Antibodies that block the KIR2DL receptors of NK cells can be produced by methods comprising: i) immunizing a non-human mammal with an immunogen comprising a KIR2DL polypeptide; ii) preparing monoclonal antibodies from said immunized animal, wherein said monoclonal antibodies bind said KIR2DL polypeptide; iii) selecting monoclonal antibodies from step ii) that cross react with at least two different serotypes of KIR2DL polypeptides; and iv) selecting monoclonal antibodies of (iii) that inhibit KIR2DL-mediated inhibition of NK cells.

[0078] The order of steps (iii) and (iv) can be changed. Optionally, the method may further comprise additional steps of making fragments or derivatives of the monoclonal antibody, as disclosed below.

[0079] In another embodiment, the method comprises: i) selecting, from a library or repertoire, a monoclonal antibody or a fragment or derivative thereof that cross reacts with at least two different serotypes of KIR2DL polypeptides; and ii) selecting an antibody from step i) that inhibits KIR2DL-mediated inhibition of NK cells.

[0080] In a preferred aspect, the non-human animal used in these methods, or used in the production of any of the herein-described antibodies, is a mammal, such as a rodent (e.g., mouse, rat, etc.), bovine, porcine, horse, rabbit, goat, sheep, etc.

[0081] Also, any of the herein-described antibodies can be genetically modified or engineered to be human-suitable, e.g., humanized, chimeric, or human antibodies. Methods for humanizing antibodies are well known in the art. Generally, a humanized antibody according to the present invention has one or more amino acid residues introduced into it from the original antibody. These murine or other non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al. (1986) Nature 321:522; Riechmann et al. (1988) Nature 332:323; Verhoeyen et al. (1988) Science 239:1534 (1988)). In some cases, such "humanized" antibodies are chimeric antibodies (Cabilly et al., U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from the original antibody. In practice, humanized antibodies according to this invention are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in the original antibody.

[0082] Another method of making "humanized" monoclonal antibodies is to use a XenoMouse® (Abgenix, Fremont, CA) as the mouse used for immunization. A XenoMouse is a murine host that has had its immunoglobulin genes replaced by functional human immunoglobulin genes. Thus, antibodies produced by this mouse or in hybridomas made from the B cells of this mouse, are already humanized. The XenoMouse is described in United States Patent No. 6,162,963. An analogous method can be achieved using a HuMAb-Mouse™ (Medarex).

[0083] Human antibodies may also be produced according to various other techniques, such as by using for immunization other transgenic animals that have been engineered to express a human antibody repertoire (Jakobovitz et al., Nature 362 (1993) 255), or by selection of ant body repertoires using phage display methods. Such techniques are known to the skilled person and can be implemented starting from monoclonal antibodies as disclosed in the present application.

[0084] The antibodies of the present application may also be derivatized to "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in the original antibody, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly et al., supra; Morrison et al. (1984) Proc. Natl. Acad. Sci. 81:6851).

[0085] The antibody may by polyclonal or, preferably, monoclonal. The antibody may be produced by a hybridoma or by a recombinant cell engineered to express the desired variable and constant domains. The antibody may be a single chain antibody or other antibody derivative retaining the antigen specificity and the lower hinge region or a variant thereof. The antibody may be a polyfunctional antibody, recombinant antibody, humanized antibody, or a fragment or derivative thereof. Said fragment or a derivative thereof is preferably selected from a Fab fragment, a Fab'2 fragment, a CDR and a ScFv. The fragment is an antigen-binding fragment. An antibody which comprise an antibody fragment may also include but are not limited to bispecific antibodies.

COMPOSITION AND ADMINISTRATION

[0086] The application also provides a composition comprising at least one antibody or a fragment thereof that blocks the inhibitory receptor of an NK cell as claimed and a therapeutic antibody, the use of said composition for increasing the efficiency of the therapeutic antibody, for increasing ADCC in a subject treated with a therapeutic antibody, or for treating a subject having a disease, more particularly a disease requiring the depletion of the targeted cells, preferably diseased cells such as virally-infected cells, tumor cells or other pathogenic cells. Preferably, the disease is selected from the group consisting of a cancer, an auto-immune disease, an inflammatory disease, and a viral disease. The disease also concerns a graft rejection, more particularly allograft rejection, and graft versus host disease (GVHD).

[0087] More particularly, the treatment of the disease requires the depletion of the targeted cells, preferably the diseased cells such as virally-infected cells, tumor cells or other pathogenic cells. Preferably, the disease is a cancer, infectious disease or immune disease. More preferably, the disease is selected from the group consisting of a cancer, an auto-immune disease, an inflammatory disease, and a viral disease. The disease also concerns a graft rejection, more particularly allograft rejection, and graft versus host disease (GVHD).

[0088] Said diseases include neoplastic proliferation of hematopoietic cells. Optionally, said diseases are selected from the group consisting of lymphoblastic leukemia, acute or chronic myelogenous leukemia, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, myelodysplastic syndrome, multiple myeloma, and chronic lymphocytic leukemia. Said diseases also include ENT cancers, colorectal cancers, breast cancer, and epithelial cancer. Said diseases include CMV infection and hepatitis B. Said diseases include Crohn disease, rheumatoid arthritis, asthma, psoriasis, multiple sclerosis or diabetes. In particular, any disease listed in the table provided *supra* can be treated.

[0089] Said therapeutic antibody can be bound by CD16, preferably through its Fc region. Preferably, said therapeutic antibody has a human IgG1 or an IgG3 Fc portion, particularly a monoclonal antibody or a fragment thereof, further preferably a human antibody, a humanized antibody, a chimeric antibody, or a fragment thereof, for instance rituximab.

[0090] The antibody binds a common determinant of KIR2DL1, KIR2DL2, and KIR2DL3 human receptors and inhibits KIR2DL1-, KIR2DL2-, and KIR2DL3-mediated inhibition of NK cell cytotoxicity. In a particular aspect, said antibody inhibits the binding of a HLA-C allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2 and KIR2DL3 receptors. In another particular embodiment, this antibody binds to the same epitope as monoclonal antibody DF200 produced by hybridoma DF200. Optionally, this antibody competes with monoclonal antibody DF200 produced by hybridoma DF200 for binding to a KIR receptor at the surface of a human NK cell. In one preferred embodiment, the antibody is monoclonal antibody DF200 produced by hybridoma DF200.

[0091] The composition according to the present disclosure can comprise a combination of several antibodies or a fragment thereof that block different inhibitory receptors of NK cells. Preferably, antibodies or fragments thereof that block inhibitory receptors on NK cells are specific for an inhibitory receptor selected from KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1, KIR3DL2, NKG2A, and NKG2C, and are able to inhibit the related KIR- or NKG2A/C-mediated inhibition of NK cell cytotoxicity. More preferably, the combination of "neutralizing" antibodies is able to inhibit the KIR2DL1-, KIR2DL2-, and KIR2DL3-mediated inhibition of NK cell cytotoxicity. By providing a combination of antibodies, a maximum number of different inhibitory receptors will be blocked in a maximum number of patients. As described below, in a preferred embodiment, a sample of NK cells can be obtained from a patient prior to the application of the present methods, and the responsiveness of the NK cells to different combinations of antibodies, e.g., in the presence of target cells and the therapeutic antibody, can be assessed.

[0092] Compositions of this invention may comprise any pharmaceutically acceptable carrier or excipient, typically buffer, isotonic solutions, aqueous suspension, optionally supplemented with stabilizing agents, preservatives, etc. Typical formulations include a saline solution and, optionally, a protecting or stabilizing molecule, such as a high molecular weight protein (e.g., human serum albumin).

[0093] Kits are also provided comprising any combination of one or more therapeutic antibodies, one or more NK cell potentiating antibodies, and, preferably, instructions for their use.

[0094] According to the methods and compositions of the present disclosure, antibodies or a fragment thereof that block an inhibitory receptor of a NK cell and therapeutic antibodies are administered in an "efficient" or "therapeutically effective" amount.

[0095] The efficient amount of therapeutic antibodies administered to the recipient can be between about 0.1 mg/kg and about 20 mg/kg. The efficient amount of antibody depends, however, on the form of the antibody (whole Ig or fragments), affinity of the mAb, and pharmacokinetics parameter that must be determined for each particular antibody.

[0096] The efficient amount of antibodies or a fragment thereof that blocks the inhibitory receptor of a NK cell administered to the recipient can be between about 0.1 mg/kg and about 20 mg/kg. The efficient amount of antibody depends, however, on the form of the antibody (whole Ig or fragments), affinity of the mAb, and pharmacokinetics parameters that must be determined for each particular antibodies.

[0097] In an important aspect of the disclosure, the use of the present antibodies can allow therapeutic efficacy to be achieved with reduced doses of therapeutic antibodies. The use (e.g., dosage, administration regimen) of therapeutic antibodies can be limited by side effects, e.g., in the case of rituximab, fever, headaches, wheezing, drop in blood pressure, and others. Accordingly, while in many patients a standard dose of the therapeutic antibodies will be administered in conjunction with the herein-described NK cell potentiating antibodies (i.e., the recommended dose in the absence of any other compounds), thereby enhancing the efficacy of the standard dose in patients needing ever greater therapeutic efficacy, in other patients, e.g., those severely affected by side effects, the administration of the present antibodies will allow therapeutic efficacy to be achieved at a reduced dose of therapeutic antibodies, thereby avoiding side effects. In practice, a skilled medical practitioner will be capable of determining the ideal dose and administrative regimen of the therapeutic antibody and the NK cell potentiating antibody for a given patient, e.g., the therapeutic strategy that will be most appropriate in view of the particular needs and overall condition of the patient. Numerous references are available to guide in the determination of proper dosages, for both the therapeutic antibodies and the NK cell potentiating antibodies, e.g., Remington: The Science and Practice of Pharmacy, by Gennaro (2003), ISBN: 0781750253; Goodman and Gilmans The Pharmacological Basis of Therapeutics, by Hardman , Limbird & Gilman (2001), ISBN: 0071354697; Rawlins E. A., editor, "Bentley's Textbook of Pharmaceutics", London: Bailliere, Tindall and Cox, (1977); and others.

[0098] In one aspect, a medical practitioner can gradually lower the amount of the therapeutic antibody given in conjunction with the administration of any of the present NK cell potentiating antibodies, either in terms of dosage or frequency of administration, and monitor the efficacy of the therapeutic antibody, e.g., monitor NK cell activity, monitor the presence of target cells in the patient, monitor various clinical indications, or by any other means, and, in view of the results of the monitoring, adjust the relative concentrations or modes of administration of the therapeutic antibodies and/or NK potentiating antibody to optimize therapeutic efficacy and limitation of side effects.

[0099] In another set of aspects, NK cells will be obtained from the patient prior to the administration of the therapeutic antibody and NK cell potentiating antibodies (and, if appropriate, during the treatment), and assessed to determine the ideal antibody or suite of antibodies to be used for maximum efficacy. For example, the identity of the inhibitory or activating receptors present on the NK cells can be determined, and antibodies administered that specifically targeted the most prominent receptors. Alternatively, the obtained NK cells can be incubated with the therapeutic antibody and target cells, and the ability of one or more antibodies to enhance target cell depletion can be assessed. Whichever one or more antibodies is most effective at enhancing depletion in vitro can then be selected for use in the present treatment methods.

[0100] Suitable doses of the antibodies and/or therapeutic antibodies can also generally be determined in vitro or in animal models, e.g., in vitro by incubating various concentrations of a therapeutic antibody in the presence of target cells, NK cells (preferably human NK cells), optionally other immune cells, and varying concentrations of one or more NK cell potentiating antibodies, and assessing the extent or rate of target cell depletion under the various conditions, using standard assays (e.g., as described in the examples section). Alternatively, varying dosages of the therapeutic antibodies can be given to animal models for diseases treatable with the antibodies (e.g., an animal model for NHL in the case of rituximab), along with varying dosages of the herein-described antibodies, and the efficacy of the antibodies (e.g., as determined by any suitable clinical, cellular, or molecular assay or criterion) in treating the animals can be assessed.

[0101] The composition according to the present disclosure may be injected directly to a subject, typically by intra-venous, intra-peritoneal, intra-arterial, intra-muscular or transdermic route. Several monoclonal antibodies have been shown to be efficient in clinical situations, such as Rituxan (Rituximab) or Xolair (Omalizumab), and similar administration regimens (i.e., formulations and/or doses and/or administration protocols) may be used with the composition of this invention.

[0102] Furthermore, the compositions of this disclosure may further comprise or may be used in combination with other active agents or therapeutic programs, such as chemotherapy or other immunotherapies, either simultaneously or sequentially.

[0103] In certain preferred example, the method of the invention further comprises one or several injections of two or more antibodies that block an inhibitory receptor of a NK cell. Thus, these two or more antibodies can be used in combination. This can serve to cause an even greater augmentation of ADCC and efficacy of therapeutic antibodies, and/or can serve to reduce the dosage of a particular antibody that block an inhibitory receptor of a NK cell. For example, compounds such as IL-2 are known to be toxic at increased doses. For example, a preferred regimen is where said two compounds are (i) a first compound selected from the group consisting of an antibody which blocks an inhibitory KIR receptor, and (ii) a second compound selected from the group consisting of IL-12, IL-15, IL-18 and IL-21. The disclosure, therefore, further provides a method of treatment of a disease in a subject in need thereof comprising: (a) administering to said subject a compound according to the disclosure, an antibody or a fragment thereof, that blocks an inhibitory receptor of a NK cell; (b) administering to said subject a therapeutic antibody; and (c) administering to said subject IL-2. IL2 is available from Research Diagnostics, NJ, RDI-202, or Chiron Corp. (Emeryville, CA).

[0104] The cytokine can be administered according to any suitable administration regimen, and may be administered before, simultaneously and/or after administration of the compound which blocks an inhibitory receptor or stimulates an activating receptor of a NK cell, and before, simultaneously and/or after administration of therapeutic antibody. In a typical example, the cytokine is administered daily for a period of 5-10 days, the cytokine(s) being first injected on the same day as the first injection of the compound which blocks an inhibitory receptor or stimulates an activating receptor of a NK cell. Said method preferably comprises one or two injections/day of cytokine(s) by subcutaneous route.

[0105] The dosage of the cytokine will be chosen depending on the condition of the patient to be treated. In preferred examples, a relatively low dose of cytokine can be used. For example, an effective dose is typically lower than 1 million units/square meters/day of cytokine(s), when the cytokine-containing pharmaceutical composition is used for daily subcutaneous injection. In a preferred example, IL-2 is injected subcutaneously at daily doses below 1 million units/m^2 for 5 to 10 days. Further detail of the use of cytokines is described in International Patent publication WO 2004/056392 and U.S. patent application no. 60/435,344 titled "Pharmaceutical compositions having an effect on the proliferation ofNK cells and a method using the same".

[0106] It will also be appreciated that the therapeutic antibodies and NK cell potentiating antibodies can be coadministered, e.g., co-injected, or can be administered simultaneously but in different formulations, or can be independently administered, e.g. the compound is administered hours, days, or weeks before or after the administration of the antibody.

[0107] Further aspects and advantages of this invention are disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of this application.

EXAMPLES

Example 1 : Generation of a pan KIR2DL antibody.

Purification of PBLs and generation of polyclonal or clonal NK cell lines.

[0108] PBLs were derived from healthy donors by Ficoll Hypaque gradients and depletion of plastic adherent cells. To obtain enriched NK cells, PBLs were incubated with anti-CD3, anti-CD4 and anti-HLA-DR mAbs (30 min at 4°C), followed by goat anti-mouse magnetic beads (Dynal) (30 min at 4°C) and immunomagnetic selection by methods known in the art (Pende et al., 1999). CD3 minus, CD4 minus DR minus cells are cutivated on irradiated feeder cells and 100 U/ml Interleukin 2 (Proleukin, Chiron Corporation) and 1.5 ng/ml Phytohemagglutinin A (Gibco BRL) to obtain polyclonal NK cell populations. NK cells are cloned by limiting dilution and clones of NK cells are characterized by flow cytometry for expression of cell surface receptors.

[0109] The following clones were used in this study:

CP11, CN5 and CN505 are KIR2DL1 positive clones and are stained by EB6 or XA-141 antibodies. CN12 and CP502 are KIR2DL3 positive clones and are stained by GL183 antibody.

Flow cytometry analysis

[0110] mAbs used were produced in the laboratory JT3A (IgG2a, anti CD3), EB6 and GL183 (IgG1 anti KIR2DL1 and KIR2DL3 respectively), XA-141 IgM anti KIR2DL1 (same specificity as compared to EB6, anti CD4 (HP2.6), anti DR (D1.12, IgG2a). Instead of JT3A, HP2.6, and DR1.12, commercially available mAbs of the same specificities can be used for example from Beckman Coulter Inc, Fullerton, CA. EB6 and GL183 are commercially available in Beckman Coulter Inc, Fullerton, CA. XA-141 is not commercially available but EB6 can be used for control reconstitution of lysis as described in (Moretta et al., 1993).

Flow cytometry

[0111] Cells were stained with the appropriate antibodies (30 min at 4°C) followed by PE or FITC conjugated polyclonal anti mouse antibodies (Southern Biotechnology Associates Inc). Samples were analysed by cytofluorometric analysis on a FACSAN apparatus (Becton Dickinson, Mountain View, CA).

Cytotoxicity experiments

[0112] The cytolytic activity of NK clones was assessed by a standard 4hr ^{51}Cr release assay, in which effector NK cells were tested on Cw3 or Cw4 positive cell lines known for their sensitivity to NK cell lysis. All the targets are used at 5000 cells per well in microtitration plate and the Effector on target ratio is indicated in the figures (usually 4 effectors per target cells). The cytolytic assay is performed with or without supernatant of indicated monoclonal antibodies at a ½ dilution. The procedure is essentially the same as described in (Moretta et al., 1993).

Generation of new mAbs

[0113] mAbs have been generated by immunizing 5 week old Balb C mice with activated polyclonal or monoclonal NK cell lines as described in Moretta et al., 1990. After different cell fusions, the mAbs were first selected for their ability to cross react with EB6 and GL183 positive NK cell lines and clones. Positive monoclonal antibodies were further screened for their ability to reconstitute lysis by EB6 positive or GL183 positive NK clones of Cw4 or Cw3 positive targets, respectively.

DF200, a novel monoclonal antibody against a common determinant ofKIR2DL human NK receptors

[0114] One of the monoclonal antibodies, the DF200 mAb, was found to react with various members of the KIR family including KIR2DL1 and KIR2DL2/3. Regarding the staining of NK cells with DF200mAb both KIR2DL1+ and KIR2DL2/3+ cells were stained brightly (figure 1).

[0115] NK clones expressing one or another (or even both) of these HLAclass I-specific inhibitory receptors were used as effectors cells against target cells expressing one or more HLA-C alleles. As expected, KIR2DL1+ NK clones displayed little if any cytolytic activity against target cells expressing HLA-Cw4 and KIR2DL3+ NK clones displayed little or no activity on Cw3 positive targets. However, in the presence of DF200mAb (used to mask their KIR2DL receptors) NK clones became unable to recognize their HLA-C ligands and displayed strong cytolytic activity on Cw3 or Cw4 targets.

[0116] For example, the C1R cell line (CW4+ EBV cell line, ATCC n°CRL 1993) was not killed by KIR2DL1+ NK clones (CN5/CN505), but the inhibition could be efficiently reverted by the use of either DF200 or a conventional anti KIR2DL1 mAb. On the other hand, NK clones expressing the KIR2DL2/3+ KIR2DL1- phenotype (CN12) efficiently killed C1R and this killing was unaffected by the DF200mAb (Figure 2). Similar results can be obtained with KIR2DL2- or KIR2DL3-positive NK clones on Cw3 positive targets.

Biacore analysis of DF200 mAb/ KIR2DL1 and DF200 mAb/ KIR2DL3 interactions.

Materials and Methods

[0117] Production and purification of recombinant proteins. The KIR2DL1 and KIR2DL3 recombinant proteins were produced in *E. coli.* cDNA encoding the entire extracellular domain of KIR2DL1 and KIR2DL3 were amplified by PCR from pCDM8 clone 47.11 vector (Biassoni et al, 1993) and RSVS(gpt)183 clone 6 vector (Wagtman et al, 1995) respectively, using the following primers:

Sense: 5'-GGAATTCCAGGAGGAATTTAAAATGCATGAGGGAGTCCACAG-3'

Anti-sense: 5'-CCCAAGCTTGGGTTATGTGACAGAAACAAGCAGTGG-3'

[0118] They were cloned into the pML1 expression vector in frame with a sequence encoding a biotinylation signal (Saulquin et al, 2003).

[0119] Protein expression was performed into the BL21(DE3) bacterial strain (Invitrogen). Transfected bacteria were grown to OD_{600}=0.6 at 37°C in medium supplemented with ampicillin (100 µg/ml) and induced with 1 mM IPTG.

[0120] Proteins were recovered from inclusion bodies under denaturing conditions (8 M urea). Refolding of the recombinant proteins was performed in Tris 20 mM, pH 7.8, NaCl 150 mM buffer containing L-arginine (400 mM, Sigma) and β-mercaptoethanol (1 mM), at RT, by decreasing the urea concentration in a six step dialysis (4, 3, 2, 1, 0.5 and 0 M urea, respectively). Reduced and oxidized glutathion (5 mM and 0.5 mM, respectively, Sigma) were added during the 0.5 and 0 M urea dialysis steps. Finally, the proteins were dialyzed extensively against Tris 10 mM, pH 7.5, NaCl 150 mM buffer. Soluble refolded proteins were concentrated and then purified on a Superdex 200 size-exclusion column (Pharmacia; AKTA system).

[0121] Biacore analysis. Surface plasmon resonance measurements were performed on a Biacore apparatus (Biacore). In all Biacore experiments HBS buffer supplemented with 0.05% surfactant P20 served as running buffer.

[0122] Protein immobilization. Recombinant KIR2DL1 and KIR2DL3 proteins were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5 (Biacore). The sensor chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimidehydrochloride and N-hydroxysuccinimide, Biacore). Proteins, in coupling buffer (10 mM acetate pH 4.5) were injected. Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH8 (Biacore).

[0123] Affinity measurements. For kinetic measurements, various concentrations of the soluble antibody (10^{-7} to $4x10^{-10}$ M) were applied onto the immobilized sample. Measurements were performed at 20 µl/min continuous flow rate. For each cycle, the surface of the sensor chip was regenerated by 5 µl injection of 10 mM NaOH pH 11.

[0124] The BIAlogue Kinetics Evaluation program (BIAevaluation 3.1, Biacore) was used for data analysis.

Results

[0125] BIAcore analysis of DF200 mAb binding to immobilized KIR2DL1 and KIR2DL3.

	KD (10^{-9} M)
KIR2DL1	10.9+/-3.8
KIR2DL3	2.0 +/- 1.9

KD: Dissociation constant.

[0126] The soluble analyte (40 µl at various concentrations) was injected at a flow rate of 20 µl/min in HBS buffer, on a dextran layers containing 500 or 540 reflectance units (RU), and 1000 or 700 RU of KIR2DL1 and KIR2DL3 respectively. Data are representative of 6 independent experiments.

Example 2 : Enhancement of ADCC by using a combination of Rituxan and anti KIR mAb.

[0127] Preparation of human NK clones. Blood mononuclear cells depleted of T cells by negative anti-CD3 immuno-magnetic selection (Miltenyi) are plated under limiting-dilution conditions, activated with phytohemagglutinin (PHA) (Biochrom KG, Berlin, Germany), and cultured with interleukin (IL)-2 (Chiron B.V., Amsterdam, Netherlands) and irradiated feeder cells. Cloning efficiencies are equivalent in all donors and range between 1 in 5 and 1 in 10 plated NK cells. Cloned NK cells are screened for alloreactivity by standard ^{51}Cr release cytotoxicity against Epstein-Barr virus-transformed B lymphoblastoid cell lines of known HLA type at an effector to target ratio of 10:1. Clones exhibiting ≥ 30% lysis were scored as alloreactive. As a rule, clones either exhibit < 5% or > 40% lysis.

Enhancement of ADCC mediated by Rituxan by a KIR2DL1 positive NK cell clone

[0128] The cytolytic activity of NK clone is assessed by a standard 4hr ^{51}Cr release assay, in which effector NK cells were tested on Cw4 or Cw3 positive EBV cell lines (CD20 positive), known for their sensitivity to NK cell lysis. All the targets are used at 5000 cells per well in microtitration plate and the Effector (NK cell clone) on target ratio is indicated in the Figure 3. In certain experiments, the therapeutic chimeric anti CD20 rituximab (Rituxan, Idec) is added at 5 µg/ml is added to the effector target mixture. In certain experiments, the EB6 antibody (anti KIR2DL1) at 10 µg/ml is added to the effector target mixture.

[0129] This experiment showed that Rituxan alone mediates essentially no ADCC by the KIR2DL1 positive NK clone on Cw4 positive target. ADCC of KIR2DL1 positive clone is greatly enhanced in the presence of anti KIR2DL1 antibody.

Example 3 - Enhancement of ADCC mediated by Campath by a KIR2DL1 positive NK cell clone

[0130] In a similar experiment to that described in Example 2, autologous Cw4+ PHA blasts were incubated in the presence of NK cells plus alumtuzumab (Campath, Berlex), the EB6 antibody (at 100ug/ml), or Campath and EB6. The results, shown in Figure 4, show that the presence of EB6 dramatically enhances the ability of the NK cells to deplete the autologous cells: approximately 4% of the target cells were lysed in the presence of Campath alone, whereas more than 30% of the cells were lysed in the presence of Campath plus EB6.

REFERENCES

[0131] Biassoni R, Verdiani S, Cambiaggi A, Romeo PH, Ferrini S, Moretta L. (1993) Human CD3-CD16+ natural killer cells express the hGATA-3 T cell transcription factor and an unrearranged 2.3-kb TcR delta transcript. Eur J Immunol.;23(5):1083-7.

[0132] Karre K, Ljunggren HG, Piontek G, Kiessling R, (1986) Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy.Nature 319:675-8.

[0133] Lanier LL (1998) NK cell receptors. Annu Rev Immunol 16:359-93.

[0134] Moretta, A., Bottino, C., Pende, D., Tripodi, G., Tambussi, G., Viale, O., Orengo, A., Barbaresi, M., Merli, A., Ciccone, E., and et al. (1990) Identification of four subsets of human CD3-CD16+ natural killer (NK) cells by the expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognition. J Exp Med 172:1589-1598.

[0135] Moretta, A., Vitale, M., Bottino, C., Orengo, A. M., Morelli, L., Augugliaro, R., Barbaresi, M., Ciccone, E., and Moretta, L. (1993) P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med 178:597-604.

[0136] Moretta A, Moretta L (1997) HLA class I specific inhibitory receptors. Curr Opin Immunol 9:694-701.

[0137] Ohlen C, Kling G, Hoglund P, Hansson M, Scangos G, Bieberich C, Jay G, Karre K (1989) Prevention of allogeneic bone marrow graft rejection by H-2 transgene in donor mice. Science 246:666-8.

[0138] Pende, D., Parolini, S., Pessino, A., Sivori, S., Augugliaro, R., Morelli, L., Marcenaro, E., Accame, L., Malaspina, A., Biassoni, R., et al. (1999) Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. J Exp Med 190:1505-1516.

[0139] Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W. D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., et al. (2002) Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 295:2097-2100.

[0140] Saulquin X, Gastinel LN, Vivier E. (2003) Crystal structure of the human natural killer cell activating receptor KIR2DS2 (CD158j) J Exp Med. 197(7):933-8.

[0141] Valiante NM, Lienert K, Shilling HG, Smits BJ, Parham P (1997) Killer cell receptors: keeping pace with MHC class I evolution. Immunol Rev 155:155-64.

[0142] Wagtmann N, Biassoni R, Cantoni C, Verdiani S, Malnati MS, Vitale M, Bottino C, Moretta L, Moretta A, Long EO. (1995) Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. Immunity. 2(5):439-49.

[0143] Ward ES, Güssow D, Griffiths AD, Jones PT, Winter G. (1989) Binding activities of a repertoire of single immunoglobulin variable domains secreted from Eschericia coli. Nature 341:544-546.

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- WO2004016750A **[0050] [0051]**
- US2003025399W **[0050]**
- WO99158572A **[0050]**
- WO99151642A **[0050]**
- WO98123289A **[0050]**
- WO89107142A **[0050]**
- WO88107089A **[0050]**
- US5843597A **[0050]**
- US5642821A **[0050]**

- US6194351B [0052]
- WO00142072A [0052]
- US6194551B [0052]
- US20020106324A [0055]
- WO0250122A [0055]
- WO2005003172A [0063] [0064]
- US4816567A [0081]
- US6162963A [0082]
- WO2004056392A [0105]
- US435344P [0105]

Non-patent literature cited in the description

- **SIVORI et al.**J. Exp. Med., 1997, vol. 186, 1129-1136 [0037] [0069] [0069]
- **VITALE et al.**J. Exp. Med., 1998, vol. 187, 2065-2072 [0037] [0069]
- **PESSINO et al.**J. Exp. Med., 1998, vol. 188, 953-960 [0037] [0069]
- **NERI et al.**Clin. Diag. Lab. Immun., 2001, vol. 8, 1131-1135 [0037] [0069]
- **KUFER P et al.**TRENDS in Biotechnology, 2004, vol. 22, 5238-244 [0048]
- **BAEUERLE et al.**Current Opinion in Molecular Therapeutics, 2003, vol. 5, 4413-419 [0048]
- **CARRINGTONNORMAN**The KIR Gene Cluster, 2003, [0055]
- **PENDE et al.**J. Exp. Med., 1999, vol. 190, 1505-1516 [0069]
- **HARLOW et al.**Antibodies: A laboratory ManualCSH Press19880000 [0074]
- **GODING**Monoclonal Antibodies: Principles and PracticeAcademic Press19860000 [0074]
- **JONES et al.**Nature, 1986, vol. 321, 522- [0081]
- **RIECHMANN et al.**Nature, 1988, vol. 332, 323- [0081]
- **VERHOEYEN et al.**Science, 1988, vol. 239, 1534- [0081]
- **JAKOBOVITZ et al.**Nature, 1993, vol. 362, 255- [0083]
- **MORRISON et al.**Proc. Natl. Acad. Sci., 1984, vol. 81, 6851- [0084]
- **GENNARO**Remington: The Science and Practice of Pharmacy, 2003, 0781750253 [0097]
- **GOODMANGILMANS**The Pharmacological Basis of Therapeutics20010000 [0097]
- **BAILLIERETINDALLCOX**Bentley's Textbook of Pharmaceutics19770000 [0097]
- **BIASSONI RVERDIANI SCAMBIAGGI AROMEO PHFERRINI SMORETTA L**Human CD3-CD16+ natural killer cells express the hGATA-3 T cell transcription factor and an unrearranged 2.3-kb TcR delta transcriptEur J Immunol., 1993, vol. 23, 51083-7 [0131]
- **KARRE KLJUNGGREN HGPIONTEK GKIESSLING R**Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategyNature, 1986, vol. 319, 675-8 [0132]
- **LANIER LL**NK cell receptorsAnnu Rev Immunol, 1998, vol. 16, 359-93 [0133]
- **MORETTA, A.BOTTINO, C.PENDE, D.TRIPODI, G.TAMBUSSI, G.VIALE, O.ORENGO, A.BARBARESI, M.MERLI, A.CICCONE, E. et al.**Identification of four subsets of human CD3-CD16+ natural killer (NK) cells by the expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognitionJ Exp Med, 1990, vol. 172, 1589-1598 [0134]
- **MORETTA, A.VITALE, M.BOTTINO, C.ORENGO, A. M.MORELLI, L.AUGUGLIARO, R.BARBARESI, M.CICCONE, E.MORETTA, L.**P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificitiesJ Exp Med, 1993, vol. 178, 597-604 [0135]
- **MORETTA AMORETTA L**HLA class I specific inhibitory receptorsCurr Opin Immunol, 1997, vol. 9, 694-701 [0136]
- **OHLEN CKLING GHOGLUND PHANSSON MSCANGOS GBIEBERICH CJAY GKARRE K**Prevention of allogeneic bone marrow graft rejection by H-2 transgene in donor miceScience, 1989, vol. 246, 666-8 [0137]
- **PENDE, D.PAROLINI, S.PESSINO, A.SIVORI, S.AUGUGLIARO, R.MORELLI, L.MARCENARO, E.ACCAME, L.MALASPINA, A.BIASSONI, R. et al.**Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cellsJ Exp Med, 1999, vol. 190, 1505-1516 [0138]
- **RUGGERI, L.CAPANNI, M.URBANI, E.PERRUCCIO, K.SHLOMCHIK, W. D.TOSTI, A.POSATI, S.ROGAIA, D.FRASSONI, F.AVERSA, F. et al.** Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplantsScience, 2002, vol. 295, 2097-2100 [0139]

- **SAULQUIN XGASTINEL LNVIVIER E**Crystal structure of the human natural killer cell activating receptor KIR2DS2 (CD158j)J Exp Med., 2003, vol. 197, 7933-8 [0140]
- **VALIANTE NM LIENERT KSHILLING HGSMITS BJPARHAM P**Killer cell receptors: keeping pace with MHC class I evolutionImmunol Rev, 1997, vol. 155, 155-64 [0141]
- **WAGTMANN NBIASSONI RCANTONI CVERDIANI SMALNATI MSVITALE M BOTTINO CMORETTA LMORETTA A LONG EO**Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domainsImmunity, 1995, vol. 2, 5439-49 [0142]
- **WARD ESGÜSSOW DGRIFFITHS ADJONES PTWINTER G**Binding activities of a repertoire of single immunoglobulin variable domains secreted from Eschericia coliNature, 1989, vol. 341, 544-546 [0143]

FREMGANGSMÅDER OG SAMMENSÆTNINGER TIL ØGNING AF EFFEKTIVITETEN AF TERAPEUTISKE ANTISTOFFER VED ANVENDELSE AF FORBINDELSER, DER POTENSERER NK-CELLER

PATENTKRAV

1. Anvendelse af (a) et anti-NK-cellereceptor (NKR)-antistof, der bindes til og hæmmer aktiviteten af en hæmmende KIR2DL-receptor af en NK-celle, og (b) et terapeutisk antistof, der kan bindes ved hjælp af CD16 gennem dets Fc-region, og som depleterer målceller ved hjælp af ADCC, til fremstilling af et lægemiddel til behandling af en sygdom, hvor anti-NKR-antistoffet øger effektiviteten af det terapeutiske antistof ved forstærkning af ADCC, hvor anti-NKR-antistoffet bindes til en almindelig determinant af humane KIR2DL1-, KIR2DL2- og KIR2DL3-receptorer og hæmmer KIR2DL1-, KIR2DL2- og KIR2DL3-medieret inhibition af NK-celle-cytotoksicitet.

2. Anvendelse ifølge krav 1, hvor det terapeutiske antistof har en human IgG1 eller en IgG3 Fc-del.

3. Anvendelse ifølge kravene 1 eller 2, hvor anti-NKR-antistoffet omfatter et antigenbindingsfragment deraf.

4. Anvendelse ifølge kravene 1 til 3, hvor det terapeutiske antistof er et monoklonalt antistof eller omfatter et antigenbindingsfragment deraf.

5. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor det terapeutiske antistof ikke er konjugeret med en radioaktiv eller toksisk del.

6. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor anti-NKR-antistoffet er et humant, humaniseret eller kimærisk antistof eller omfatter et antigenbindingsfragment deraf.

7. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor det terapeutiske antistof er et humant, humaniseret eller kimærisk antistof eller omfatter et antigenbindingsfragment deraf.

8. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor det terapeutiske antistof er rituximab eller alemtuzumab.

9. Anvendelse ifølge krav 8, hvor det terapeutiske antistof er rituximab og administreres i en dosis på mindre end 375 mg/m^2 pr. uge.

10. Anvendelse ifølge krav 8, hvor det terapeutiske antistof er alemtuzumab og administreres i en dosis på mindre end 90 mg pr. uge.

11. Anvendelse ifølge et hvilket som helst af kravene 1 til 10, hvor anti-NKR-antistoffet bindes til det samme epitop som det monoklonale antistof DF200 fremstillet ved hybridom DF200, der er deponeret under registreringsnummer CNCM I-3224.

12. Anvendelse ifølge et hvilket som helst af kravene 1 til 11, hvor anti-NKR-antistoffet konkurrerer med det monoklonale antistof DF200 fremstillet ved hybridom DF200, der er deponeret under registreringsnummer CNCM I-3224, til binding til en KIR-receptor på overfladen af en human NK-celle.

13. Anvendelse ifølge et hvilket som helst af kravene 1 til 11, hvor anti-NKR-antistoffet er det monoklonale antistof DF200 fremstillet ved hybridom DF200, der er deponeret under registreringsnummer CNCM I-3224, eller et fragment eller derivat deraf.

14. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor det terapeutiske antistof og anti-NKR-antistoffet administreres samtidigt til individet.

15. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor anti-NKR-antistoffet administreres til individet inden for én uge efter administration af det terapeutiske antistof.

16. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvor sygdommen er en cancer, eller en infektions- eller immunsygdom.

17. Anvendelse ifølge et hvilket som helst af de ovenstående krav, hvilken anvendelse endvidere omfatter vurdering af aktiviteten eller antallet af NK-celler i individet forud for eller efter administration af anti-NKR-antistoffet.

18. Anvendelse ifølge krav 17, hvor vurderingen af aktiviteten eller antallet af NK-celler involverer

i) inkubation af NK-celler, der er opnået fra individet forud for administration i nærvær af den ene eller flere målceller, der er genkendt af det terapeutiske antistof, i nærvær eller fravær af anti-NKR-antistoffet; og

ii) vurdering af effekten af anti-NKR-antistoffet på evnen for NK-cellerne til at depletere målcellerne;

hvor en påvisning af, at anti-NKR-antistoffet øger evnen for NK-cellerne til at depletere målcellerne indikerer, at anti-NKR-antistoffet er egnet til anvendelse i fremgangsmåden og fremgangsmåden er egnet til anvendelse til individet.

19. Farmaceutisk sammensætning, der omfatter:

(a) et terapeutisk antistof, der kan bindes ved hjælp af CD16 gennem dets Fc-region, og som depleterer målceller ved hjælp af ADCC, (b) et anti-NK-cellereceptor (NKR)-antistof, der bindes til og hæmmer aktiviteten af en hæmmende KIR2DL-receptor af en NK-celle, og (c) en farmaceutisk acceptabel bærer, hvor anti-NKR-antistoffet bindes til en almindelig determinant af humane KIR2DL1-, KIR2DL2- og KIR2DL3-receptorer og hæmmer KIR2DL1-, KIR2DL2-, og KIR2DL3-medieret inhibition af NK-celle-cytotoksicitet.

20. Sammensætning ifølge krav 19, hvor det terapeutiske antistof har en human eller ikke-human primat IgG1 eller IgG3 Fc-del.

21. Sammensætning ifølge kravene 19 eller 20, hvor anti-NKR-antistoffet omfatter et antigenbindingsfragment deraf.

22. Sammensætning ifølge kravene 19 eller 20, hvor anti-NKR-antistoffet er et monoklonalt antistof eller omfatter et antigenbindingsfragment deraf.

23. Sammensætning ifølge kravene 19 til 22, hvor anti-NKR-antistoffet er et humant, humaniseret eller kimærisk antistof eller omfatter et antigenbindingsfragment deraf.

24. Sammensætning ifølge kravene 19 til 23, hvor det terapeutiske antistof er et monoklonalt antistof eller omfatter et antigenbindingsfragment deraf.

25. Sammensætning ifølge krav 24, hvor det terapeutiske antistof er et humant, humaniseret eller kimærisk antistof eller omfatter et antigenbindingsfragment deraf.

26. Sammensætning ifølge kravene 24 eller 25, hvor antistoffet ikke er konjugeret med en radioaktiv eller toksisk del.

27. Sammensætning ifølge kravene 19 til 26, hvor det terapeutiske antistof er rituximab eller alemtuzumab.

28. Sammensætning ifølge krav 19, hvor anti-NKR-antistoffet bindes til det samme epitop som det monoklonale antistof DF200 fremstillet ved hybridom DF200, der er deponeret under registreringsnummer CNCM I-3224.

29. Sammensætning ifølge krav 19, hvor anti-NKR-antistoffet konkurrerer med det monoklonale antistof DF200 fremstillet ved hybridom DF200, der er deponeret under registreringsnummer CNCM I-3224, til binding til en KIR-receptor på overfladen af en human NK-celle.

30. Sammensætning ifølge krav 19, hvor anti-NKR-antistoffet er det monoklonale antistof DF200 fremstillet ved hybridom DF200, deponeret under registreringsnummer CNCM I-3224, eller et fragment eller derivat deraf.

31. Anvendelse af et anti-NK-cellereceptor (NKR)-antistof, der blokerer for en hæmmende KIR2DL-receptor af en NK-celle, hvor anti-NKR-antistoffet bindes til en almindelig determinant af humane KIR2DL1-, KIR2DL2- og KIR2DL3-receptorer og hæmmer KIR2DL1-, KIR2DL2- og KIR2DL3-medieret inhibition af NK-celle-cytotoksicitet til fremstilling af et lægemiddel til øgning af effektiviteten af en behandling, der involverer administration til et individ af et terapeutisk antistof, der kan bindes ved hjælp af CD16 gennem dets Fc-region, og som depleterer målceller hos individet ved hjælp af ADCC, hvor det terapeutiske antistof administreres til individet forud for, samtidigt med, eller efter en terapeutisk effektiv mængde af anti-NKR-antistoffet, og hvor anti-NKR-antistoffet øger effektiviteten af behandlingen ved forstærkning af ADCC hos individet.

DRAWINGS

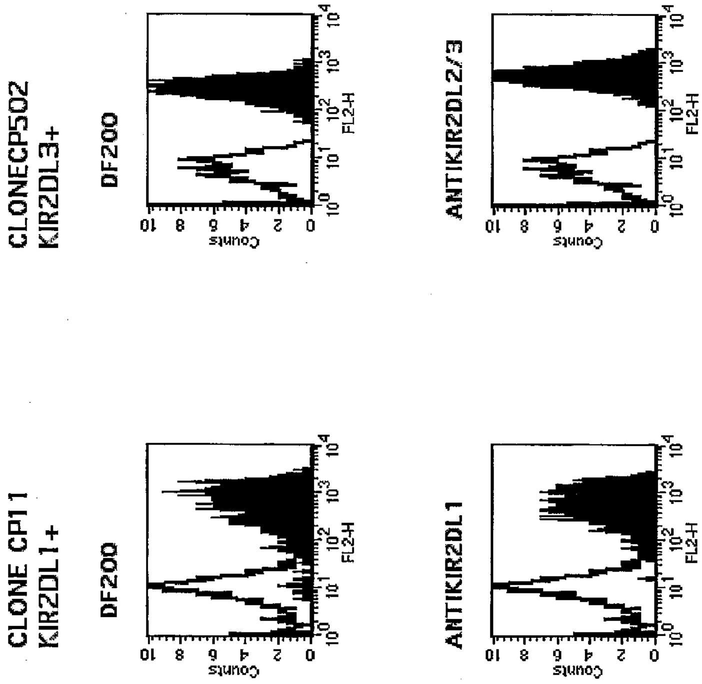

Figure 1

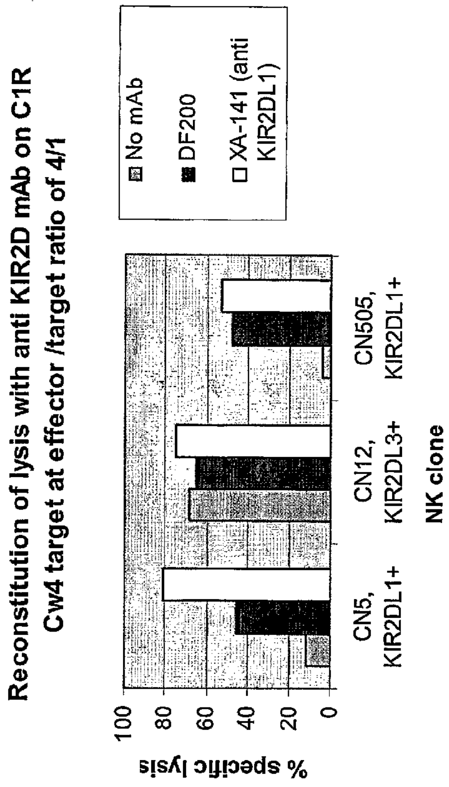
Reconstitution of lysis with anti KIR2D mAb on C1R Cw4 target at effector /target ratio of 4/1
No mAb
DF200
XA-141 (anti KIR2DL1)
% specific lysis
100
80
60
40
20
0
CN5, KIR2DL1+
CN12, KIR2DL3+
CN505, KIR2DL1+
NK clone

Figure 2

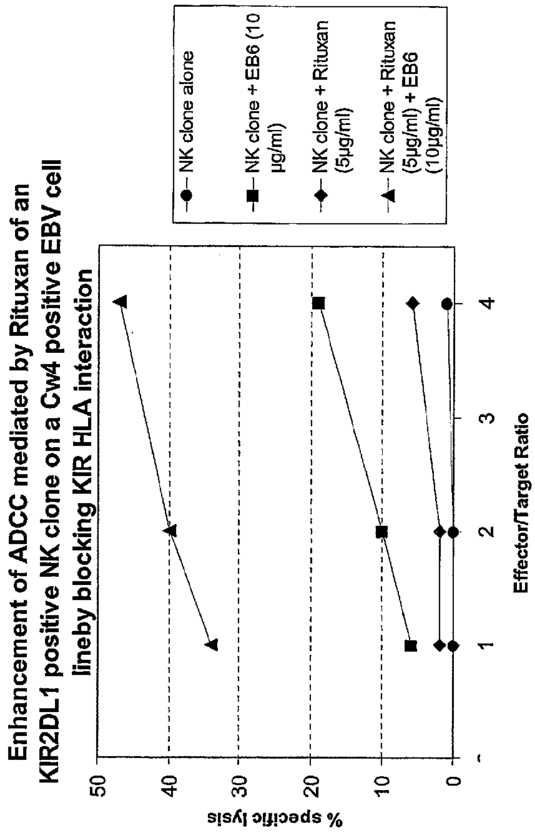
Enhancement of ADCC mediated by Rituxan of an KIR2DL1 positive NK clone on a Cw4 positive EBV cell lineby blocking KIR HLA interaction
% specific lysis
50
40
30
20
10
0
1
2
3
4
Effector/Target Ratio
NK clone alone
NK clone + EB6 (10 µg/ml)
NK clone + Rituxan (5µg/ml)
NK clone + Rituxan (5µg/ml) + EB6 (10µg/ml)

Figure 3

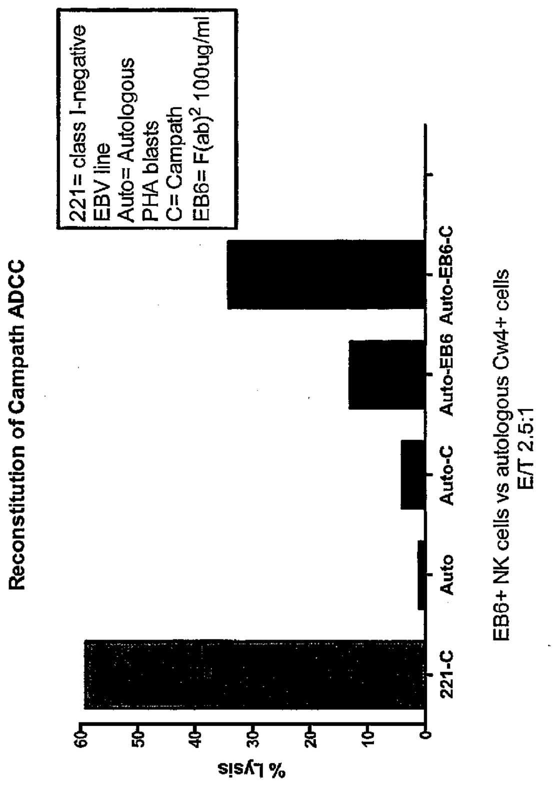
Reconstitution of Campath ADCC
221= class I-negative EBV line
Auto= Autologous PHA blasts
C= Campath
EB6= F(ab)2 100ug/ml
% Lysis
0
10
20
30
40
50
60
221-C
Auto
Auto-C
Auto-EB6
Auto-EB6-C
EB6+ NK cells vs autologous Cw4+ cells
E/T 2.5:1

Figure 4